Journal of Pharmaceutical Research International

26(6): 1-12, 2019; Article no.JPRI.48311
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Exploration of Antidiabetic Activity of *Stephania japonica* Leaf Extract in Alloxan-Induced Swiss Albino Diabetic Mice

Md. Dobirul Islam[1], Syeda Farida Akter[1], Md. Amirul Islam[1] and Md. Salim Uddin[1*]

[1]Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. Author MDI designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author SFA managed the literature searches and carried out the tests. Authors MAI and MSU managed the analyses of the study and reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2019/v26i630154
Editor(s):
(1) Dr. Jongwha Chang, University of Texas, College of Pharmacy, USA.
Reviewers:
(1) Rajibul Islam, Gono Bishwabidyalay, Bangladesh.
(2) Dr. Dennis Amaechi, Veritas University Abuja, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48311

Original Research Article

Received 24 January 2019
Accepted 07 April 2019
Published 20 April 2019

ABSTRACT

Aims: Presently the medicinal world is rapidly turning more on the therapeutic health benefits of natural product and medicinal plants in the management of major crucial disease and their complications. Medicinal plant, *Stephania japonica* has been studied for exploring antidiabetic potentiality as an alternative source of medicine against the global threat of Diabetes mellitus (DM).
Methods: The extraction of *S. japonica* leaf was carried out by acetone and ethanol. Phytochemical screening and quantitative analysis of *S. japonica* leaf extracts were evaluated through chemically forming characterized color formation and calibration method respectively, by using standard reference substances (ascorbic acid, gallic acid, and quercetin) to assess the probable compounds present in the extract. Anti-diabetic potentiality of highest phytochemicals containing two extracts were investigated in *in vitro* as a α-amylase inhibitors and *in vivo* through alloxan-induced Swiss albino diabetes mice model.

**Corresponding author: E-mail: msuddin75@yahoo.com; msuddin75@ru.ac.bd;*

Results: Alkaloids, carbohydrates, steroids, flavonoids, resins, saponins, tannins and coumarins were present in the leaf extracts. The estimated amount of total phenolics, flavonoids, flavonols and proanthrocyanidins contents of acetone and ethanol extract were 92.12±0.64 and 56.54±1.05 mg of gallic acid equivalent (GAE)/gm of dry extract, 66.02±1.42 and 46.17±0.54 mg of catechin equivalent (CAE)/gm of dry extract, 7.05±0.108 and 5.26±0.083 mg of quercetin equivalent (QUE)/gm of dry extract, 35.19±0.67 and 9.55±1.11 mg CAE/gm of dry extract, respectively. In 3, 5-dinitrosalicylic acid method, acetone and ethanol extract showed α-amylase inhibition of 51.02% and 46.62%, respectively at the concentration of 1000 µg/mL whereas in starch iodine color assay, acetone and ethanol extract showed inhibition of 57.32% and 52.12%, respectively at the concentration of 800 µg/mL. In contrast, both of the leaf extracts significantly ($p<0.05$ to $p<0.001$) improved the lipid profile parameters, blood glucose level and serum hepatic marker proteins in alloxan-induced diabetic mice.
Conclusion: The present study strongly concluded that *S. japonica* leaf extracts process potent antidiabetic potentiality that might be significance for the management of diabetes and its complications.

Keywords: Diabetic mellitus; phytochemicals; flavonoids; Stephania japonica; hypolipidemic effect.

1. INTRODUCTION

Diabetes mellitus, which is reflected as a group of metabolic disease is considered as one of the main leading threat to human lives in the 21st century. The chronic metabolic disorder, DM is caused due to deficiency or inadequate function of insulin that leads to abnormalities in glucose metabolism resulting in ketoacidosis, thirst, polyuria, blurring of vision, and weight loss [1,2]. Diabetes also dramatically increases the risk of various devastating complications such as cardiovascular diseases, peripheral vascular disease, stroke, neuropathy, renal failure, retinopathy complications and blindness [3,4]. Recently, the number diabetic patients are increasing apprehensively. There are now 415 million adults aged from 20 to 79 years with diabetes worldwide and moreover, another 318 million adults are estimated to have impaired glucose tolerance, which puts them at high risk of developing the disease. If this augmentation is continued for 2040 almost 642 million people will be suffering from the diabetes complication [5].

Inhibitors of amylase based on alkaloids, glycosides, galactomannan, terpenoids, peptidoglycans and steroids, are ordinarily prescribed to diabetic patients to decrease postprandial hyperglycemia induced by the digestion of starch in the small intestine [6]. These inhibitors are designed to primarily target of exo-acting starch hydrolyzing enzymes (α-amylase) found in the intestinal tract. The inclusive consequence of inhibition is to reduce the flow of starch monomers from complex dietary carbohydrates into the bloodstream, diminishing the postprandial effect of starch consumption on plasma glucose levels [7]. The life threaten effect and detrimental complication of DM are mediated by oxidative stress. In diabetic condition, large number of free radicals that are produced impaired anti-oxidant defense system leading to oxidative damaged through the reactive oxygen species mediated diabetic pathogenesis [8,9]. Presently many commercial synthetic drugs such as biguanides, acarbose, glitazones and sulfonylureas are used in the treatment of DM for reduction of blood glucose level. For management of DM and its complication many commercial synthetic drugs used throughout the prolong period of life and hence produced many adverse side effects [10]. Therefore, researchers are looking for safe and effective medications to overcome the devastating effects of diabetes. Many medicinal plants such as *Ficus racemosa, Momordica charantia* etc. contain large amount of bioactive secondary metabolites that are used in the treatment of diabetes have high potency of activity, non-toxic, with less or no side effects. Antioxidant compounds specially, polyphenols in the medicinal plants have been found prolific activity against diabetes [11].

