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# *In vitro* and *In vivo* evaluation for anti-diabetic activity of *Eupatorium triplinerve* vahl in streptozotocin induced diabetic model

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**Abstract**
Diabetes mellitus, characterized by hyperglycaemia due to insulin production deficiency, affects macromolecule metabolism and blood sugar regulation. It has multiple causes, including genetics and lifestyle, and can lead to severe complications like nerve and organ damage. Over 537 million adults worldwide are affected. Flavonoids, polyphenolic compounds in plant-based foods, have various bioactivities, including anti-diabetic effects. The present study investigates the anti-diabetic potential of methanolic extract of *Eupatorium triplinerve* Vahl (MEETV) at doses of 200, 400, and 800 mg/kg body weight. Phytochemical screening of MEETV revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, tannins, and phenolic compounds, which possess antioxidant properties. The study utilized streptozotocin to induce diabetes in animals, with elevated glucose levels and weight as indicators. Serum parameters such as albumin, creatinine, total protein, uric acid, and urea were measured to assess anti-diabetic activity. Results indicated that MEETV significantly reduced glucose levels and exhibited notable anti-diabetic effects compared to control groups.

**Keywords:** Diabetes mellitus, *Eupatorium triplinerve* Vahl, streptozotocin-induced diabetes, Anti-oxidant activity, anti-diabetic activity

## Introduction

Diabetes mellitus is a medical condition of hyperglycemia either during fasting or after devouring food, which is characterized by a deficiency in insulin production [1]. It is a macromolecule metabolism condition that affects the body's capacity to regulate blood sugar levels through endocrine responses [2]. Although pancreatic beta cells and insulin play a key role in diabetes, the causes of hyperglycemia vary significantly. Diabetes has multiple causes, including genetics, environmental circumstances and lifestyle decisions. Some types of diabetes are caused by insulin insufficiency or a genetic abnormality, while others are due to insulin resistance [3]. The prolonged duration of this condition can cause polygenic illness and harm nerves, eyes, kidneys and other organs failure and dysfunction [4]. Diabetes mellitus is the most common endocrine illness, affecting over 537 million (10.5%) in undiagnosed individuals those who aged between 20-79 years worldwide, which make up 10.5% of global population [5, 6]. Streptozotocin (STZ) is a nitrosourea analogue which is well recognized to induce diabetes by destruction of pancreatic beta cells and it causes DNA damage. It causes DNA alkylation which leads to fragmentation of DNA in beta cells Vitamin B3 (niacin) comes in the amide form as nicotinamide. Rats are administered nicotinamide to help partly shield insulin-secreting cells from streptozotocin. Through GLUT2, STZ penetrate beta cells and damage DNA, which is then repaired by PAPR-1. Over activity of this enzyme can cause insulin-secreting cells to necrotize by depleting intracellular NAD+ and ATP. In cells exposed to STZ, nicotinamide inhibits the enzyme that depletes NAD+ and ATP. Moreover, nicotinamide increases intracellular NAD+ levels by acting as a precursor to NAD+ [10].

*Eupatorium triplinerve vahl* widespread to South America, namely Brazil's Amazonian region. Also discovered there are the Mascarene Island, Hawaii, India & Vietnams. Plant: this species of plant is a beautiful tall perennials herbs that can reach a height of one meter & semi woody towards its bottom. Leaves: The leaves of the plant 4.5-10.5cm in length & 0.8-

1.7 cm in width are lance shaped, fragrant, silky smooth, simple, reverse, sub-sessile, three nerved, acuminated, shiny. stems: the stems appear in brownish red flowers, Cultivation: the seeds are used to grow the plant, & it is collected in the time of summer. chemical constituents: Hespertin, glyciten [7], 4-hydroxyph, azelaic acid, gentisicacid, salicylic acid, ursolicacid, linoleic acid, luteolin, suberic acid etc. A broad body purifying analysis is included by ayapana. All the body organs are cleansed & the liver function is restored. *Eupatorium triplinerve Vahl* has medicinal qualities that can be used to treat colds, coughs, & fever effectively [8]. It has antibacterial & anti-inflammatory properties, are part of the plant defence against abiotic & biotic stressors [9].

