Evaluation of Pedilanthus Tithymaloides Ethanolic Leaf Extract on Serum Lipid Profile Changes in Normal and Alloxan Induced Diabetic Albino Wistar Rats

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Abstract: Type 1 diabetes is associated with damage to the liver, kidney and pancreas of patients. The damage varies in proportion and susceptibility among diabetic patients of type 1 class. This study assessed the hypoglycaemic and antihyperlipidemic activities of whole ethanolic leaf extract of Pedilanthus tithymaloides in alloxan-induced diabetic albino Wistar rats. Extraction of the ethanolic extract of Pedilanthus tithymaloides was performed by maceration. Thirty rats were divided into five groups. Group I consists of normal rats that were given only normal saline solution and served as a control group. Group II consists of normal rats that were given alloxan monohydrate (150mg/kg B.W). Group III consists of alloxan induced diabetic rats that were given daily sterile solution, ethanolic leaf extract of Pedilanthus tithymaloides (500 mg/kg), Group IV consists of alloxan induced diabetic rats that were given daily sterile solution and glibenclamide(5mg/kg) respectively for 21 days by an instragastric tube with free access of food and water. Several biochemical parameters were assessed. Oral administration of the extract resulted in significant reduction in mean values of blood glucose, cholesterol, triglycerides, LDL-C, VLDL accompanied by an increase in the mean values of the HDL in diabetic rats. The effects produced by this extract were closely similar to a standard anti diabetic drug, glibenclamide. In conclusion, the present study indicates that the ethanolic extract of Pedilanthus tithymaloides to exhibit antihyperlipidemic and antihyperglycemic activities in alloxan induced diabetic rats.

Keywords: Alloxan monohydrate 150mg/kg.b.w, Glibenclamide 5mg/kg.b.w, Pedilanthus tithymaloides, water.

I. Introduction

Diabetes mellitus is a syndrome associated with hyperglycemia, hyperlipidemia, oxidative stress, polyurea, polyphagia, polydypsia, ketosis, nephropathy, neuropathy, cardiovascular disorders. From the literature review it has been noted that hyperlipidemia is associated with diabetes. In modern medicine no satisfactory effective therapy is yet available to cure diabetes mellitus but there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver. Chronic treatment with sulfonylureas and biguanides are also associated with serious side effects. In India, use of herbal drugs based on ayurveda is very commonly practiced from time immemorial and is less expensive. The herbal drugs which we are considered frequently are of less toxicity and fewer side effects. For such reasons, at present traditional and complementary medicine has seen an upsurge popularity for the treatment of different diseases.

Pedilanthus tithymaloides family Euphorbiaceae is a low tropical american shrub with a reported wide range of healing properties namely emetic, anti-inflammatory, antibiotic, antiseptic, antihemorrhagic, antiviral, antitumoral, abortive. In search for the bioactive principles, a new cancer cell growth inhibitor designated Pedilstain was isolated from tithymaloides. A new proteolytic enzyme with oral anti-inflammatory, designated pedilanthin and a galactose specific lectin possessing mitogenic activity with murine spleen lymphocytes were isolated from the latex. The lectin was also assayed in biomedical research to study the hemagglutination pattern in patients with diabetes mellitus and tuberculosis.

II. Review Of Drug Under Study

Pedilanthus tithymaloides belongs to family Euphorbiaceae. It is found throughout the India, Origin is in Tropical America.

Common names:
Japanese poinsettia, red bird flower, slipper flower, devil’s backbone
Vernacular names: Nallajilledu in telugu

Description:
It is a succulent shrub with milky juice, stems green, often zigzag, leaves alternate, simple, pointed, green or white edged, flowers red and clusters at the end of branches, fruit a capsule.

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Preliminary phytochemical investigation and pharmacological screening of Pedilanthus tithymaloides states that sterols, triterpenes, carbohydrates, flavonoids and tannins were found to be present in leaf extract\(^7\). Proteolytic enzyme pedilanthin, pedilstatin\(^5\) have been reported to have an anti-inflammatory activity. Lakshmi VS, sheshanka M, Himani Reddy K, and Sandhya B reported alcoholic and ethanolic extract significantly reduced glucose levels when compared to petroleum ether and chloroform extract\(^7\). Sterols main action is to lower LDL cholesterol level hence we may assume that the plant may have antihyperlipidemic activity.\(^91,92\) was reported by Peter J.H Jones.

Pedilanthus tithymaloides family Euphorbiaceae (ornamental plant) is a low tropical shrub\(^74\) with a repored range of healing properties.

Pharmacological actions:

**Antiinflammatory:** Medicinal tincture from Pedilanthus tithymaloides was evaluated for in vivo anti-inflammatory activity and for its in vivo scavenging effect on ROS, RNS and DPPH radical. The intra peritoneal administration of the tincture inhibited carrageenan induced rat paw edema whereas in the scavenging assays the tincture showed to be effective against all the assayed ROS, RNS. These results provide scientific support for empirical use of pedilanthus tithymaloides tincture as an anti-inflammatory medicine by Pedro abrew, Susan Mathew, et.al\(^5\).

In the search for the bioactive principles, a new proteolytic enzyme with oral anti-inflammatory activity designated pedilanthin and a galactose specific lectin possessing mutagenic activity with murine spleen lymphocytes were isolated from latex.

**Diabetes:** The usefulness of galactose specific lectin Pedilanthus tithymaloides was examined to study the hemagglutination pattern in patients with diabetes mellitus Significantly low titer was seen in patients with insulin dependant diabetes and no significant changes in non insulin dependant diabetes\(^72\).