Stephania japonica is an evergreen slender twining shrub with greenish yellow flowers and large tubers. The leaf, stem and tuberous root are astringent, bitter and acrid used in the treatment of wide variety of ailments such as diarrhea, dysentery, fevers, stomach ache, dyspepsia, hepatitis, and urinary diseases [12,13]. So far, not enough scientific data has been found regarding antidiabetic properties of *S. japonica* leaf. Therefore, the present study was to explore the antidiabetic activity of *S.*

japonica leaf extract in alloxan-induced diabetic mice and the effect on lipid profile and liver function biochemical markers were also investigated to ensure the antidiabetic activity of *S. japonica* leaf extract. The screening and quantification of phytochemicals were performed to compare and support these findings.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials and Authentication

The fresh leaves of *S. japonica* were collected from well growth healthy plants from the area of Rajshahi University campus, Rajshahi (north-western part of Bangladesh) and authenticated by Prof. Dr. A. H. M. Mahbubur Rahman, Taxonomist, Department of Botany, University of Rajshahi, Bangladesh. The leaves were washed with fresh water and then dried under the shade. After complete shade drying, the leaves were then pulverized into a coarse powder with the help of a grinding machine (FFC-15, China) and were stored in an airtight container for further use.

2.2 Preparation of Plant Extracts

Approximately, 200 g of powdered leaf material was kept immersed in 500 mL of each solvent, ethanol and acetone separately in an aspirator bottle at room temperature for 2 days with occasional shaking and stirring. The extract was then filtered through a filter paper (Whatman No.1) and concentrated with a rotary evaporator under reduced pressure at 40°C to obtain different extracts (about 3 gm of each) of *S. japonica* leaf. Then, the extracts were stored at 4°C for further analysis.

2.3 Qualitative and Quantitative Analysis of Phytochemicals

Phytochemical screening with suitable analytical reagents was carried out to detect bioactive phytoconstituents present in the extracts. The main bioactive groups (polyphenols, terpenoids, steroids, saponins, tannins, flavonoids, alkaloids and glycosides) were identified in two extract using different standard methods [14,15]. The total quantities of phytochemicals such as polyphenolics, flavonoids, flavonols and proanthrocyanidines were estimated spectrophotometrically using standard protocols [16-19].

2.4 *In vitro* Antidiabetic (α-amylase inhibition) Assay

In vitro antidiabetic assay was performed by measuring α-amylase inhibition activity. α-amylase inhibition assay was carried out using 3, 5-dinitrosalicylic acid (DNSA) method [20] and starch-iodine color assay [21].

2.4.1 DNSA method

Ethanol and acetone extracts of *S. japonica* leaf and standard (acarbose) at the concentration of 100 µg/mL to 1000 µg/mLwere added to 500 µL of 0.02 M sodium phosphate buffer (pH-6.9) containing 6 mM NaCl and 0.5mg/mL of α-amylase solution. Exactly, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH-6.9) were added to the mixture and then incubated at 37°C for 10 min. The reaction was stopped with 1.0 mL of DNSA reagent and heated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by the addition of 10 mL distilled water and absorbance was measured at 540 nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of extracts. The results were expressed as % inhibition calculated using the formula:

$$\alpha\text{-amylase inhibition activity} = [1 - \{(A_1 - A_2)/A_0\}] \times 100$$

Where A_1 is the absorbance of test sample, A_2 is the absorbance of product control (sample without α-amylase solution) and A_0 is the absorbance of negative control (α-amylase without extract).

2.4.2 Starch iodine color assay

Different concentration (50 µg/mL to 800 µg/mL) of 500 µl extracts and acarbose were added to 500 µL of 0.02 M sodium phosphate buffer (p^H 6.9 containing, 6 mM sodium chloride) containing 0.5 mg/mL of α-amylase solution were incubated at 37°C for 10 min. The reaction mixture of each test tube was incubated at 37°C for 15 min after adding 500 µL soluble starch (1%, w/v) then 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent (5 mM I_2 and 5 mM KI). The color change was noted and the absorbance was recorded at 620 nm. The results were expressed as % inhibition calculated using the formula:

α-amylase inhibition activity = [1 – {(A_1-A_2)/A_0}] ×100

Where A_1 is the absorbance of test sample, A_2 is the absorbance of product control (sample without α-amylase solution) and A_0 is the absorbance of negative control (α-amylase without extract).

2.5 *In vivo* Antidiabetic Assay

2.5.1 Animals

Male Swiss albino mice of weighting range of 26-30 g were purchased from the Department of pharmacy, Jahangirnagar University, Savar, Bangladesh. Animals were accommodated at standard laboratory conditions (relative humidity 55±5 %, room temperature 25±3°C, and 12h light dark cycle)with free access to water and food. All animals were acclimatized one week prior to the experiments. The approval and permission of using mice model in this study were obtained from the Institute of Biological Sciences, University of Rajshahi, Bangladesh.

2.5.2 Induction of diabetes

Diabetes was induced in overnight fasted mice by a single intra-peritoneal injection (i.p.) of a freshly prepared solution of alloxan monohydrates (90 mg/kg of body weight) in a 0.1M sodium citrate buffer (pH-4.5). The development of hyperglycemia in mice was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood with a portable glucometer after 48 hours of alloxan administration. The animals exhibited fasting glucose level greater than 16.0 mmol/L after 7 days were screened as diabetic mice and used for the experiment. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

2.5.3 Experimental design

The animals were randomly divided into seven groups and each group belonged to six mice. The animal grouping and dose selection are shown in Table 1.

