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# Antidiabetic, Antihyperlipidemic and Antioxidant Properties of Roots of *Ventilago Maderaspatana* Gaertn. On Streptozotocin-Induced Diabetic Rats

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***Abstract**: Different extracts (Alcoholic, Hydroalcoholic and Chloroform) of Ventilago maderaspatana (V.maderaspatana; Family- Rhamnaceae) were evaluated for toxicity unto 3000 mg $kg^{-1}$. In oral glucose tolerance test chloroform extract did not produce significant glucose lowering effect. Alcoholic and hydroalcoholic extracts of V.maderaspatana (VMAE and VMHAE) elicited significant glucose tolerance effect. Hence VMAE and VMHAE were screened further by streptozocin induced diabetic model. VMAE and VMHAE significantly lowered blood glucose, triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol, creatinine, urea and increased HDL cholesterol, serum insulin and liver glycogen levels when compared to standard drug glibenclamide (10 mg $kg^{-1}$). V.maderaspatana also increased catalase levels and decreased lipid peroxidase and glutathione reductase. VMAE and VMHAE elicited significant dose-dependent antidiabetic, antihyperlipidemic and antioxidant activity. VMHAE at 500 mg $kg^{-1}$ induced more significant antidiabetic activity than VMAE (500 mg $kg^{-1}$). VMAE at 500 mg $kg^{-1}$ elicited more antihyperlipidemic and antioxidant activity compared to VMHAE (500 mg $kg^{-1}$).*

***Keywords:** Oral Glucose tolerance test, Streptozotocin, Glibenclamide, Serum Insulin, Liver Glycogen, Catalase.*

## I. Introduction

Diabetes mellitus is characterized by chronic hyperglycemia, disorder of carbohydrates, lipids, proteins and microvascular pathology in the retina, renal glomerulus and peripheral nerves [1]. According to the diabetes Atlas 2009, India has the largest number of people with diabetes in the world; with an estimated 50.8 million people diabetic. This figure is slated to increase to 87 million by 2030 [2]. Diabetes aggravates the generation of tissue damaging reactive oxygen species (ROS) [3] by glucose oxidation and/or non enzymatic protein glycosylation [4]. ROS involve in the pathogenesis of complications such as neuropathy, retinopathy and nephropathy [5]. Numerous therapies have been developed for effective regulation of blood glucose levels. Synthetic hypoglycemic agents were more expensive and produce various side effects. The management of diabetes without side effects is yet a challenge to the medical system. Therefore there is a need to search for effective and safer hypoglycemic agents. Demand is increasing for natural products with antidiabetic activity and antioxidant activity as they are non narcotic, easily biodegradable and produce lesser side effects [6]. Therefore it is essential to develop and introduce medicinal plants for dreadful illnesses.

*Ventilago maderaspatana* is a woody climbing plant commonly called Red creeper. It is widely distributed in jungles of papavinasanam, Tirumala horseley hills [7]. Leaves are oblong-lanceolate. Flowers are terminal or axillary. Fruits are subglobose nut 5 to 7 mm in diameter, yellow to grey. Seeds are globose, thin walled brown [8-11]. The powdered stem bark is mixed with gingelly oil and applied externally for skin diseases and itch [12]. Whole plant of *Ventilago maderaspatana* showed potent antibacterial activity against Proteus vulgaris, Bacillus thuringiensis, Streptococcus faecalis, Staphylococcus aureus, Salmonella paratyphi and Serratia marcescens by agar diffusion method [13]. Ethanolic extract of *Ventilago maderaspatana* have shown mild to moderate anti-denaturation and antioxidant activity [14]. Aqueous extract of *Ventilago maderaspatana* root bark have shown hepato protective effect against $ccl_4$ induced liver damage. Ethanolic extract of bark showed moderate cytotoxicity towards mcf -7 cell lines by MTT assay method [15]. Bioassay guided fractionation of *Ventilago maderaspatana* yielded physcion and emodin. These compounds have shown anti-inflammatory as well as anti cancer potential [16]. Ventiloquinone A isolated form *Ventilago maderaspatana* was the most effective antifeedant against Henosepilachna vigintioctopunetata and spodoptera litura [17]. Endophytic fungi isolated from *Ventilago maderaspatana* have indicated the presence of highly active protease in fusarium sporotrichioides and quorum sensing inhibitors in fusarium graminearum and lasidiplodia species [18]. Methanolic extract of whole plant *Ventilago maderaspatana* was found to possess cardioprotective effect against Isoproterenol induced myocardial infarction [19]. Root bark of *Ventilago*

*maderaspatana* (Fig.1) has shown the presence of various anthraquinones like ventinone-A.B, chrysophanol, physcion, emodin, islandicin, xanthorin and xanthorm-5-methyl ether [20], Napthalene derivatives and Napthaquinones like cordeauxione and isocardeauxione [21]. Traditionally it is used for treating many disorders like skin problems, fever, leprosy, scabies, prurities, diabetes, carminative, digestive, stomachic [22] and vitiated conditions of kapha, dyspepsia, colic, flatulence and erysipelas.

*Ventilago maderaspatana* is a medicinal plant with many therapeutic properties. Since the antidiabetic activity of this plant has not yet scientifically evaluated, the present study was undertaken to evaluate antidiabetic antihyperlipidemic and antioxidant activity of Ventilago maderaspatana roots in streptozotocin-induced diabetic rats.

