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Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of *Amaranthus*

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ABSTRACT

Objective: To investigate the anti-diabetic and anti-cholesterolemic activity of methanol extracts of leaves of *Amaranthus caudatus*, *Amaranthus spinosus* and *Amaranthus viridis* in normal and streptozotocin (STZ) induced diabetic rats. **Methods:** In this study, the anti-diabetic and anti-cholesterolemic activity of methanol extracts of leaves of all three plants was evaluated by using normal and STZ induced diabetic rats at a dose of 200 mg/kg and 400 mg/kg *p.o.* daily for 21 days. Blood glucose levels and body weight were monitored at specific intervals, and different biochemical parameters, serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein and very low density lipoprotein were also assessed in the experimental animals. Histology of pancreas was performed. **Results:** It was found that all the three plants at 400 mg/kg dose showed significant anti-diabetic and anti-cholesterolemic activity ($P<0.01$), while at 200 mg/kg dose less significant anti-diabetic activity ($P<0.05$) was observed. **Conclusions:** Methanol extracts of *Amaranthus caudatus*, *Amaranthus spinosus* and *Amaranthus viridis* showed significant anti-diabetic and anti-cholesterolemic activity, which provides the scientific proof for their traditional claims.

1. Introduction

Dependence on herbs as medicines in the treatment of disease is common among a large proportion of population of the rural populace because of its availability and affordability[1]. Due to the increasing awareness of the importance of traditional medicine in human and animal healthcare, researches into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile. WHO[2] supports the use of effective and safe remedies and accepts traditional medicine as a valuable resource for primary healthcare.

Amaranth is herbaceous annually growing up to 15-100 cm in height. It was once nearly as important food as maize and beans in central and South America. The *Amaranthus* plants (Amaranthaceae) are spread throughout the world, growing under a wide range of climatic conditions and they are able to produce grains and leafy edible vegetables.

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Amaranthus spinosus L. (Amaranthaceae) (*A. spinosus*) commonly known as Kantabhaji in Hindi is an annual or perennial glabrous herb, native to tropical America, found in tropical and sub-tropical regions of India. In Ayurvedic system (traditional) of medicines, the plant is used as diuretic, anti-diabetic, analgesic, anti-pyretic, anti-leprotic and in the treatment of bronchitis and piles[3]. *A. spinosus* water extract directly stimulates proliferation of β-lymphocytes *in vitro*. *In vivo* anti-malarial activities of extracts of *A. spinosus* were reported[4]. It is also reported to be used as anti-inflammatory and immunomodulatory[5]. It contains amaranthoside, a lignan glycoside amaricin, a coumaryl adenosine along with stigmasterol glycoside, betaine, such as glycine betaine and trigonelline[6].

Amaranthus viridis L. (Amaranthus) (*A. viridis*) commonly called as Chauraiya in Hindi is an erect much branched glabrous herb, 30-60 cm high, distributed in all tropical countries[3,7]. The traditional uses are in diuretic, analgesic, anti-pyretic, vermifuge, anti-ulcer, hypoglycemic, hypolipidemic, laxative, asthma and veneral diseases. Anti-oxidant activity and phenolic content of raw and branched *A. viridis* were reported[3,7-9]. *A. viridis* also possesses antiviral activity[10].

Amaranthus caudatus (Amaranthaceae) (*A. caudatus*)

commonly known as Chulai is an annual herb growing to 2 m by 0.45 m. Its well known medicinal uses are for anthelmintic, astringent, diuretic. *In vitro* anti-oxidant effect and inhibition of β - amylase of *A. caudatus* seeds were reported[11]. *A. caudatus* showed anti-atherosclerotic[12] and anthelmintic[13].

A. spinosus, *A. viridis*, *A. caudatus* were selected since they were used to reduce blood glucose level and lipid profile in Indian traditional system of medicine. Our aim of investigation was to provide scientific data for traditional use.