Blood samples were collected from the tail veins and the body weight of each mouse was measured before the start of the treatment and 5 days interval (up to 21 days) during the treatment.

2.5.4 Biochemical parameter analysis

At the end of 21th days of treatment, mice were sacrificed after overnight fasting by anesthetizing with chloroform. Blood was collected from the heart and serum was separated by centrifugation at the rate of 2000 rpm for 10 minutes. Serum glucose level was estimated by glucometer. Lipid profile parameters such as total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were estimated by commercially available kits (Vitro Scient, Cairo, Egypt). Serum low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were also determined according to the Friedewald et al. [22].

VLDL = TG/5; LDL = TC─ (HDL + VLDL)

Liver function test were carried out by the measurement of some biochemical markers such as, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and hepatic inflammatory marker, C-reactive protein (CRP). The measurements of these biochemical markers were done by bioanalyzer utilizing commercially available kits (Plasmatec Laboratory Products Ltd, UK).

Table 1. Animal grouping and dose selection

Groups	Alloxan (mg/kg b.w.)	Glibenclamide (mg/kg b.w.)	Acetone extract (mg/kg b.w.)	Ethanol extract (mg/kg b.w.)
Group-1 Untreated normal control	-	-	-	-
Group-2	90	-	-	-
Group-3	90	-	100	-
Group-4	90	-	200	-
Group-5	90	-	-	100
Group-6	90	-	-	200
Group-7	90	5	-	-

2.5.5 Histophathologyical studies

Histopathological studies were carried out at Rajshahi Medical College Hospital, Rajshahi, Bangladesh. After sacrificed of animals, livers were separated, excised and rinsed in ice-cold saline solution. A small part of liver tissue was fixed in 10% formalin solution for 12 hours at room temperature. After fixation, selected tissues were dehydrated in acetone/ethanol (80–100%) solution, incubated in xylene and finally impregnated in paraffin at 56-58°C for 4-20 hours. The sample of 5-6 µm diameters were obtained by using a microtome machine. The sections were stained with haemotoxiline-eosin and histopathological observations were carried out under a light microscope.

2.5.6 Statistical analysis

Data were statistically calculated by operating one-way ANOVA followed by post-hoc Bonferroni was performed using SPSS software ver-16. Comparisons with p values<0.05, <0.01and <0.001 were considered to be statistically significant. Results are expressed as means ± standard deviation followed by computerized Dunnett's t-test.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Major phytoconstituents present in different parts of plant are responsible for various biological activities. The preliminary screening of *S. japonica* leaf extracts (acetone and ethanol) showed the presence of diversity of phytochemical constituents. Table 2 shows the phytochemical constituents of *S. japonica* leaf extract. It was found that alkaloids, flavonoids, saponins, sugars, steroids, resins, caumarin, tannins and glycosides were the major phytoconstituents of *S. japonica* leaf extract. Alkaloids, carbohydrates, steroids, flavonoids, resins, saponins, tannins and coumarins were detected in both of the leaf extracts. Glycosides, cardioglycosides and anthracenosides were present only in acetone extract, triterpenoids were not found in both acetone and ethanol extract of *S. japonica* leaf.

The quantitative analysis of secondary metabolites- total phenolics, flavonoids, flavonols and proanthocyanidin content of leaf extracts were shown in Fig. 1. Acetone extract showed

Table 2. Phytochemical screening of *S. japonica* leaf extracts obtained by acetone and ethanol

Name of test compounds		Name of sample extracts	
		Acetone extract	Ethanol extract
Alkaloids test	Dragendorff's test	+	+
	Hager's test	+	+
	Wagner's test	+	+
	Mayer's test	+	+
Carbohydrate test	Anthrone's test	+	+
	Benedict's test	+	+
	Fehling's test	+	+
	Molisch's test	+	–
Steroids test	Liebermann-Burchard's test	+	+
	Salkowski reaction	–	–
Flavonoid test		+	+
Glycosides		+	–
Triterpenoids		–	–
Resins		+	+
Saponins		+	+
Tannins		+	+
Coumarins test		+	+
Cardio glycoside test		+	–
Anthracenosides test		+	–

Key: (+) indicate the presence and (-) indicate the absence of phyto-constituents

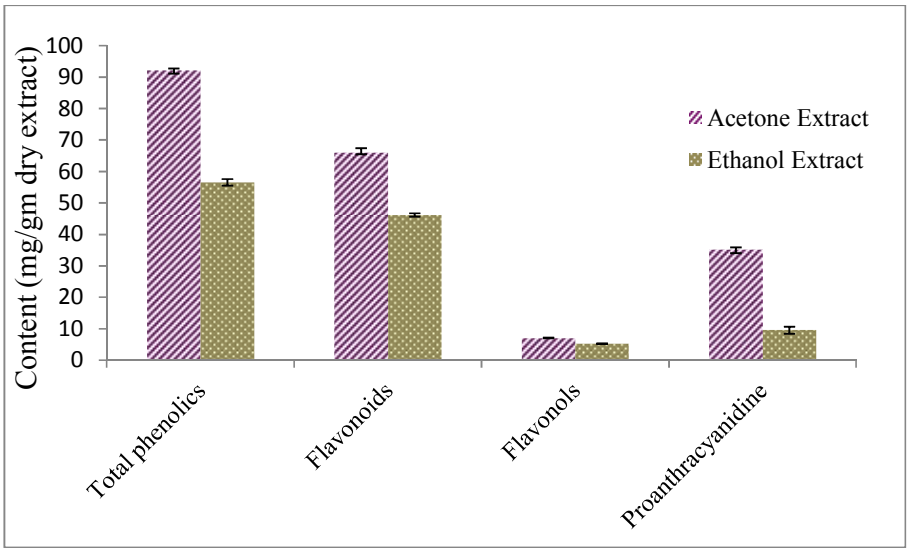

Fig. 1. Quantitative estimation of phytochemicals in *S. japonica* leaf extracts

Result was expressed as mean ± standard deviation (n=3)

