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Document heading

## Antidiabetic and antihyperlipidaemic potential of *Amaranthus viridis* (L.) Merr. in streptozotocin induced diabetic rats

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### ABSTRACT

**Objective:** The present study is planned to investigate the antidiabetic and antihyperlipidaemic potential of *Amaranthus viridis* stem aqueous extract (AVSAE) in STZ-induced diabetic rats. **Methods:** Diabetes was induced in rats by single intraperitoneal injection of STZ (55mg/kg b.wt.). After 72 h rats with marked hyperglycaemia (fasting blood glucose  $\geq 250$  mg/dl) were selected and used for the study. Antidiabetic activity was evaluated by administration of AVSAE orally at the doses of 100, 200 and 400 mg/kg body weight for 30 days. Glibenclamide (500 ug/kg) was used as the reference drug. Fasting blood glucose and lipid parameters, viz. triglycerides, total cholesterol, high density lipoprotein and low density lipoprotein levels were measured. **Results:** In STZ-induced diabetic rats, repeated administration of AVSAE significantly ( $P < 0.05$ ) decreased the blood glucose level in a dose-dependent manner during the 30 days of treatment period. AVSAE modulated lipid profile changes in STZ-diabetic rats in a dose-dependent manner. **Conclusions:** The significant control of serum lipids levels in the AVSAE treated diabetic rats may be directly attributed to improvement in glycemic control upon AVSAE therapy. Hence, these findings demonstrate that *Amaranthus viridis* has the potential to treat diabetes mellitus and complications owing to its antidiabetic and antihyperlipidaemic effect.

## 1. Introduction

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism [1]. Globally, the estimated incidence of diabetes and projection for year 2030, as given by International Diabetes Federation is 350 million [2]. Currently available pharmacotherapies for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs do not restore normal glucose homeostasis and they are not free from side effects [3]. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored [4]. Over 400 traditional plants have been reported for the treatments of diabetes [5]. Furthermore, after WHO recommendation,

investigation of hypoglycemic agents from medicinal plants has become more important [6]. Also, diabetes has been treated orally with several medicinal plants or their extracts based on folklore medicine since ancient times.

*Amaranthus viridis* Linn. (Amaranthaceae) is an annual herb, erect, 10 to 75 (–100) cm stem; slender, branched, angular, glabrous leaves. Commonly called as 'Cholai' in Hindi, which is grown in all regions of India, has been used in Indian and Nepalese traditional system to reduce labor pain and act an antipyretic [7]. Other traditional uses range from an anti-inflammatory agent of the urinary tract, in venereal diseases, vermifuge, diuretic, antirheumatic, antidiabetic, antiulcer, analgesic, antiemetic, laxative, improvement of appetite, antileprotic, treatment of respiratory and eye problems and treatment of asthma [8–10].

Furthermore, *Amaranthus viridis* contains antiproliferative and antifungal lactin properties as well as ribosome inactivating protein,  $\beta$ -carotene [11–12] and antiviral potential [13]. Experimentally the plant evaluated for analgesic and antipyretic activities [14], in vitro anthelmintic [15] anti-inflammatory [16], antidiabetic, antihyperlipidaemic and antioxidant properties [17], Pharmacognostic study [18], antinociceptive [19], antioxidant & nutrient [20],

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hepatoprotective activity [21].

However, the literature survey revealed that there is no experimental evidence of antidiabetic effect of the stem part of the plant. Therefore, the present work was undertaken to explore the antidiabetic and antihyperlipidaemic potentials of *Amaranthus viridis* stem aqueous extract (AVSAE) in streptozotocin (STZ) induced diabetic rats.

## 2. Materials and methods

### 2.1. Plant material

Plant specimen of *Amaranthus viridis* were collected during July 2010 from the Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India. The plant was authenticated by Dr. P.G. Diwakar, Joint Director, Botanical Survey of India, Pune as *Amaranthus viridis* Linn. (Amaranthaceae) with a voucher specimen (BSI/WRC/Tech/2010/462) kept in herbarium, BSI, Pune.

### 2.2. Chemicals

Streptozotocin was purchased from Sigma Chemical Company, Bangalore. All other chemicals used in the experiments were purchased locally (Merck and S D fine Chemicals) and were of analytical grade. Standard kits obtained from Span Diagnostics, India.