## II. Materials And Methods

### 2.1 Plant material

Roots of *Ventilago maderaspatana* were collected from Tirumala forest area, Tirupathi, Andhrapradesh during the mon of June. The plant was authenticated by Dr. Madhava chetty, Assosciate Professor, Department of botany, Sri Venkateshwara University, Tirupathi. A vocher specimen (Spn No: 1162) was deposited in the herbarium.

### 2.2 Extraction process

Roots were washed, shade dried and finely powdered using a mechanical grinder. Powdered drug was extracted using soxhlet apparatus with ethanol (90%) and ethanol: water (3:1) for 3 days. Obtained extracts were concentrated under reduced pressure using rota evaporator and stored in the refrigerator at 2-8 ºc. Percentage yield was found to be 16.49% and 15.80%.

### 2.3 Phytochemical Investigation

Preliminary photochemical screening was carried out for qualitative identification of phytochemical constituents employing standard methods. Chemicals and reagents used were of analytical grade [23, 24].

### 2.4 Animals

Healthy Sprague-dawley rats (200-300 g) were purchased from Albino Research Center, Hyderabad. They were housed under standard laboratory conditions of temperature (22±3 ºc), humidity (30% - 70%), light dark cycles (12 h:12 h), standard diet (Hindustan Lever Ltd.,) and water *ad libitum.* Experiments were performed according to the Institutional Anima ethics committee (IAEC) guidelines of Albino research center with the CPCSEA Regn No: 1722/PO/A/13/IAEC/CPCSEA EXP-049).

### 2.5 Chemicals

Streptozotocin (STZ) was procured from loba chemie, Mumbai (India). Glibenclamide, a standard antidiabetic drug was purchased from Apollo pharmacy, B. N Reddy Avanthi pharmaceuticals (Hyderabad). Glucose was obtained from Qualigens fine chemicals (Mumbai). Kits for estimation of TG, CH and HDL were obtained from Span diagnostics pvt ltd, Surat (India). Estimations were carried out using ELICO semiauto CL 380.

### 2.6 Acute toxicity study ($LD_{50}$) [23]

This study was performed according to the OECD guidelines 423, using acute toxic class method. Male rats were divided into groups containing three each. After overnight fast, alcoholic, hydroalcoholic and chloroform extracts of *Ventilago maderaspatana* were administered at doses of 300 mg $kg^{-1}$, 2000 mg $kg^{-1}$ and 3000 mg $kg^{-1}$ b.wt. Rats were observed individually for first 30 min, periodically during first 24 h and daily thereafter for 14 d. At the end they were observed for autonomic, neurological and behavioural profiles.

### 2.7 Antihyperglycemic activity

#### 2.7.1 Assessment of plant extracts in normal rats: oral glucose tolerance test (OGTT) [24]

Rats were divided into groups (n=5). After overnight fast Group I received Vehicle (1% Na.cmc). Group II received standard drug, glibenclamide (10 mg $kg^{-1}$). Group III, IV, V and VI received alcoholic extract (125, 250 and 500 mg $kg^{-1}$ b.wt) and hydroalcoholic extract (125, 250 and 500 mg $kg^{-1}$ b.wt) orally. 30 min later rats of all groups were loaded with glucose (3 gm $kg^{-1}$ b.wt). Blood samples were collected from retroorbital plexus prior to and after the drug administration at intervals of 0h, 1h, 3h and 5 h intervals

**2.7.2 Assessment of plant extracts in streptozotocin induced diabetic rats**

Diabetes was induced by single intraperitoneal injection of streptozotocin (50 mg kg-1 b.wt). Streptozotocin was freshly prepared in 0.01M ice cold citrate buffer (pH 7.4). Streptozotocin induces fatal hypoglycemia as a result of massive pancreatic insulin release. To avoid hypoglycemic effect rats were provided with 5% dextrose solution after 6 h of streptozotocin administration. The threshold glucose level to diagnose diabetes was taken as greater than 200 mg $dl^{-1}$ and only those rats were included in the experiment. Diabetic rats were divided into nine groups of five each.

Group I Normal control - 1% Na.cmc (1 ml $kg^{-1}$ $day^{-1}$, p.o)
Group II Diabetic control - Streptozotocin+1% Na.cmc (1 ml $kg^{-1}$ $day^{-1}$, p.o)
Group III Streptozotocin+ Glibenclamide (10 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days
Group IV Streptozotocin+ VMAE (125 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days
Group V Streptozotocin+ VMAE (250 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days
Group VI Streptozotocin+ VMAE (500 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days
Group VII Streptozotocin+ VMHAE (125 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days
Group VIII Streptozotocin+ VMHAE (250 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days
Group IX Streptozotocin+ VMHAE (500 mg $kg^{-1}$ $day^{-1}$, p.o) for 21 days

Blood samples were withdrawn on $1^{st}$, $7^{th}$, $14^{th}$ and $21^{st}$ d. Serum separated by centrifugation (3000 r/m; 20 min) was used for biochemical estimations.

**2.8 Biochemical estimations**

Blood glucose estimation was done by Glucocheck® (Major Biosystem Corporation. Taipei, Taiwan 235). Triglycerides (TG) were estimated by GPO-POD method using span diagnostic kit [25]. Total serum cholesterol (CH) was measured by CHOD-POD method using span diagnostic kit [26]. High density lipoproteins (HDL), was assayed by Phosphotungstate magnesium acetate reagent method using Agappe diagnostic kit [27]. Very Low density lipoproteins (VLDL) and Low density lipoproteins (LDL) cholesterol were calculated by Friedewald formulae [28]. VLDL cholesterol = TG/5. LDL cholesterol = Total cholesterol-HDL cholesterol –TG/5. Serum creatinine and serum urea were estimated by span diagnostic kit, India [29, 30]. Body weight measurement was done by digital weighing balance. Liver glycogen levels were estimated by Anthrone reagent method [31]. Serum insulin measurement was done by radioimmunoassay method [32]. On $21^{st}$ d rats were sacrificed by cervical dislocation. Pancreas was isolated, washed with saline and homogenized by Tris-Hcl buffer. Supernatant was used for measuring the activity of catalase, lipid peroxidation and glutathione reductase.