2. Materials and methods

2.1. Collection of plant material and extraction

The fresh leaves of *A. spinosus*, *A. viridis* and *A. caudatus* were collected from GKVK, Agricultural University, Bangalore, and were authenticated by the taxonomist Dr. Rajanna, GKVK, Bangalore. The voucher specimen (PESCP-26, 27, 28) was deposited in College Herbarium. The leaves of *A. spinosus*, *A. viridis* and *A. caudatus* were shade dried and coarsely powdered. The coarse powder (60 g each) was subjected to extraction with methanol by soxhlet apparatus and extracts were concentrated using rotary evaporator under reduced pressure (Yield - 4.8, 4.4, 4.6% w/w). Then the extract was stored in a refrigerator at 4 ℃ until use for the biological testing and phytochemical screening.

2.2. Preliminary phytochemical screening

The methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* were screened for the presence of various phytoconstituents[14].

2.3. Animals

Healthy Wistar rats between 2-3 months of age of either sex and weighing 180-200 g were acclimatized to the laboratory condition at temperature (25±1) ℃, relative humidity (50±15)%, 12 h light-dark cycles, kept in standard polypropylene cages of maximum 2 animals each, and given standard diet (Kamadhenu Enterprises, Bangalore) and water *ad libitum* in accordance with the instructions given by Institutional Animal Ethical Committee, CPCSEA[15].

2.4. Acute toxicity studies

Methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* were studied for the acute oral toxicity according to the guidelines set by OECD (Organization for Economic Cooperation and Development) guidelines No. 423[16]. Healthy Wistar rats (150-180 g) were used for the study. The two doses of 2 000 mg/kg (*p.o.*) and 5 000 mg/kg (*p.o.*) of the test samples were given to two groups with 5 in each group for three plants. The mortality and general behaviour of treated groups were monitored for 14 days. The extract was devoid of any toxicity in rats when the dose up to 5 000 mg/kg was given orally. Hence, for further studies 200-400 mg/kg doses of extract were selected.

2.5. Effect of methanol extracts in normal rats

Animals in the normal control group received normal saline 10 mL/kg orally. Vehicle control received 3% v/v Tween 80 in water 10 mL/kg orally. Standard group received glibenclamide at 10 mg/kg orally. The test groups of animals were treated with the methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* at a predetermined therapeutic doses of 200 and 400 mg/kg per orally for 21 days. The blood samples were withdrawn from retro-orbital plexus at 1st, 7th, 14th, 21st day, and blood glucose levels were estimated using GOD-POD kit (Acuurex, India)[17].

2.6. Oral glucose tolerance test in normal rats (OGTT)

Rats were divided into five groups with 6 each and were administered normal saline and dose of 200 mg/kg and 400 mg/kg per oral of methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus*. Glucose solution 2 g/kg was administered 30 min after the administration of the extract. Blood samples were withdrawn from the retro-orbital plexus at intervals of 60, 120, 180 min of glucose administration. Blood glucose levels were estimated using GOD-POD kit (Acuurex, India) [OECD 2 001-guideline on (AOT) No. 425][18].

2.7. Evaluation of antidiabetic activity

2.7.1. Induction of diabetes

The streptozotocin (STZ) diabetic rat model was performed following the methods described by Kadnur and Goyal, 2005[19]. STZ was injected into rats (Sigma chemical Co. USA) intraperitoneally (i.p.) with 70 mg/kg bw. The diabetic state was assessed by measuring the serum blood glucose levels 48 h after STZ administration. The rats with fasting glucose levels in the range of 275-300 mg/100 mL were considered diabetic and were included in the study.

2.7.2. Experimental design and treatment schedule

The effect of the extracts was studied in STZ-induced diabetic rats for 21 days. The rats (n=6 per group) were divided into 9 groups: group 1: normal rats treated with vehicle alone, saline 10 mL/kg *p.o.*; group 2: diabetic control treated with STZ, 70 mg/kg bw dissolved in 0.1 M cold citrate buffer (pH 4.5); group 3: diabetic rats treated with glibenclamide (Ranbaxy, India); group 4 and 5: diabetic rats administered orally with methanol extract of *A. spinosus* at doses of 200 mg/kg and 400 mg/kg *p.o.*, respectively; group 6 and 7: diabetic rats administered orally with methanol extract of *A. viridis* at doses of 200 mg/kg and 400 mg/kg *p.o.*, respectively; group 8 and 9: diabetic rats administered with methanol extract of *A. caudatus* at doses of 200 mg/kg and 400 mg/kg *p.o.*, respectively. Blood glucose levels and body weight were measured on day 1, 7, 14 and 21 of the study. Finally on day 21, blood was drawn by retro-

orbital puncture. Blood samples were collected, allowed to clot and then centrifuged at 2 500 rpm for 10 min to obtain serum. Blood glucose levels were estimated by GOD-POD kit (Accurex, India). All the lipid profile parameters were determined. Total cholesterol, HDL, LDL and VLDL were analysed from serum[19]. Triglycerides were determined using Hantzsch condensation method[20].

