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ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *AMARANTHUS TRISTIS* LINN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes is a chronic disorder, which occurs when the pancreas not produces sufficient insulin, or when the body cannot utilize the insulin it produced. The anti-diabetic activities of methanolic extract of *Amaranthus tristis* Linn. It's evaluated by both invitro and in vivo tests. *In vitro* method alpha-glycosidase inhibitory activity model and Alpha amylase inhibitory model methanolic extract of *Amaranthus tristis* Linn showed the best inhibitory activity. In vivo model methanolic extract of *Amaranthus tristis* Linn was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract for 15 days treatment at the dose of 400mg/kg body weight. The effect was compared with standard drug of Glibenclamide 0.5mg/kg body weight. Fasting blood glucose level determined by GOD-POD kit method. The result shows the methanolic extract of *Amaranthus tristis* Linn significantly lowered the blood glucose of hyperglycemic rats. Based on Acute toxicity study OECD guidelines (423) it was observed that nontoxic up to 2000g/kg body weight, preliminary phytochemical screening showed the presence of carbohydrates, bioflavonoid, phytosterols and glycosides. Concluded that methanolic extract *Amaranthus tristis* Linn plant extract has significant antidiabetic activity, which effectively lowered the fasting blood glucose level in Streptozotocin induced diabetic rats.

KEYWORDS: *Amaranthus tristis* Linn *Invitro* model, alpha-glycosidase, alpha-amylase, *Invivo* model, Streptozotocin.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder characterized by decrease in both insulin secretion and insulin action. It is commonly associated with the development of micro and macro vascular diseases like neuropathy, nephropathy, and cardiovascular diseases. The disease is associated with reduced quality of life and also increased risk factors of Heart, kidney, brain etc.[1] The worldwide prevalence of diabetes for all age groups was estimated to be 3.8% in 2000 and it is increases at 5.6% in 2025. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylurea's, biguanides, and α-glycosidase inhibitors having high cost and side effect, but herbal drugs are easily available and long term history of treatment of diseases.[2]

Amaranthus tristis Linn family Amaranthaceae, have long history of Leafy vegetables are essential sustenance that major component in traditional diet due to its health benefits,[3] which is mainly because of the presence of more phytoconstituents with potential antioxidant properties. *Amaranthus tristis* Linn have been used as food as well as for combating various disorders traditionally. Both the plants have been reported to possess unique bioactive compounds that contribute to the pharmacological activities of that plant.[4] *Amaranthus tristis* Linn is a common edible plant that contains Amarantin, Isoamarantin, Betaine, amino acids and sterols. It has great medicinal value, as it is used as an astringent in dysentery, diarrhea, and hemorrhagic colitis and also used in cough and bronchitis.[5]

MATERIALS AND METHODS

(i) Plant material and extraction

Fresh whole plant were collected in the month of October from pottari village, kanchipuram dist Tamil nadu India, and authentified by pro. jeyaraman plant anatomy research institute west Tambaram Chennai, Herbarium was submitted to pharmacognosy department for further reference. The plants are shade dried and made coarse powder by passed through sieve number 60.[6, 7] The Extraction of powdered plant materials were extracted with help of soxlet apparatus with continuous hot percolation method. The course power of Amaranthus tristis Linn using defatted with pet ether (60-80°C) for 72 hours. Marc are subject to methanol for extraction at 72 hours, the extracts were concentrated to dryness and perform preliminary phytochemical screenings.[8]

(ii) INVITRO ANTIDIABETIC ACTIVITY

Inhibition of alpha-Amylase enzyme

In vitro methods employed in antidiabetic studies to inhibition of alpha-amylase enzyme and starch solution (0.1% w/v), preparation of 0.1g of potato starch stirred within 100 ml of 16 mM of sodium acetate buffer solution. The preparation of enzyme solution 27.5mg of alpha-amylase mixed with 100 ml of distilled water. Colorimetric reagent preparation by added the sodium potassium tartarate solution and 96 mM 3, 5 di nitro salicylic acid solution. Control and methanolic extract was added with starch solution and left to react with alpha- amylase solution made alkaline conditions temperature at 25ºC. The reaction was taken within three minutes. The generation of maltose was quantified by the formation of 3- amino-5- nitro salicylic acid from 3, 5 dinitro salicylic acid. This reaction is measured at 540 nm.[9]