The main aim of this research is to evaluate the anti-oxidant and anti-diabetic potential in the selected plant extract using various *in-vitro* and *in-vivo* methods. As this can lead to prove the potentiality of the plant for certain anti-diabetic activity and can be used as herbal medicine.

## Materials and Methods

### Plant Collection and Authentication

*Eupatorium triplinerve Vahl* was obtained from local sources in Tirupati, Andhrapadesh. The plant was authenticated by Dr. K. Madhav Chetty, M.Sc., M.ED., M.Phil., Ph.D., PG., DPD. Plant taxonomist, Assistant Professor Department of Botany Sri Venkateshwara University-Tirupati.

### Extraction by Maceration

To weigh the 25grams of coarse powder of *Eupatorium triplinerve* Vahl were extracted with polar solvents like 250ml of methanol, ethyl acetate, petroleum ether in 3 different beakers. Kept for a side at ambient temperature for a while seven days & stir every day at least twice a day. Cover the beaker with aluminium foil for prevention of evaporation of solvents from beaker this process is known as maceration. At 8th day the solvents are filtered by using Whatman filter paper. the filtrate solvents are put on an electronic warming device maintain 60 degree C temperature or kept for shade drying at room temperature for 2-4 days during this time evaporation of solvent takes place & phytochemical constituents are remains in the extract container finally extract was kept in a sealed container [11].

### Qualitative Phytochemical Screening

Numerous phytoconstituents, such as carbohydrates, tannins, flavonoids, alkaloids, and others, were analysed in the MEETV.

### HRLC-MS analysis

Identification of Phytoconstituent the plant extract was sent to the IT MUMMBAI Laboratory for the analysis of compounds identification by using High-resolution liquid chromatography-mass spectroscopy technique.

### *In vitro* Antioxidant Activities

A single anti-oxidant test model may not be used to draw conclusions about anti-oxidant activity. Additionally, a number of *in vitro* test protocols are really used to assess the antioxidant activity of the target sample. Anti-oxidant testing procedures also vary in various ways, which is a further factor. Consequently, it is challenging to completely comparing a single model to another.

### Ferric reducing anti-oxidant power activity

Different concentration Of MEETV (1-500 µg/ml). & ascorbic as standard were combined with 2.5 ml of 200 mM, phosphate buffer at pH 6.6 and 2.5 ml of 1% potassium ferric cyanide, 2.5 ml of 10% TCA was added, and the mixture was incubated at 50 degrees C for 20 mins, then test tubes were centrifuged at 1000 rpm. 5 ml distilled water & 1ml of 0.1% ferric chloride were added to 5 ml of supernatant liquid at 700 nm, the absorption was tested [12].

### Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (40 mM). was prepared in addition to phosphate buffer (50 Mm). At PH-7.4. The plant extracts are 15-50 micrograms/mL, in di-ionized water & the different concentrations are added to hydrogen peroxide & and kept for a side up to 10 minutes then absorbance at 230 nm by using a UV spectrophotometer against a blank solution which contains phosphate buffer only.

The percentage of hydrogen peroxide scavenging is calculated by using the formula [13].

$$\% \ H_2O_2 \text{ scavenging activity} = \frac{\text{control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

### *In vitro* Anti-Diabetic Activity

### Alpha amylase assay

After pre-incubating 1 ml of MEETV with alpha-amylase 1 U/ML for 30 minutes, then 1mL 1% W/V of the starch solution was added. The solution was then incubated for an additional 10 minutes at room temperature. Add 1 mL Dinitro salicylic acid reagent (12 grams of sodium potassium tartrate tetrahydrate in 8 mL of 2 molar Sodium hydroxide & 96 mM 3, 5-Dinitro salicylic acid). & the mixture's warmed in a pot of boiling water for 5-10 minutes. Equal amount of sodium phosphate buffer-20 mM with sodium chloride 6.7 mM at PH 6.9 the solution is considered as blank. Absorbance was determined at 540 nm using UV spectrophotometer [14].

$$\% \text{ Inhibition of } \alpha - \text{Amylase} = \frac{\text{Absorbance of control} - \text{Abosrbance of sample}}{\text{Abosrbance of control}} \times 100$$

### Statistical Analysis

The data are shown as mean +/- SEM, and each test was run in triplicate. The statistical analysis was carried out with GraphPad Prism 6. The Pearson correlation test was employed to analyze the relationship between the means. For antioxidant experiments, the correlation was determined using the $IC_{50}$ value. For anti-diabetic test, the $IC_{50}$ value was used to determine statistical significance. Analysis of variance was used to examine the statistical significance of the assay means (ANOVA).