### 2.3. Preparation of extract

The stems of plant were washed with distilled water, shed dried and latter powdered. This powder was then defatted with petroleum ether which was further macerated with distilled water for 72 h with occasional shaking. It was then filtered and evaporated. The yield of AVSAE was 2.2% w/w.

### 2.4. Preliminary phytochemical Screening

The preliminary phytochemical screening of AVSAE was carried out for qualitative identification of type of phytoconstituents present [22].

### 2.5. Animals

Healthy adult male wistar rats weighing 150–200g were obtained from in house breed at the animal house of M.E.S. College of Pharmacy, Sonai and were housed in polypropylene cages lined with husk in standard environmental conditions (Temperature  $25 \pm 2$  °C; relative humidity  $55 \pm 10\%$ ; and 12:12 light: dark cycle.). The animals were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) and water ad libitum.

Animals were acclimatized to the laboratory condition for at least 8 days prior to the experiment and were maintained in a well ventilated animal house. The experimental protocol was approved by the Institutional animal Ethical Committee

(MES/COP/IAEC/2010–11/03) and the care of the laboratory animals was taken as per the current CPCSEA regulations.

## 2.6. Experimental design

### 2.6.1. Acute toxicity study (OECD 420, 2001)

The present study was conducted according to the organization for economic cooperation and development (OECD) revised fixed dose procedure for acute toxicity testing (OECD guideline 420, 2001). Two groups of five healthy albino wistar rats of either sex (3–month old, 150–200 g b.wt.) were administered a limit dose of 2000 and 5000 mg/kg of the AVSAE and animals were observed for mortality and clinical signs for the first hour, then hourly for 3 h and finally periodically until 48 h. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD50 was predicted to be above 2000 or 5000 mg/kg if three or more rats survived [23].

### 2.6.2. Effect of AVSAE on normoglycaemic rats

The rats were divided into four groups of 6 animals (n=6) each. Group I served as control and received vehicle. Group II, III and IV received AVSAE orally at doses 100,200 and 400 mg/kg/day b.wt. Blood glucose levels were determined at 0,1,2,3 and 4 h following treatment by retro-orbital plexus of the eye under mild ether anaesthesia.

### 2.6.4. Induction of diabetes

Diabetes was induced in rats by single intraperitoneal injection of STZ (55mg/kg b.wt.) dissolved in freshly prepared 0.01M citrate buffer, pH 4.5 [24] after 72 h rats with marked hyperglycemia (fasting blood glucose  $\geq 250$  mg/dl) were selected and used for the study.

## 2.7. Antidiabetic and antihyperlipidaemic effect

30 wistar rats of either sex (25 diabetic surviving and 05 nondiabetic) were divided into six groups of 6 animals (n=6) each. The solution of AVSAE was prepared with 1% gum acacia, an emulsifying agent. Glibenclamide was served as a reference standard. Group-I (nondiabetic control) animals were received only 1% gum acacia (1ml/kg/day, p.o.), Group-II (diabetic control) animals were diabetic and received 1% gum acacia (1ml/ kg/day, p.o.), Group-III (diabetic+ glibenclamide) animals were diabetic and received glibenclamide (0.25 mg/kg/day, p.o.) and Groups IV, V, VI animals were diabetic and received graded doses of AVSAE 100,200 and 400mg/kg, p.o. respectively. All the animals received above treatment daily up to 30 days.

### 2.7.1. Evaluation of antidiabetic activity

Antidiabetic activity of AVSAE was evaluated by estimation of blood glucose levels and body weight measurement on 1st, 10th, 20th and 30th day of the study by using Glucometer (Optium omega, Abbott Diabetes Care Ltd).

### 2.7.2. Evaluation of Antihyperlipidaemic activity

At the end of the experiment, the animals from each group were sacrificed by cervical dislocation and blood samples were collected to estimate various biochemical parameters [34]. Blood was collected from the heart and allowed to clot and the serum was separated by centrifugation at 3500 rpm for 10 minutes. Serum was assayed either immediately or stored at  $-200\text{ C}$ . The tissue like pancreas was collected and used for histological studies. Serum samples were analyzed spectrophotometrically for triglycerides, total cholesterol, high density lipoprotein (HDL-C), using their respective kits using UV-visible spectrophotometer (Jasco V-630, Japan), VLDL-C and LDL-C were calculated as per Friedwald's equation [25].

$$\text{VLDL} = \text{TG}/5$$

$$\text{LDL} = \text{TC} - (\text{HDL} + \text{VLDL}) \text{ [26-28].}$$

### 2.7.3. Oral glucose tolerance test (OGTT)

All the animals were given glucose (2 g/kg) 30 min after daily dosing. Blood samples were collected from the retro-orbital plexus of the eye just prior (0 h) and 1, 2, 3 and 4 hr. after the glucose loading and blood glucose levels were estimated.