2.7.3. Histopathology of the pancreas of STZ induced diabetic rats

On the last day of the study, the animals were sacrificed and quickly dissected, and small slices of pancreas samples were fixed in 10 % formalin. Thin sections of the tissue, 5-7 μm, were cut and stained with haematoxylin-eosin. The tissue sections were subjected to rehydration by exposing them to the decreasing concentrations of alcohol (10%-30%) and then stained with haematoxylin. The sections were dehydrated by using increasing concentrations of alcohol and then stained with eosin. They were then treated with diphenyxylene (DPX) and examined under microscope[21,22].

2.8. Statistical analysis

The results were expressed as mean±SEM. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnetts test. A difference in the mean P value < 0.05 was considered as significant.

3. Results

3.1. Preliminary phytochemical screening

The percentage yield of MEAV, MEAS and MEAC was found to be 4.4% w/w, 4.8% w/w, 4.6% w/w, respectively. The methanol extracts of the three species of *Amaranthus* contained glycosides, saponins, flavonoids, proteins, amino acids and carbohydrates.

3.2. Acute toxicity study

The acute oral toxicity test showed the normal behaviour of the treated rats. No toxic effects were observed at a higher dose of 5 g/kg bw. Hence there were no lethal effects, which indicated that it may have a reasonable safety margin with regards to acute toxicity.

3.3. Oral glucose tolerance test in normal rats (OGTT)

OGTT in normal rats showed that blood glucose level in rats administered with 2 g/kg glucose was significantly decreased with methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* at 2 h with 400 mg/kg (P<0.01) while *A. viridis* at 400 mg/kg suppressed the rise in blood glucose at 1 h as compared with standard group on the 21st day (Table 1).

3.4. Effect on normoglycemic rats

Treatment with methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* showed that the fall in blood glucose levels of normal rats was significant (P<0.05) at 400 mg/kg *p.o.* on the 14th day of the study. However, rat treated with *A. viridis* at 200 mg/kg also showed significant fall in blood glucose level (P<0.01) on the 21st day of the study (Table 2).

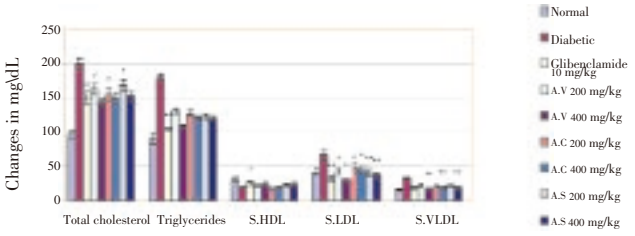

Figure 1. Anti-hyperlipidemic effect of MEAV, MEAC, MEAS in STZ induced diabetic rats.
S.HDL: Serum high density lipoproteins; S.LDL: Serum low density lipoproteins; S.VLDL: Serum very low density lipoproteins.

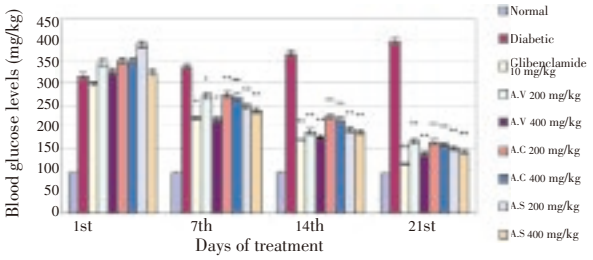

Figure 2. Antidiabetic activity of MEAV, MEAC and MEAS in STZ induced diabetic rats.