### Experimental Animals

The animals were kept in standard lab conditions, which included a 14/10 h light/dark cycle, 25 ±2 °C, and 50±5% humidity. They were fed regular rat pellets and allowed unlimited access to water. Before the trial, all the rats were given 14 days to get used to the laboratory environment. The Institutional Animal Ethics Committee (IAEC) of CMR College of Pharmacy, located in Kandlakoya village, Medchal Road, Hyderabad, Telangana, reviewed the experimental methods and procedures employed in this

study to ensure that they complied with IAEC guidelines (CPCSEA/1657/IAEC/CMRCP/COL-22/108).

## Experimental Design

### Preparation of STZ dose

Preparation of acetate buffer: weight about anhydrous sodium acetate (0.82 g) is dissolved in water, and then distilled water is added to bring the volume up to 50 ml (solution A). Measure out approximately 0.7775 ml of glacial acetic acid, then add distilled water to bring the amount up to 50 ml (Solution B).

Mix 9.75 ml of solution A with 15.25 ml of solution B, add distilled water to make the volume equal 50 ml, and set the pH to 4.5 by adding NaCl to the above mixture (0.45 g of NaCl is added approximately). To the above buffer STZ is added [15].

### Induction of diabetic mellitus

STZ (50 mg/kg) is administered as a single dosage through *I.P* route. As it alters blood glucose levels and causes hyperglycemia as there is drop in insulin levels in body (16). This STZ solution is administered through *I.P* route. Type 2 diabetes was identified in rats with blood glucose levels more than 350 mg/dL on the third day after STZ injection. Rats with less than 350 mg/dL of glucose will be monitored for additional 2 days to see if diabetes was induced. Rats were separately cages based on their disease condition under supervision.

### Design of the study

The research was carried out over a period of fourteen days. Group 1 will receive distilled water, which will serve as the control group. Group 2 will be toxic, consisting of rats with diabetes who will receive doses of 50 mg/kg/BW of STZ. Group 3 will receive a standard drug of 2 mg/kg/BW. Group 4 will receive a low dose of 200 mg/kg/BW of extract, Group 5 will receive a medium dose of 400 mg/kg/BW, and Group 6 will receive a low dose of 800 mg/kg/BW of extract [17].

### Oral glucose tolerance test

After an overnight fast on the 14th-day trail, all animals are administered 5% glucose orally [16]. Glibenclamide (2 mg/kg) is administered intraperitoneally to standard group 3, Then the MEETV different doses like 200 mg to group-4 & group-5 is 400 and 800 mg/kg body weight administered orally to those receiving glucose (2 mg/kg). Group-1 & group-2 also receives glucose. A blood collected from vein of tail's rat glucose was determined with a time range at 0, 30, 60, 90, 120minutes by using Glucose meter [18].

### Estimation of Glucose

### By tail pricking method

To estimate glucose levels, tail pricking method was used. 25-G needle is used to prick the tail vein at the end of tail, followed by pressing the tail then collect blood drop put on glucose strip & wait a second blood glucose value display on the glucometer [19, 20].

## Results and Discussion

### Percentage yield of crude extracts

25 grams of *Eupatorium triplinerve* Vahl was used for the extraction with methanol, ethyl acetate & and petroleum ether. The percentage yield for individual solvent extracts is shown in Table. The percentage yield of individual extracts was found in the sequence Methanol ≥ ethyl acetate ≥ petroleum ether. Percentage yield of *Eupatorium triplinerve* extract is more in methanol than ethyl acetate & and petroleum ether. From these *Eupatorium triplinerve* Vahl is rich in bioactive compounds.

