Original Research Article

An Assessment of Anti-Diabetic Potentility of Ethanolic Extract of *Andrographis paniculata*in Alloxan Induced Rat Model.

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Abstract

Background. Diabetes is one of the most frequent noncommunicable illnesses in the world and the fourth leading cause of mortality in the most industrialized countries. Plants have long been used as a source of medicine, and preclinical and clinical studies have shown that *Andrographis paniculata* has considerable anti-diabetic properties. The historical and current state of research on *Andrographis paniculata* in terms of medicinal usage, phytochemistry, pharmacological activity, toxicity profile, and therapeutic usage, in order to bridge the gap necessitating future research prospects.

Methods. Alloxan was used to induce hyperglycaemia in rats, and an extract of *Andrographis paniculata* was steeped in ethanol. Metformin is a frequently used anti-diabetic medicine that has been shown to be helpful at reducing blood sugar levels while not inducing hypoglycaemia. Antidiabetic action was assessed primarily by assessing pre- and postprandial blood glucose levels, as well as other parameters such as cholesterol, LDL, triglyceride, and body weight, as well as liver and kidney functions.

Results & Discussion. When compared to controls, the purified leaves extract substantially (P<0.05) reduced blood glucose, triglyceride, SGPT, SGOT, creatinine, urea, and LDL levels. Serum cholesterol, on the other hand, showed no changes. Metformin had comparable impacts on these parameters. Anti-diabetic activity is demonstrated by bioactive compounds andrographolide and 14-deoxy11,12-didehydroandrographolide.

Conclusion. Active compounds from *Andrographis paniculata* have a hypoglycaemic impact in alloxan-induced rats. It was successful in lowering blood glucose, triglyceride, and LDL levels. However, they had no effect on cholesterol levels, and more research is needed to determine their methods of action. According to the study's discoveries, *Andrographis paniculata* leaf extract offers great therapeutic potential for the action of diabetes.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic condition of glucose, lipid, and protein that is defined by a lack of insulin synthesis and action and affects a huge number of people globally [1]. Because of microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke, and peripheral vascular disease) consequences, it has caused severe morbidity and death [2]. In 2010, it was projected that over 200 million individuals globally suffered from diabetes, and this figure is predicted to rise to 300 million by 2025 [3].

Currently, treatment for diabetes is primarily based on several approaches aimed at reducing hyperglycaemia, including Sulphonylureas, which increase insulin release from pancreatic islets; Metformin, which reduces hepatic glucose production; peroxisome proliferator-activated receptor-agonists (Thiazolidinediones), which enhance insulin action; α-glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself. These medicines are ineffective, have low tolerance, and have severe mechanism-based adverse effects. [4].

Traditional medicine (herbal) is utilized to treat diabetes in impoverished nations where the cost of mainstream treatments is prohibitively expensive [5]. Despite great breakthroughs in health sciences and medical treatment, many people use alternative therapies alone or in addition to prescription medicine [6]. One of the most significant advantages of medicinal plants is that they are widely available and have relatively few negative effects. Plants have long been an excellent source of medications, with many currently accessible pharmaceuticals produced directly or indirectly from them. According to ethnobotanical data, around 800 plants may have anti-diabetic properties [7].

Few medicinal plants with antidiabetic activity and elucidating their mechanisms of action such as *Brassica juncea*[8], *Eugenia jambolana* [9], *Coccina grandis* [10], *Alangium lamarckii* [11], *Albizia odoratissima* [12], *Artemis sphaerocephala Krasch* [13], *Xonopus compressus* [14], *Berberis vulgaris* [15], *Caesalpinia digyna* [16], *Catharanthus roseus* [17], *Centaurium erythrea* [18], *Chaenomeles sinensis* [19] etc. and method of experiment on animals and therapeutic efficiency of plant extracts were exploited.

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Andrographis paniculata (AP) is a significant medicinal plant that is utilized in traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan, the Philippines, Malaysia, Indonesia, and Thailand [20, 21]. It is also known as King of Bitters in English, Mahatikta in Sanskrit, Kiryato in Gujarati, Chirayetah in Hindi, Kalmegh in Bengali and Urdu, and Fah Talai Jone in Thai [22].

