Original Research Article

An Evaluation of Anti-Diabetic Activity of Ethanolic Extract of *Asparagus racemosus*in Alloxan Induced Rat Model.

Abstract:

From traditional and widely used medications in every culture to standardized and tritated herbal extracts, all fall under the umbrella term "herbal medicine," which is defined as the use of medicinal plants for the prevention and treatment of illness. In this research, rats were given *Asparagus racemosus* extract to test for anti-diabetic activity, liver, kidney, and lipid profiles. After receiving alloxan and extract at low, medium, and high doses, there was a dose-dependent drop in body weight. This weight reduction is minimal when compared to the positive metformin, alloxan control, and alloxan + metformin groups. Groups 8, 9, and 10 will see an increase in body weight. Only metformin with a large dose of extract resulted in a statistically significant ($p<0.05$) decrease in blood glucose levels. Groups 3 and 4 had decreased blood sugar levels, but the findings were not statistically significant. The SGPT level did not reduce across all groups in the case of SGPT and SGOT. Only the high dose, however, resulted in a statistically significant ($p<0.05$) decrease in SGOT levels. Throughout the renal function test, creatinine levels decreased in all groups, which was statistically significant ($p<0.05$). At medium and high dosages, the quantity of urea in the blood statistically significant ($p<0.05$) decrease. Only the medium and high dosages lowered cholesterol levels across all groups. Triglyceride levels decreased in all groups, although they were not statistically significant. Only the highest dose resulted in a statistically significant($p<0.05$) decrease in LDL levels. HDL values increased significantlysignificant ($p<0.05$) at medium and high dosages. The findings indicate that *A. racemosus* has potential for the creation of standardized phytomedicine for the treatment of patients with diabetes, cardiovascular disease, liver disease, and renal illness.

Keywords: Herbal medicine,Antidiabetic, Cholestererol, Creatinine, LDL, HDL.

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Introduction

High amounts of glucose (also known as sugar in the blood) characterize the disease known as diabetes. In this condition, either insulin production is insufficient, or insulin action is impaired. An international epidemic, diabetes mellitus, shows itself when glucose (a sugar) levels in the blood become too high, and the kidneys generate an excessive volume of urine due to a lack of insulin production or ineffective insulin consumption [1]. About 4.4% of people aged 15–49, 15% aged 50–69, and 22.0% of those aged 70+ had type 2 diabetes in 2017, making up about 462 million people with the disease worldwide and yielding a prevalence rate of 6059 cases per 100,000. Diabetes is the tenth greatest cause of death, responsible for over a million fatalities annually. Worldwide, the prevalence of diabetes mellitus is increasing, although the pace of increase is highest in Western Europe and other industrialized countries [2]. Many oral medications, such as sulphonylureas, meglitinides, biguanides, thiazolidinedione, alpha-glucosidase, acarbose, etc., are used to treat diabetes[3]. However, these medications may cause serious side effects, including hypoglycemia, heart failure, stomach problems, broken bones, and liver damage [4].In addition to the potentially fatal adverse effects, the high expense of these synthetic pharmaceuticals may make it impossible for the patient to complete the whole course of treatment. Herbalism is the practical use of plants having therapeutic properties, whereas phytotherapy is the study of such plants scientifically. Because plants contain a broad array of compounds with therapeutic properties, they have been used as a source of medicine for thousands of years. Phenols, glycosides, alkaloids, saponins, terpenoids, tannins, polysaccharides, flavonoids, plant lipids, resins, and essential oils are only some of the many chemically active chemicals found in plants [5,6]. Again, the genetic alteration of the plant may provide the desired therapeutic effect by increasing or lowering the concentration of the plant's chemical components. For instance, reverse genetics may boost the biosynthesis of secondary metabolites like alkaloids [7]. Important antidiabetic action was shown in*Gynura procumbens*, *Terminalia arjuna*, *Azadirachta**Indica*, and *Dioscoreaalata*[8-11].The *Asparagus racemosus*, also known as Satawar, Satamuli, and Satavari, grows at low elevations all throughout India and is a member of the Liliaceae family. The range of *A. racemosus* extends from Sri Lanka to India and the Himalayas. It is widespread in Asia, Australia, and Africa at low elevations in shady, tropical climes. *Asparagus racemosus* is the most widely utilized of the several Asparagus