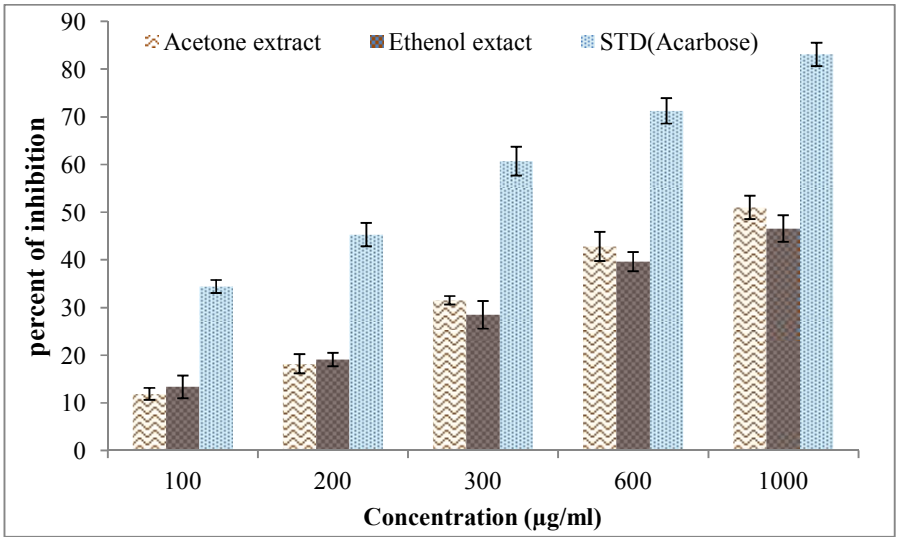

Fig. 2. α-amylase inhibition activity of *S. japonica* leaf extracts in 3,5-dinitrosalicylic acid (DNSA) method

Result was expressed as mean ± standard deviation (n=3)

maximum amount of phenolics, flavonoids, flavonols and proanthocyanidin content as compared to ethanol extract. Among these phytochemicals, total phenolic contents were higher i.e. 92.12±0.64 mg of GAE (gallic acid equivalent)/gm and 56.54±1.05 mg of GAE/gm of dry extract, respectively. Flavonoids, flavonols and proanthocyanidin content of acetone and ethanol extracts were 66.02±1.42 and 46.17±0.54 mg of CAE (catechin equivalent)/gm of dry extract,7.05±0.108 and 5.26±0.083 mg of QUE (quercetin equivalent)/gm of dry extract,35.19±0.67 and 9.55±1.11 mg CAE/gm of dry extract, respectively. The quantities of phenolics and flavonoids, flavonols and proanthocyanidin content of plant extract depend on the biological factors (genotype and cultivars) as well as environmental (temperature, salinity, water stress and light intensity) conditions. Moreover, the extraction of phenolic compounds rely on the type of solvent used, polarity of solvents, the degree of polymerization of phenolics and their internal organization [23,24].

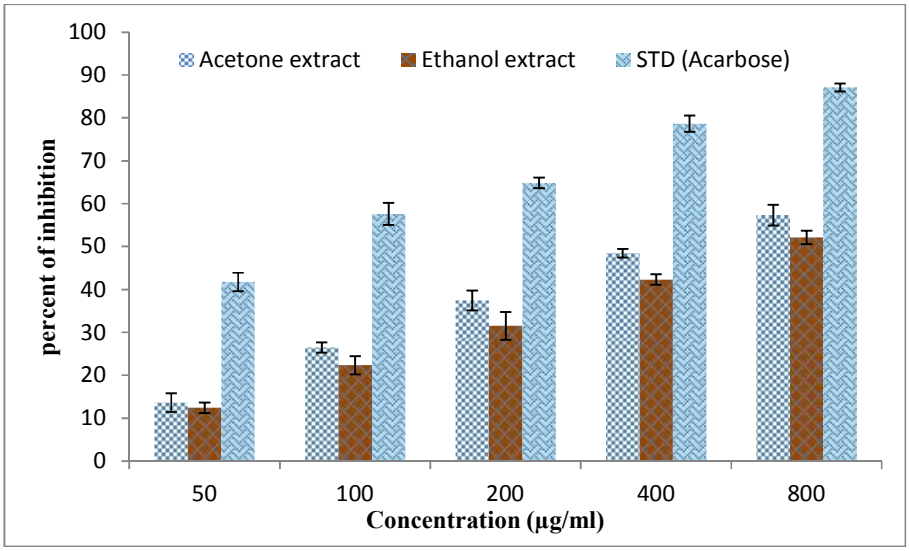

Fig. 3. α-amylase inhibition activity of *S. japonica* leaf extracts in starch iodine color assay
Result was expressed as mean ± standard deviation (n=3)

Table 3. Hypoglycemic effect of acetone and ethanol extracts of *S. japonica* leaf in alloxan-induced diabetic mice

