

Full Length Research Paper

***Amaranthus viridis* modulates anti-hyperglycemic pathways in hemi-diaphragm and improves glycogenesis liver function in rats**Shihab Uddin¹, Md. Mahmodul Islam^{2*}, Md. Mynul Hassan³, Amrita Bhowmik⁴ and Begum Rokeya⁵¹Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Bangladesh.²Department of Pharmacy, Dhaka International University, Banani, Dhaka, Bangladesh.³Dept of Biotechnology and Genetic Engineering, Khulna University, Khulna, Bangladesh.⁴Department of Applied Laboratory Sciences, Bangladesh University of Health Sciences, Dhaka, Bangladesh.⁵Department of Pharmacology, Bangladesh University of Health Sciences, Dhaka, Bangladesh.

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Amaranthus viridis is an ecumenical species in the botanical family of Amaranthaceae, which has been traditionally used to treat several skin diseases along with some antilipidemic activities. The present study was carried out to investigate the anti-hyperglycemic effect of 75% ethanolic extract of *A. viridis* in Neonatal streptozotocin (N-STZ) induced rats' hemi-diaphragm, including screening for secondary plant metabolites. Qualitative phytochemical studies were done by various conventional methods for the possible secondary metabolites. For antidiabetic assay via hemi-diaphragm, Long-Evan rats were used in the study. Type 2 diabetes was induced by a single *ip* injection of streptozotocin to 48 h old pups (N-STZ) and after 3 months, rats were confirmed by an oral glucose tolerance test and further selected for the experiment. Studies to evaluate the glucose utilization capacity of *A. viridis* in isolated rat hemi-diaphragm were done. The data were analyzed by appropriate statistical analysis. *In vitro* glucose uptake by hemi-diaphragm study showed glucose uptake increased significantly in left diaphragm of type 2 diabetes mellitus with insulin alone treated and *A. viridis* alone treated group, where *A. viridis* alone treated group showed very highly significance ($p=0.000$). Treatment with both insulin and *A. viridis* increased the glucose uptake also very significantly ($p=0.004$). *A. viridis* extract acted more significantly compared to insulin in T2DM rats. In the normal rats at left hemi diaphragm, *A. viridis* extract also increased glucose uptake more significantly ($p=0.009$) compared to insulin ($p=0.013$). At the right diaphragm, glucose uptake increased in all treated groups compared to control group but not significantly. This plant may contain potential anti-hyperglycemic agents which possibly act through some extra pancreatic mechanism that include glucose uptake by diaphragm and increased glycogenesis by liver.

Key words: *Amaranthus viridis*, antidiabetic, hemi-diaphragm, streptozotocin, glucose, Long-Evan rats.

INTRODUCTION

It is staggering to consider the threat that diabetes poses to our current healthcare system. Recent technological

and therapeutical advancement in the management of diabetes mellitus includes pancreas regeneration, islet

transplantation, pancreas transplantation, glucose monitoring at continuous basis, uninterrupted subcutaneous insulin infusion and assorted medication (George, 2009). For mortals with T2DM mellitus (T2DM), an assortment of treatments is available. Most of the pharmacological aid schemes for T2DM are typically grounded on efficacy. Hence, prosperous responses to such therapeutics are frequently variable and unmanageable to predict. In this circumstances, delineation of drug reaction is expected to considerably heighten our ability to provide patients with the most effective treatment strategy given their individual backgrounds. Hence pharmacogenetic analysis of medications against diabetes is still in its early stage. Up to date, major pharmacogenetic acquisitions have focused on biguanides, TZDs and sulfonylureas (Distefano et al., 2010). Most recently researchers have focused on the management diabetes and its associated complications. A variety of approaches have been taken for this purpose.

The plant is usually known as green amaranth or slender amaranth. Possible origin is South America, although widely distributed in tropical weed, foreign to hot-temperate regions and distributed in the tropical and subtropical regions of the world. It is an annual herb with erect or ascending habit, growing to 1 m tall. Leaves are light green and the fruit are obviously wrinkled. It has prominent axillary spines and its leaves can have an obvious reddish or purplish tinge (Stanley et al., 1984). *A. viridis* is found to be a very common garden weed. Also it is found in areas such as roadsides, parks, pastures and other disturbed sites, but seldom cropped, often flattened and prostrate, vacant lots, sometimes crevices of sidewalks and edge of asphalt strips, etc. (Stone, 1970), casual in croplands and waste places too (Whistler, 1988).