Inhibition of alpha-glycosidase enzyme

Inhibition of alpha-glycosidase enzyme inhibitory activity was determined by incubated a solution of starch substrate (2%w/v maltose) with 1 ml of 0.2 M Tris buffer at the pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction mixture was initiated by added 1 ml of alpha-glycosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. This reaction was measured at 540nm.

Determination of the Calculation of Inhibitory Concentration (IC50) of *Amaranthus tristis Linn*

Calculation of 50% Inhibitory Concentration (IC50) The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by used the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by.

$$I\ \% = (Ac-As)/Ac\ X\ 100$$

Where control (Ac) absorbance of the control and sample absorbance (As). There was a dose-dependent increase in percentage inhibitory activity against alpha- amylase enzyme. At a concentration of 0.2 ml of plant extract showed a percentage inhibition 27.8% and for 1.0 ml plant extract showed inhibition of 96.3%.[10]

(iii)In vivo studies

Animals

Swiss albino mice of female sex weighing 20-25gms were employed for toxicity study by acute oral class method OECD guidelines (423). And wistar albino rats of male sex weighing 150-200 gms were used for antidiabetic study. Animals were housed in clean and maintain aseptic environment condition and fed with standard rodent diet of ablibitum with water. Ethical clearance for the animal study was obtained from Institutional Animal Ethical Committee (09MP03AUG2009) of CPCSEA (887/ac/CPCSEA).

Induction of diabetes

All the rats were fasted overnight before the administration of Streptozotocin. Wister albino rats injected streptozotocin by intra peritoneal route. Drug dissolved with ice cold buffer solution of 0.1M sodium citrate (pH4.5) at the dose of 50mg/kg body weight. After injected animals has free access to food and water. The development of diabetes was confirmed by glucometer after 48hrs of Streptozotocin injection.[11, 12] To measured the fasting blood glucose level more than 200mg/dl were considered as diabetic rats and used for the experimentation. Diabetic animals were grouped after five days induction of diabetes Effect of methanolic Extract of *Amaranthus tristis* Linn in streptozotocin induced diabetes in rats.[13]

EXPERIMENTAL DESIGN

In the experiment design rats were divided into the following groups with six animals each

Group I: Normal control of Wister rats received 1% gum acacia for 15 days orally

Group II: Diabetic control of Wister rats received 1% gum acacia for 15 days orally.

Group III: Diabetic rats received 400mg/kg body methanolic extract of *Amaranthus tristis* Linn weight once a day orally for 15 days.

Group IV: Diabetic rats received 0.5mg/kg of standard drug of Glibenclamide orally once a day for 15 days.

Rats were fasted overnight and the blood was withdrawn from the orbital sinus of the eye on the 5th day, 15th day and 20th day post induction of diabetes to determine blood glucose by GOD-POD kit method. In diabetes rats change body weight also observed throughout treatment period in experimental animals.[14, 15]

STASTISTICAL ANALYSIS

All values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. P<0.05 were considered significant.

RESULTS

The preliminary phytochemical studies shown the presence of bio active substance carbohydrates, bioflavonoid phytosterols and glycosides, the methanolic extract *of Amaranthus tristis* Linn non toxic up to the dose level of 2000mg/kg ,by oral acute toxic class method OECD (423).The invitro studies percentage inhibition zone at 0.2-1.0 ml concentrations of Amaranthus tristis Linn extract showed a dose dependent increase in percentage inhibition.