**Antimalarial and antituberculosis:** Study yielded six new poly-O- acylated jatrophone diterpenes along with five known compounds from white latex of P.tithymaloides :Compounds 1,3,4 and 5 showed antiplasmodial activity against mycobacterium tuberculosis\(^68\).

**Antifungal:** Of 58 malaysian plants screened, Pedilanthus tithymaloides was one of the 34 plants that showed selective antifungal activity\(^73\).

**Antioxidants:** Study yielded principles identified as kaempferol 3-O-B-D glucopyranoside-6'', (3-hydroxy-3-methylglutarate), quercetin, isoquercetin and scopoletin.\(^5\)

**Larvicidal activity** of some euphorbeaceae plant extracts against Aedes aegypti and culex quinquefasciatus of ethylacetate, butanol and pet ether extracts of 5 species including jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta and euphorbea tirucalli were tested. All the extracts showed low larvicidal effects however the highest larval mortality was found in pet ether extract.\(^71\)

### III. Materials & Methods

#### 3.1 Collection Of Drug:

Dried leaf powder of Pedilanthus tithymaloides family euphorbiaceae was obtained from Chittoor district and was authenticated by Dr. MADHAV SHETTY, Assistant Professor of Botany, Department of Pharmacognosy, Sri Venkateshwara University, Tirupathi.

#### 3.2 Chemicals

The following chemicals were used during the experiment carried out to analyze and interpret the effect of Pedilanthus tithymaloides leaf extract on serum lipid profile in alloxan induced diabetic rats.

1. Alloxan monohydrate 150mg/kg.b.w
2. Glibenclamide 5mg/kg.b.w
3. Ethanol

#### 3.3 Preparation Of Plant Extract

Pedilanthus tithymaloides leaf extract was prepared by maceration in the following way 1000g/1kg powder was soaked in distilled ethanol in the ratio with solute : solvent = 1:10 by vigorous shaking and left for 7 days at room temperature. The filtrate, thus obtained, was evaporated to complete dryness on water bath. The residue thus obtained is ethanolic plant extract.\(^77\)

Whenever required the solution was mixed with 0.9% normal saline as a vehicle in the experiment.
3.4 Animals:
Thirty albino rats of Wister strain of either sex weighing between 200-250g were taken under study and housed with a 12 hour light/dark cycle. The rats were provided with a diet of standard pellets and were allowed to free access to water.

3.5 Acute Toxicity Studies (Oecd Guideline 423)
Animals were fasted prior to dosing, food but not water was withheld overnight. Following the period of fasting, the animals were weighed and the test substance administered orally. After the substance has been administered, food was withheld for a further 3-4 hrs. As a dose was administered in fractions over a period, it was necessary to provide the animals with food and water depending on the length of the period.

Three animals were used for each step. The dose level to be used as the starting dose was selected from one of the four fixed levels, 500,1000,2000 mg/kg body weight. The starting dose level was that which was most likely to produce mortality in some of the dosed animals. After administration of test sample, the animals were observed continuously for first 4 hrs for behavioural changes and at the end of 48 hr for mortality rate, if any.

3.6 Experimental Design:
Evaluation Of Pedilanthus Tithymaloides Ethanolic Leaf Extract On Serum Lipid Profile In Alloxan Induced Diabetic Rats.
The study included 30 animals among which six animals were separated and served as a normal control group. The blood glucose level of all the animals was recorded.

Animals were fasted for 24 hrs and diabetes was induced by intraperitoneal injection of alloxan monohydrate 150mg/kg.b.w using normal saline as a vehicle. Blood glucose levels were checked and animals with blood glucose levels >272±17mg/dl were selected and divided into 5 groups of six animals each, accounting 30 animals. 15 day study with the desired therapeutic dose of the extract was given in alloxan induced diabetic rats by administering the extract orally using normal saline as a vehicle.
Experimental Design:

30 albino rats of either sex were segregated into 5 groups with 6 rats in each group.

**Group I:** Normal control rats received normal saline as a vehicle p.o

**Group II:** Alloxan induced diabetic control received normal saline p.o

**Group III:** Alloxan induced diabetic rats + Pedilanthus tithymaloides leaf extract 500mg/kg.b.w p.o

**Group IV:** Alloxan induced diabetic rats + Pedilanthus tithymaloides leaf extract 1000mg/kg.b.w in normal saline p.o.

**Group V:** Alloxan induced diabetic rats + Glibenclamide 5mg/kg.b.w in normal Saline i.p

The normal and diabetic control groups were given 1ml normal saline, i.p. Animals in the third group were treated with the ethanolic leaf extract of Pedilanthus tithymaloides at a dose of 500mg/kg.b.w. fourth group were given Pedilanthus tithymaloides leaf extract at a dose 1000mg/kg.b.w. and fifth group were treated with Glibenclamide at a dose of 5mg/kg.b.w, for 21 days. Body weight and blood glucose levels were checked for every three days intervals during the duration of the experiment. The blood glucose levels were determined by tail tipping method using Accu-check active glucometer. On twenty first day blood from all the groups was collected by retro-orbital puncture under mild anesthesia, serum was separated quickly for estimating

Blood glucose levels, Total cholesterol levels, (TC) Low density lipoproteins cholesterol (LDLc) levels, High density lipoproteins cholesterol (HDLc) levels, Very low density lipoproteins cholesterol (VLDLc) level, Triglycerides levels and Cholesterol ratio.

### 1.7 Accu-Chek Sensor Glucometer

**Indications:** Blood glucose monitoring in diabetic patients, persons suspected of diabetes, emergency diagnosis and self-testing by diabetes.