Its perceived "blood purifying" property results in its use in diseases & has been reported to have a broad range of pharmacological effects including anticancer [23], antidiarrheal [24], antihepatitis [25], anti-HIV [26], antihyperglycemic [27], anti-inflammatory [28], antimicrobial, antimalarial [29, 30], antioxidant [31], cardiovascular [32], cytotoxic [33], hepatoprotective [34], immunostimulatory [35], and sexual dysfunctions [36].

The aerial portion of the plant includes a great variety of chemical elements, primarily lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides, while the leaves contain two bitter principles, andrographolide and kalmeghin [37]. Andrographolide, a primary Ent-labdane diterpenoid of AP, contributes the most to numerous pharmacological actions. Anti-diabetic action demonstrated by andrographolide and 14-deoxy11,12-didehydroandrographolide [38-40].

Andrographolide reduced plasma glucose levels in streptozotocin-induced diabetic rats, the animal model used to study Type 1 diabetes [41]. However, the mechanism(s) by which andrographolide affects glucose homeostasis remain unknown. Exogenous -endorphin has been shown to raise circulating insulin in adults with or without diabetes [42]. Indeed, stimulation of opioid -receptors by exogenous -endorphin or pharmacological agents such as loperamide and tramadol has been shown to enhance glucose homeostasis in diabetic rats in the absence of insulin [43,44]. Furthermore, stimulation of 1A-adrenoceptors in the adrenal medulla may increase -endorphin production from the rat adrenal gland [45,46].

Andrographolide activates the 1A-adrenoceptor, increasing glucose absorption in cultured mouse myoblast C2C12 cells [47]. In order to evaluate the potential antihyperglycemic potential of *Andrographis paniculata* (AP) and the main active constituent andrographolide (AG), in vitro -glucosidase and -amylase enzyme inhibition studies, as well as in vivo studies to confirm the activity in live animals, were carried out [48]. He and coworkers showed that after being converted to 14-deoxy-11,12-didehydroandrographolide, a -glucosidase inhibitor in vivo, AG may exhibit an anti-diabetic effect by blocking -glucosidase. Furthermore, AP ethanol extract displayed an anti-diabetic effect via -glucosidase inhibitory action [49]. 14-

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deoxy-11,12-didehydroandrographolide inhibits DN phenotypes more effectively than andrographolide. Both of these drugs lower ROS levels but lack antioxidative action in the acellular environment, indicating that their anti-diabetic nephropathy impact is mediated through intracellular signaling pathways [50].

It is essential to discover an integrated therapy for diabetes by employing alternative medicine such as herbal medicine or a mix of synthetic medications and herbal medicine. Numerous therapeutic qualities of *A. paniculata* have been documented. Furthermore, *A. paniculata* is included as a major medicinal plant in treating diabetes, high blood pressure, and a variety of other illnesses in the Compendium of Medicinal and Aromatic Plants [51].

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This study will go through the anti-diabetic action of *Andrographis paniculata*, which has been employed as an anti-diabetic treatment in traditional medical systems. As a result, the dry powder of *A. paniculata* was chosen for the current investigation in order to standardize its antidiabetic function in Diabetes mellitus.

Materials and Methods

2.1. Drugs, Chemicals, and Instruments

The ethanol and alloxan were purchased from Sigma Aldrich in Germany. Healthcare Pharmaceutical Limited provided us with a free sample of the common diabetic medicine metformin. The blood serum analysis kits for total cholesterol, HDL, LDL, triglycerides, SGOT, SGPT, and creatinine were purchased from Plasmatic Laboratory Product Ltd. in the United Kingdom. The glucometer, Alere GI from Alere Inc., USA, was obtained from Shahbag in Dhaka, Bangladesh, and the biochemical parameters were evaluated using the Humalyzer 3000 (a semiautomated clinical chemistry analyzer).

2.2. Plant Collection and Extract Preparation.

A. paniculata leaves were collected in three separate areas of Bangladesh: North Bengal, a hill track area, and a low land area. The following stage was authentication and taxonomic identification. The plant specimen was maintained at Bangladesh's National Herbarium in accordance with their laws. The leaves were dried in the shade for seven to ten days before being finely ground. The powdered leaves were forcefully agitated for 96 hours while

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steeping in 70% ethanol. The extract was filtered when it had finished soaking, and the filtered liquid was collected. Once the extracted solution was brought there, it was concentrated using a rotating evaporator. The dried extract was then carefully collected and stored for future use.