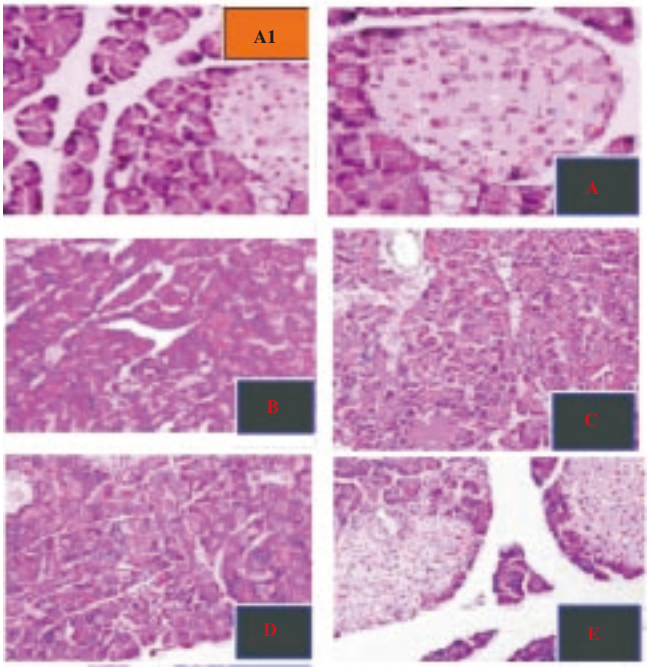

Figure 3. Photomicrographs of histological changes of rat pancreas of islets of langerhans.
A1: Normal rats showing many round and elongated islets evenly distributed throughout the cytoplasm, with their nucleus more lightly stained than the surrounding acinar cells; A: Diabetic control rat showing irregular cells, not well defined necrosis of cells being very clear; B: Glibenclamide (10 mg/kg *p.o.*) treated rat pancreas showing mild protection from STZ-induced changes in the pancreatic islets; C: MEAV extract (400 mg/kg); D: MEAC (400 mg/kg); E: MEAS extract (400 mg/kg) showing slight regeneration of β-cells when compared with diabetic control.

Table 1
Effects of methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* on OGTT in normal rats (mean±SEM).

Treatment (mg/kg bw)	Blood glucose level (mg/mL)			
	0 min	60 min	120 min	180 min
Normal control	98.74±1.98	128.26±2.2	106.32±1.28	98.62±1.28
Diabetic + glibenclamide (10)	99.24±2.01	114.68±1.36**	102.41±1.19**	96.28±1.32**
MEAV (200) *p.o.*	98.82±2.19	136.21±1.21	122.22±1.52	112.98±1.52**
MEAV (400) *p.o.*	98.27±1.83	121.68±1.09**	121.68±1.09**	99.56±2.18**
MEAC (200) *p.o.*	97.92±1.82	142.48±1.92	124.22±1.21	108.92±1.34
MEAC (400) *p.o.*	98.92±1.91	139.36±1.82	119.42±1.94**	102.28±1.28**
MEAS (200) *p.o.*	99.29±2.42	137.18±1.28	122.19±1.76	109.68±1.58**
MEAS (400) *p.o.*	98.22±1.82	131.64±1.12	108.78±1.46**	102.92±1.31**

** $P<0.01$ standard group *vs* all groups.

Table 2
Effects of methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* on normal rats (mean±SEM).

Treatment (mg/kg bw)	Blood glucose level (mg/mL)			
	1st day	7th day	14th day	21st day
Normal control	90.49±2.99	88.78±1.29	87.88±1.12	86.67±1.68
Diabetic + glibenclamide (10)	88.98±1.29	61.72±2.01	56.78±1.67**	52.52±1.31**
MEAV (200) *p.o.*	92.16±1.93	84.55±1.72	71.89±1.77	69.69±1.81*
MEAV (400) *p.o.*	91.97±3.01	80.98±1.97	65.72±1.62*	61.56±1.88**
MEAC (200) *p.o.*	95.62±1.48	89.29±.152	74.21±6.2	69.92±1.9
MEAC (400) *p.o.*	92.78±3.12	82.81±2.12	69.28±2.2	63.46±2.2
MEAS (200) *p.o.*	93.32±1.63	85.55±1.82	72.91±1.86	68.28±1.72*
MEAS (400) *p.o.*	91.68±2.97	80.68±1.68	66.68±1.68	60.69±1.62*

* $P<0.05$; ** $P<0.01$ standard group *vs* all groups.