**Test Principle:**

The glucose dehydrogenase converts the glucose in the blood sample to gluconolactone. This reaction liberates an electron that reacts with a coenzyme electron acceptor, the oxidized form of the mediator hexacyanoferrate (III), forming the reduced form of mediator, hexacyanoferrate(II). The test strip employs the electrochemical principle of bioamperometry. The monitor applies a voltage between two identical electrodes, which causes the reduced mediator formed during the incubation period to be reconverted to an oxidized mediator. This generates a small current that is read by monitor. The meter detects electrical current change and with the aid of the code key, converts signal obtained into a displayed reading.

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**Product specifications:**

<table>
<thead>
<tr>
<th>1. Meter storage conditions</th>
<th>-25°C to 70°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;85% humidity</td>
</tr>
<tr>
<td></td>
<td>≥53.5kPa atmospheric pressure</td>
</tr>
<tr>
<td>2. System Operating Conditions</td>
<td>Use test strips between 14°C to 40°C(57°F to 104°F) and less than 85% humidity</td>
</tr>
<tr>
<td>3. Measuring Range</td>
<td>System measuring range is 0.6-33.3mmol/L(10-600mg/dL)</td>
</tr>
<tr>
<td>4. Memory capacity</td>
<td>480 blood glucose results with time and date</td>
</tr>
<tr>
<td>5. Display</td>
<td>LCD display</td>
</tr>
<tr>
<td>6. Blood volume</td>
<td>4µL</td>
</tr>
<tr>
<td>7. Size</td>
<td>3.3”×2.2”×0.8”(84mm×56mm×20mm)</td>
</tr>
<tr>
<td>8. Weight</td>
<td>1.8oz(57g)</td>
</tr>
<tr>
<td>9. Sample types</td>
<td>Capillary, venous, arterial and neonatal(including cord) blood</td>
</tr>
<tr>
<td>10. Power supply</td>
<td>One three volt lithium battery 2032(CR 2032,DL2032) or equivalent</td>
</tr>
<tr>
<td>11. Test strips storage conditions</td>
<td>Store test strips between 2°C to 32°C(36 to 90°F)</td>
</tr>
</tbody>
</table>

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Limitations:
- Intravenous infusion of ascorbic acid
- Galactosaemia
- Bilirubin concentrations above 20mg/dL (e.g., jaundice) may interfere with the detection reaction.
- Ketoadidosis and dehydration may produce false results
- Plasma expanders and dialyzing fluids may interfere with the results.

Advantages:
- Simple method of estimating glucose
- Results are reproducible
- Large volume of the blood need not be drawn
- Separation is not required
- Gives accurate values thereby minimizing manual results
- Sugar level in the blood can be known within 30 sec

Disadvantages:
- Cost of the glucometer and test strips is high
- Strips are provided for single use.
- Many mechanical errors are encountered
- Glucose values can be obtained only in a specific range of 10-600mg/dL.

Estimation Of Serum Glucose, Serum Lipid Profile Including Serum Cholesterol, Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL), Very Low Density Lipoproteins (VLDL), Serum Triglycerides.

The concentration of serum glucose was measured by GOD-POD. End point Assay and Kinetic Assay method; Serum cholesterol by CHOD-PAP method, HDL cholesterol by Phosphotungstate method, LDL cholesterol and VLDL cholesterol by using formulae, serum triglycerides by enzymatic GPO method.

3.8 Estimation Of Serum Glucose:
Method: GOD-POD. End point Assay and Kinetic Assay.82

Principle: Glucose oxidase (GOD) oxidizes glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505nm and is directly proportional to glucose concentration in the sample.

\[
\text{Glucose} + \text{Oxygen} + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{D-gluconic acid} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 4\text{AAP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + \text{H}_2\text{O}
\]

Assay Parameters For End Point Method:

<table>
<thead>
<tr>
<th>Mode</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>505nm (490-550nm)</td>
</tr>
<tr>
<td>Flow cell temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>Optical path length</td>
<td>1 cm</td>
</tr>
<tr>
<td>Blank</td>
<td>Reagent Blank</td>
</tr>
<tr>
<td>Sample volume</td>
<td>10µL</td>
</tr>
<tr>
<td>Working Reagent Volume</td>
<td>1000µL</td>
</tr>
<tr>
<td>Incubation time</td>
<td>10min at 37°C/30min, at room temperature (15-30°C)</td>
</tr>
<tr>
<td>Concentration of standard</td>
<td>100mg/dL</td>
</tr>
<tr>
<td>Stability of colour</td>
<td>1 hour</td>
</tr>
<tr>
<td>Permissible Reagent Blank absorbable</td>
<td>&lt;0.2AU</td>
</tr>
<tr>
<td>Linearity</td>
<td>Upto 500mg/dL</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dL</td>
</tr>
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</table>

Procedure:

<table>
<thead>
<tr>
<th>Pipette into the marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum or Plasma</td>
<td>-</td>
<td>10µL</td>
<td></td>
</tr>
<tr>
<td>Glucose Standard</td>
<td>-</td>
<td>10µL</td>
<td>10µL</td>
</tr>
<tr>
<td>Working Glucose Reagent</td>
<td>1000µL</td>
<td>1000µL</td>
<td>1000µL</td>
</tr>
</tbody>
</table>

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Mixed well and incubated at 37°C for 10 min. or at room temperature. Absorbance was measured at 505 nm against reagent blank.

