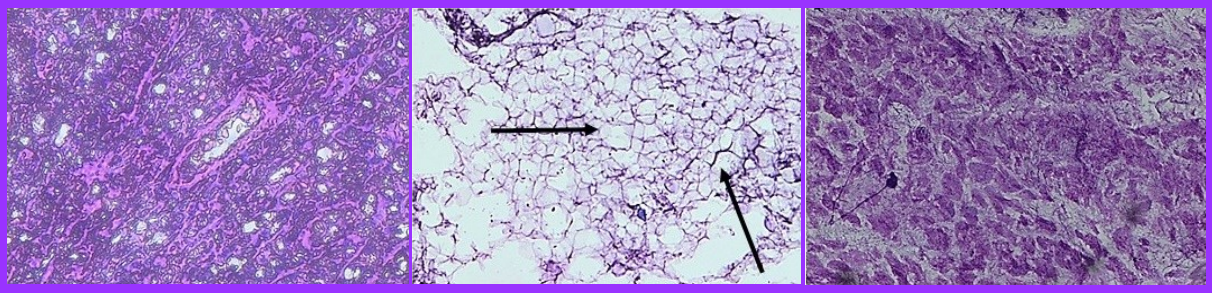

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Anti-diabetic activity of *Allium wallichii* on streptozotocin-nicotinamide-induced type-2 diabetes in rat

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Abstract

The aim of the present study was to investigate the effect of *Allium wallichii* leaves extract against streptozotocin-nicotinamide-induced type-2 diabetes in rats. The hydroalcohol extract of *A. wallichii* leaves (200 and 400 mg/kg) was tested for oral glucose tolerance test and in non-insulin-dependent diabetes mellitus-induced elevated serum glucose levels in the rat. Interestingly, single oral administration of *A. wallichii* caused a significant ($p<0.05$) reduction in the serum glucose level (mg/dL). On other hand, *A. wallichii* normalized plasma glucose levels after repeated oral administration for up to 21 days in diabetic rats ($p<0.05$). The *A. wallichii* in the dose of 400 mg/kg showed significant effects on streptozotocin-nicotinamide-induced type-2 non-insulin-dependent diabetes mellitus in rats. In conclusion, therapeutic potential of *A. wallichii* as per claims is validated towards anti-diabetic potential.

Introduction

Type-2 diabetes mellitus is majorly caused due to insulin resistance and accounts for 90% of the total number of cases, which continues to rise daily due to the overburden of associated factors such as aging, rapid urbanization, and obesity (Khan et al., 2020). Some of the common factors responsible for causing diabetes include living a sedentary lifestyle, drinking alcohol, smoking, and inactivity, which may result in a reduction of insulin sensitivity and glucose tolerance, leading to obesity (Chatterjee et al., 2017).

Worldwide, ethnobotanical and plant-based traditional medicines research leads to the identification of several bioactive and nutraceutical leads for the prevention and treatment of various diseases including diabetes (Raghav et al., 2022). In this context, some of the important high valued traditional ethnomedicinal plants along with their bioactive such as *Aegle marmelose* (Sabu and Kuttan, 2004), *Allium cepa* (Akash et al., 2014), *Allium sativum* (Eidi et al., 2006), *Aloe vera* (Bunyapraphatsara et al., 1996), *Azadirachta indica* (Satyanarayana et al., 2015), *Gymnema sylvestre* (Baskaran et al., 1990), *Hibiscus rosa-sinesis* (Venkatesh et al., 2008), *Mangifera indica* (Irondi et al., 2016), *Momordica charantia* (Joseph and Jini, 2013), *Tinospora cordifolia* (Sharma et al., 2015) and *Trigonella foenum-graecum* (Geberemeskel et al., 2019) have been already validated for anti-diabetic activities in recent past.

Allium wallichii Kunth (Family: Amaryllidaceae) was documented for medicinal remedies such as dysentery, cholera, blood cholesterol levels, hypertension, intestinal pain, liver diseases, high altitude sickness, etc (Rana et al., 2022a). Among phytochemicals such as diosgenin, 1,2 bis (methylthio) ethene, tigogenin, 2,4 dimethyl thiophene, dimethyl disulfide, and trisulfide are some few identified compounds among flavonoids, glycosides, steroids, reducing sugars, organosulfur and terpenoids (Kamal and Sharma, 1984; Kattel and Maga, 1995; Bhandari et al., 2017). The plant has antimicrobial, antioxidant, anti-cancer, and anti-inflammatory activities via *in vitro* and *in vivo* studies (Acharya et al., 2011;

Bhandari et al., 2017; Wang et al., 2016; Iqbal et al., 2018; Sørnes et al., 2021). Since ancient times this plant have a rich traditional and medicinal application. However, only a few activities have been performed in modern medicine. Therefore, the present work has been designed to investigate the effect of *A. wallichii* leaves extract against streptozotocin-nicotinamide-induced type-2 diabetes in rats.