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species produced in India for medicinal purposes [12]. This plant is rich in steroid, flavonoid, saponin, phenolic, and carbohydrate compounds[13]. It is effective as an antibacterial, hypolipidemic, antidiabetic, enzyme inhibitor, hepatoprotective, diuretic, anti-parasitic, cytotoxic, antidepressant, antiulcerogenic, antioxidant, and in a variety of other uses[14-23]. The presence of these phenolic compound plats shows antidiabetic activity via alpha-amylase and alpha-glucosidase enzyme inhibition [24], and total phenolic content (TPC) in plants may be responsible for this potential antidiabetic activity [25]. The phytochemical analysis of this plant identified a phenolic substance that, by inhibiting these enzymes, may have antidiabetic effects.

Comment [KB6]: plants

The current research aimed to determine whether or not an *A. racemouses* L. ethanolic extract had hypoglycemic effects.

Method and materials:

Drugs, Chemicals and Instruments

The ethanol and alloxan were purchased from Sigma Aldrich in Germany. Healthcare Pharmaceutical Limited was kind enough to provide us with a complimentary sample of the diabetic drug metformin. The total cholesterol, HDL, LDL, triglyceride, SGOT, SGPT, and creatinine analysis kits were purchased from Plasmatic Laboratory Product Ltd. in the United Kingdom. The biochemical parameters were analyzed using the Humalyzer 3000 (a semiautomated clinical chemistry analyzer) and an Alere GI glucometer manufactured by Alere Inc., USA, acquired from Shahbag in Dhaka, Bangladesh.

Plant Collection and Extract Preparation

North Bengal, a hilly track area, and a low-land area of Bangladesh were all scoured for *A. racemosus*. The next stage was taxonomic classification and authentication. The plant specimen was well preserved at Bangladesh's Countrywide Herbarium. The leaves were left to dry in the shade for seven to ten days before being finely ground. The powdered leaves were steeped in 70% ethanol with vigorous stirring for 96 hours. Once the extract's soaking time was over, it was filtered, and the resulting liquid was saved for later use. Once the extracted solution had been transported to the location, it was concentrated using a rotary evaporator. The concentrated extract was then dried and stored for further use.

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Experimental Animal Handling

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Male Wistar rats weighing 125–200 g were obtained from the Dhaka, Bangladesh, Jahangirnagar University Zoology Department and housed at the Institute of Nutrition and Food Science, University of Dhaka, on a 12-hour light/dark cycle with a constant temperature of 25 °C. Standard pellet food and potable water were provided on a consistent basis. The rats were kept there to become acclimated to the environment before the investigation started.

Experimental Guidelines

The 2013 Declaration of Helsinki served as the basis for all experiments' ethical conduct. The "3R" standards, which form the basis of Swiss and worldwide legislation regulating the use of animals in research, were adhered to throughout the study. All forms of "replacement" are represented by the letter "R," whether they are complete (such as the replacement of animal models with computer-generated models) or partial (such as the replacement of live animals by cell or tissue cultures, or the replacement of vertebrates with invertebrates). Our research started with an in silico evaluation to make sure it fit the definition of "replacement." Unfortunately, this model did not provide satisfactory results. To further investigate, an animal model was employed. In order to study the antidiabetic potential of a substance, it was decided to conduct the experiments on rats since, unlike invertebrates, they possess specialized pancreas and beta cells. The second "R" refers to "reduction," or any method that will allow for fewer animals to be used to gather adequate data to answer the research objectives, or that will maximize the information gained from each animal. The sample size of 10 rats per group was determined using the "power analysis method" to guarantee that this suggestion would be followed. The third "R" stands for "refinement," which refers to the concept of easing the suffering of the test animals. Before and after measuring blood glucose levels, isopropyl alcohol was rubbed into the ends of the rats' tails to lessen the discomfort of the pinching. The rats were well fed during the trial and euthanized in accordance with the revised Guidelines for the Euthanasia of Animals (2013), so their deaths were quick and painless.