The *A. viridis* is a good source of vitamins B and C, taken as vegetables (Sayed et al., 2007). Leaves and seeds are also edible. Previous experiments ascertained it to be a superior source of protein (Macharla et al., 2011). Traditionally it is used to cure eczema, psoriasis and rashes including antinociceptive and antipyretic properties, reported by Kumar (Kumar et al., 2009). Besides these, it is reported by Krishnamurthy that *A. viridis* has anti-inflammatory, antihyperglycemic, hypolipidemic activity as well as acne and skin cleansing property (Krishnamurthy et al., 2011). It has a wide application over diuresis, for snake bites, scorpion stings, dysentery, constipation, eczema, bronchitis, anemia, leprosy and stomach problems like many incidences (Pandhare et al., 2012; Macharla et al., 2011). According to Syed et al. it is quite beneficial to pregnant women to subside labor pains and diabetes (Syed et al., 2007). Its pharmacological study also reveals that it is antiviral (Obi et al., 2006). Meanwhile, its anti-allergenicity is claimed by Sayed et al. (2007). According to Kumar, *A. viridis* is

a potent hepatoprotective and antioxidant plant (Kumar et al., 2011). Studies also claimed that it is a good source of anthelmintic and isoproterenol-induced cardiac toxicity inhibitory plant (Ashok et al., 2011; Kumar et al., 2012).

Plants that exhibit activity against hyperglycemia are mainly owing to their ability to bushel the function of pancreatic tissues by causing an alleviation in insulin output or conquer the intestinal assimilation of glucose or aid of metabolites in insulin subordinate processes. Most plants contain carotenoids, terpenoids, glycosides, flavonoids, alkaloids etc. that are usually entailed as having antidiabetic effect (Jung et al., 2006). Type 2 diabetes represents a progressing decline in beta-cell function. Regarding the restrictions of being therapies in fixing the quality of life to normal as well as reducing the risk of chronic diabetic complications by maintaining normal blood glucose level, the search for alternating sources of oral hypoglycemic agents is a requirement. Due to the limitation of recent therapies to control all the metabolic defects of diabetes as well as their possible pathological outcomes with the great expense, there is a clear need for the development of alternative strategies for diabetes treatment.

There has been a possibility of anti-hyperglycemic potentialities of *A. viridis* reported by Krishnamurthy et al. (2011). However, so far hemi-diaphragm pathways of *A. viridis* on Long Evan rats against the hypoglycemic activities, has not been done. So in this study, an attempt was made to evaluate the anti-hyperglycemic activity of ethanolic extract of *A. viridis* plant and also to find out the chemical factors present therein causative for the biological activity. The pharmacological study was carried out on streptozotocin induced type 2 Neonatal model in Long Evan rats. Glucose utilization capacity of bioactivity guided fractions of *A. viridis* in isolated rat hemi-diaphragm in both normal and N-STZ rats was performed and the observed activity was identified, characterized and quantified.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol (PubChem CID: 702), Ferric chloride (PubChem CID: 24380), potassium ferrocyanide (PubChem CID: 11963580), Chloroform (PubChem CID: 6212), sulphuric acid (PubChem CID: 1118), sodium dihydrogen phosphate (PubChem CID: 23672064) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). 4,6-Ethylidene glucose streptozotocin (PubChem CID: 3081692) were purchased from Merck (Darmstadt, Germany) and Human insulin (PubChem CID: 16131099) from Sanofi Bangladesh (Bangladesh). All other chemicals used were from the laboratory stock of the Department of Pharmacology, Bangladesh University of Health Sciences, Dhaka, Bangladesh and were of the highest grade

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available.

Plant material collection and identification

In this study, whole plant part of *A. viridis* (family: amaranthaceae, local name: Notey shak) was used. The plants were collected from Pabna, Bangladesh (Geographic coordinates: Latitude: 24°00'23" N, Longitude: 89°14'13" E and elevation above sea level: 19 m = 62 ft) available sources from the field in the month of June, 2015. The plant was identified by the Bangladesh National Herbarium, Dhaka (Accession No: DACB-38568).