Materials and Methods

Plant materials

Plant samples of *A. wallichii* were collected from the outskirts of Pitthoragarh (Uttarakhand, India) from August to September 2021 and identified by Dr. M. C. Bharti (Department of Botany, Hemwati Nandan Bahuguna Central Garhwal University, Srinagar, Uttarakhand, India). Voucher specimens of the plant (No. Herbarium/bot./1070) had been deposited at the Department of Botany, Hemwati Nandan Bahuguna Central Garhwal University, Srinagar, Uttarakhand, India.

Animals

Thirty-six Sprague-Dawley (SD) rats of either sex (Average weight 200 g and 6 weeks old) were purchased from the National Institute of Biologicals, Noida, India and maintained under standard animal husbandry conditions (temperature 23 ± 2°C, relative humidity 55% ± 10% and 12 hours light-dark cycle). The animals were fed a standard rat pellet diet and had free access to water.

Preparation of leaves extract

Powdered leaves of *A. wallichii* were subjected to maceration in hydroalcohol (30:70 v/v) for 72 hours. After complete maceration, the filtrate was evaporated under reduced pressure, and the percentage yield, color, and consistency were determined (Bhatt et al., 2010).

Acute toxicity studies

Six Sprague Dawley rats were fasted overnight without withholding water supply. A single dose of 2 mL of 2000 mg/kg of the test sample was administered via a gavage needle to each of the rats. After dosing, rats were individually observed once every 30 min periodically during the first 24 hours, and daily thereafter for 14 days. The animals were observed for any change in fur (fur lost), eyes and mucous, breathing pattern (panting), tremors, convulsion, abnormal defecation (diarrhea), lethargy, severe pain, distress, and moribund (Perumal et al., 2022).

Anti-diabetic activity

The *A. wallichii* extract was screened by the oral glucose tolerance test and streptozotocin-nicotinamide-induced type-II diabetic rat model with slight modification as standard reported methods (Agrawal et al., 2013).

Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight (18 hours) fasted normal rats. A total of 30 Sprague Dawley rats were divided into five groups of six rats in each group. Group I (positive control) rats received only saline water and the remaining groups 30 min after the administration of the test sample were administered with glucose (2 g/kg) orally. Group II (vehicle control) rats treated with saline water followed by glucose. Group III rats were treated with standard drug (glibenclamide, 1 mg/kg; East West Pharma India) followed by glucose. Group IV and Group V rats were treated with *A. wallichii* extract at a dose (200 or 400 mg/kg) followed by glucose. Blood samples were collected from the tail vein of all the test animals after a different time interval (30, 60, and 120 min) of glucose administration and the blood glucose levels were estimated using a blood glucose monitoring system (BG-03; Morepen Laboratories Limited, India).

Streptozotocin-nicotinamide-induced type-2 diabetes

After the oral glucose tolerance test, rats were taken to carry out streptozotocin-nicotinamide (National Chemicals, India)-induced type-2 diabetes studies for 21 days. In this study, Group I (positive control) rats received normal saline only and the remaining groups 30 min after the administration of the test sample were administered with a single dose of streptozotocin-nicotinamide on day 1. Group II (vehicle control) rats treated with saline water followed by streptozotocin-nicotinamide. Group III rats were treated with the standard drug glibenclamide (1 mg/kg) followed by streptozotocin-nicotinamide. Group IV and Group V rats were treated with *A. wallichii* extract at a dose (200 or 400 mg/kg) followed by streptozotocin-nicotinamide.

Type-2 diabetes was induced by a single intraperitoneal injection of nicotinamide (230 mg/kg) followed by streptozotocin (65 mg/kg; Otto Chemie Pvt. Ltd. India) 15 min afterward on day 1 of the study. Nicotinamide (National Chemicals, India) was dissolved in normal saline and streptozotocin was dissolved in 0.1 M citrated buffer (pH 4.5) immediately before use

Histological procedures

The histological procedure was performed as per the standard reported method (Balamash et al., 2018). In brief, excised pancreas tissues from all the groups were fixed in 10% neutral buffered formalin and processed with an automatic tissue processor. Embedding was done in paraffin wax and sectioned at 5 µm on a rotatory microtome. The sections were stained with hematoxylin and eosin and mounted on distyrene plasticizer xylene. Photomicrographs at magnifications of ×250 were taken from all the groups with the aid of a digital microscope camera.