Dose Selection

A preliminary investigation using plant extracts obtained from the same three regions of Bangladesh was performed before the main research started. Based on these findings, the

minimum effective concentration (MEC) for the plant extract (A. racemosus) was 500 mg/kg. This suggests that a larger dosage would have a greater pharmacological impact. This effect was shown to get increasingly stronger when higher doses were administered. No appreciable shift in pharmacological effect was seen when the dosage was raised from 1,100 mg/kg to 1,200 mg/kg. This showed that the receptors associated with the plant's pharmacological activity began to become saturated at a dosage of 1,100 mg/kg. We chose a middle ground for the final trial at 750 mg/kg since the least and highest efficacious dosages of the plant extract were determined to be 500 mg/kg and 1100 mg/kg, respectively. Metformin (a frequent medicine) dosages were also determined in this manner.

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Biological Sample Collection

Blood samples were obtained by puncturing the rat's tail with the aim of determining glucose levels. On the other hand, after the heart was punctured in the slaughtered animal, blood was immediately extracted and placed in a microcentrifuge tube. After centrifuging the samples at 5,000 rpm for 5 minutes, the supernatant fluid was recovered. After that, biochemical assays were conducted by transferring the fluid to a different microcentrifuge tube. For kidney and liver function testing, the organs were removed from the animal as soon as possible after sacrifice and washed thoroughly in ice-cold saline.

Experimental Design

Anti-hyperglycemic action was measured in rats that were categorized by their weight (Table 1). There were 10 rats in each of the groups, which were created based on the weight of the rodents. In Table 1, alloxan-only-treated rats stand in for the alloxan control group. N/A indicates that no therapeutic intervention was provided for these animals.

Evaluation of Antidiabetic Properties

Using alloxan, a diabetic rat model was created. A cold citrate buffer (0.1 M; pH = 4.5) was used to first dissolve the alloxan. The rats received 150 mg/kg of body weight of alloxan via intraperitoneal injection. After being dosed with alloxan, in order to detect hyperglycemia, blood glucose levels were checked four times daily at six-hour intervals. All of the rats were found to be hyperglycemic or diabetic within 72 hours of initiating alloxan therapy, as shown by an

average blood glucose level over 15 mmol/L. Rats were given an oral dose of metformin together with a *A. racemosus* extract.

Table 1. Anti-hyperglycemic activity analysis

Group number	**Group status**	**Treatment specimen**	**Dose of treatment specimen (mg/kg)**
1	Control	Physiological saline	
2	Alloxan control	Alloxan control	
3	Alloxan + metformin	Alloxan + metformin	
4	Alloxan+low dose	Alloxan+low dose	
5	Alloxan+medium dose	Alloxan+medium dose	
6	Alloxan+high dose	Alloxan+high dose	
7	Metformin	metformin	
8	*A. racemosus*	*A. racemosus*	
9	*A. racemosus*	*A. racemosus*	
10	*A. racemosus*	*A. racemosus*	

Estimation of Biochemical

Parameters

The blood sugar level was checked using a glucometer. A lipid profile, as well as tests of kidney and liver function, were performed in addition to the use of the Humaluzer 3000. The levels of gluconeogenic and glycolytic enzyme activity in the kidney and liver samples were also analyzed. Each group's mean and SD are shown for all research parameters.

Statistical Analysis

The statistical significance of intergroup heterogeneity was evaluated using the "one-way ANOVA test" to compare the groups across a range of biological characteristics. The "SPSS 16" application was used for the analysis. Results were considered statistically significant when the

"p" value was less than 0.05 ($p < 0.05$) and highly significant when the "p" value was less than 0.01 ($p < 0.01$).

Results and discussion:

Both the control and plant extract groups of our experimental rats have gained weight as a result of answering nature's call. Although alloxan had a devastating impact and caused significant weight loss, the alloxan-induced treatment group did not show the same degree of weight loss due to the antioxidant properties of the plant (*A. racemosus*).