2.3. Experimental Animal Handling.

Adult healthy male Wistar rats (125-200 grams) were recruited from the Pharmacy Department of Jahangirnagar University in Dhaka, Bangladesh, and kept on a 12-hour dark/light cycle at a constant temperature of 25° C at the University of Dhaka's Institute of Nutrition and Food Science. On a regular basis, a normal pellet meal and clean water were provided. The rats were kept there to adapt before the investigation began. All rat trials followed the guidelines provided by the Institutional Animal Ethics Committee (IEAC). Animals were treated and maintained in accordance with the guidelines of the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

2.4. Experimental Guidelines

All tests were carried out in accordance with the ethical principles outlined in the 2013 Helsinki Declaration. The "3R" standards, which are a cornerstone of Swiss and global legislation governing the use of animals in research, were scrupulously observed throughout the duration of this study. The term "replacement" is symbolized by the prefix "R," which encompasses both absolute replacements (such as replacing animal models with computer-generated models) and relative replacements (such as replacing live animals with cell or tissue cultures or vertebrates with invertebrates). Our research began with an in-silico examination to determine that it adhered to the concept of "replacement." However, this model was unable to generate adequate data. In order to do more study, an animal model was employed. Rats were selected as test animals because, unlike invertebrates, mammalian vertebrates have particular pancreas and beta cells for antidiabetic research.

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The second "R" stands for "reduction," which refers to any method that results in the use of fewer animals to acquire adequate data to answer the research objectives or in maximizing the information collected from each animal. Ten rats were gathered for this investigation based on the "power analysis method" sample size estimate, which was utilized to guarantee compliance with this suggestion.

The third "R" stands for "refinement," and it refers to lowering the amount of pain inflicted on the experimental animals by alleviating their anguish. To make the surgery more tolerable and reduce discomfort from pinching, the tail tips of rats were rubbed with isopropyl alcohol before and after each blood glucose level measurement. The rats were fed adequately throughout the trial, and they were painlessly killed at the end in accordance with the 2013 amendment of the Guidelines for the Euthanasia of Animals.

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2.5. Experimental Design

Individually weighed rats were separated into groups based on body weight and then tested for anti-hyperglycemic action (Table 1). The rodents were divided into groups based on body weight, with 10 rats in each group. Table 1 depicts the alloxan control group as rats that solely received alloxan therapy. N/A indicates that no therapeutic treatment was administered to the rats in this group.

Table 1: Anti-hyperglycemic activity analysis

Group number	Group Status	Treatment specimen	Dose of treatment specimen (mg/kg)	Group Abbreviation
1	Negative Control	Physiological Saline	10 mL/kg	N
2	Alloxan control	Alloxan	150 mg/kg	A
3	Alloxan + Metformin	Alloxan + Metformin	150 mg/kg + 100mg	A + M100
4	Alloxan + *A. paniculata*	Alloxan + *A. paniculata* leaves extract low dose	150 mg/kg + 500 mg/kg	A + AP_{500}
5	Alloxan + *A. paniculata*	Alloxan + *A. paniculata* leaves extract medium dose	150 mg/kg + 1000 mg/kg	A + AP_{1000}
6	Alloxan + *A. paniculata*	Alloxan + *A. paniculata* leaves extract high dose	150 mg/kg + 1500 mg/kg	A+AP_{1500}

7	Metformin	Metformin	100 mg/kg	M
8	*A. paniculata*	*A. paniculata* leaves extract low dose	500 mg/kg	AP_{500}
9	*A. paniculata*	*A. paniculata* leaves extract medium dose	1000 mg/kg	AP_{1000}
10	*A. paniculata*	*A. paniculata* leaves extract high dose	1500 mg/kg	AP_{1500}

2.6 Biological Sample Collection.

Blood samples were collected by puncturing the tip of the rat's tail in order to measure blood glucose levels. In contrast, blood was immediately collected from the slaughtered animal after a heart puncture and transported to a microcentrifuge tube. The supernatant fluid was produced by centrifuging the collected samples for 5 minutes at 5,000 rpm. This fluid was then transferred to another microcentrifuge tube for biochemical testing. The kidney and liver were promptly removed from the animal body after sacrifice and meticulously washed in ice-cold saline for kidney and liver function testing.