**Table 1:** Percentage yield of *Eupatorium triplinerve* Vahl

| Solvent | Amount of crude extracts obtained in grams | % yield |
|---|---|---|
| Methanol | 3.26 g | 13.04% |
| Ethyl acetate | 2.06 g | 8.24% |
| Petroleum ether | 0.97 g | 3.88% |

**Table 2:** Qualitative tests for preliminary phytochemical constituents of *Eupatorium triplinerve* Vahl

| S. No. | Phytoconstituent | MEETV |
|---|---|---|
| 1 | Test for primary Alkaloid's | + |
| 2 | Test for secondary Alkaloid's | + |
| 3 | Test for Amino acids | - |
| 4 | Test for carbohydrates | + |
| 5 | Test for Flavonoid's | + |
| 6 | Test for Glycoside | + |
| 7 | Test for Phenolic compounds | + |
| 8 | Test for proteins | + |
| 9 | Test xanthoproteins | - |
| 10 | Test for Reducing sugars | + |
| 11 | Test for Tannins | + |
| 12 | Test for Triterpenoid's | + |

The performed phytochemical screening resulted in showing that the selected extract contains the various phytochemicals that include primary alkaloids, secondary alkaloids, carbohydrates, flavonoids, glycoside, phenolic compounds, proteins, reducing sugars, tannins and triterpenoids.