### 2.7.4. Estimation of glycated hemoglobin

After 30 days of treatment, the 12hr fasted rats were sacrificed by cervical decapitation, blood was withdrawn by retro orbital puncture under light ether anesthesia and the glycated hemoglobin was estimated [29].

### 2.7.5. Statistical analysis

The results were expressed as mean $\pm$ S.E.M., statistical difference was done by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A difference in the mean  $P$  value  $<0.05$  was considered as statistically significant.

## 3. Results

### 3.1. Preliminary phytochemical Screening

The study showed the presence of steroids, terpenoids, alkaloids, tannins, phenolic compounds, flavonoids, Sugars and amino acids.

### 3.2. Acute toxicity study (OECD 420, 2001)

Oral administration of AVSAE was found safe up to dose of 2,000 mg/kg; p.o. and produced no signs of toxicity. However, from 5g/kg APSAE caused slow movement of animal, decreased aggressiveness, altered touch and pain sensibility but did not cause any negative behavioral changes such as excitement, respiratory distress, convulsions or coma. No mortality was observed up to 14 days. Hence, the median lethal dose ( $\text{LD}_{50}$ ) of the AVSAE was then greater than 2000

mg/kg body weight. Therefore doses 100,200 and 400 mg/ kg b.wt. were selected for all in vivo experiments.

### 3.3. Effect of AVSAE in normoglycaemic rats

The results from the study shows that there was no any significant effect observed on normoglycaemic rats when treated with the single dose of AVSAE at 100,200 and 400 mg/ kg b.wt. (Table 1).

### 3.4. Antidiabetic activity

On repeated administration of AVSAE daily up to 30 days exhibited significant antidiabetic activity in stz-induced diabetic rats, whilst there was no significant effect observed on normoglycaemic rats. However, at the end of 30 days of treatment, there was a 70.50%, 66.19%, 67.99% and 69.63% ( $P<0.01$ ) decrease of serum glucose levels with the glibenclamide and AVSAE (100,200 and 400 mg/kg) respectively when compared with diabetic control group (Table 2).

### 3.5. Antihyperlipidaemic activity

On repeated administration of AVSAE daily up to 30 days exhibited significant reduction in lipid profile in stz-induced diabetic rats. Lipid profile of animals showed significant reductions ( $P<0.01$ ) of 8.76%, 16.61% and 21.08% CHL (cholesterol), 34.46%, 42.07% and 52.72% LDL, 8.70%, 17.40% and 16.70% VLDL (Very Low density lipoproteins) and 23.83%, 29.30% and 30.66% TG after treatment with AVSAE 100,200 and 400 mg/kg respectively when compared with diabetic control rats. There was also a significant ( $P<0.01$ ) increase of 20.75%, 27% and 39.50% HDL in the AVSAE treated diabetic rats in comparison of diabetic control rats, where a fall in HDL level (Table 3).

### 3.6. Oral glucose tolerance test (OGTT)

The results from the study indicated that the AVSAE at 100, 200 and 400 mg/kg and glibenclamide (0.25 mg/kg) reduced the blood glucose level (hyperglycemia due to glucose load 2 g/kg p.o.) significantly after 3hrs of oral administration, when compared to diabetic control group (Table 4).

### 3.7. Changes in body weight

At the end of 30 days treatment, the body weight of normal rats, AVSAE and standard drug treated group, increased significantly, whereas body weight of diabetic control group rats decreased (Table 5).

### 3.8. Changes of serum glycosylated hemoglobin

After 30 days of treatment with AVSAE, it was observed that animals treated with AVSAE showed a significant decrease in glycosylated hemoglobin levels when compared to

diabetic control groups (Table 5).