Preparation of ethanol extracts of *Amaranthus viridis*

Mature and fresh whole plants were washed thoroughly after collection and air dried. The weights of plants before and after dry were recorded. After then, the whole plants were grinded and again weighed. Finally, these grind portions were extracted by using 75% ethanolic solvent. The ethanolic extract was prepared by using Soxhlet (Beijing Getty glassware Co. Ltd. maintained at 70°C) and following the completion of extraction was concentrated by using water evaporator (Fujian Snowman Co. Ltd. 5 Litters, asserted at 80°C). The ethanolic extracts at semi dried state were encouraged to dry in a freeze drier (HETOSICC, Heto Lab Equipment, Denmark) at -55°C temperature and preserved in a reagent bottle at -8°C in a freezer for analysis.

Preparation of animals for treatment

Adult Long Evans rats weighting 160 to 220 g were included in the study. The animals were bred at Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM) animal house, Dhaka, Bangladesh, maintained at a constant room temperature of 22±5°C with humidity of 40 to 70% and the natural 12 h day-night cycle. Animal housing and handling were performed in accordance with Good Laboratory Practice (GLP) mentioned in US guidelines (NIH publication # 85-23, revised in 1985). The experimental protocols were critiqued and sanctioned by the Institutional Animal Ethics Committee prior to initiation of the experiment. The rats were fed upon a stock lab pellet diet and water supplied *ad libitum*. Standard rat diet contained wheat (40%), wheat bran (20%), fish meal (10%), germ (3.9%), oil cake (10%), milk (3.8%), pulses (3.9%), soyabean oil (1.5%), rice polishing (5%), molasses (0.95%) and salt (0.95%). Embavit GS (vitamin mixture) 250 g was added per 100 kg of rat food. The influence of circadian rhythm was avoided by starting all experiments at 7:30 am. The experiments were conducted according to the ethical guidelines approved by Bangladesh Association for Laboratory Animal Science.

Preparation of type 2 diabetes model rats

Type 2 diabetes was hastened by a single *ip* injection of streptozotocin (STZ) dissolved in citrate buffer (10 ml), at a dose of 90 mg/kg of body weight into the rat pups (48 h old, average weight 7 g) as described by Weir and Bonner-Weir et al. (2013). Following 3 months of STZ injection, rats were examined by oral glucose tolerance test (OGTT) for their blood glucose level. Diabetic model rats with blood glucose level >7.00 mmol/L, at fasting condition were selected for studying the effects of *A. viridis* extracts.

Phytochemical screening of *A. viridis*

Three (3) g of *A. viridis* 75% ethanolic extract was boiled with 30 ml

distilled water for 5 min in a water bath and was filtered while hot. The extract sample or filtrate was taken for the experiments wherever applicable using standard protocols (Sharmistha et al., 2012) to test the presence of bioactive compounds.

In vitro glucose uptake study of *A. viridis* by isolated rat hemi-diaphragm

Glucose uptake by rat hemi-diaphragm was estimated by the methods described elsewhere (Walaas and Walaas, 1952; Chattopadhyay et al., 1992) with some modifications. 32 male and female Long Evans normal and Type 2 rats were weighed and rats weighing between 170 to 210 g were used in the study. The weight of the rats were measured before and after fasting. Four sets containing graduated test tubes (n=4) for each hemi-diaphragm were taken. Group I served as a control which contained 2 ml of Tyrode (NaCl (8 gm/L), KCl (0.20 gm/L), CaCl₂ (0.20 gm/L), MgCl₂ (0.10 gm/L), NaH₂PO₄ (0.05 gm/L), NaHCO₃ (1 gm/L), Glucose (1 gm/L) having pH 6.5) solution with 2% glucose, Group II contained 2 ml Tyrode solution with 2% glucose and regular insulin (Novo Nordisk) 0.62 ml of 0.4 units per ml solution. Group III contained 2 ml Tyrode solution with 2% glucose and 1.38 ml of *A. viridis* extract (30 mg extract dissolved in 3 ml H₂O and adjust PH to 7.4) and the Group IV contained 2 ml Tyrode solution with 2% glucose and regular insulin 0.62 ml of 0.4 units per ml solution and 1.38 ml of *A. viridis* extract. The volumes of all the test tubes were made up to 4 ml with distilled water to match the volume of the test tubes of Group IV. Long Evans rats were tested overnight and killed by decapitation. The diaphragms were dissected out quickly with minimal trauma and divided into two halves. Two diaphragms from the same animal were not used for the same set of experiment. Four numbers of diaphragms were used for each group. The hemi-diaphragms were placed in test tubes and incubated for 30 min at 37°C in an atmosphere of 100% oxygen with shaking at 140 cycles/min. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

Estimation of glucose level

The glucose oxidase (GOD-PAP) test was performed using the established method published by Trinder (Trinder, 1969) without modification.