**2.8.1 Estimation of Catalase [33]**

Catalase was assayed as described by Aebi (1974). Supernatant (0.1 ml) was added to cuvette containing 1.9 ml f 50 mM phosphate buffer (pH 7.0). To this mixture, 1.0 ml of freshly prepared 30 mM $H_20_2$ was added. The rate of decomposition of $H_20_2$ was measured spectrophotometrically at 240 nm for 3 min at an interval of 30 s.

**2.8.2 Estimation of Lipid Peroxidation [34]**

Lipid peroxidation in pancreas was estimated by measuring malonaldehyde (MDA) using the method of ohkawa (1979). In brief, 0.2 ml of tissue homogenate was treated with 1.5 ml of 0.8% TBA (thio barbituric acid), 0.2 ml of 8.1% SDS (Sodium dodecyl sulphate), 1.5 ml of 20% acetic acid. The mixture was made upto 5 ml with distilled water and heated in an oil bath at 95 °C for 1 h. Later n- butanol and pyridine (15:1, v/v) was added. After centrifugation at 4000 r/m for 10 min organic layer was taken and absorbance was measured at 532 nm calorimetrically.

**2.8.3 Estimation of Reduced Glutathione [35]**

Reduced glutathione was estimated by the method of Ellman (1959). The homogenate (in 0.25 M Tris buffer) was added with equal volume of 10% Trichloro acetic acid (TCA). The mixture was centrifuged at 5000 r/m for 10 min. The supernatant (0.1 ml) was added with DTNB (5, 5´ Dithio bis-2-nitrobenzoic acid, 2 ml of 0.6 mM) and phosphate buffer (1.9 ml of 0.2 M, PH 9.0). The absorbance was measured at 412 nm against blank.

**2.9 Histopathological studies**

Liver and pancreas were dissected out, rinsed in ice cold saline solution and fixed in 10% formalin. These were dehydrated in alcohol and embedded in paraffin. Microtome sections (4-5µm in thickness) were cut and stained with haematoxylin and eosin for histopathological examination. The evaluation was represented (Fig.2 and Fig. 3).

## III. Statistical Analysis

Results were expressed as mean ± SEM. The significance of data was evaluated by graph pad in stat version 3.2. P value of analysis less than 0.05 was considered to be statistically significant.

## Results

**TABLE 1: Phytochemical screening of VMAE and VMHAE Roots**

| Phyto Constituents | Test | Alcoholic | Hydroalcoholic |
|---|---|---|---|
| **Alkaloids** | Mayers test | ++ | ++ |
| | Hagners test | ++ | ++ |
| | Wagners test | ++ | ++ |
| | Dragendroff"s test | ++ | ++ |
| **Glycosides** | Borntrager's test | ++ | ++ |
| | Dam-karrer test | ++ | ++ |
| | Juglone test | ++ | ++ |
| **Emodin** | Specific test | ++ | ++ |
| **Cardiac glycosides** | Legal test | ++ | ++ |
| | Baljet test | -- | ++ |
| **Terpenoids** | Salkowsky test | -- | ++ |
| **Carbohydrates** | Molich test | ++ | ++ |
| | Fehling's test | ++ | ++ |
| **Flavonoids** | Shinoda test | ++ | ++ |
| **Steroids** | Leibermann-burchards test | ++ | -- |
| **Proteins** | Biuret test | -- | -- |
| | Millons test | -- | -- |
| **Tannins** | $Fecl_3$ test | ++ | ++ |
| **Saponins** | Frothing test | ++ | ++ |

Present (++); Absent (--)

**TABLE 2: Effect of VMAE, VMHAE and $VMCHCl_3$ in normal glucose loaded rats**

| Group | Treatment (mg/kg b.wt) | Serum glucose (mg/dl) (mean± SEM) | | | |
|---|---|---|---|---|---|
| | | 0 h | 1 h | 3 h | 5 h |
| **I** | **Normal Control** | 73.50±2.75 | 75.40±3.44 | 79.50±4.44 | 77.80±3.10 |
| **II** | **Glucose (10 g/kg)** | 63.03±2.70** | 102.40±3.03** | 96.41±1.67** | 93.31±2.85** |
| **III** | **Glibenclamide(10)** | 69.80±3.30** | 100.32±2.55 | 69.66±2.01** (30.56%) | 66.13±2.55** (34.08%) |
| **IV** | **VMAE (125)** | 73.55±3.11 | 117.33±3.55** | 80.75±2.30** (31.18%) | 78.63±4.23* (32.98%) |
| **V** | **VMAE (250)** | 71.40±2.11** | 115.22±3.42* | 77.31±2.50** (32.90%) | 73.33±3.55** (36.36%) |
| **VI** | **VMAE (500)** | 73.40±3.04** | 102.50±3.81 | 75.14±3.21** (26.69%) | 63.55±4.03** (38%) |
| **VII** | **VMHAE (125)** | 75.67±2.45 | 110.03±3.01 | 80.32±3.10** (27.00%) | 76.37±3.95**(30.59%) |
| **VIII** | **VMHAE (250)** | 77.60±2.55** | 103.36±3.11 | 85.52±2.80* (17.26%) | 72.66±3.10** (29.7%) |
| **IX** | **VMHAE (500)** | 67.89±2.75* | 105.66±2.45 | 74.66±3.45** (29.33%) | 67.35±2.44** (36.26%) |
| **X** | **VMCHCl3 (250)** | 77.80± 3.03** | 124.65±4.10** | 115.8±3.25** (7.09%) | 89.83±2.77 (27.93%) |
| **XI** | **VMCHCl3 (500)** | 73.20± 2.91** | 106.73±3.99 | 91.34±3.71 (14.41) | 90.82±3.15 (14.90%) |

n=5; Group II is compared with Group I. Groups III-XI were compared with Group II. *P<0.05. **P<0.01.