### HRLC-MS

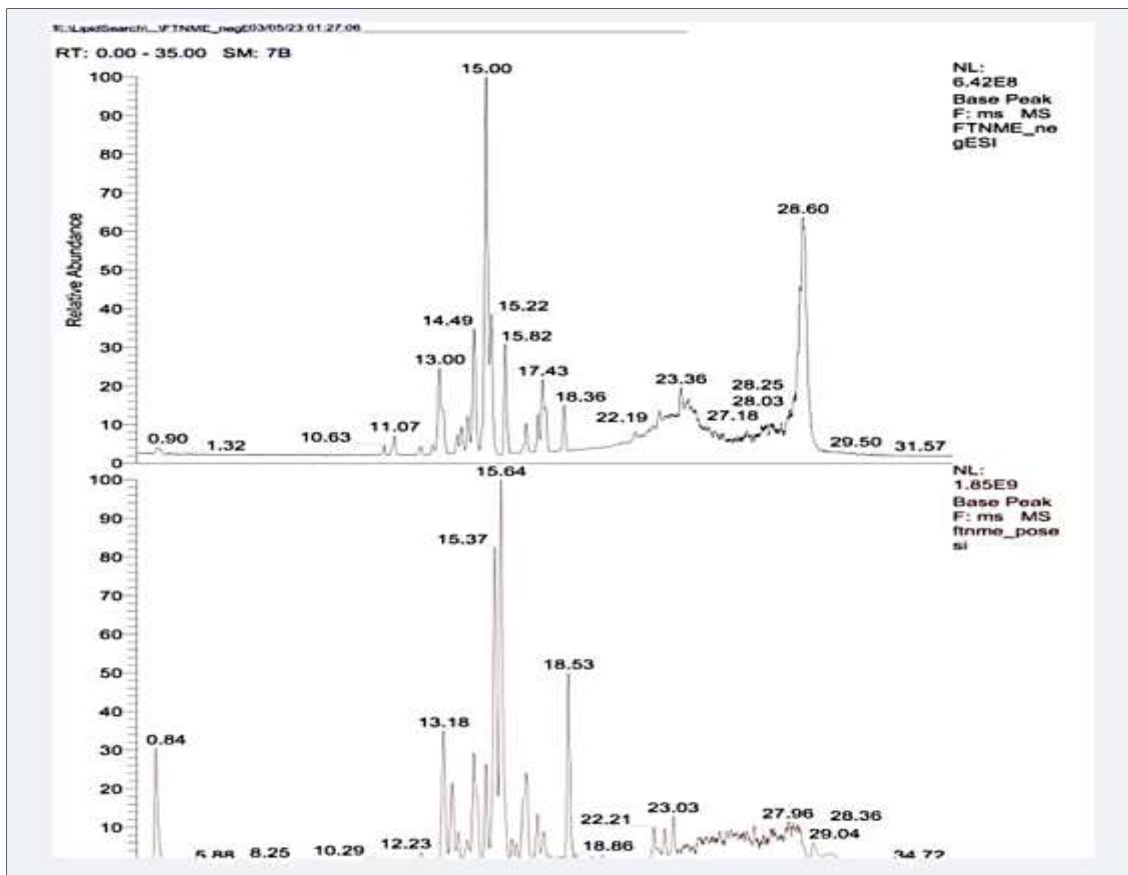


**Fig 4:** Relative abundance graph

**Table 3:** Compounds present in the *Eupatorium triplinerve* Vahl

| S. No. | Compound Name | Formula |
|---|---|---|
| 1 | Azelaic acid | $C_9H_{16}O_4$ |
| 2 | Catechol | $C_6H_6O_2$ |
| 3 | D-ribose | $C_5H_{10}O_5$ |
| 4 | Glycitein | $C_{16}H_{12}O_5$ |
| 5 | Gentisic acid | $C_7H_6O_4$ |
| 6 | Gallic acid | $C_7H_6O_5$ |
| 7 | Genistein | $C_{15}H_{10}O_5$ |
| 8 | Hesperetin | $C_{16}H_{14}O_6$ |
| 9 | 2-hydroxycin | $C_9H_8O_3$ |
| 10 | Isoferulic acid | $C_{10}H_{10}O_4$ |
| 11 | Linoleic acid | $C_{18}H_{32}O_2$ |
| 12 | Luteolin | $C_{15}H_{10}O_6$ |
| 13 | Naringenin | $C_{15}H_{12}O_5$ |
| 14 | Phenol | $C_6H_6O$ |
| 15 | Quercetin | $C_{15}H_{10}O_7$ |
| 16 | Salicylic acid | $C_7H_6O_3$ |
| 17 | Suberic acid | $C_8H_{14}O_4$ |
| 18 | Trifolin | $C_{21}H_{20}O_{11}$ |
| 19 | 4-Oxoproline | $C_5H_7NO_3$ |

**Ferric-reducing antioxidant power assay**

**$IC_{50}$ values of the extract by FRAP inhibition assay**

The antioxidant capabilities were investigated and represented as IC50 values. Lower $IC_{50}$ values suggest more anti-oxidant activity.

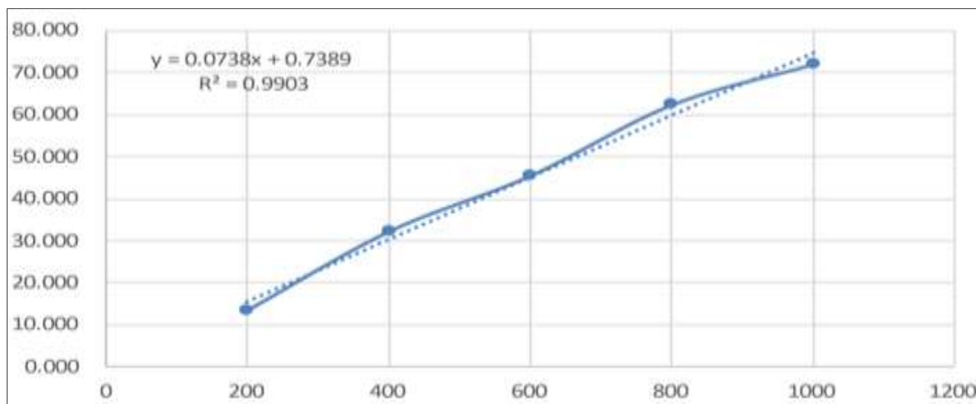


**Fig 5:** $IC_{50}$ calculation Graph by FRAP method

$IC_{50}$ values of extract were calculated by FRAP inhibition assay. The extract $IC_{50}$ value was calculated by extrapolating the concentration and inhibition percentage graph. $IC_{50}$ stands for half-maximal inhibitory concentration, and it's a measure of how much of a drug or compound is needed to inhibit a biological process by half. It's a common way to measure a drug's potency in pharmacological research