#### 4. Discussion

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes [30–32], but only a few have been scientifically evaluated. Therefore, we have investigated the antidiabetic and antihyperlipidaemic effect of *Amaranthus viridis* stem aqueous extract in STZ-induced diabetic rats. AVSAE showed a dose dependent effect on fasting blood glucose at 100,200 and 400 mg/kg b.wt. in diabetic rats. So, detailed studies were carried out with the graded doses of 100, 200 and 400 mg AVSAE mg/kg b.wt. The capacity of AVSAE to decrease the elevated blood glucose to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. Lower levels of total hemoglobin observed in diabetic rats might be due to the increased formation of HbA1c. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin and crystalline of lens [33]. HbA1c was found to increase in patients with diabetes mellitus and the amount of increase was directly proportional to the fasting blood glucose levels [34] therefore, measurement of HbA1c is supposed to be very sensitive index for glycemic control. Treatment with AVSAE showed a significant decrease in the glycated hemoglobin levels,

which could be due to an improvement in insulin secretion. Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting [35], and due to loss of tissue proteins [36]. Diabetic rats treated with the AVSAE showed an increase in body weight when compared to the untreated diabetic rats which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in glycemic control. Increased levels of triglycerides and cholesterol during diabetes lead to cardiovascular complications. In this study, STZ-induced diabetic mellitus characterized by hyperglycemia caused a significant rise in serum lipids. These findings indicate that diabetes mellitus is accompanied by increased risk of atherosclerosis and coronary artery diseases. In the present study, the AVSAE significantly reduced the triglyceride, total cholesterol, LDL and VLDL cholesterol levels with an increase of HDL cholesterol in treated diabetic rats as compared to untreated diabetic rats. These changes are beneficial in preventing diabetic complications as well as in improving lipid metabolism in diabetics [37]. The significant control of serum lipids levels in the AVSAE treated diabetic rats may be directly attributed to improvement in glycemic control upon AVSAE therapy. Hence, these findings demonstrate that *Amaranthus viridis* has the potential to treat diabetes mellitus and complications owing to its antidiabetic and antihyperlipidaemic potential. Further studies are necessary to substantiate above claim and to work out exact mechanism of action involved in antidiabetic and antihyperlipidaemic potential of this plant.

**Table 1**

Effect of AVSAE on blood glucose level (BGL) of normoglycaemic rats.

| Groups Treatment<br>(n=6) | Blood glucose level (mg/dl) at (hrs) |            |            |             |             |
|---------------------------|--------------------------------------|------------|------------|-------------|-------------|
|                           | 0                                    | 1          | 2          | 3           | 4           |
| I Normal                  | 92.50±1.54                           | 95.00±0.63 | 91.66±0.71 | 91.50±0.42  | 91.50±0.22  |
| II Glibenclamide          | 94.83±0.87                           | 94.00±0.44 | 90.50±0.84 | 88.66±0.49* | 87.83±0.47* |
| III AVSAE                 | 93.16±0.70                           | 92.33±1.08 | 92.66±0.55 | 90.83±0.30  | 90.83±0.16  |
| IV AVSAE                  | 93.66±0.61                           | 93.16±0.70 | 92.16±0.60 | 90.50±0.42  | 90.16±0.30  |
| V AVSAE                   | 93.00±1.06                           | 92.33±0.21 | 91.33±0.61 | 90.00±0.25  | 89.66±0.42* |

\*  $P < 0.05$ , \*\* $P < 0.01$  Values are mean±SEM,  $n=6$ , when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test.

**Table 2**

Antidiabetic effect of AVSAE on blood glucose level (BGL) of stz-induced diabetic rats.

| Groups Treatment (n=6) | Blood glucose level (mg/dl) at (days) |              |               |               |
|------------------------|---------------------------------------|--------------|---------------|---------------|
|                        | 1st                                   | 10th         | 20th          | 30th          |
| I Normal               | 99.16±0.94                            | 99.33±0.95   | 99.83±0.94    | 101.17±0.79   |
| II Diabetic control    | 254.00±1.21                           | 274.00±2.03  | 313.50±3.49   | 371.67±5.99   |
| III Glibenclamide      | 257.83±1.24                           | 213.17±3.68* | 154.83±0.94** | 109.83±1.16** |
| IV AVSAE               | 258.83±1.40                           | 238.67±1.30  | 175.17±1.01*  | 125.67±0.49** |
| V AVSAE                | 259.83±0.94                           | 236.17±1.40  | 165.50±0.99*  | 119.00±1.06** |
| VI AVSAE               | 257.50±2.14                           | 225.17±1.49* | 160.67±0.71*  | 114.00±1.06** |

\*  $P < 0.05$ , \*\* $P < 0.01$  Values are mean±SEM,  $n=6$ , when compared with diabetic control by using one way ANOVA followed by Dunnett's multiple comparison test.