**TABLE 3: Effect of VMAE and VMHAE on serum glucose levels in STZ induced diabetic rats**

| Treatment (mg/kg b.wt) | Serum glucose (mg/dl) (mean± SEM) | | | |
|---|---|---|---|---|
| | 1st day | 7th day | 14th day | 21st day |
| **Normal Control** | 105.1±2.0 | 105.22±3.13 | 99.68±2.15 | 102.38±1.74 |
| **Diabetic Control** | 341.93±3.40** | 350.58±4.53** | 361.02±5.95** | 379.16±6.03** |
| **Glibenclamide(10)** | 337.5±4.34 | 278.44±3.75** (17.49%) | 197.5±2.10** (41.48%) | 153.12±1.59** (54.63%) |
| **VMAE (125)** | 337.55±4.25 | 310.43±4.11** (08.03%) | 278.63±3.33**(17.45%) | 222.11±3.15** (34.19%) |
| **VMAE (250)** | 339.2±5.11 | 306.58±4.10** (9.61%) | 271.76±3.45** 19.88%) | 213.15±3.33** (37.16%) |
| **VMAE (500)** | 325.10±3.10** | 286.5±2.77** (11.87%) | 207.5±3.01** (36.17%) | 159.45±2.40** (50.95%) |
| **VMHAE (125)** | 340.45±3.23 | 311.37±3.33** (08.54%) | 249.72±3.13** (26.65%) | 218.32±4.10** (35.87%) |
| **VMHAE (250)** | 336.56±3.51 | 304.18±4.44** (9.62%) | 250.94±2.65** (25.44%) | 204.10±3.23** (39.35%) |
| **VMHAE (500)** | 353.46±3.50** | 283.42±5.10** (19.81%) | 199.72±2.55** (43.50%) | 157.42±2.10** (55.46%) |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

**TABLE 4: Effect of VMAE and VMHAE on serum TC, TG, HDL, LDL, VLDL levels in STZ induced diabetic rats on 1st day**

| Treatment (mg/kg b.wt) | TC (mg/dl) | TG (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|
| Control | 90±2.10 | 105.2±3.35 | 35.94±1.45 | 33.02±2.01 | 21.04±2.10 |
| Diabetic Control | 145.75±1.72** | 172.10±3.051** | 34.48±1.57** | 76.85±2.11** | 34.42±1.34** |
| Glibeclamide (10) | 140.62±2.01** | 166.90±3.51* | 37.44±2.32 | 69.80±2.07** | 33.38±2.10 |
| VMAE (125) | 151.33±2.55 | 205.06±3.01** | 30.55±2.01 | 79.77±3.45 | 41.01±2.18 |
| VMAE (250) | 149.55±2.22** | 199.70±2.45** | 34.68±1.72 | 74.93±3.33 | 39.94±1.79** |
| VMAE (500) | 141.05±2.00** | 157.80±1.75** | 36.28±1.14 | 73.21±3.04 | 31.56±2.53 |
| VMHAE (125) | 149.12±2.01 | 198.32±1.55** | 31.60±1.09 | 77.86±2.77 | 39.66±3.10 |
| VMHAE (250) | 147.08±3.10 | 189.50±2.56** | 33.94±1.26 | 75.24±2.95 | 37.90±2.27 |
| VMHAE (500) | 145.05±1.05** | 158.90±2.03** | 36.42±2.05 | 73.85±3.05 | 31.78±2.33 |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

**TABLE 5: Effect of VMAE and VMHAE on serum TC, TG, HDL, LDL, VLDL levels in STZ induced diabetic rats on 21st day**

| Treatment (mg/kg b.wt) | TC (mg/dl) | TG (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|
| Control | 88.32±2.71 | 143.7±3.11 | 37.24±1.34 | 22.34±2.55 | 28.74±2.0 |
| Diabetic Control | 253.15±4.10** | 271.7±3.32** | 29.16±1.75** | 169.65±3.11** | 54.34±2.73** |
| Glibeclamide (10) | 98.68±2.35** (29.82%) | 127.7±3.10** (23.48%) | 46.62±3.10** (24.52%) | 26.52±4.35** (62.01%) | 25.54±2.80** (23.48%) |
| VMAE (125) | 130.65±4.10** (13.66%) | 160.23±4.37** (21.86%) | 38.43±2.31* (20.50%) | 60.17±3.05** (24.57%) | 32.05±1.57** (21.84%) |
| VMAE (250) | 125.77±3.10** (15.90%) | 155.7±3.91** (22.03%) | 42.88±2.34** (23.64%) | 51.75±3.42** (30.94%) | 31.14±2.0** (22.03%) |
| VMAE (500) | 110.40±2.89** (21.72%) | 112.9±4.89** (28.45%) | 46.10±1.54** (27.07%) | 41.72±2.92** (43.01%) | 22.58±1.55** (28.45%) |
| VMHAE (125) | 132.55±3.11** (11.11%) | 156.13±3.19** (21.27%) | 37.33±1.95* (15.34%) | 63.99±3.01** (17.81%) | 31.23±2.07** (21.25%) |
| VMHAE (250) | 128.62±3.75** (12.55%) | 147.9±5.20** (21.95%) | 42.22±2.10** (24.4%) | 56.82±3.55** (24.48%) | 29.58±2.07** (21.95%) |
| VMHAE (500) | 110.33±3.10** (22.33%) | 119.8±4.15** (24.60%) | 44.12±1.77** (21.14%) | 42.25±2.66** (94.54%) | 23.96±3.10** (24.60%) |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