Group	Blood glucose level (mmol/L)				
	0 day	6th day	11th day	16th day	21st day
Normal control	6.04±0.58	5.71±0.52	5.97±0.62	6.2±0.28	6.86±0.35
Diabetic control	20.24±0.94[a]	21.44±1.11[a]	22.68±1.01[a]	23.18±1.00[a]	23.58±0.36[a]
Standard treated	19.97±0.77[a]	15.28±0.80[c]	13.56±0.71[c]	11.28±0.38[c]	9.39±0.67[c]
Acetone extract (100 mg/kg b.w.)	20.23±0.47[a]	19.28±0.94	17.74±0.85[b]	15.96±1.08[b]	15.74±0.39[c]
Acetone extract (250 mg/kg b.w.)	20.86±1.25[a]	18.21±0.78[b]	16.26±0.81[b]	14.45±0.47[c]	13.65±1.03[c]
Ethanol extract (100 mg/kg b.w.)	22.17±0.53[a]	21.34±0.65	20.13±0.53	18.09±1.02[b]	17.22±1.43[b]
Ethanol extract (250 mg/kg b.w.)	21.24±1.33[a]	19.86±0.79	18.13±0.86[b]	16.34±0.32[b]	15.04±0.76[c]

Result are expressed as mean ± standard deviation, (n=6) [a]p<0.001 compared with normal control, [b]p<0.05, and [c]p<0.001 compared with diabetic control

3.2 *In vitro* Antidiabetic (α-amylase inhibition) Assay

α-amylase inhibitor such as acarbose in the intestinal lumen can delay carbohydrate digestion, absorption and strongly useful to treat non-insulin-dependent diabetes mellitus and obesity. Pancreatic α-amylase content and secretion is straightly proportional to the amount of carbohydrate absorption [25]. α-amylase inhibitory activity of crude extract obtained by acetone and ethanol are shown in Figs. 2 and 3. In DNSA method, it was found that the administration of acetone and ethanol extract exhibited the inhibition of α-amylase activity from 11.91% to 51.02% and 13.39% to 46.62%, respectively at various concentrations (100 µg/mL to 1000 µg/mL) of extracts. The standard drug acarbose exhibited 34.45% to 83.11% inhibition of α-amylase activity at the same concentrations. In starch-iodine color assay method, almost similar α-amylase inhibition activity was observed by the treatment of acetone and ethanol extract of *S. japonica* leaf. However, it was observed that both of the extracts exhibited significant inhibition of α-amylase activity.

The administration of acetone extract showed high α-amylase inhibition activity compared with

the ethanol extract. This observation confirms the fact that acetone extract is generally known for their high contents of chemical compounds capable of producing more biological activities. The presence of different types of bioactive phytoconstituents in leaf extract such as steroids, saponins, terpinoids, cumarine and polyphenols which may be responsible for this therapeutic activity. Aromatic ingredient, terpinoids such as oleanane, ursane and lupine and polyphenols showed strong inhibition of α-amylase activity [26]. Thilagam et al. [27] also reported the inhibition of α-amylase activity by ethanolic extract of the leaves of *Senna surattensis*.

3.3 *In vivo* Antidiabetic Assay

Pyrimidine derivative hydrophilic chemical compound, alloxan, structural similarities with glucose can generate toxic free radicals through redox cycling reaction that can be toxic for pancreatic β-cell and partially destroyed its [28,29]. Table 3 shows the hypoglycemic effect of acetone and ethanol extracts of *S. japonica* leaf on alloxan-induced diabetic mice. Intra-peritoneal administration of alloxan monohydrate in mice gradually increased the blood glucose levels (P<0.01 and P<0.001) as compared to the normal group. It might be happened due to muscle wasting and loss of tissue proteins as compared to normal control mice [30,31]. The oral treatments of acetone extract of *S. japonica* leaf at the dose of 100 and 250 mg/kg body weight showed a significant (p<0.01 and p<0.001) reduction of blood glucose level about 4.70% to 22.19% and 15.06% to 43.54%, respectively on the day 6th to 21st compared to blood glucose level of diabetic control group. On the other hand, at the dose of 100 and 250 mg/kg body weight, the leaf extract obtained by ethanol decreased blood glucose level of 3.74% to 22.32% and 7.37% to 37.80%, respectively on the day 6th to 21st.

Table 4. Hypolipidemic effect of acetone and ethanol extracts of *S. japonica* leaf in alloxan-induced diabetic mice

Group	Serum lipid profile (mg/dL)				
	TG	TC	HDL	LDL	VLDL
Normal control	106.96±4.22	89.10±3.79	55.56±4.19	12.14±3.78	21.39±0.84
Diabetic control	146.78±5.31^{a}	134.43±2.17^{a}	31.47±3.52^{a}	73.60±4.90^{a}	29.36±1.06^{a}
Glibenclamide treated	90.44±2.62^{d}	110.84±1.94^{d}	70.99±3.13^{d}	21.76±3.29^{d}	18.09±0.52^{d}
Acetone extract (100 mg/kg b.w.)	130.65±0.97^{b}	121.37±1.36^{d}	40.26±2.15^{b}	54.98±3.58^{d}	26.13±2.62^{c}
Acetone extract (250 mg/kg b.w.)	122.60±3.7^{d}	118.48±3.08^{d}	48.43±5.25^{d}	45.53±6.89^{d}	24.52±0.74^{d}
Ethenol extract (100 mg/kg b.w.)	134.43±1.50	127.04±1.10^{b}	35.42±0.78	64.73±1.84^{b}	26.89±0.30^{b}
Ethanol extract (250 mg/kg b.w.)	126.29±7.73^{b}	119.52±6.61^{d}	38.41±4.49	55.85±7.14^{c}	25.26±1.55^{c}

Table 5. Effect of acetone and ethanol extracts of *S. japonica* leaf in SGPT, SGOT and CRP level