**Table 3**

Antihyperlipidaemic effect of AVSAE in stz-induced diabetic rats.

| Groups Treatment<br>(n=6) | Lipid levels (mg/dl) |              |              |              |               |
|---------------------------|----------------------|--------------|--------------|--------------|---------------|
|                           | Body Cholesterol     | LDL          | HDL          | VLDL         | TG            |
| I Normal                  | 66.50±0.56           | 23.33±0.42   | 11.50±0.42   | 16.16±0.30   | 66.66±0.88    |
| II Diabetic control       | 93.33±0.98           | 95.50±0.76   | 8.00±0.51    | 23.00±0.36   | 109.33±1.19   |
| III Glibenclamide         | 69.33±0.55**         | 39.16±1.30** | 11.33±0.33** | 17.83±0.65** | 73.83±1.101** |
| IV AVSAE                  | 85.16±1.01*          | 62.66±2.72*  | 9.66±0.21*   | 21.00±0.36*  | 83.66±1.05*   |
| V AVSAE                   | 77.83±0.94*          | 55.33±2.87*  | 10.16±0.30*  | 19.00±0.25*  | 77.66±0.91*   |
| VI AVSAE                  | 73.66±0.33**         | 45.16±1.42** | 11.16±0.30** | 19.16±0.16*  | 76.16±0.79    |

\*  $P < 0.05$ , \*\* $P < 0.01$  Values are mean±SEM,  $n=6$ , when compared with diabetic control by using one way ANOVA followed by Dunnet's multiple comparison test.

**Table 4**

Effect of AVSAE on oral glucose tolerance test (OGTT) in stz-induced diabetic rats.

| Groups Treatment<br>(n=6) | Blood glucose level (mg/dl) at (hrs) |             |             |               |             |
|---------------------------|--------------------------------------|-------------|-------------|---------------|-------------|
|                           | 0                                    | 1           | 2           | 3             | 4           |
| I Normal                  | 100.17±1.35                          | 125.71±0.84 | 136.17±0.79 | 145.83±0.60   | 156.00±0.57 |
| II Diabetic control       | 257.33±1.25                          | 268.67±1.28 | 280.83±0.94 | 290.17±0.90   | 295.50±0.76 |
| III Glibenclamide         | 256.83±1.19                          | 266.67±1.05 | 276.50±0.76 | 284.50±0.99** | 266.83±0.49 |
| IV AVSAE                  | 259.83±0.94                          | 269.83±0.94 | 278.83±0.87 | 289.33±0.88   | 274.83±1.10 |
| V AVSAE                   | 258.00±0.57                          | 286.33±0.66 | 277.67±0.66 | 287.67±0.80   | 271.83±1.38 |
| VI AVSAE                  | 259.83±0.47                          | 269.33±0.60 | 276.83±0.60 | 285.50±0.42** | 267.33±0.49 |

\*  $P < 0.05$ , \*\* $P < 0.01$  Values are mean±SEM,  $n=6$ , when compared with diabetic control by using one way ANOVA followed by Dunnet's multiple comparison test.

**Table 5**

Effect of AVSAE on body weight and glycosylated Hb of stz-induced diabetic rats.

| Groups Treatment<br>(n=6) | Changes in body wt. (gm) |               |  | HbA1c mg/gm Hb |
|---------------------------|--------------------------|---------------|--|----------------|
|                           | Initial                  | Final         |  |                |
| I Normal                  | 160.00±0.85              | 185.17±0.70   |  | 6.45±0.17      |
| II Diabetic control       | 140.67±1.72              | 10.81±0.10    |  | 161.17±0.94    |
| III Glibenclamide         | 159.50±1.89              | 178.33±0.70** |  | 7.55±0.07**    |
| IV AVSAE                  | 159.83±2.72              | 176.33±0.71** |  | 8.70±0.10*     |
| V AVSAE                   | 158.67±2.06              | 177.17±0.70** |  | 8.18±0.04*     |
| VI AVSAE                  | 159.17±1.13              | 179.67±0.42** |  | 7.76±0.06*     |

\*  $P < 0.05$ , \*\* $P < 0.01$  Values are mean±SEM,  $n=6$ , when compared with diabetic control by using one way ANOVA followed by Dunnet's multiple comparison test.

### Conflict of interest statement

Authors declare that, they have no conflict of interest.

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