Statistical analysis

All results were reported as mean ± SD. The variation in a set of data has been estimated by performing Turkey multiple comparison post-test to measure one-way ANOVA using non-parametric methods in Graph pad prism.

Results

Preparation of leaves extract

The percentage yield of leaves extract was found to be 11.9% with dark green color.

Acute toxicity studies with pharmacological behavioral screening

A. wallichii extract (2000 mg/kg) did not show any toxicity and was found to be non-toxic and safe as evidenced by observations study of 14 days.

Anti-diabetic activity

Oral glucose tolerance test

A single administration of *A. wallichii* extract (200 and 400 mg/kg) and glibenclamide (1 mg/kg) significantly reduced serum glucose levels at 30, 60, and 120 min after administration. In the oral glucose tolerance test, the administration of the glucose load (2 g/kg) increased serum glucose levels significantly after 30 min in non-diabetic rats. Glibenclamide (1 mg/kg) and *A. wallichii* extract (200 and 400 mg/kg) produced a significant increase in the glucose threshold within 60 min, which was then reversed at 120 min after glucose loading. The reductions in serum glucose from basal value (before) to 120 min after glibenclamide (1 mg/kg) and *A. wallichii* extract (200 and 400 mg/kg) were shown in Figure 1A.

Streptozotocin-nicotinamide-induced type-2 diabetes

Positive control rats were found to be stable in their body weight, but diabetic rats (vehicle control) showed a significant reduction in body weight. Streptozotocin followed by nicotinamide caused body weight reduction, which was reversed by both glibenclamide and *A. wallichii* extract-treated groups after day 7, 14, and 21 of the treatment (data not shown). Similarly, administration of glibenclamide (1 mg/kg) and *A. wallichii* extract (200 and 400 mg/kg) caused significant reductions in the serum glucose level, which was observed on day 0, 7, 14, and 21 (Figure 1B).

Histological findings

Figure 2 indicates changes in the quality of the islet cell structure in all experimental groups. The pancreatic tissue sections showed that there was a distortion of the normal histo-architecture of the cells in the islet area of the streptozotocin-nicotinamide-treated group when compared with the control group. Such distortion was reduced and islet cell quality was restored to near normal in the *A. wallichii* extract-treated groups.

Discussion

A single administration of *A. wallichii* extract significantly reduced serum glucose levels during the course of study suggesting potential application as an anti-hyperglycemic agent in a dose-dependent manner. Moreover, a significant reduction in the consistent serum glucose level was observed during 21 days by *A. wallichii* extract. Histological evidence, where recovery