Table1: In vitro anti diabetic study methanolic extract of *Amaranthus tristis* Linn

S.No	Concentration of sample	% of inhibition OF alpha-amylase	% of inhibition OF alpha glycosidase
1	0.2	27.8	31.7
2	0.4	48.9	53.9
3	0.6	62.5	68.7
4	0.8	87.2	76.6
5	1.0	96.3	89.4

The methanolic extract of showed that significant action of zone of inhibition using enzymes alpha-glycosidase and alpha amylase. The percentage inhibition at 0.2-1.0 ml concentrations of extract showed a dose dependent increase in percentage inhibition.

Table- 2: Effect of methanolic extract *Amaranthus tristis* Linn on body weight in Streptozotocin induced diabetic rates

Values are expressed as Mean ± S.E. n=6.

Groups	Body weight in gms(Mean±SEM)		
	Post induction days		
	5^{th} day	15^{th} day	20^{th} day
Control	167.2±3.25	173±3.54	181±3.34
Diabetic control	161.8±3.34	138.8/±2.10*	127.3±2.39*
Diabetic rats+ extract	164.3±1.98	165.3±1.76*	175.8±1.47*
Diabetic rats+ glibenclamide	166.5±2.77	169.8±4.62*	178.5±2.37

P*<0.05 Experimental groups were compared with diabetic control.

P*<0.05 Diabetic groups were compared with control group.

In the antidiabetic activity, the effects of methanolic extract *Amaranthus tristis* Linn on body weight is measured on 5th, 15th and 20th day of post induction diabetes were compared with normal and diabetic control groups. Streptozotocin induced diabetic rats showed a significant decrease (P<0.05) in body weight compared to normal rats As per shown Table No-2 Oral administration of extract of *Amaranthus tristis* Linn the dose of 400mg/kg showed a significant increase (P<0.05) in body weight on 15th and 20th day of post induction when compared to untreated diabetic rats.

Table- 3: Effect of methanolic extract of Amaranthus tristis Linn on blood sugar level in streptozotocin induced diabetic rats.

Values are expressed as Mean ± S.E. n=6.

Groups	**Blood glucose level in mg/dl (Mean±SEM)**		
	Post induction days		
	5th day	15th day	20th day
Control	62.2±1.22	61.05±1.11	60.47±1.16
Diabetic control	273.46±1.70	260.2±1.34*	269.8±1.88*
Diabetic rats+ plant extract	269.10±3.04	145.4±3.99*	72.61±2.24*
Diabetic rats+ glibenclamide	263.20±3.59	126.06±4.07*	64.06±1.28*

P*<0.05 Experimental groups were compared with diabetic control.

P*<0.05 Diabetic groups were compared with control.

The effect Amaranthus tristis Linn extract on fasting blood glucose level is measured on 5th, 15th and 20th day of post induction of diabetics and compared with normal and plant extract groups. Streptozotocin induced rats showed a significant increase (P<0.05) in fasting blood glucose level compared to normal rats. Oral administration of methanolic extract of *Amaranthus tristis* Linn at the dose of 400mg/kg body weight showed a significant decreased (P<0.05) in blood glucose level in 10 and 15 days of treatment.

DISCUSSION

In this present study evaluated *invitro* alpha amylase and alpha glucosidase activity of crude methanol extract of *Amaranthus tristis* Linn. The plant showed significant inhibition activity, so further the isolated compound to purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent. hypoglycemic activity of methanolic extract of *Amaranthus tristis* Linn was evaluated in Streptozotocin induced diabetic rats. The continuous administration of *Amaranthus tristis* Linn extract for a period of 15 days produced a significant decrease in blood glucose level in diabetic rats which is

comparable to that of standard drug Glibenclamide which is used in treatment of type II diabetes mellitus.

CONCLUSION

The methanolic extract of *Amaranthus tristis* Linn exhibited significant hypoglycemic activity in both *invitro* and *invivo* method. Streptozotocin induced diabetic rats. From the phytochemical test it was found that the major chemical constituents of the plant extract were bioflavonoid and glycosides. On the basis of above study realy on it is possible that the presence of bioflavonoid may be responsible for the observed antidiabetic activity.

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