2.7. Estimation of Biochemical Parameters.

The blood glucose level was measured using a glucometer. Aside from the Humaluzer 3000, lipid profile, kidney, and liver function tests were performed. In addition, the gluconeogenic and glycolytic enzyme activity of kidney and liver samples was examined.

2.8. Statistical Analysis.

For each group, the mean and standard deviation (SD) of each research parameter are displayed. The "one-way ANOVA test" was used to examine the statistical significance of intergroup heterogeneity by evaluating differences across groups in terms of different biological parameters. The application "SPSS 16" was used for the analysis. The result was considered statistically significant when the "p" value was less than 0.05 (p 0.05), and highly significant when it was less than 0.01 (p 0.01).

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Results and Discussion

Both the negative control and plant extract-treated groups of our experimental rats gained weight as a result of the call of nature. Alloxan had a devastating impact and significantly reduced body weight; however, because to the antioxidant properties of the plant (*A. paniculata*), the alloxan-induced treatment group did not lose the same amount of weight. However, as seen in Table 2, metformin led to an additional reduction of body weight in Group 3 since this group received both metformin and alloxan, both of which lowered weight significantly. In contrast, form group 4-6 plant extract administered with alloxan, and weight does not fall much. As a result, increasing dosages of plant extract have the potential to prevent animal weight loss.

100 rats were separated into ten groups to see how the extract altered blood glucose levels. Blood glucose levels were examined before giving alloxan to rats in groups 2-6. After three days, diabetes was induced. They went 14 days without treatment. The treatment then began on day 14. The drugs were likewise given to the rats in groups 7 through 10, but no alloxan was provided to them. They were treated for 14 to 42 days. Furthermore, rats in Group 1 were fed and water on a regular basis. Alloxan is one of the most commonly utilized drugs to induce diabetes in experimental animals. As shown in Fig. 1, the blood glucose levels of the group 1 rats were normal. However, due to alloxan, the blood glucose level in the positive control group was greater than in all other groups. When compared to a positive control, metformin appears to lower blood glucose levels. On the contrary, all dosages of plant extract considerably lower blood glucose levels, while medium and high doses outperform low ones. As a result, its anti-diabetic effects will be naturally greater than metformin's.

As shown in Table 3, the treatment group had lower creatinine and urea levels during the renal function test than the alloxan control group. In groups 4-6, all dosages of plant extract significantly lowered creatinine levels when compared to the Alloxan Control group. Group 6 (Alloxan + *A. paniculata* leaves extract high dosage) beat the Alloxan Control group in terms of urea levels. There was no significant difference in creatinine deduction between metformin and placebo.

In liver functional test, alloxan-induced diabetic rats had substantially higher SGOT and SGPT values. The treatment groups' SGOT and SGPT levels were lower than all other groups, including the alloxan control group. The hepatoprotective effect of *A. paniculata* leaves extract was seen in the reduction of liver enzymes. The high dose lowered the excessively high level of SGOT, whereas the medium and high doses reduced SGPT levels.

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All dosages of *A. paniculata* leaves extract demonstrate no reduction in cholesterol equivalency to the alloxan-controlled groups, as shown in Table 3, because alloxan also increased the levels of these indicators. According to Table 3, the negative control group had the greatest HDL level, whereas the alloxan control group had the lowest. Thus, the quantity of HDL was increased in both the metformin and high dosage*A. paniculata* plant extract instances. The drug-treated groups 5 and 6 (Alloxan + *A. paniculata* leaves extract medium dosage and Alloxan + *A. paniculata* leaves extract high dose) outperformed the positive control group in terms of LDL levels. In the case of triglycerides, a higher dose of plant extract has a greater effect and significantly lowers triglycerides.

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Human fasting blood glucose levels were dramatically reduced after oral treatment of ethanol extracts of AP. Another bioactive molecule, 14-deoxy-11,12-didehydroandrographolide, was shown to have antihyperglycemic properties. We discovered that groups 5 and 6 performed better in terms of blood glucose-lowering impact, SGOT, SGPT, creatinine, total cholesterol, LDL, triglyceride, and HDL levels in our study. As a result, researchers will work on identifying new antihyperglycemic components of AP and combining AP with other medicinal plants for a better therapy option for diabetic patients.