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD or as Median (Range) as appropriate. Statistical analysis of the results was performed by using the student's t-test (paired and unpaired), ANOVA (analysis of variance) followed by Bonferroni and Dunnett post hoc test and Mann Whitney (u) test. GraphPad Prism (Version 5) software was used for all statistical analysis and P<0.05 was considered as significance.

RESULTS

Phytochemical screening of 75% ethanolic extract of *A. viridis*

The present investigation was carried out to assess the

Table 1. Phytochemical screening for the possible secondary metabolites of the *A. viridis* extract.

Serial no	Phytochemicals	Results
1	Tannin	++
2	Saponin	++
3	Alkaloid	++
4	Flavonoid	++
5	Phenol	++
6	Steroid	-
7	Terpenoid	+
8	Carbohydrate	-

++ = Presence; + = trace; - = absence.

qualitative phytochemical analysis of 75% ethanolic extract of *A. viridis*. The phytochemical screening reveals the presence of various plants' secondary metabolites as shown in Table 1. Tannin, saponin, alkaloid, flavonoid and phenols were found in the extract of *A. viridis*. Terpenoid was in trace amount but carbohydrate and steroids were not found in our phytochemical studies.

Check values of STZ induced Type 2 diabetic rats in different groups

To generate a rat model mimicking human type 2 diabetes with impaired insulin secretion and insulin resistance, we used STZ injection (90 µg/kg, body weight) to 48 h old pulps. STZ injection to neonates led to the injury of the pancreas resulting in destruction of the functional β -cells. At the age of 3 months, when an oral glucose challenge (500 mg/kg, body weight) was done, the remaining β cell could not cope with the load, which reflected in the postprandial rise of serum glucose level at 30 min (Table 2). The rise was significant among all rats compared to baseline value. On the basis of this experiment these rats were selected and sorted into three distinct groups to carry out the experiments with feeding of different extracts.

Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (left) diaphragm on normal rats in vitro assay

In order to explore the mechanism underlying the anti-hyperglycemic activity of *A. viridis* in vitro glucose uptake study of rat hemi-diaphragm was done. The results of glucose uptake by the left hemi-diaphragm in normal rats are presented in Table 3 and Figure 1. The results showed that glucose uptake was enhanced by hemi-diaphragm when the normal rats were treated with insulin alone (glucose uptake $m \pm SD$, mg/g/30 min, control 2.02 ± 0.62 vs insulin 6.59 ± 2.01 ; $p=0.013$). When hemi-diaphragm was treated with *A. viridis* extract, glucose

uptake was also increased significantly (glucose uptake $m \pm SD$, mg/g/30 min, control 2.02 ± 0.62 vs plant extract 6.93 ± 0.62 ; $p = 0.009$). When the hemi-diaphragm of normal rats was exposed to both insulin and *A. viridis* extract, it did not increase glucose uptake significantly.

Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (right) diaphragm on normal rats in vitro assay

The results of glucose uptake by rat right hemi-diaphragm in normal rats is presented in Table 4 and Figure 2. The results showed that glucose uptake was enhanced by hemi-diaphragm when the normal rats were treated with insulin alone (glucose uptake $m \pm SD$, mg/g/30 min, control 0.568 ± 0.350 vs insulin 2.684 ± 2.527 ; $p = 0.148$). When hemi-diaphragm was treated with *A. viridis* extract, glucose uptake was also increased but not-significantly (glucose uptake $m \pm SD$, mg/g/30 min, control 0.568 ± 0.350 vs plant extract 1.893 ± 1.704 ; $p = 0.178$). When the hemi-diaphragm of normal rats was exposed to both insulin and *A. viridis* extract, it also did not increase glucose uptake significantly.

Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (left) diaphragm on STZ induced type 2 rats in vitro assay

The results of glucose uptake by rat left hemi-diaphragm in T2DM rats is presented in Table 5 and Figure 3. When the T2DM rats were treated with insulin alone the glucose uptake was significantly increased in compared to control (glucose uptake $m \pm SD$, mg/g/30 min, control 2.55 ± 0.36 vs insulin 5.26 ± 1.21 ; $p = 0.037$). In the treatment with *A. viridis* extract, glucose uptake was significantly higher in comparison to control (glucose uptake $m \pm SD$, mg/g/30 min, control 2.55 ± 0.36 vs plant extract 9.74 ± 0.87 ; $p = 0.000$) as well as insulin alone (glucose uptake $m \pm SD$, mg/g/30 min, insulin 5.26 ± 1.21 vs plant extract 9.74 ± 0.87 ; $p = 0.002$), respectively.

Insulin with *A. viridis* extract was also exposed to a significant increased glucose uptake when it compared with control (glucose uptake $m \pm SD$, mg/g/30 min, control 2.55 ± 0.36 vs insulin with plant extract 6.46 ± 0.93 ; $p = 0.004$) as well as plant extract alone (glucose uptake $m \pm SD$, mg/g/30 min, plant extract 9.74 ± 0.87 vs insulin with plant extract 6.46 ± 0.93 ; $p = 0.012$), respectively.

Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (right) diaphragm on STZ induced type 2 rats in vitro assay

The results of glucose uptake by rat right hemi-diaphragm in T2DM rats is presented in Table 6 and

Table 2. Check values of STZ induced Type 2 diabetic rats in different groups.

Groups	Fasting (0 min)	After OGTT (30 min)
WC (n=6)	7.44±0.57 (100%)	14.62±1.92 (197%)
Glc (n=6)	7.71±0.55 (100%)	14.82±1.35 (192%)
AVEtE (n=6)	7.72±0.87 (100%)	14.73±1.39 (191%)

Paired samples T test	
Groups	0 min vs 30 min
WC (n=6)	0.000
Glc (n=6)	0.000
AVEtE(n=6)	0.000

Results are expressed as Mean ±SD. OGTT= Oral Glucose Tolerance Test, STZ= Streptozotocin; WC = Type 2 Water Control; Glc = Type 2 Glibenclamide treated group and AVEtE= 75% ethanol extract of *A. viridis*.

Table 3. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (Left) diaphragm on normal rats *in vitro* assay.

Group (G)	Treatment	Incubation medium	Glucose uptake (mg/g/30 min)	p value
G1	Control	Tyrode solution with Glucose (2%)	2.02±0.62 (100%)	-
G2	Insulin	Tyrode solution with Glucose and Insulin (Actrapid 40 u/ml)	6.59±2.01 (326%)	G1 Vs G2, p=0.013*
G3	Plant extract	Tyrode solution with Glucose and plant extract (30 mg/3 ml H ₂ O)	6.93±0.62 (343%)	G1 Vs G3, p=0.009**
G4	Insulin+ plant extract	Tyrode solution with Glucose, Insulin and plant extract	4.38±1.25 (211%)	G1 Vs G4, p= 0.021*

Results were expressed as Mean ±SD. Statistical analysis between group comparison was done by using one-way ANOVA with post hoc Bonferroni test. *= p<0.05; **=p<0.01.

Table 4. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (Right) diaphragm on normal rats *in vitro* assay.

Group (G)	Treatment	Incubation medium	Glucose uptake (mg/g/30 min)	p value
G1	Control	Tyrode solution with Glucose (2%)	0.568±0.350 (100%)	
G2	Insulin	Tyrode solution with Glucose and Insulin (Actrapid 40 u/ml)	2.684±2.527 (471%)	G1 Vs G2, p=0.148
G3	Plant extract	Tyrode solution with Glucose and plant extract (30mg/3ml H ₂ O)	1.893±1.704 (333%)	G1 Vs G3, p=0.178
G4	Insulin+ plant extract	Tyrode solution with Glucose, Insulin and. plant extract	1.898±0.977 (334%)	G1 Vs G4, P=0.067

Results were expressed as Mean ±SD. Statistical analysis between group comparison was done by using one-way ANOVA with post hoc Bonferroni test. *= p<0.05; **=p<0.01.

Figure 4. When the T2DM rats were treated with insulin alone the glucose uptake was increased by 59% compared to control group but not significantly. In the

treatment with *A. viridis* extract, glucose uptake was 84% higher in comparison to control group. Glucose uptake was found to be increased up to 119% in case of

Table 5. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (Left) diaphragm on STZ induced type 2 rats *in vitro* assay.