Table 3
Changes in body weight in the treatment of MEAV, MEAC and MEAS in STZ induced diabetic rats (mean±SEM).

Treatment (mg/kg bw)	Changes in body weight (g)		
	Initial	7th day	21st day
Normal control	169.0±0.6	179.0±0.6	192.2±2.8
Diabetic + glibenclamide (10)	161.0±0.3	154.3±0.9*	161.2±2.1*
MEAV (200) *p.o.*	168.0±0.8	141.2±1.1**	148.1±1.4**
MEAV (400) *p.o.*	163.0±0.3	162.1±2.1**	188.1±2.9**
MEAC (200) *p.o.*	161.0±0.1	145.8±1.4**	150.8±1.4**
MEAC (400) *p.o.*	162.0±0.1	159.2±1.5**	175.0±2.2**
MEAS (200) *p.o*	162.0±0.4	148.2±1.4**	151.8±1.5**
MEAS (400) *p.o.*	164.0±0.4	161.1±1.9**	179.1±2.4**

* $P<0.05$; ** $P<0.01$ diabetic control *vs* all groups.

3.5. Hypoglycaemic effect of methanolic extracts of three plants of Amaranthus

The results from the study clearly indicated that the methanol extract exhibited significant hypoglycaemic activity in STZ induced diabetic rats, while there was no significant effect observed on normoglycemic rats. However, at the end of the 21 days of treatment, there was a 64.13%, 65.00%, 59.60% decrease of serum glucose level with the methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* at 400 mg/kg, while at 200 mg/kg it showed 61.92%, 57.68% and 58.38% reduction of serum glucose levels, respectively and the standard drug glibenclamide at 10 mg/kg group had the effect of 70.80% decrease on serum glucose level (Figure 1). Effects of methanolic extracts of three plants on OGTT in STZ induced diabetic rats were showed in Figure 2.

3.6. Changes in body weight

At the end of 21 days´ treatment, the body weight of normal rats in groups treated with methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* at 200 mg/kg and 400 mg/kg and in standard drug treated group increased significantly ($P<0.01$) by 48.9%, 45.3%, 47.9% (200 mg/kg), 75.7%, 84.5%, 71.7% (400 mg/kg), and 58.1%, respectively (Table 3).

3.7. Changes of histopathology of the pancreas

After 21 days' treatment, it was observed that the normal animals showing many round and elongated islets evenly distributed throughout the cytoplasm, their nucleus being more lightly stained than that of the surrounding acinar cells. The cells (Figure 3A) of diabetic animals were irregular, not well defined and defect in cell membrane. Necrosis of the cell was very clear (Figure 3A).

The standard group showed a mild protection from STZ

induced changes in the pancreatic islets (Figure 3B). Methanolic extract of *A. viridis*, *A. caudatus*, *A. spinosus* at 400mg/kg showed slight regeneration of beta cells when compared with the diabetic control (Figure 3C, 3D, 3E).

3.8. Lipid profile

When compared with the diabetic control rats at the end of 21 days treatment, significant (P<0.01) reductions of cholesterol (CHL), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) were observed at 400 mg/kg and 200 mg/kg of extracts of *A. spinosus*, *A. viridis* and *A. caudatus*. The reductions of CHL by methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* at 400 mg/kg were 23.7%, 28.7% and 25.7%, respectively, while at 200 mg/kg they were 15.5%, 18.6% and 22.4%, respectively. Triglyceride (TGL) reductions at 400 mg/kg were found to be 33.3%, 39.4% and 32.6%, respectively, while at 200 mg/kg the reductions were 31.8%, 27.3% and 28.9%, respectively. LDL reductions at 400 mg/kg were found to be 45.3%, 51.3% and 31.7%, respectively, while at 200 mg/kg they were 39.7%, 36.7% and 28.7%, respectively. VLDL at 400 mg/kg were found to be 42.3%, 39.8% and 41.7%, respectively while at 200 mg/kg they were 34.3%, 31.2% and 36.5%, respectively. Also, there was a significant (P<0.05) increase of HDL at 400 mg/kg 20.9%, 18.3%, and 3.57%, respectively while at 200 mg/kg that was 15.3%, 10.2% and 7.14%, respectively in treated diabetic rats. In cases of untreated diabetic rats, there was a fall in HDL level compared with diabetic control group. The standard drug group significantly reduced CHL by 25.2%, TGL by 41.4%, LDL by 51.8%, VLDL by 42.9% and significantly increased HDL by 28.5 %, respectively (Figure 1).