**TABLE 6: Effect of VMAE and VMHAE on body weight in STZ induced diabetic rats (mean± SEM)**

| Group | Treatment (mg/kg b.wt) | Body weight (g) | | | |
|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 |
| I | Normal Control | 209.00±7.45 | 209.80±11.75 | 202.20±6.15 | 204.60±5.35 |
| II | Diabetic Control | 298.00±8.32** | 256.33±5.25** | 233.66±4.11** | 207.00±7.89** (30.53%) |
| III | Glibenclamide(10) | 288.40±3.45* | 250.25±5.55 | 231.50±4.70 (19.09%) | 224.50±4.52** (22.15%) |
| IV | VMAE (125) | 295.35±4.12 | 265.35±5.15 | 250.57±4.32* (15.16%) | 246.45±5.32** (16.55%) |
| V | VMAE (250) | 299.40±6.71 | 267.66±3.04* | 258.33±6.15** (13.11%) | 248.33±5.55** (18.47%) |
| VI | VMAE (500) | 296.40±5.19 | 264.66±5.15 | 236.60±8.10 (11.48%) | 237.00±6.10** (16.13%) |
| VII | VMHAE (125) | 290.20±5.15 | 266.22±4.32 | 243.63±3.25 (13.65%) | 235.47± 6.32*(16.04%) |
| VIIII | VMHAE (250) | 304.80±4.23 | 280.00±5.20** | 267.00±5.55** (14.51%) | 247.50±6.95** (17.39%) |
| IX | VMHAE (500) | 283.00±5.10** | 265.66±6.31 | 248.00±5.34** (19.97%) | 222.33±7.10** (25.10%) |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

**TABLE 7: Effect of VMAE and VMHAE on serum creatinine and serum urea levels in STZ induced diabetic rats (mean± SEM)**

| Group | Treatment (mg/kg b.wt) | Serum Creatinine (mmol/L) 1st day | Serum Creatinine (mmol/L) 21st day | Serum Urea (mg/dl) 1st day | Serum Urea (mg/dl) 21st day |
|---|---|---|---|---|---|
| I | Control | 0.546±0.01 | 0.594±0.01 | 31.55±1.57 | 31.8±1.75 |
| II | Diabetic Control | 0.534±0.02 | 2.36±0.05** | 30.82±2.11 | 61.55±5.10** |
| III | Glibenclamide (10) | 0.536±0.03 | 0.585±0.05** (75.21%) | 30.46±3.75 | 31.55±3.54** (48.74%) |
| IV | VMAE(125) | 0.515±0.04 | 1.25±0.04** (47.03%) | 33.15±3.15 | 53.23±3.05 (13.52%) |
| V | VMAE (250) | 0.524±0.03 | 0.98±0.06** (58.47%) | 31.64±2.18 | 43.6±3.56** (29.16%) |
| VI | VMAE (500) | 0.537±0.03 | 0.63±0.03** (73.31%) | 32.14±3.37 | 32.5±3.89** (47.19%) |
| VII | VMHAE (125) | 0.523±0.01 | 1.23±0.05** (47.88%) | 32.01±3.55 | 50.15±4.35 (18.52%) |
| VIII | VMHAE (250) | 0.538±0.01 | 0.946±0.02** (59.92%) | 30.46±2.45 | 42.7±5.33** (30.62%) |
| IX | VMHAE (500) | 0.542±0.05 | 0.62±0.05** (73.73%) | 31.44±2.10 | 32.3±4.10** (47.52%) |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

**TABLE 8: Effect of VMAE and VMHAE on serum insulin and liver glycogen levels in STZ induced diabetic rats (mean± SEM)**

| Group | Treatment (mg/kg b.wt) | Serum insulin (IU/ml) | Liver glycogen (mg/gm of wet tissue) |
|---|---|---|---|
| **I** | **Control** | 92.57±4.0 | 55.32±3.42 |
| **II** | **Diabetic Control** | 48.3±3.95** | 30.36±4.10** |
| **III** | **Glibenclamide (10)** | 91.63±5.10** (89.71%) | 45.17±3.36** (48.71%) |
| **IV** | **VMAE(125)** | 78.30±3.84**(38.31%) | 38.23±2.46 (20.59%) |
| **V** | **VMAE (250)** | 82.90±3.67** (71.64%) | 40.53±2.52** (33.49%) |
| **VI** | **VMAE (500)** | 88.17±3.42** (82.55%) | 44.50±3.54** (46.57%) |
| **VII** | **VMHAE (125)** | 75.40±3.53** (35.94%) | 36.54±3.57 (16.91%) |
| **VIII** | **VMHAE (250)** | 81.33±4.11** (68.39%) | 41.73±4.41** (37.41%) |
| **IX** | **VMHAE (500)** | 87.41±5.10** (80.97%) | 43.71±3.11** (43.97%) |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