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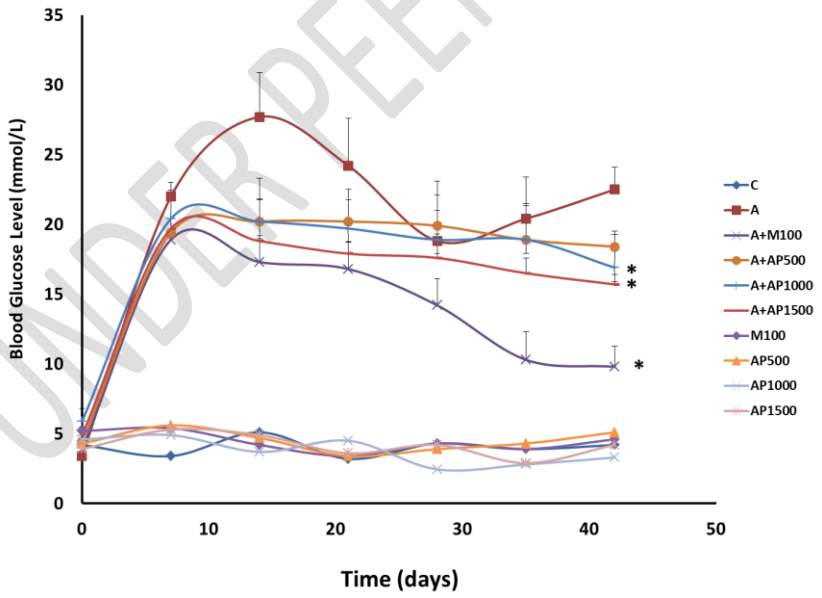

Fig. 1. Blood glucose levels of rats in ten groups over the course of treatment

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,

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M = metformin, A + M = alloxan + metformin, A + AP = alloxan + *Andrographis paniculata*, and AP= *Andrographis paniculata*)

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

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Group number	Group status	Body Weight (gm)	
		Initial	Final
1	Negative Control		
2	Alloxan control		
3	Alloxan + Metformin		
4	Alloxan + *A. paniculata*		
5	Alloxan + *A. paniculata*		
6	Alloxan + *A. paniculata*		
7	Metformin		
8	*A. paniculata*		
9	*A. paniculata*		
10	*A. paniculata*		

Table 3. Effect of *A. paniculata* on the kidney, liver, and lipid function of control and experimental rats

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Group	Group	Kidney	Liver Function		Lipid Profile Function Test

no.	status	Function test		test					
		Creatinine (mg/dl)	Urea	SGOT (u/l)	SGPT (u/l)	Cholesterol (mg/dl)	Trigliseride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1	Negative Control	**0.6±0.02**							
2	Alloxan control	**2.3±0.9253**							
3	Alloxan + Metformin	**1.3±0.235**							
4	Alloxan + *A. paniculata*	**1.8±0.23**							
5	Alloxan + *A. paniculata*	**1.4±0.12**							
6	Alloxan + *A. paniculata*	**0.9±0.09**							
7	Metformin	**0.5±0.03**							
8	*A. paniculata*	**0.7±0.359**							
9	*A. paniculata*	**0.7±0.069**							
10	*A. paniculata*	**0.6±0.03**							

Conclusion

The anti-diabetic effect of *Andrographis paniculata* was observed in the ethanolic extract and the fractional compound (i.e., andrographolide or its analogue14-deoxy11,12-didehydroandrographolide) in alloxan-induced diabetic rats. As a result, the folk usage of this herb to treat diabetes is warranted. Because of its wide pharmacological actions, the AP may be fairly classified as a modern catholicon. However, the identified pharmacological actions of AP require clinical confirmation. Verification of the efficacy of additional biological activities of AP, such as anti-cancer, anti-inflammatory, and hepatoprotective actions, on human research participants would provide significant advantages to the world's biggest population. We believe that in the near future, the AP might be used as extremely effective therapeutic agents for a range of ailments in order to treat human diseases. As a result,

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Andrographis paniculata may be developed as an alternate anti-diabetic medication. Further research may be conducted to investigate the mechanism of action of *Andrographis paniculata* as an anti-diabetic in its extract, fractional component, and combination with a synthetic medicine or another herbal.

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