4. Discussion

Diabetes mellitus is a chronic disorder which results in increased concentration of glucose in the blood and in turn damages many of the body's system, particularly the blood and the nerves. A number of natural products are used in various traditional medicinal systems to relieve symptoms of disorders. The methanol extracts of three plants of *Amaranthus* demonstrated significant anti-diabetic and anti-cholesterolemic effects at two different dose levels on normal and STZ induced diabetic rats. Acute toxicity studies revealed the non-toxic nature of the methanol extract of leaves of *A. viridis*, *A. caudatus* and *A. spinosus*. There was no lethality or any toxic reactions found with the selected dose until the end of the study period. The basal food intake of normal group rats were found to be (14.3±0.2) g/rat/day whereas the food intake were significantly (18.4±0.2) increased in the diabetic group of rats (compared with normal), but no change in food intake was observed (14.5±0.2) in the standard and samples of treated rats.

Treatment with methanol extracts of *A. viridis*, *A. caudatus* and *A. spinosus* showed that the fall in blood glucose levels in normal rats were significant (P<0.05) at 400 mg/kg (*p.o.*) on the 14th day of the study. However, the rats treated with extracts of *A. viridis* at 200 mg/kg also showed significant fall in blood glucose level (P<0.01) on the 21st day of the study. The fundamental mechanism in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and the decreased utilization of glucose by the tissues[23]. STZ, slightly cytotoxic agent of pancreatic β-cells[24], selectively destroys the pancreatic insulin secreting beta cells, thus leaving less active cells and resulting in a diabetic state[25]. In the present study, we observed an increased level of blood glucose in STZ induced rats. The anti-diabetic activity of methanol extracts of three plants was significant (P<0.01) in decreasing blood glucose level from the 7th day onwards at both the doses of 200 mg/kg and 400 mg/kg (*p.o.*). STZ-induced diabetes is characterized by a severe loss in body weight[26], and this reduction is due to loss or degradation of structural proteins, as the structural proteins are known to contribute to body weight. In our study, a significant weight loss was observed in the diabetic group and significant gain in body weight was observed in the groups treated with methanol extracts of three plants. This may be due to the presence of 20% protein, all 8 essential amino acids (high in lysine, threonine and tryptophan), vitamins, calcium and minerals[27] in methanol extracts of three plants to reduce hyperglycemia.

Flavonoids are one of the most numerous and wide spread groups of phenolic compounds in higher plants[28]. Some of them, due to their phenolic structure, are known to be involved in the healing process of free radical mediated diseases including diabetes[29]. The methanol extracts of three plants possess flavonoids as the anti-diabetic principles. In diabetes, hyperglycaemia is accompanied with dyslipidemia[30] representing risk factor for coronary heart diseases. The abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hyper triglyceridemia[31], and insulin deficiency is also associated with hyper cholesterolemia due to metabolic abnormalities[32]. The dyslipidemia is characterized by increase in TC, LDL, VLDL, TG and fall in HDL. This altered serum lipid profile was reversed towards normal after treatment with methanol extracts of three plants. The hypothesis is further supported by the pancreatic histology which showed protection of pancreatic β-cells from toxic effects of STZ, and focal necrosis was observed in the diabetic rat pancreas but was less obvious in treated groups. From the above result, we can confirm that the methanolic extract of leaves of three plants at doses of 200 mg/kg and 400 mg/kg possesses significant anti-diabetic activity on long-term (21 day) treatment in rats.

On the basis of the current investigation, it is noted that the methanolic extracts of *A. viridis*, *A. caudatus* and *A. spinosus* act in a similar fashion to the glibenclamide (standard drug) in reducing the elevated blood glucose level and lipid profile of STZ induced diabetic rats (thus, justifying the claim made in Ayurvedic classics). Therefore, the results provide pharmacological evidence for its folklore

claim as an anti-diabetic and hypolipidemic agent. Further studies on its isolation, identification and characterization of the active principles are in progress. The anti-diabetic activity may be attributed to some of its active principles.

Conflict of interest statement

We declare that we have no conflict of interest.

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