Group	SGPT(U/L)	SGOT(U/L)	CRP(U/L)
Normal control	42.54±0.93	39.07±0.78	1.62±0.05
Diabetic control	75.43±1.78^{a}	82.54±0.67^{a}	3.19±0.03^{a}
Glibenclamide treated	45.56±1.00^{c}	54.27±1.24^{c}	2.16±0.05^{c}
Acetone extract (100 mg/kg b.w.)	59.62±0.73^{b}	72.47±1.05c	2.78±0.04^{c}
Acetone extract (250 mg/kg b.w.)	52.67±1.07^{c}	61.16±1.93^{c}	2.64±0.10^{c}
Ethanol extract (100 mg/kg b.w.)	68.34±1.12^{b}	77.16±1.30^{b}	2.91±0.14^{c}
Ethanol extract (250 mg/kg b.w.)	63.45±0.53^{c}	73.45±1.15^{c}	2.86±0.07^{c}

Result are expressed as mean ± standard deviation, (n=6) ^{a}p<0.001 compared with normal control, ^{b}p<0.01 and ^{c}p<0.001 compared with diabetic control

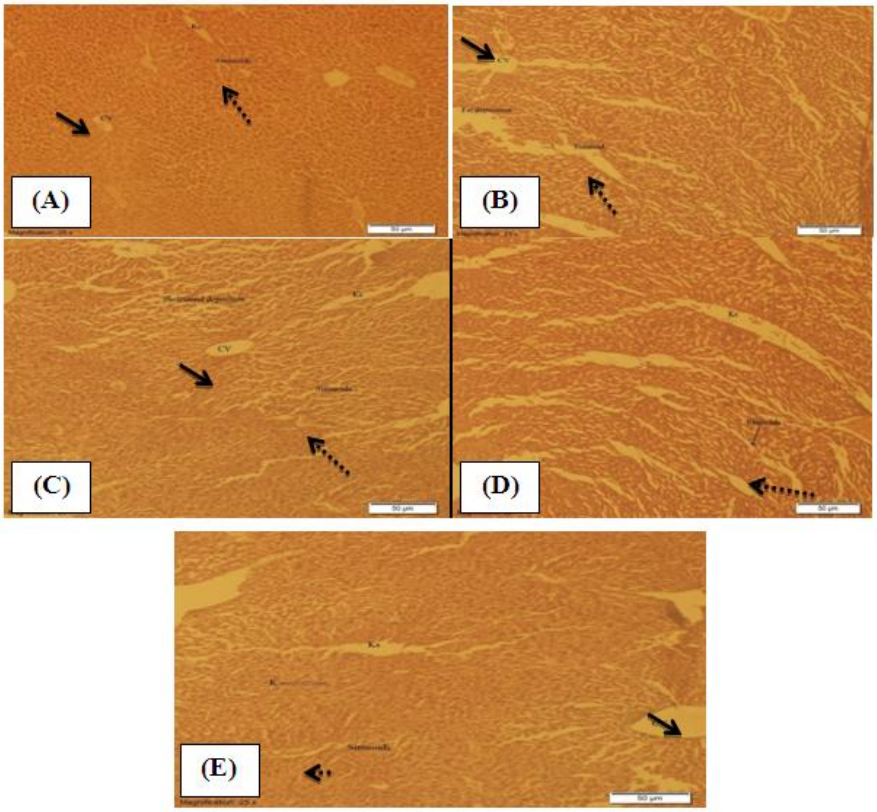

Fig. 4. Photomicrographs of mice liver tissues under normal and diabetic condition

(A) Normal control, (B) Diabetic control, (C) Diabetic treated with acetone extract, (D) Diabetic treated with ethanol extract and (E) Diabetic treated with glibenclamide (All figures are in 25x magnification, CV= central vain, Kupffer cells (K), Ks=Kiernan's spaces)

The hypoglycemic effects of the both extracts were found to be in dose dependent manner. The standard oral hypoglycemic agent glibenclamide (5 mg/kg) exhibited maximum reduction of blood glucose level of diabetic mice about 28.73% to 61.16% ($p<0.001$) on the day 6^{th} to 21^{st} compared to diabetic control group. Junejo *et al.* [32, 33] also reported that the plant extracts of *Callicarpa arborea* Roxb. and *Diplazium esculentum* significantly reduced blood glucose level in streptozotocin-induced diabetic rats.

3.4 Hypolipidemic Effect of Acetone and Ethanol Extracts of *S. japonica* Leaf

The effect of *S. japonica* leaf extract obtained by acetone and ethanol on the lipid profile parameters in alloxan-induced diabetes mice are shown in Table 4. Except HDL all the lipid profile parameters such as TG, TC, LDL and VLDL in diabetic mice increased significantly ($P<0.01$ and $P<0.001$) compared to the normal control group. The administration of both acetone and ethanol extracts at the dose of 250 mg/kg body weight in diabetic mice significantly reduced the lipid parameters (TG, TC, LDL and VLDL) compared with the diabetic control mice, whereas HDL level increased significantly. Administration of acetone and ethanol extracts decreased TG of16.47% and 13.96%, TC of11.86% and 11.09%, LDL of 38.14% and 24.12%and VLDL of 16.49% and 13.96%, respectively at the dose of 250 mg/kg body weight whereas lower dose (100 mg/kg) of both extracts (acetone and ethanol) reduced lipid profile parameter significantly ($p<0.05$) with TG of 11.08% and 8.41%, TC of 9.44% and 5.50%, LDL of 24.48% and 12.05%, and VLDL of 11.10% and 8.43%, respectively in diabetic mice compared to the diabetic control mice. In contrast, HDL increased significantly (35.02% by acetone extract and 18.07% by ethanol extract treatment) in diabetic mice compared with the diabetic control mice. Standard drug glibenclamide (5 mg/kg body weight) treated diabetic mice showed a reduction of TG by

38.38%, LDL by 70.43%, VLDL by 38.39% and an increase in HDL by 55.70%. Several studies reported the similar hypoglycemic effect of mice obtained by various plants extracts [33-35].