Table 2. Body weight of rats before the initiation and after the termination of the experiment

Group no	Group status	Body weight	
		Initial	Final
1	Control	126.28±5.39	144.57±6.34
2	Alloxan control	128.46±6.44	104.53±5.39
3	Alloxan + metformin	124.22±5.38	98.37±4.73
4	Alloxan+low dose	130.57±7.64	107.44±3.37
5	Alloxan+medium dose	127.38±4.82	113.84±6.35
6	Alloxan+high dose	131.93±6.55	122.77±7.31
7	Metformin	124.33±4.50	144.80±5.33
8	*A. racemosus*	127.32±5.57	146.69±6.37
9	*A. racemosus*	132.93±6.48	155.50±7.40
10	*A. racemosus*	128.88±4.63	160.60±6.63

On the other hand, Table 1 shows that metformin aided Group 3 in losing much more weight.Body weight (bw) will go up in the negative control group, whereas it will go down in the positive metformin, alloxan control, and alloxan+metformin groups. There was a dose-dependent decrease in body weight after receiving alloxan as well as extract at low, medium, and high dosages. In comparison to the positive metformin, alloxan control, and alloxan+ metformin groups, this wt loss is modest. The body weight will rise in groups 8, 9, and 10.

Comment [KB10]: table 2

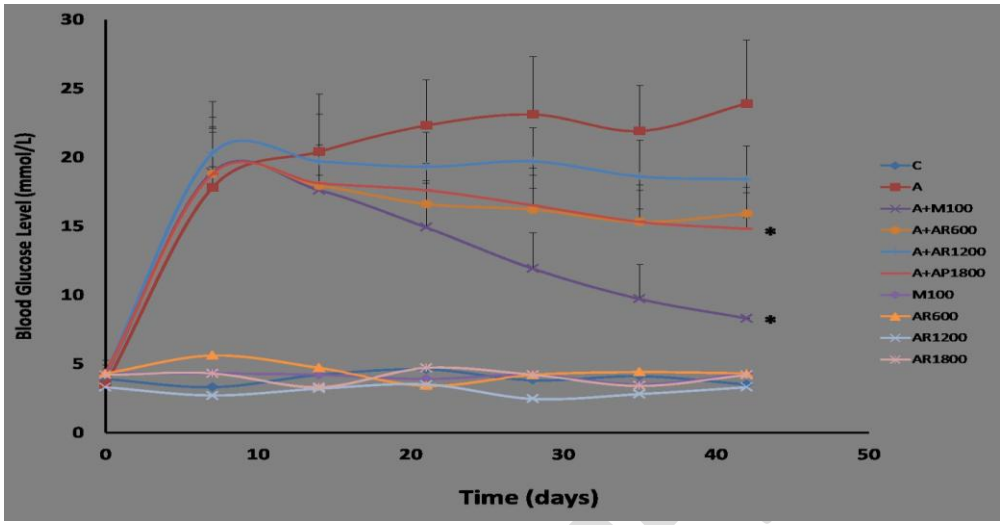

Fig. 1. Blood glucose levels of rats in ten groups over the course of treatment

Values were presented as the mean ± SD (n = 10/group). *p < 0.05 and **p < 0.01 indicate significant difference from the disease group (C = control group, A = alloxan-treated group, M = metformin, A + M = alloxan + metformin, A +AR = alloxan + extract, AR.

One hundred rats were utilized in a ten-group study to see what effect the extract had on blood sugar levels. The blood glucose levels of the rats in groups 2–6 were checked before alloxan was given to them. After three days, diabetes was induced. For 14 days, they received no medical attention. On day 14, treatment officially began. From days 3 to 42, rats in Groups 3-6 received the prescribed drugs. Rats in groups 7–10 likewise received the drugs but were not exposed to alloxan. They were under care for between 14 and 42 days. Also, the rats in group 1 had unrestricted access to both food and water.In the lab, alloxan is often used to induce diabetes in mice. The statistically significant reduction in blood glucose levels was seen only with metformin and the high dosage of extract. There was a trend toward lower blood sugar in Groups 3 and 4, but the results were not statistically significant. Anti-diabetic action was also found in two more investigations of *A. racemosus* [26, 27].