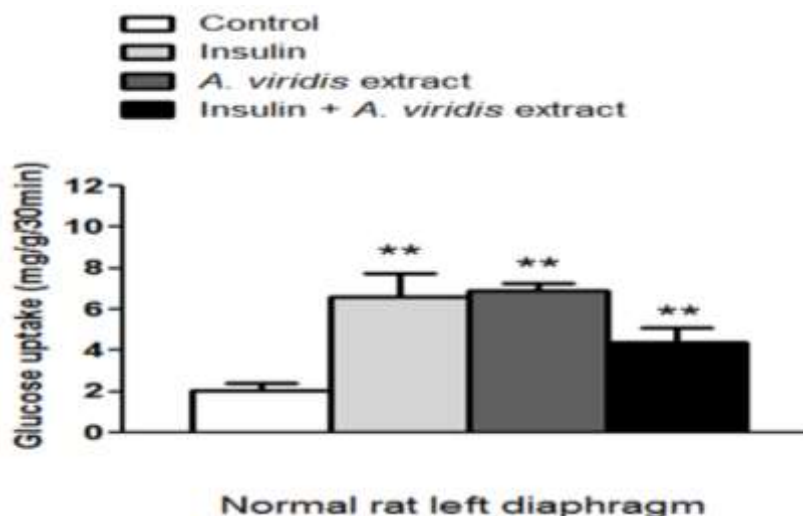
Group (G)	Treatment	Incubation medium	Glucose uptake (mg/g/30 min)	p value
G1	Control	Tyrode solution with Glucose (2%)	2.55±0.36 (100%)	
G2	Insulin	Tyrode solution with Glucose and Insulin (Actrapid 40 u/ml)	5.26±1.21 (206%)	G1 Vs G2, p=0.037*
G3	Plant extract	Tyrode solution with Glucose and plant extract (30 mg/3 ml H ₂ O)	9.74±0.87 (381%)	G1 Vs G3, p=0.000** , G2 Vs G3; p=0.002**
G4	Insulin+ plant extract	Tyrode solution with Glucose, Insulin and plant extract	6.46±0.93 (253%)	G1 Vs G4, p=0.004** , G3 Vs G4, p=0.012*

Results were expressed as Mean ±SD. Statistical analysis between group comparison was done by using one-way ANOVA with post hoc Bonferroni test. * = p<0.05; ** = p<0.01.

Table 6. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (right) diaphragm on STZ induced type 2 rats *in vitro* assay.

Group (G)	Treatment	Incubation medium	Glucose uptake (mg/g/30 min)	p value
G1	Control	Tyrode solution with Glucose (2%)	1.385±0.980 (100%)	
G2	Insulin	Tyrode solution with Glucose and Insulin (Actrapid 40 u/ml)	2.195±1.293 (159%)	G1 Vs G2, p=0.359
G3	Plant Extract	Tyrode solution with Glucose and plant extract (30mg/3ml H ₂ O)	2.548±1.423 (184%)	G1 Vs G3, p=0.233
G4	Insulin+ Plant Extract	Tyrode solution with Glucose, Insulin and. plant extract	3.033±0.823 (219%)	G1 Vs G4, P=0.043*

Results were expressed as Mean ±SD. Statistical analysis between group comparison was done by using one-way ANOVA with post hoc Bonferroni test. * = p<0.05; ** = p<0.01.

**Figure 1.** Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (Left) diaphragm on normal rats *in vitro* assay. * = p<0.05; ** = p<0.01.