**TABLE 9: Effect of VMAE and VMHAE on Catalase, Lipid peroxidase (LPO) and glutathione on pancreas in STZ induced diabetic rats (mean± SEM)**

| Group | Treatment (mg/kg b.wt) | Catalase (U/mg protein) | LPO (U/mg protein) | Glutathione (U/mg protein) |
|---|---|---|---|---|
| **I** | **Normal Control** | 20.49±1.50 | 95.19±3.55 | 102.69±1.05 |
| **II** | **Diabetic Control** | 8.06±0.98** | 189.47±3.02** | 209.40±2.07** |
| **III** | **Glibenclamide(10)** | 20.47±2.0** (60.63%) | 103.38±1.77** (45.44%) | 114.64±2.77** (45.25%) |
| **IV** | **VMAE (125)** | 16.21±2.45*(50.28%) | 116.72±3.11** (38.39%) | 135.73±2.51** (35.18%) |
| **V** | **VMAE (250)** | 17.5±2.75** (53.94%) | 114.61±2.51** (39.51%) | 137.5±3.0** (34.33%) |
| **VI** | **VMAE (500)** | 14±1.77** (42.43%) | 103.14±3.0** (45.56%) | 121.33±2.83** (42.05%) |
| **VII** | **VMHAE (125)** | 15.35±1.97* (47.49%) | 125.43±2.72** (33.79%) | 143.21±3.11** (31.61%) |
| **VIIII** | **VMHAE (250)** | 16±2.3** (49.63%) | 120.33±2.33** (36.49%) | 146.66±4.6** (29.96%) |
| **IX** | **VMHAE (500)** | 12±2.0*(32.83%) | 106.55±3.15** (43.76%) | 123.11±3.9** (41.20%) |

n=5; Group II is compared with Group I. Groups III-IX were compared with Group II. *P<0.05. **P<0.01.

Results enumerated active principles like napthaquinones (emodin), alkaloids, glycosides, cardiac glycosides, terpenoids, carbohydrates, flavonoids, steroids, proteins, tannins and saponins as indicated in TABLE 1. Acute toxicity studies revealed VMAE and VMHAE were safe upto 3000 mg $kg^{-1}$. Hence the doses 125 mg $kg^{-1}$, 250 mg $kg^{-1}$ and 500 mg $kg^{-1}$ were selected.

### 3.1 Oral glucose tolerance test

In oral glucose tolerance test, glibenclamide (10 mg $kg^{-1}$) produced significant (P<0.01) glucose lowering effect at 3rd and 5th h compared to glucose loaded group. The normal group showed an unchanged profile of glycemia at all time intervals as shown in TABLE 2. VMAE and VMHAE at doses of 125, 250 and 500 mg $kg^{-1}$ produced a significant reduction (P<0.05) at 3rd h and 5th h. VMAE at a dose of 500 mg $kg^{-1}$ produced greater antihyperglycemic effect (38%) when compared to other groups. $VMCHCl_3$ did not produce significant reduction at doses of 250 and 500 mg $kg^{-1}$.

### 3.2 Serum glucose levels

Diabetic rats treated with 125, 250 and 500 mg kg-1 of VMAE and VMHAE showed a significant reduction (P<0.01) in glucose levels (34.19%, 37.16%, 50.95%); (35.87%, 39.35%, 55.96%) at the end of 21st day treatment as indicated in TABLE 3. At the same time glibenclamide (10 mg kg-1) also produced a significant reduction (P<0.01) of glucose levels (54.63%). Among these groups VMHAE at a dose of 500 mg kg-1 showed higher antihyperglycemic activity.

### 3.3 Serum lipid levels

Serum lipid levels of TG, TC, HDL, LDL and VLDL on 1st and 21st day of the study were given in TABLE 4 and TABLE 5. The diabetic rats showed significantly increased lipid levels and decreased HDL levels on 1st day. VMAE and VMHAE (125, 250 and 500 mg kg-1) significantly reduced (13.66%, 15.9%, 21.71%, 11.11%, 12.5% and 22.3%) (P<0.01) enhanced level of lipids on 21st day and increased HDL levels (P<0.05).

### 3.4 Body weight

Control group manifested increase in the body weight (5.25%) as given in TABLE 6. In diabetic control group a significant decrease (30.53%) (P<0.01) was observed. Standard drug glibenclamide, VMAE, VMHAE (125, 250 and 500 mg kg-1) also manifested decreased body weight (16.55%, 22.15%, 18.47%, 16.13%, 16.04%, 17.39%, 25.10%). The percentage decrease was less when compared to diabetic control group.

### 3.5 Serum creatinine and urea levels

VMAE and VMHAE (125, 250 and 500 mg kg-1) produced a significant decrease (47.03%, 58.47%, 73.31%, 47.88%, 59.92%, 73.73%) (P<0.01) in increased serum creatinine levels of diabetic control group (TABLE 7). VMAE and VMHAE (250 and 500 mg kg-1) caused a significant reduction (29.16%, 47.19%,

30.62%, 47.52%) (P<0.01) in the increased serum urea levels of diabetic control group as indicated in TABLE 7. Decrease in serum urea levels was not significant at 125 mg kg-1 of both VMAE and VMHAE (13.52% and 18.52%).