**Calculation**

Serum/Plasma Glucose (mg/dl) = \( \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100 \)

**4.9 Estimation Of Triglycerides**

**Method:** Enzymatic (GPO/Trinder), End point Colorimetry, Single Reagent Chemistry with LCF (Lipid Clearing Factor).

**Principle:** The estimation of Triglycerides involves the following enzymatic reactions:

\[
\text{Triglycerides} \rightarrow \text{Glycerol + FFA}
\]

\[
\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol-3-Phosphate + ATP}
\]

\[
\text{Glycerol-3-Phosphate + O}_2 \rightarrow \text{DHAP + H}_2\text{O}_2
\]

\[
\text{2H}_2\text{O} + \text{4-AAP} \rightarrow \text{Quinoneimine dye + 4H}_2\text{O}
\]

LPL = Lipoprotein Lipase
FFA = Free Fatty Acids
GK = Glycerol Kinase
GPO = Glycerol - 3-phosphate Oxidase
POD = Peroxidase
ATP = Adenosine Triphosphate
ADP = Adenosine Diphosphate
DHAP = Dihydroxyacetone phosphate
4-AAP = 4-Aminoantipyrine

**Procedure:**

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>10µL</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10µL</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides Reagent</td>
<td>1000 µL</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
</tbody>
</table>

Mixed well and incubated at 37°C for 10 min. Absorbance of standard & sample was measured against reagent blank at 505 nm within 60 min.

**Calculation:**

Triglycerides concentration (mg/dL) = \( \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200 \)

Triglycerides concentration (mmol/L) = Concentration (mg/dL) \( \times 0.0114 \)

**4.10 Estimation Of Total Cholesterol**

**Method:** Enzymatic, (Cholesterol Oxidase-Peroxidase), Endpoint colorimetry, Single Reagent Chemistry, with LCF (Lipid Clearing Factor).

**Principle:** The estimation of Cholesterol involves the following enzymatic reactions:

\[
\text{Cholesterol esters} \rightarrow \text{cholesterol + free fatty acids}
\]

\[
\text{Cholesterol + O}_2 \rightarrow \text{cholesten-3-one + H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Phenol + 4AAP} \rightarrow \text{Quinoneimine dye + H}_2\text{O}_2
\]

CE = Cholesterol esterase
POD = Peroxidase
4-AAP = 4-Aminoantipyrine

Absorbance of quinoneimine measured at 505 nm is proportional to cholesterol concentration in the specimen.
Evaluation of Pedilanthus Tithymaloides Ethanolic Leaf Extract on Serum Lipid Profile Changes

### Assay Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>End point</td>
</tr>
<tr>
<td>Wavelength</td>
<td>505nm (490-530nm)</td>
</tr>
<tr>
<td>Temperature</td>
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<td>Optical path length</td>
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<td>Blanking</td>
<td>Reagent Blank</td>
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<tr>
<td>Sample Volume</td>
<td>10µL</td>
</tr>
<tr>
<td>Working Reagent Volume</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Incubation time</td>
<td>10min at 37°C</td>
</tr>
<tr>
<td>Concentration of Standard</td>
<td>200mg/dL</td>
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<tr>
<td>Stability of color</td>
<td>1 hour</td>
</tr>
<tr>
<td>Maximum Absorbance Limit</td>
<td>2.0</td>
</tr>
<tr>
<td>Linearity</td>
<td>750mg/dL</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

### Procedure:

1. Pipette into tubes marked Blank Standard Test
2. Serum - - 10µL
3. Standard - 10 µL -
4. Cholesterol Reagent 1000 µL 1000 µL 1000 µL

Mixed well and incubated at 37°C for 10 min. Absorbance of standard & sample was measured against reagent blank at 505nm within 60min.

### Calculation:

Cholesterol concentration (mg/dL) = \( \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200 \)

Cholesterol concentration (mmol/L) = Concentration (mg/dL) \( \times 0.0259 \)

### 4.11 Estimation Of Hdl Cholesterol

**Method:** Phosphotungstate

**Principle:** Chylomicrons, VLDL and LDL fractions in serum and plasma are separated from HDL by phosphotungstic acid and magnesium chloride.

After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen 4-Aminooantipyrene/Phenol.

### Assay parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>End point</td>
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<tr>
<td>Reaction slope</td>
<td>Increasing</td>
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<tr>
<td>Wavelength</td>
<td>500nm (492-550nm)</td>
</tr>
<tr>
<td>Flow cell temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Incubation time</td>
<td>5min at 37°C</td>
</tr>
<tr>
<td>Sample volume (Supernatant)</td>
<td>20µL</td>
</tr>
<tr>
<td>Standard Concentration</td>
<td>100mg/dL</td>
</tr>
<tr>
<td>Blank</td>
<td>Reagent blank</td>
</tr>
<tr>
<td>Working reagent volume</td>
<td>1.0ml</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

### Procedure:

1. Dispensed into test tubes marked Blank Standard Test
2. Reconstituted reagent 1ml 1ml 1ml
3. Standard - 20µL -
4. Supernatant - - 20µL

Mixed well and incubated at 37°C for 5min

### 4.12 Estimation of LDL Cholesterol

The value of LDL cholesterol was calculated as follows

If the value of Triglycerides was known LDL Cholesterol can be calculated by
Evaluation of Pedilanthus Tithymaloides Ethanolic Leaf Extract on Serum Lipid Profile Changes

Friedewald’s equation:

\[
LDL \text{ CHOLESTEROL} = \text{TOTAL CHOLESTEROL} - \frac{\text{TRIGLYCERIDES}}{5} - \text{HDL CHOLESTEROL}
\]

4.13 Estimation of VLDL cholesterol

VLDL Cholesterol was calculated using the formula:

\[
\text{VLDL} = \frac{\text{TRIGLYCERIDES}}{5}
\]

4.14 Estimation of Cholesterol Ratio

\[
\text{CHOLESTEROL RATIO} = \frac{\text{TOTAL CHOLESTEROL}}{\text{HDL CHOLESTEROL}}
\]

Dose selection: Different doses of the plant Pedilanthus tithymaloides were selected on the basis of acute toxicity studies.