Table 3: kidney function, liver function and lipid profile of the extract

Group no	Group status	Kidney function test		Viver function test		Lipid profile test			
		Creatinine (mg/dl)	Urea	SGPT(µ/l)	SGOT(µ/l)	TG(mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1	Control	0.63±0.08	26.40±2.08	38.34±2.70	45.40±4.41	54.14±2.41	97.75±3.14	37.33±1.72	74.42±1.73
2	Alloxan control	2.6±0.94	97.39±8.34	79.47±5.39	94.91±8.39	113.46±3.63	131.32±7.71	72.45±4.62	53.16±2.47
3	Alloxan + metformin	1.8±0.06	71.37±6.81	64.84±4.84	73.46±6.48	84.14±7.30	110.43±5.46	56.40±3.32	64.50±3.30
4	Alloxan +low dose	1.9±0.94	94.93±5.28	78.73±4.39	92.34±7.40	108.49±6.30	130.44±6.83	70.75±3.31	55.93±4.14
5	Alloxan +mediu	1.4±0.63	88.63±4.63	76.33±6.94	91.45±6.80	104.34±4.69	128.46±8.34	63.22±4.50	57.17±3.10

Comment [KB11]: Liver

	m dose								
6	Alloxan +high dose	1.1±0.11	83.86±5.30	75.48±6.47	86.83±7.41	98.41±3.89	125.30±7.30	59.83±3.41	60.25±4.66
7	Metformin	0.70±0.03	27.80±3.08	34.82±3.33	42.46±3.87	50.57±2.33	95.50±3.30	35.52±2.73	74.14±4.5
8	*A. racemosus*	*0.51*±0.08	29.30±2.89	37.70±4.47	47.16±4.01	58.32±3.93	99.93±4.10	33.18±3.16	71.30±3.24
9	*A. racemosus*	*0.71*±0.06	26.46±3.8	39.39±5.04	44.46±3.80	49.82±3.84	92.14±3.38	38.30±4.40	73.91±2.48
10	*A. racemosus*	*0.52*±0.05	24.17±4.73	37.80±2.89	43.77±4.08	56.94±4.10	94.38±4.16	36.36±4.01	75.75±3.32

In the case of SGPT and SGOT, the SGPT level did not drop statistically significantly across all groups. However, only the high dosage resulted in a statistically significant reduction in SGOT levels. As a result, we may conclude that *A. racemosus* possesses liver-protective properties. Another two investigations evaluating *A. racemosus* cardioprotective effects found a substantial reduction in SGPT and SGOT [28, 29].

Creatinine levels fell in all groups throughout the renal function test, which was statistically significant. Only at medium and high doses does the amount of urea in the blood decrease statistically significantly. Another study on *A. racemosus*of creatinine had similar results [30].

Comment [KB12]: significant

Among all the groups, only the medium and high doses reduced cholesterol levels. Triglyceride levels fell in all groups but were not statistically significant. Only the high dosage resulted in a statistically significant reduction in LDL levels. HDL levels rose in medium and high doses,

which is statistically significant. An ethanolic and aqueous extract of *A. racemosus* had similar results [31].

More study is needed to identify and isolate the exact component responsible for the pharmacological actions that may introduce a fresh drug into the treatment system.

Conclusion:

The results of this research show that a plant called A. racemosus, when extracted using ethanol, may protect against diabetes, hyperlipidemia, liver damage, and renal function. Despite having anti-hyperlipemic and anti-diabetic activities, the plant extract does not have a remarkable impact on its own. Therefore, more research is needed to screen for the active ingredient in the entire extract that produces the desired antidiabetic and anti-hyperlipedemic action. Once the active molecules have been isolated, further thorough research may be conducted.

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Comment [KB15]: All the references Have to correct properly

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