3.5 Effect of *S. japonica* Leaf Extracts on SGPT, SGOT and CRP Level in Diabetic Mice

Some important markers of liver function are SGPT, SGOT and CRP. An increase in the blood plasma level of SGPT, SGOT, and CRP of diabetic mice may be due to the leakage of these enzymes and protein from the cytosol of liver cell into the blood stream which is an indicator of the hepatotoxic effect of diabetic-induced compound, streptozotocin [36]. Type 2 diabetes causes a high incidence of abnormalities of liver function. Table 5 shows the effect of acetone and ethanol extract of *S. japonica* leaf on SGPT, SGOT and CRP level. Liver function test enzymes, SGPT and SGOT were significantly (P<0.001) increased during diabetic stage. In contrast, the oral administration of acetone and ethanol extract of *S. japonica* leaf at higher dose (250 mg/kg) reduced the SGPT level by 30.17% and 15.88% and SGOT by 25.90% and 11.01%, respectively in diabetic mice compared with the diabetic control mice. Hepatic marker protein, CRP was also increased in diabetic condition. By the treatment of acetone and ethanol extract, the CRP level reduced significantly at 21st day of administration (P<0.001). Compared to diabetic control mice, the reduction of CRP level in treated diabetic mice by acetone and ethanol extract at the dose of 100 and 250 mg/kg body weight were 10.03% to 17.24% and 8.78% to10.34%, respectively on the day 6th to 21st.

The results were compared with standard oral hypoglycemic agent glibenclamide (5 mg/kg) which exhibited maximum reduction of SGPT, SGOT and CRP by 39.60%, 34.25% and 32.29%, respectively on the 21st day. The present study was in agreement with the finding of Junejo et al. [32] demonstrating the reduction of serum hepatic marker protein in diabetic mice by *Callicarpa arborea* Roxb. stem bark extract.

3.6 Histopathological Finding on Hepatic Cells in Diabetic Mice

The structures of liver tissue under the fluorescence light microscopy of normal, diabetic, extract/drug treated mice are shown in Fig. 4. Photomicrographs of liver showed normal hepatic cells with well preserved cell organelles. Liver tissue of diabetic mice showed distortion in the arrangement of liver cells, a probable consequence of insulin dependent fat metabolism disorders. In diabetic control mice, the liver cells were more vacuolated around the central veins, cloudy swelling as a result lymphocyte infiltration was observed. Liver cell showed degenerated parenchymatous cells with severe necrosis and dilation of sinusoids.

Administration of acetone and ethanol extract in diabetes mice at 250 mg/kg body weight showed a smaller number of vacuolated cells with a lower degree of vacuolization, lymphocyte infiltration, and also mild congestion of the portal tract were observed. It was found that acetone extract treated hepatocytes showed nearly normal appearance, minimal necrosis and mild sinusoidal congestion. In contrast, liver of diabetic mice after the treatment of ethanol extract showed moderate necrosis and sinusoidal congestion. Liver of diabetic mice after treatment with glibenclamide exhibited almost similar of normal hepatocyte arrangement. Acetone and ethanol extract treated group was comparable with glibenclamide treated group and the treated groups showed significant changes compared to normal control group. Junejo *et al.* [32] reported the similar effects of stem bark extract of *Callicarpa arborea* Roxb. on streptozotocin-induced rat liver cell.

4. CONCLUSION

Active biological components containing *S. japonica* leaf extract showed potent hypo-glycemic, hypo-lipidimic properties and subsequently reduced the diabetic marker (enzymes) levels that are directly indicate the improvement of liver function. Between the extracts, acetone extract of *S. japonica* leaf contained higher amount of phytochemicals that exhibited maximum antidiabetic activity in *in vitro* α-amylase inhibition and *in vivo* alloxan-induced diabetes mice. Further extensive studies are required to explore the underlying mechanism of antidiabetic activity by isolation and purification of the bioactive compounds from *S. japonica* leaf.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Approval and permission of using mice model were obtained from Institute of Biological