Statistical Analysis:

Results were expressed as mean±SEM. Statistical analysis were performed with INSTAT Software using One Way Analysis of Variance (ANOVA) followed by Dunnett’s t-test. P values less than *P>0.05, **P<0.01, ***P<0.001 was considered to be statistically significant when compared with control and toxicant group as applicable.

IV. Results

Acute Toxicity Studies:

In acute toxicity study, there were no behavioural changes seen up to 4 hrs and no mortality was observed up to the end of 48hrs even at the maximum tested dose level of 2000mg/kg.b.w per oral. As a result an effective doses of 500mg/kg.b.w and 1000mg/kg.b.w was taken.

Effect of Pedilanthus tithymaloides Ethanolic Leaf Extract on blood glucose and serum lipid profile in Alloxan Induced Diabetic Rats.

The effect of Pedilanthus tithymaloides ethanolic leaf extract on the blood glucose level of the experimental animals was determined for 21 days after oral administration at doses of 500mg/kg.b.w and 1000mg/kg.b.w.

The Effect of Pedilanthus tithymaloides Ethanolic Leaf Extract on Blood Glucose Levels is Presented in Table 1.

There was a significant elevation in the blood glucose levels in Group II, III, IV and Group V after alloxan administration when compared to normal Group I.

Mean blood glucose levels in the diabetic control was 279.16 ± 2.98mg/dL after induction of diabetes with alloxan monohydrate 150mg/kg.b.w. In group I, value was 82.53 ± 1.37mg/dL. In comparison with the diabetic control group Group II, The group III which was treated with Pedilanthus tithymaloides ethanolic extract 500mg/kg.b.w lower mean glucose level 117.33 ± 3.22mg/dL) was observed and significant decreased level of blood glucose level was observed in Group IV which was administered Pedilanthus tithymaloides at the rate of 1000mg/kg.b.w 110.66 ± 2.18mg/dL, almost comparable to the standard group i.e glibenclamide treated group V 94.83 ± 2.60mg/dL.

Hence the administration of pedilanthus tithymaloides leaf extract for 21 days decreased the elevated blood glucose level.

The above result suggests that Pedilanthus tithymaloides at a dose of 1000mg/kg.b.w was more effective than 500mg/kg.b.w was almost comparable with the effect produced by standard drug glibenclamide treated group. The results were represented by means of a Graph 1 by using ANOVA followed by Dunnett’s t-test.
Effect of Pedilanthus tithymaloides Ethanolic Leaf Extract on Serum Lipid Profile Changes in Alloxan Induced Diabetic Rats.

The effect of Pedilanthus tithymaloides ethanolic leaf extract on the serum lipid profile of the experimental animals was determined. On the fifteenth day after oral administration of the extract at doses of 500mg/kg.b.w and 1000mg/kg.b.w.

It was observed that the alloxanised animals showed an increase in Total Cholesterol level (T.C), Triglycerides level, cholesterol ratio, Low Density Lipoprotein Cholesterol (LDLc), Very Low Density Lipoprotein (VLDLc) Cholesterol, and decreased levels of High Density Lipoprotein Cholesterol (HDLc) levels.

Table 2 represents the effect of Pedilanthus tithymaloides ethanolic leaf extract on serum lipid profile which includes LDLc, VLDLc, and HDLc and Table 3 represents Total cholesterol, triglycerides and cholesterol ratio levels.

Low Density Lipoprotein cholesterol (LDLc)

Mean LDLc in Group I was 46 ± 0.80mg/dL. It was significantly elevated to 210.63 ± 2.89mg/dL in the diabetic control group, GP II. Administration of pedilanthus tithymaloides at a dose of 500mg/kg.b.w lowered LDL level to 68 ± 2.95mg/dL. In group treated at a dose of 1000mg/kg.b.w there was a significantly lowering LDL to 40.033 ± 1.66 almost comparable to standard drug 20.9 ± 5.73 mg/dL. The results were represented by means of a Graph 2. by using ANOVA followed ny Dunnett’s t-test.

Effect of Pedilanthus tithymaloides ethanolic leaf extract on Very Low Density Lipoprotein cholesterol (VLDLc)

The same effect was observed with VLDLc. Administration of Pedilanthus tithymaloides 500mg/kg.b.w reduced elevated level of serum VLDLc from 34.53 ± 0.35mg/dL to 22.73 ± 0.48mg/dL in the diabetic control group. Pedilanthus tithymaloides extract at a dose of 1000mg/kg.b.w significantly lowered VLDLc 19.13 ± 0.458 almost comparable to glibenclamide treated Group V 18.9 ± 0.43mg/dL. The results were represented by means of a Graph 3. by using ANOVA followed ny Dunnett’s t-test.

High Density Lipoprotein cholesterol (HDLc)

Induction of diabetes resulted in reduction of HDLc to 30 ± 1.31mg/dL compared to 51.33 ± 0.8819mg/dL in the normal group. In comparison with the diabetic control group, Group III which was administered Pedilanthus tithymaloides at a dose of 500mg/kg.b.w showed increased level 49.66 ± 1.202mg/dL and extract treated with 1000mg/kg.b.w, significantly increased HDLc level to 66.83 ± 2.638mg/dL. which was almost comparable to the standard drug 70.33 ± 2.290mg/dL. The results were analysed by means of a (Graph 4) by using ANOVA followed ny Dunnett’s t-test.