**Hydrogen peroxide assay**

**$IC_{50}$ values of the extract by $H_2O_2$ inhibition assay**

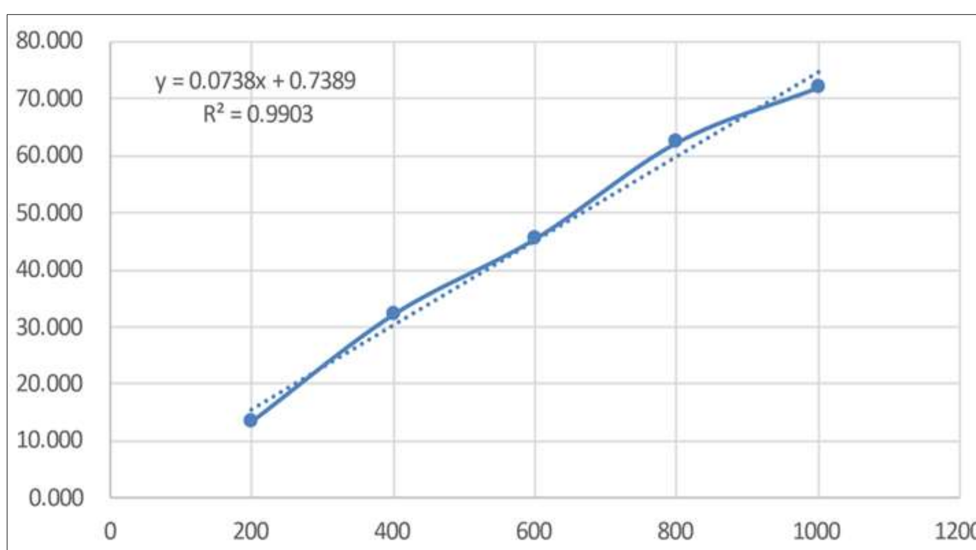


**Fig 6:** $IC_{50}$ calculation Graph by $H_2O_2$ method

$IC_{50}$ values of extract were calculated by $H_2O_2$ inhibition assay. The extract $IC_{50}$ value was calculated by extrapolating the concentration and inhibition percentage graph. $IC_{50}$ stands for half-maximal inhibitory concentration, and it's a measure of how much of a drug or compound is needed to inhibit a biological process by half. It's a common way to measure a drug's potency in pharmacological research.

**Anti-diabetic activity (*in-vitro*)**

IC50 values of the extract by Alpha amylase method

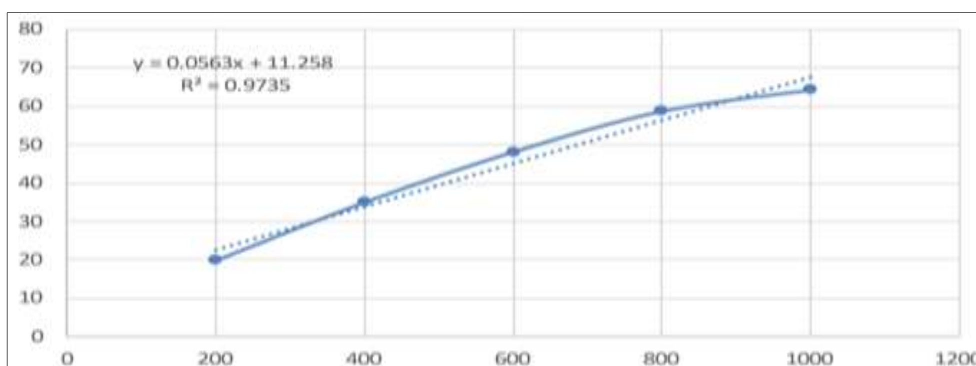


**Fig 7:** $IC_{50}$ Calculation Graph by Alpha amylase method

**Estimation of changes in body weight (g)**

All the animals which are grouped were weighed using analog weighing machine on daily basic.

**Table 4:** Change in body weight from 0th, 3rd,7 th and 14th day

| Days | Normal control | Toxic control | Positive control | ETELD (200 mg/kg/BW) | ETEID (400 mg/kg/BW) | ETEHD (800 mg/kg/BW) |
|---|---|---|---|---|---|---|
| 0 | 220.83±2.38 | 238.33±1.66 | 218.33±1.05 | 257.5±7.15 | 211.66±2.10 | 235.83±10.11 |
| 3 | 213.33±3.80 | 590.83±351.83 | 221.66±1.66 | 239.16±9.16 | 197.5±2.14 | 230.83±6.50 |
| 7 | 202.5±3.59 | 249.16±3.96 | 228.33±2.47 | 249.16±10.03 | 212.5±2.81 | 209.16±15.13 |
| 14 | 181.66±3.07 | 273.33±3.80 | 235±2.88 | 215.83±12.74 | 218.33±3.57 | 224.16±19.21 |