Sciences (Memo no: 97/320/IAMEBBC/IBSc), University of Rajshahi, Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: Can the doomsday scenario be averted? J Internal Med. 2000;247(3):301-310.
2. Abbas AK, Lichtman AH, Pober JS. Cellular and Molecular Immunology, Fourth Edition, W.B. Saunders Co; 2000.
3. Bajaj JS, Madan R. Diabetes in tropics and developing countries. IDF Bull. 1995;38:5-6.
4. Nathan DM. The pathophysiology of diabetic complications; How much does the glucose hypothesis explain? Ann Intern Med. 1996; 174: 286-289.
5. International Diabetes Federation (IDF). IDF Diabetes Atlas, 7th Edition, 2015.
6. Bolen S, Feldman L, Vassy J, Wilson L, Yeh HC, Marinopoulos S, Wiley C, Selvin E, Wilson R, Bass EB, Brancati FL. Systematic review: Comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Ann Intern Med. 2007;147:386-399.
7. Bhat M, Zinjarde SS, Bhargava SY, Kumar AR, Joshi BN. Antidiabetic Indian plants: A good source of potent amylase inhibitors. Evid Based Complement Alternat Med. 2011;2011:810207.
8. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes. 1998;47(6):859–866.
9. Pitozzi V, Giovannelli L, Bardini G, Rotella CM, Dolara P. Oxidative DNA damage in peripheral blood cells in type 2 diabetes mellitus: higher vulnerability of polymorphonuclear leukocytes. Mutat Res. 2003;529:129–133.
10. Eshrat MH. Effect of *Coccinia indica* L. and *Abroma augusta* L. on glycemic, lipid profile and on indicators of end organ damage in streptozotocin induced diabetic rats. Indian J Clin Biochem. 2003;18(2):54-63.
11. Shokeen P, Anand P, Murali YK, Tandon V. Antidiabetic activity of 50% ethanolic extract of Ricinus communis and its purified fractions. Food Chem Toxicol. 2008;46(11):3458-3466.
12. Senthamarai R, Ismail AM, Kiurbha TSV, Balasubramanian P. Pharmacognostical and phytochemical studies on leaves of *Stephania japonica* Linn. J Chem Pharmaceutical Res. 2012;4(3):1457-1464.
13. Hossan MS, Hanif A, Agarwala B, Sarwar MS, Karim M, Rahman MT, Jahan R, Rahmatulla M. Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. Ethnobotany Research & Applications. 2010;8:61-74.
14. Kodangala C, Saha S, Kodangala P. Phytochemical studies of aerial parts of the plant *Leucas lavandulaefolia*. Der Pharma Chemica. 2010;2(5):434-437.
15. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum books Ltd., Ibadan; 1993.
16. Meda A, Lamien CE, Romito J, Nacoulma OG. Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005;91: 571-577.
17. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51(3):609–614.
18. Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452-458.
19. Kwon GJ, Choi DS, Wang MH. Biological activities of hot water extracts from *Euonymus alatus* leaf. J Korean Food Sci Technol. 2007;39:569-574.
20. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426-428.
21. Xiao Z, Storms R, Tsang A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Ana. Biochem. 2006;351(1):146-148.
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6): 499-502.
23. del Baño MJ, Lorente J, Castillo J, Benavente-García O, del Río JA, Ortuño A, Quirin KW, Gerard D. Phenolic diterpenes, flavones, androsmarinic acid distribution during the development of leaves, flowers, stems and roots of

Rosmarinus officinalis Antioxidant activity. J Agric Food Chem. 2003;51(15):4247–4253.

24. Djeridane A, Yousfi M, Boubekeur N, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006; 97:654-660.
25. Daisuke koike, Kazushi yamadera and Eugene P. Dimagno, Effect of a wheat amylase inhibitor on canine carbohydrate digestion, gastrointestinal function, and pancreatic growth. Gastroenterology. 1995;108(4):1221–1229.
26. Tundis R, Loizzo MR, Menichini F. Natural products asalpha-amylase and alpha-glucosidase inhibitors and their hypoglycemic potential in the treatment of diabetes: An update. Mini Rev Med Chem. 2010;10(4):315-331.
27. Thilagam E, Parimaladevi B, Kumarappan C, Mandal SC. α-glucosidase and α-amylase inhibitory activity of Senna surattensis. J Acupunct Meridian Stud. 2013;6(1):24-30.
28. Lenzen S, Munday R. Thiol-group reactivity, hydrophilicity and stability of alloxan, its reduction products and its N-methyl derivatives and a comparison with ninhydrin. Biochem Pharmacol. 1991; 42(7):1385–1391.
29. McDaniel ML, Bry CG, Fink CJ, Homer RW, Lacy PE; Studies on the site of interaction for ninhydrin, alloxan, and D-glucose in insulin secretion by isolated islets in vitro. Endocrinology. 1979; 105(6): 1446–1451.
30. Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatment for diabetes: Studies in normal and streptozotocin diabetic mice. Diabetologia. 1990;33(8):462-464.
31. Chatterjee MN, Shinde R. Textbook of Medical Biochemistry. Jaypee Brothers Medical Publishers. New Delhi; 2002.
32. Junejo JA, Rudrapal M, Nainwal LM, Zaman K. Antidiabetic activity of hydro-alcoholic stem bark extract of Callicarpa arborea Roxb. with antioxidant potential in diabetic rats. Biomed Pharmacother. 2017; 95:84–94.
33. Junejo JA, Gogoi G, Islam J, Rudrapal M, Mondal P, Hazarika H, Zaman K. Exploration of antioxidant, antidiabetic and hepatoprotective activity of *Diplazium esculentum* - A wild edible plant from North Eastern India. Future Journal of Pharmaceutical Sciences. 2018;4(1):93-101.
34. Pierre W, Gildas AJ, Ulrich MC, Modeste WN, Benoît NT, Albert K. Hypoglycemic and hypolipidemic effects of *Bersama engleriana* leaves in nicotinamide/ streptozotocin-induced type 2 diabetic rats. BMC Complement Altern Med. 2012;12:264.
35. Ramachandran S, Rajasekaran A, Adhirajan N. *In vivo* and *in vitro* antidiabetic activity of Terminalia paniculata bark: An evaluation of possible phytoconstituents and mechanisms for blood glucose control in diabetes. ISRN Pharmacology; 2013. DOI:http://dx.doi.org/10.1155/2013/484675
36. Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EGT, Swapna S, Ramesh B, Rao CA, Antihyperglycemic and antihyper-lipidemic activities of methanol: water (4:1) fraction isolated from aqueous extract of Syzgium alternifolium seeds in streptozotocin induced diabetic rats, Food Chem. Toxicol. 2010;48(4): 1078–1084.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/48311