Total cholesterol (TC)

Mean serum Total Cholesterol levels in normal group was 119.66 ± 1.39mg/dL. It was significantly elevated in diabetic control group, GPlI 275.17 ± 2.10mg/dL compared to normal group I. Administration of Pedilanthus tithymaloides 500mg/kg.b.w lowered serum total cholesterol level to 140.83 ± 2.85mg/dL and extract treated at a dose of 1000mg/kg.b.w significantly lowered serum total cholesterol levels to 124.33 ± 1.97mg/dL was comparable to the standard drug treated 107.33 ± 5.097mg/dL. The results were analyzed by means of a (Graph 5) by using ANOVA followed ny Dunnett’s t-test.

Triglycerides

The same effect was noticed with triglycerides as total cholesterol. Administration of Pedilanthus tithymaloides ethanolic leaf extract at a dose of 500mg/kg.b.w lowered the elevated mean serum triglycerides level from 172.7 ± 1.78mg/dL to 113.83 ± 2.46mg/dL in diabetic control group compared to normal control group 116.66 ± 2.99mg/dL. Group IV treated Pedilanthus tithymaloides at a dose of 1000mg/kg.b.w significantly lowered triglycerides level to 95.66 ± 2.99mg/dL which was comparable to standard drug 94.5 ± 2.17mg/dL. The results were represented by means of a Graph 6. by using ANOVA followed ny Dunnett’s t-test.

Cholesterol ratio

The mean cholesterol ratio was elevated in the diabetic control group, GPlI 9.17 ± 0.45mg/dL. after induction of diabetes when compared to the normal group 2.33 ± 0.028mg/dL. The group which had Pedilanthus tithymaloides 500mg/kg.b.w showed lowerer mean cholesterol ratio 2.84 ± 0.089mg/dL and group IV treated with pedilanthus tithymaloides 1000mg/kg.b.w showed significantly lowered mean cholesterol ratio level 1.86 ± 0.604mg/dL which was comparable to glibenclamide treated group V 1.526 ± 0.108mg/dL. The results were analyzed by means of a (Graph 7) by using ANOVA followed ny Dunnett’s t-test.
Hence the administration of Pedilanthus tithymaloides ethanolic leaf extract for 21 days reduced the elevated total cholesterol level, triglycerides level, LDLc, VLDLc, and cholesterol ratio levels and increased the low HDLc level. The above results suggest that Pedilanthus tithymaloides at a dose of 1000mg/kg.b.w was more effective than 500mg/kg.b.w which was comparable with the standard drug glibenclamide.

**Table 1:**
**Blood Glucose Levels (Mg/Dl) On 1"st, 3"rd, 7"th And 15"th Day Of The Treatment:**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>1&quot;st day mg/dL</th>
<th>3&quot;rd day mg/dL</th>
<th>7&quot;th day mg/dL</th>
<th>15&quot;th day mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>86.33 ± 5.97</td>
<td>83.5 ± 5.29</td>
<td>87.83 ± 4.86</td>
<td>82.53 ± 1.37</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>254.33 ± 2.71</td>
<td>260.16 ± 2.96</td>
<td>271.53 ± 19</td>
<td>279.16 ± 2.98</td>
</tr>
<tr>
<td>Pedilanthus tithymaloides treated 500mg/kg.b.w</td>
<td>257 ± 3.50</td>
<td>209 ± 3.50</td>
<td>169 ± 3.50**</td>
<td>117.33 ± 3.22**</td>
</tr>
<tr>
<td>Pedilanthus tithymaloides treated 1000mg/kg.b.w</td>
<td>260.66 ± 3.38</td>
<td>205.66 ± 3.34**</td>
<td>147.66 ± 3.34**</td>
<td>110.66 ± 2.18**</td>
</tr>
<tr>
<td>Glibenclamide treated</td>
<td>266.16 ± 4.17</td>
<td>201.6 ± 3.34**</td>
<td>137.66 ± 3.34**</td>
<td>94.83 ± 2.60**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for group of six animals in each group. Diabetic control rats were compared with normal rats. Diabetic + P.tithymaloides 500mg/kg.b.w, Diabetic + Pedilanthus tithymaloides 1000mg/kg.b.w and Diabetic + glibenclamide treated rats were compared with Diabetic control rats. **P<0.01 was considered significant comparing to Diabetic control group.

**Graph 1:** Blood Glucose Levels (Mg/Dl) On 1st, 3rd, 7th & 15th Day Of The Treatment

ANOVA followed by Dunnett’s t-test. **P<0.01 was considered significant comparing to Diabetic control group**
Table 2 Effect Of Pedilanthus Tithymaloides Ethanolic Leaf Extract On Serum Lipid Profile In Alloxan(150mg/Kg.B.W) Induced Diabetic Rats After 15 Days/2 Weeks Treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Lipoproteins (mg/dL) (LDL)</th>
<th>Serum Very Low Density Lipoproteins (VLDL) mg/dL</th>
<th>Serum High Density Lipoproteins (HDL) mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (NC)</td>
<td>46 ± 0.80</td>
<td>22.33±0.59</td>
<td>51.33±0.8819</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>210.63 ± 2.89</td>
<td>34.53± 0.35</td>
<td>30±1.31</td>
</tr>
<tr>
<td>Pedilanthus tithymaloides Treated 500mg/kg.b.w</td>
<td>68.4±2.95**</td>
<td>22.73±0.48**</td>
<td>49.66±1.202**</td>
</tr>
<tr>
<td>Pedilanthus tithymaloides Treated 1000mg/kg</td>
<td>40.03±1.66**</td>
<td>19.13±0.458**</td>
<td>66.83±2.638**</td>
</tr>
<tr>
<td>Glibenclamide Treated</td>
<td>20.9±5.73**</td>
<td>18.9±0.43**</td>
<td>70.33±2.290**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for group of six animals in each group. Diabetic control rats were compared with normal rats. Diabetic + P.tithymaloides 500mg/kg.b.w, Diabetic + Pedilanthus tithymaloides 1000mg/kg.b.w and Diabetic + glibenclamide treated rats were compared with diabetic control rats.