**3.6 Serum insulin and Liver glycogen levels**

Diabetic control group presented a significantly decreased serum insulin levels as given in TABLE 8. Glibenclamide, VMAE and VMHAE (125, 250 500 mg kg-1) restored significantly (89.71%, 38.31%, 82.55%, 71.64%, 35.94%, 80.97%, 68.39%) (P<0.01) decreased serum insulin levels to normal. Liver glycogen levels significantly decreased in diabetic control group. Standard drug, Glibenclamide, VMAE and VMHAE (250 and 500 mg kg-1) groups significantly increased liver glycogen levels (48.71%, 33.49%, 46.57%, 37.41%, 43.97%) (P<0.01) as tabulated in TABLE 8. Decrease in Liver glycogen levels were not significant at 125 mg kg-1 of both VMAE and VMHAE (20.59% and 16.91%)

**3.7 Catalase, LPO and Glutathione levels**

VMAE and VMHAE (125, 250 and 500 mg kg-1) exhibited a significant antioxidant effect eliciting an increased catalase levels (50.28%, 53.94%, 42.43%, 47.49%, 49.63%, 32.83%) (P<0.05) and decreased LPO and glutathione levels (38.39%, 39.51%, 45.56%, 33.79%, 36.49%, 43.76%) (P<0.01) and (35.18%, 34.33%, 42.05%, 31.61%, 29.96%, 41.20%) (P<0.01). The results were summarized in TABLE 9.

## IV. Discussion

Root bark of *Ventilago maderaspatana* contains anthraquinones like emodin and physcion, which were reported to possess glucose lowering property. This criteria was considered to evaluate antidiabetic activity of *Ventilago maderaspatana* roots. Phytochemical screening revealed the presence of constituents, alkaloids, glycosides, cardiac glycosides, terpenoids, carbohydrates, flavonoids, steroids, proteins, tannins, saponins and anthraquinones (emodin and physcion) in the V.M root. Oral glucose tolerance test is employed in most studies as preliminary screening model to assess antidiabetic activity. Both alcoholic and hydroalcoholic extracts improved glucose tolerance revealing the presence of active constituents. Chloroform extract did not show any activity. Active constituent might not be extracted by the chloroform extract. The yield of Petroleum ether was very low therefore it is not screened by this model. Streptozotocin administration induces partial destruction of pancreatic β-cells resulting in insulin deficiency [36]. Insulin deficiency leads to metabolic aberrations like increased blood glucose [37], increased cholesterol and triglyceride [38, 39]. *Ventilago maderaspatana* lowered blood glucose and lipid levels might be due to the presence of emodin and physcion. Yang and co-workers reported that emodin presents a very high binding affinity to PPAR-γ [40]. Physcion another active constituent exhibit protecting effects in stroke or cerebrovascular accident [41, 42]. Therefore physcion might have been responsible for lowering lipid levels and increasing HDL. Diabetic animals characterized by severe loss of body weight, this may be due to enhanced muscle wasting and loss of tissue proteins [43]. In VMAE and VMHAE treated rats body weight decrease was less; probably act by releasing insulin from pancreatic β-cells indicating its protective action in controlling muscle wasting. High blood glucose levels increased stress on kidneys leading to kidney disease characterized by elevated creatinine and urea levels. *Ventilago maderaspatana* extract lowered creatinine and urea levels indicating its protective role on kidney function. Treatment with the *Ventilago maderaspatana* increased serum insulin levels, due to regeneration of β-cells or increased secretion of insulin from β-cells. Decrease in glycogen content of diabetic rats was due to β-cell destruction resulting in insulin deficiency which in turn increases glycogen breakdown and decreases glycogen content [44]. VMAE and VMHAE restored glycogen levels indicating insulin secretion and thereby activating glycogen synthase enzyme system [45]. Diabetes mellitus impairs homeostasis associated with increased production of glucose and reactive oxygen species leading to depletion of antioxidant defense systems. Increased glucose levels inactivate natural antioxidant enzymes, induces oxidative stress and cause lipid peroxidation. *Ventilago maderaspatana* increased the levels of antioxidant enzymes like catalase, glutathione and deceased lipid peroxidation levels indicating its protective antioxidant effect.

## V. Conclusion

These findings enumerate antidiabetic antihyperlipidemic and antioxidant effects of *Ventilago maderaspatana* roots. *Ventilago maderaspatana* elicited dose -dependent activity. Hydroalcoholic extract (500 mg $kg^{-1}$) evidenced greater antidiabetic activity. Alcoholic extract (500 mg $kg^{-1}$) elicited slightly greater antihyperlipidemic and antioxidant activity than hydroalcoholic extract (500 mg $kg^{-1}$). Thus the study scientifically supported claimed antidiabetic and antihyperlipidemic effects of the constituents, emodin and physcion [40, 41, 42]. Further investigations are underway to better characterize the active principles and to assess the mechanism of their antidiabetic effect.

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**Fig. 1: Roots of Ventilago maderaspatana**