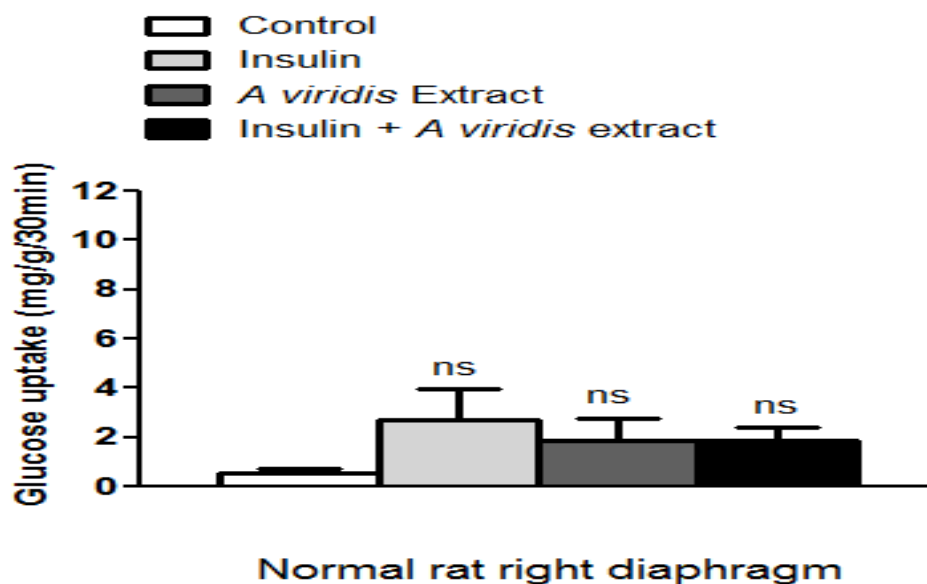


Figure 2. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (Right) diaphragm on normal rats *in vitro* assay. *= $p < 0.05$; **= $p < 0.01$ and ns= not significance.

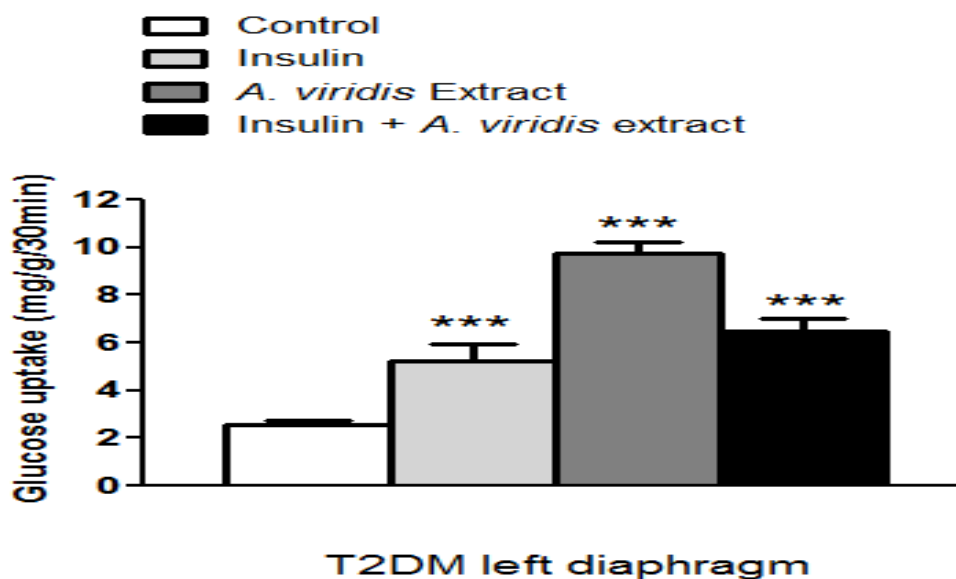


Figure 3. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (Left) diaphragm on STZ induced type 2 rats *in vitro* assay. *= $p < 0.05$; **= $p < 0.01$.

A. viridis extract with insulin, compared with control group as well as plant extract and insulin individually.

DISCUSSION

Previous studies have shown that antidiabetic plants possess the presence of alkaloids, glycosides and

polyphenols like phytoconstituents (Sharmistha et al., 2012).

Therefore in the beginning of the study, preliminary phytochemical screening of the 75% ethanolic extract of whole plant for secondary plant metabolites was performed. The results revealed the presence of saponin, tannin, flavonoids, alkaloids, terpenoids, phenol (Table 1). The presence of a significant number of secondary plant