**P<0.01 was considered significant comparing to Diabetic control group.

Graph 2

ANOVA followed by Dunnett's t-Test

**P < 0.01 was considered significant comparing to Diabetic control group.
**P < 0.01** was considered significant comparing to Diabetic control group

**Graph 3:**

ANOVA followed by Dunnett’s t-test.

**Graph 4**

ANOVA followed by Dunnett’s t-test.

**P<0.01** was considered significant comparing to Diabetic Control group
Table 3: Effect Of Pedilanthus Tithymaloides Ethanolic Leaf Extract On Serum Total Cholesterol, Triglycerides, Cholesterol Ratio In Alloxan (150mg/Kg.B.W) Induced Diabetic Rats After 15 Days/ 2 Weeks

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TOTAL CHOLESTEROL (mg/dL)</th>
<th>TRIGLYCERIDES (mg/dL)</th>
<th>Cholesterol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (NC)</td>
<td>119.66±1.39</td>
<td>111.66±2.99</td>
<td>2.33±0.028</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>275.17±2.10</td>
<td>172.7±1.78</td>
<td>9.17±0.45</td>
</tr>
<tr>
<td>Pedilanthus tithymaloides Treated 500mg/kg.b.w</td>
<td>140.83±2.85**</td>
<td>113.83±2.46**</td>
<td>2.84±0.089**</td>
</tr>
<tr>
<td>Pedilanthus tithymaloides Treated 1000mg/kg</td>
<td>124.33±1.97**</td>
<td>95.66±2.29**</td>
<td>1.86±0.0604**</td>
</tr>
<tr>
<td>Glibenclamide Treated</td>
<td>107.33±5.097**</td>
<td>94.5±2.17**</td>
<td>1.52±0.108**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for group of six animals in each group.
Diabetic control rats were compared with normal rats. Diabetic + P. tithymaloides 500mg/kg.b.w , Diabetic + Pedilanthus tithymaloides 1000mg/kg.b.w and Diabetec + glibenclamide treated rats were compared with Diabetic control rats..

**P<0.01 was considered significant comparing to Diabetic control group.

Graph 5:

ANOVA followed by Dunnett’s t-test

**P<0.01 was considered comparing to Diabetic control group.
Graph 6: Serum Total Triglycerides

ANOVA followed by Dunnett’s t-test

**P < 0.01 was considered significant comparing to Diabetic control group.

Graph 7: CHOLESTEROL RATIO

ANOVA followed by Dunnett’s t-Test

**P < 0.01 was considered significant comparing to Diabetic control group

V. Discussion

Diabetes Mellitus is a chronic disorder caused by partial or complete insulin deficiency, which produces inadequate glucose control and leads to acute and chronic complication. Premature and extensive atherosclerosis involving renal, peripheral, and cardiovascular vessels remain the major complication of Diabetes Mellitus. Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk for coronary heart disease. A reduction in serum lipids, particularly of LDL and VLDL fraction and Triglycerides, should be considered as being beneficial for long term prognosis. Lowering of blood glucose levels and serum lipid level through dietary medication and drug therapy seems to be associated with the decreased in risk of vascular disease.

Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic β cells resulting in the decrease in endogenous insulin release. Numerous studies demonstrated that the variety of plant extracts effectively lowered the glucose levels in alloxan induced diabetic animals.7
In the present study, the ethanolic extract Pedilanthus tithymaloides effectively decreased the blood glucose levels in alloxan induced diabetic rats as compared with standard group. This is in accordance with K Himani reddy, M Sheshanka et.al who reported that alcoholic and ethanolic extracts significantly reduced blood glucose levels when compared to petroleum ether and chloroform extracts. However the mechanism of the plant for lowering blood glucose levels has not been clearly defined.

The increase in serum lipid, Total cholesterol, Triglycerides, LDL, VLDL levels occurs in diabetes which is related with significant changes in lipid metabolism and structure.

In the present study, Total cholesterol, Cholesterol ratio, LDL, VLDL, Triglycerides levels increased and HDL levels decreased in alloxaanised diabetic rats, while the ethanolic extract of Pedilanthus tithymaloides in the dose of 1000mg/kg.b.w significantly lowered total cholesterol, Cholesterol ratio LDL, VLDL and Triglycerides levels and increased HDL levels than those treated with the dose 500mg/kg.b.w.

Chattopadhyay and Bandhopadhyaya reported ethanolic extract of air dried 1Kg fresh matured leaves of Azadirachta indica 500mg/kg for 7 days significantly reduced the Total Cholesterol, Total lipids, Low density lipoprotein cholesterol(LDLc), Very Low Density Lipoprotein cholesterol(VLDLc), Triglycerides, but High Density Lipoprotein cholesterol(HDLc) was unchanged of serum in streptozotocin induced diabetic rats

Ravi R, Ramachandra B et.al reported ethanolic whole jaman fruit extract 90ml/day resulted in decreased serum glucose in some individuals but not significantly, LDL was lowered and HDL was increased in 12 day. Hence jaman extract possess antidiabetic and hypolipidemic activity. Ethanolic seed extract of syzigium cumini at a dose of 100mg/kg/b.w orally produced blood sugar lowering, hypolipidemic, increased serum insulin, increased glycogen content of muscles and liver and a fall in glycosylated haemoglobin level Ethanolic seed kernels extract (100gm/kg,b.w) has been observed to improve glucose tolerance and produced hypoglycemic and hypolipidemic effects in STZ induced diabetic rats.