Effect of extract in treatment of diabetes can alter the body weight. Initial body weight, 3rd,7 th, 14 th day (final body weight) were measured after the extract treatment in all normal and diabetic rats including all groups. There is reduction of body weight in extract test groups compared to toxic group. It is observed that the change in the body weight corelate to disruptions of glucose metabolism which is clearly indicated reduction in weight among the toxic group. In contrast to the toxic group, the other groups have either maintained the body weight or slightly increase in the treatment group.

**Estimation of blood glucose effect of extract**

**Table 5: Estimation of blood glucose on 0th, 3rd, 7th and 14th day**

| Days | Normal control | Toxic control | Positive control | ETELD (200 mg/kg/BW) | ETEID (400 mg/kg/BW) | ETEHD (800 mg/kg/BW) |
|---|---|---|---|---|---|---|
| 0 | 155.5±5.75 | 457.16±19.63 | 520.33±19.04 | 505.5±32.99 | 353.66±7.39 | 367±7.48 |
| 3 | 158.83±4.2 | 489.33±20.4 | 336.66±7.60 | 444.83±22.4 | 458.83±10.3 | 341±18.04 |
| 7 | 202.5±3.59 | 249.16±3.96 | 228.33±2.47 | 249.16±10.03 | 212.5±2.81 | 209.16±15.13 |
| 14 | 181.66±3.07 | 273.33±3.80 | 235±2.88 | 215.83±12.74 | 218.33±3.57 | 224.16±19.2 1 |

Data in the table are presented as means +/- standard error of the mean (SEM). Normal group received 0.5% CMC whereas toxic group received STZ. Standard groups received glibenclamide, the test groups received extract at low dose of 200 mg/kg, medium dose 400 mg/kg and high dose 800 mg/kg BW. Data shows that, during the study, glucose levels steadily climbed in the STZ treated group compared to the normal group, where as other treatment groups had significant decrease in blood glucose levels. After 14 days of study, the glucose levels of the toxic group showed an increase in glucose levels compared to normal group, as well as low dose, medium dose and high dose group glucose levels was reduced as compared to toxic group.

## Discussion

The current study states that the phytochemical and pharmacological evaluation of the Anti- Diabetic activity of methanolic extract of *Eupatorium triplinerve* Vahl. extract in the dose of 200, 400 and 800mg/kg body weight. Preliminary phytochemical screening of MEETV indicates that presence of alkaloids, amino acids, carbohydrates, flavonoids, tannins and phenolic compounds, which have anti-oxidant property.

Streptozotocin is a widely used drug for inducing diabetes in animals. Streptozotocin induced diabetes is complexed with elevated glucose levels, weight. The anti-diabetic activity was studied and serum parameters like albumin, creatinine, total protein, uric acid, urea, were measured to assess the anti-diabetic activity of MEETV. The current study demonstrates that MEETV was shows anti-diabetic activity when compared with normal and toxic groups. These observations indicate that reduction of glucose levels.

## Conclusion

In the current study, *Eupatorium triplinerve* Vahl leaves which belong Asteraceae family were collected and authenticated. The coarse powder was subject to extraction with methanol, ethyl acetate, and petroleum ether by the maceration method. The solvent extracts were filtered and kept for drying. The dried extracts of different solvents were stored in an air-tight container. A higher percentage of yield was obtained from the methanolic solvent. Evaluation of phytochemical screening and anti-oxidant and pharmacological studies were performed. Screening of phytochemical constituents of methanolic extract of ETV showed the presence of alkaloids, amino acids, carbohydrates, tannins, phenolic compounds, and flavonoids.

According to a literature survey, MEETV was found to be safe in the dose used and there was no mortality up to 5000 mg/kg dose. MEETV showed significant anti-diabetic activity against streptozotocin-induced type-2 diabetes mellitus due to alkaloids and flavonoid compounds. Thus, we can conclude that *Eupatorium triplinerve* Vahl poses anti-diabetic activity.

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