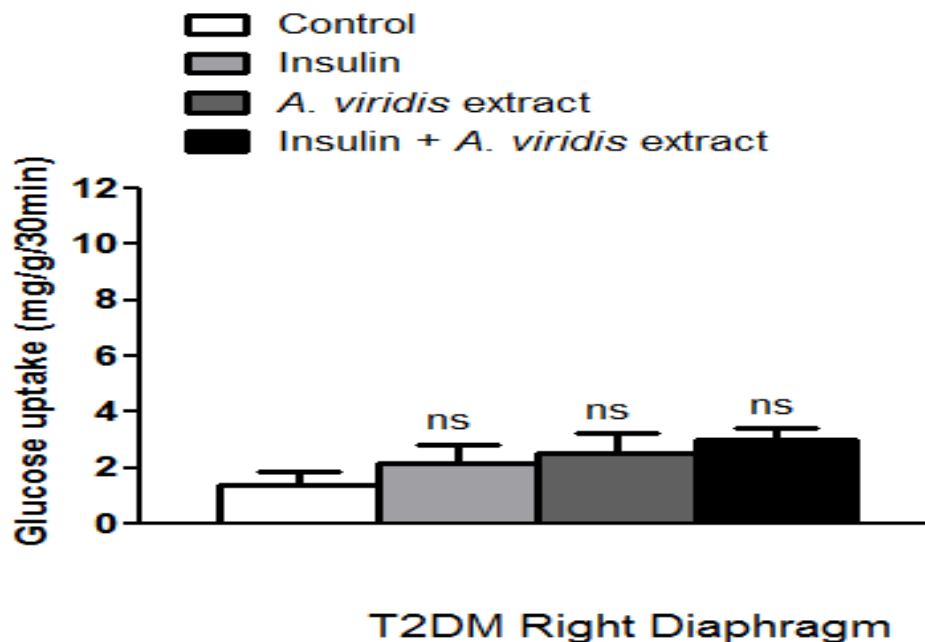


Figure 4. Effect of *A. viridis* extracts using glucose uptake by isolated rat-hemi (right) diaphragm on STZ induced type 2 rats *in vitro* assay. ns = not significance.

metabolites in *A. viridis* might be responsible for the biological activities observed later in this study.

Type 2 diabetes was developed by injecting STZ (a pancreatic β cell toxin) to 48 h old pups, which inside the β -cell dissociates into glucose and methylnitrogenase. The later alkylates and modified biomolecules breakdown DNA and destroys β cell, thereby causing diabetes. Early injury of the β cells resulted in the partial recovery of β cell leading to type 2 diabetes as a result of insulin resistance in target tissues and impaired insulin secretion, accompanied by increased adiposity.

When at the age of 3 months these rats have been challenged with an oral glucose load, all of them could not cope with the glucose load due to the defective β cells. Although their fasting glucose values were a bit higher (ranging from 7.44 to 7.72), indicating the presence of some functioning β cells but their post challenge glucose values were significantly higher which proved that these rats have developed type 2 diabetes (Table 2).

Therefore, it may be assumed that the hypoglycemic activity of *A. viridis* in type 2 model rats at least, may be partly due to increased uptake of glucose for the formation of glycogen by enhanced glycogenesis. To put further insight regarding the mechanism of anti-hyperglycemic effect of *A. viridis* extract in both normal and T2DM rats *in vitro* glucose uptake by hemi-diaphragm was performed.

In this study, individual insulin ($p = 0.013$) and *A. viridis* ($p = 0.009$) treated group showed a significant increment in glucose uptake in normal rats at left diagram (Tables 3

and 4). Hence, in case of T2DM rats $p = 0.037$ with insulin alone and $p = 0.000$ with *A. viridis* extract (Table 5). Moreover, treatment with both insulin and *A. viridis* extract caused significantly much higher glucose uptake by rat left hemi-diaphragm ($p = 0.004$). Thus it can be concluded that *A. viridis* improves hyperglycemia by extrapancreatic mechanism as the left diaphragm improves glucose uptake more than right diaphragm.

Conclusion

The present study demonstrates that phytochemical screening of 75% ethanol extract contains a number of secondary plant metabolites including flavonoids, alkaloids which might be associated with the obtained antidiabetic properties of *A. viridis*. *In vitro* glucose consumption by hemi-diaphragm study exhibited increased state of the glucose by hemi-diaphragm in the presence of *A. viridis* extract. From the findings it can be concluded that different secondary metabolites of plant materials had some extra pancreatic mechanism like glucose consumption by peripheral tissues. Thus, the plant might be considered for further chemical studies and detailed toxicological studies for future drug development.

Conflict of interest

The authors have not declared any conflict of interest.

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Abbreviations

N-STZ, Neonatal streptozotocin; **A. *viridis***, *Amaranthus viridis*; **T2DM**, type 2 diabetes mellitus; **BIRDEM**, Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders; **GLP**, good laboratory practice; **OGTT**, oral glucose tolerance test; **GOD-PAP**, glucose oxidase; **SPSS**, statistical package for social science; **ANOVA**, analysis of variance; **M $\pm$ SD**, mean $\pm$ standard deviation.

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