David O Bawak Adeyemi et.al reported methanolic leaf extract of Annona muricata 100mg/kg i.p in streptozotocin induced diabetic rats showed significant reduction in serum total cholesterol, triglycerides, Low density lipoprotein cholesterol(LDL), very low density lipoprotein cholesterol (VLDL),and significant increase in high density lipoprotein cholesterol(HDL) resulting in antihyperlipidemic activity in diabetic rats.

Chidambaram T, Kumarappan et.al reported polyphenolic extract of Ichnocarpus Frutescence leaves 150mg/kg, 300mg/kg b.w resulted in a significant reduction of fasting blood glucose . polyphenolic extract for 21 days showed significant decrease in hepatic HMG-COA reductase activity of alloxan induced diabetic rats. LDLc, VLDLc, TC, triglycerides were lowered and HDLc was increased indicating antihyperlipidemic activity. Oral administration of polyphenolic extract 100mg/kg significantly enhanced the release of lipoprotein lipase enzyme.

SharmaB, Santosh Ket.al reported ethanolic leaves extract of Aegle marmelos 300mg/kg,b.w in streptozotocin induced diabetic mice showed reduction in blood glucose, total cholesterol, LDLc, VLDLc, triglycerides and HDLc was increased in 15days. Hence Aegle marmelos possess antidiabetic and antihyperlipidemic activity. Kamalakkan and Prince S reported aegle marmelos leaf extract (1ml/100g) produced antihyperglycemic activity along with decreased cholesterol and blood urea in alloxan induced diabetic rats.

Fruit extract (125 and 250mg/kg, orally twice daily for 30 days produced antidiabetic, antihyperlipidemic, and antioxidant activity in STZ diabetic rats along with partial repair of damaged pancreatic islets.

was reported by Kamalakkan N, Ponnachan PT,Kesar AN, Gupta RK reported aegle marmelos seed extract possess significant hypoglycemic effect in normal and diabetic rats.

**Antinflammatory:** Medicinal tincture from Pedilanthus tithymaloides was evaluated for invivo antinflammatory activity and for it’s invivo scavenging effect on ROS,RNS and DPPH radical. The intra peritoneal administration of the tincture inhibited carrageenan induced rat paw oedema whereas in the scavenging assays the tincture showed to be effective against all the assayed ROS, RNS. These results provide scientific support for empirical use of pedilanthus tithymaloides tincture as an antinflammatory medicine by Pedro abrew, Susan Mathew, et.al. The usefulness of galactose specific lectin Pedilanthus tithymaloides was examined to study the hemagglutination pattern in patients with diabetes mellitus

Significantly low titer was seen in patients with insulin dependant diabetes and no significant changes in non insulin dependant diabetics.

The results of the preliminary phytochemical analysis of the leaf extract revealed the presence of steroids, alkaloids, glycosides, saponins, and flavanoids. It is therefore probable that the multiplicity of actions reflected by the broad spectrum of activity. Sterols main action is to lower LDL cholesterol levels. Hence we may assume the plant may contain sterols.

On the basis of the above mentioned results, I conclude that Pedilanthus tithymaloides family Euphorbiaceae possess a significant hypoglycemic and hypolipidemic effect in alloxan induced diabetic rats and that these effects were comparable to that of glibenclamide.

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VI. Conclusion

Pedilanthus tithymaloides family euphorbiaceae is a low tropical american shrub with a wide range of healing properties including emetic, antiinflammatory, antibiotic antiseptic, antiviral, antitumoral etc. Preliminary phytochemical investigation and screening of Pedilanthus tithymaloides states the presence of Sterols, triterpenes, carbohydrates, flavanoides, tannins etc in the leaf extract which are mainly responsible in hypolipidemid and hypoglycemic action.

The ethanolic leaf extract of Pedilanthus tithymaloides was evaluated for invivo antidiabetic and antihyperlipidemic activity against alloxan induced diabetic rats. Alloxan causes selective destruction of βcells which are involved in production of Insulin. The results of the present investigation clearly indicate that the ethanolic leaf extract of Pedilanthus tithymaloides at doses 500mg/kg/b.w and 1000mg/kg/b.w have a glucose lowering effect and lipid lowering effect in alloxan induced diabetic rats. The extract at a dose of 1000mg/kg/bw was found to be more effective in lowering blood glucose levels than serum lipid levels compared to 500mg/kg/bw.

It was also found to be highly effective in managing complications associated with diabetes mellitus such as atherosclerosis, hyperlipidemia, etc.

Therefore Pedilanthus tithymaloides leaves show therapeutic promise as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in diabetes mellitus.

The exact biological active constituents responsible for the said effect are neither reported nor the exact mode of action was reported earlier, thus further biochemical and pharmacological investigations are needed to isolate and identify active ingredients in the extract using other models.

References

[1]. Shankar S. Field tested participatory Methodology for rapid assessment of community’s therapeutic Use of Medicinal Plants. Approaches towards evaluation of medicinal plants prior to clinical trials organised by foundation for Medical Research at YASHADA. 2006 Nov 8-13

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