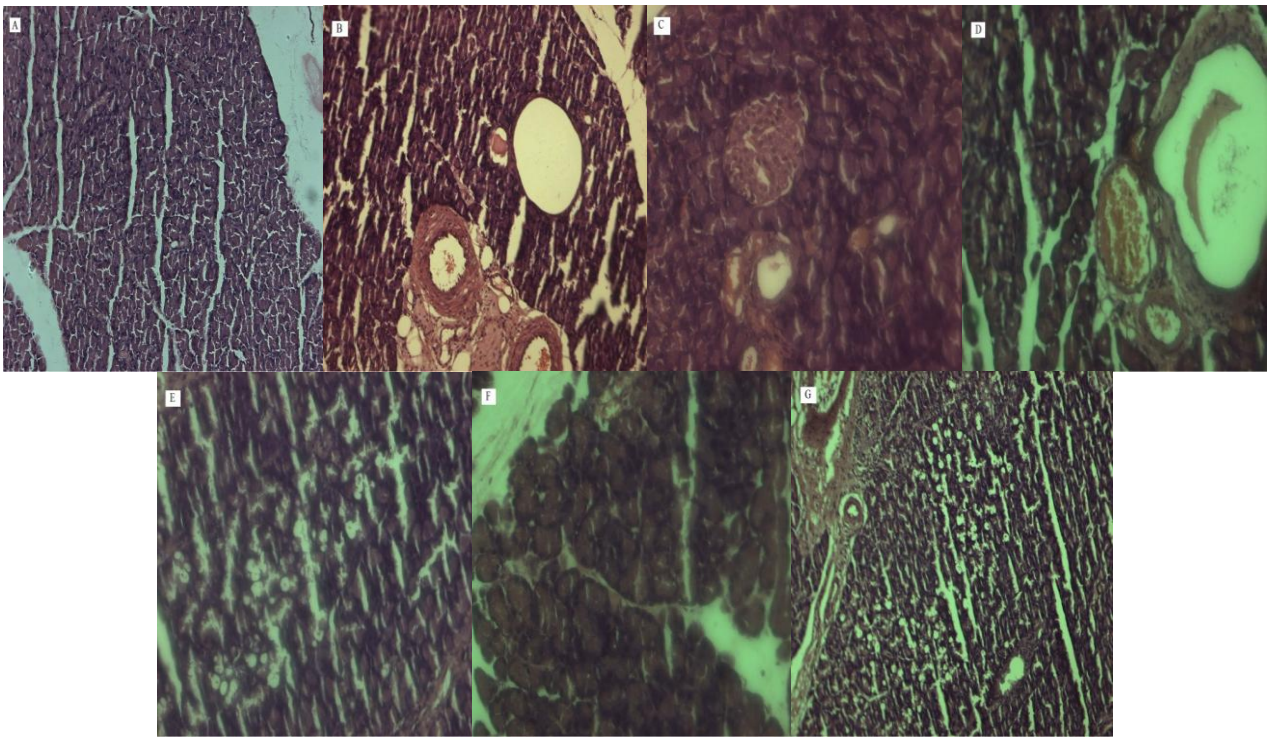


**Fig. 2: Histopathological changes showing rat pancreas:** A: Normal control group showing normal glandular and non glandular region. B: Diabetic control group manifesting cystic degeneration of non glandular region and periductular fibrosis. C: Standard, glibenclamide (10 mg/kg) treated group exhibiting glandular pancreas with islet cells normal and normal non glandular region. D. VMAE (250 mg/kg), manifesting moderate degeneration of pancreatic acini. E: VMAE (500 mg/kg), exhibiting normal glandular and non glandular region. F: VMHAE (250 mg/kg), showing glandular pancreas with islet cells normal and normal non glandular region G: VMHAE (500 mg/kg), revealed normal glandular and non glandular regions regaining normal islet cell structure.

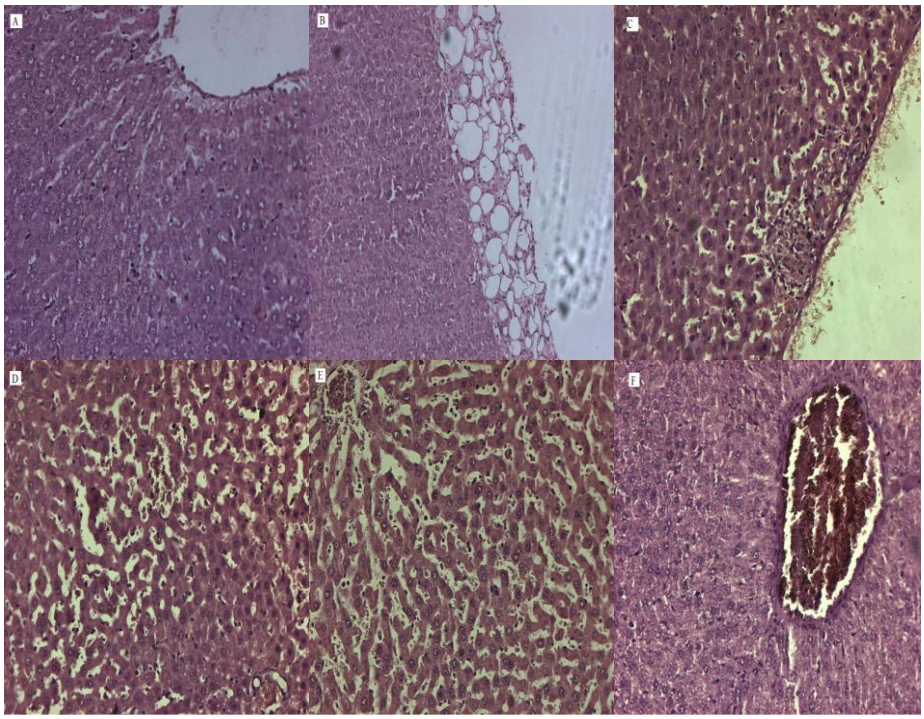

**Fig. 3: Histopathological changes showing rat liver:** A: Normal control group showing normal periportal, portal and centrilobular region with absence of inflammation and necrosis. B: Diabetic control group manifesting vacuolar degeneration in the capsules surrounding liver. C: VMAE (250 mg/kg), displayed multifocal necrosis with sinusoidal haemorrhages. D: VMAE (500 mg/kg), showed moderate sinusoidal haemorrhages in centrilobular region. E: VMHAE (250 mg/kg), exhibited moderate inflammation in periportal region of liver F: VMHAE (500 mg/kg), revealed normal periportal, portal and centrilobual region.