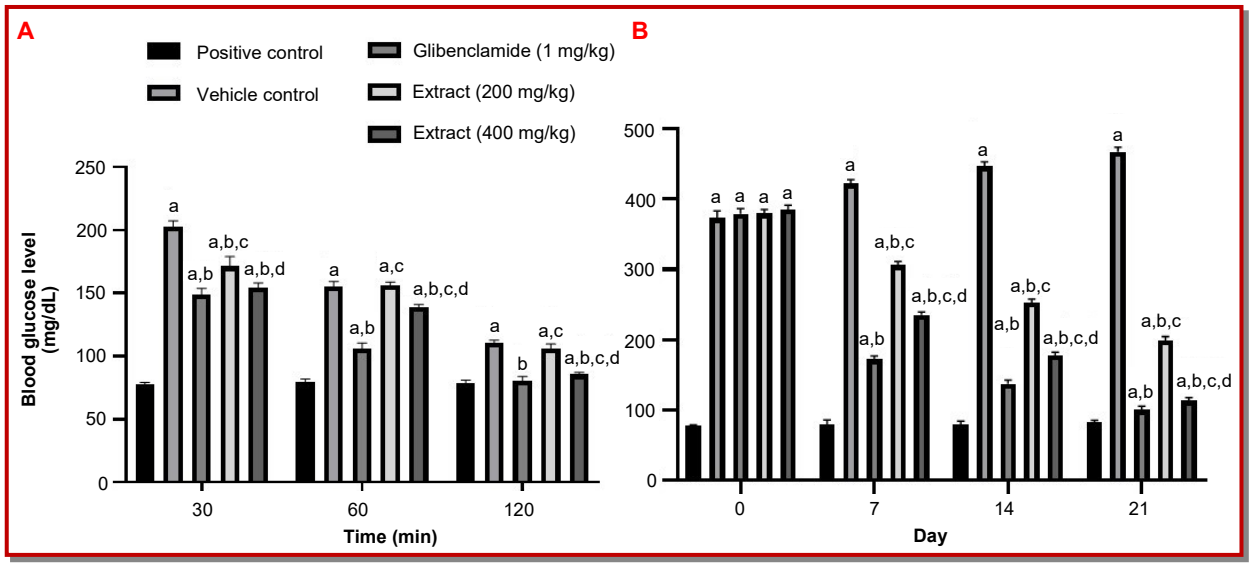

Figure 1: Effect of hydroalcohol extract of *A. wallichii* on oral glucose tolerance test (A); and anti-diabetic effect in streptozotocin-nicotinamide type-2 diabetic rat (B). Superscript a, b, c, or d denotes $p<0.05$ when compared with positive control, vehicle control, glibenclamide (1 mg/kg) and extract (200 mg/kg)

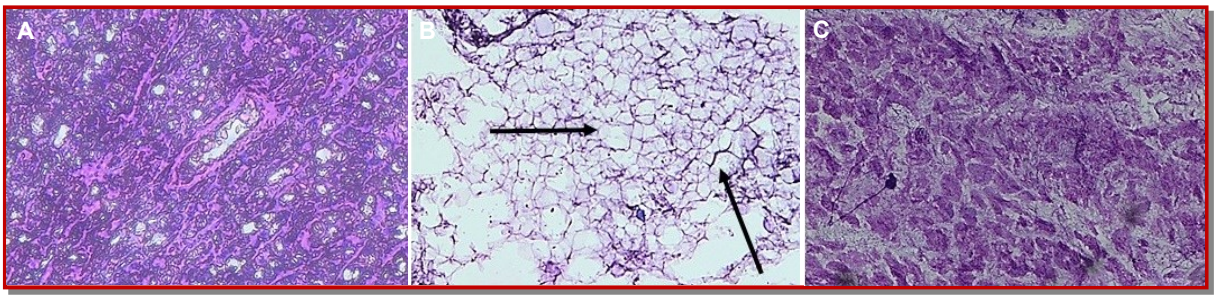

Figure 2: Histological observation of pancreas in streptozotocin-nicotinamide type-2 diabetic rat. 'A' represents the control group; 'B' represents the streptozotocin-nicotinamide-treated pancreas showing the formation of necrosis as presented by arrows; 'C' represents the *A. wallichii* extract (400 mg/kg)-treated group showing the reversal of pancreas necrosis

of pancreas towards streptozotocin-nicotinamide-induced lesion was also observed.

Induction of diabetes through streptozotocin was already established to reduce the bodyweight in diabetic animals may be due to disturbance in glucose homeostasis associated with endocrine pancreatic cells (Damasceno et al., 2014). In this connection, during current study *A. wallichii* extract was found to reversed the streptozotocin induced body weight reduction. The reversal in body weight reduction by *A. wallichii* extract may be attributed due to improvement in glucose homeostasis via normalization of pancreatic functioning as evidence from similar effect produced by other Allium species as reported earlier (Kim et al., 2011).

Past evidence shown significant contribution of several Allium species including *A. sativum* (Eidi et al., 2006), *A. saralicum*, *A. hookeri* (Fazelipour et al., 2021), *A. cepa* (Akash et al., 2014) and many more (Thomson et al., 2015) towards the prevention and treatment of diabetes. Similarly, diallyl trisulfide, which was found to be present in *A. wallachii* also exhibit anti-diabetic activities as per literature (Tsai et al., 2015). However, *A. wallichii* still lacking any similar study and not explored for anti-diabetic potential earlier (Rana et al., 2022b). Therefore, in this study, anti-diabetic potential of *A. wallichii*, on streptozotocin-nicotinamide induced type-2 diabetes in rat was established first time. Moreover, several Allium based dietary components on regular consumption have been found to exhibit multiple health benefits including lowering of blood glucose levels and improvement in the performance of pancreas as reviewed on the basis of several studies outcome (Wan et al., 2019). In brief presence of dietary organosulfur compounds in several Allium species already established for prevention and treatment of type-2-diabetes by acting as natural hydrogen sulfide donors (Melino et al., 2019). Similarly, *A. wallichii* (an underutilized dietary vegetables enriched with organosulfur compounds) as per present study able to exhibit blood glucose lowering effect followed by recovering pancreas to normal vasculature as evidence from histological finding. Based on findings this study provides supports to various traditional and ethnomedicinal dietary claims on *A. wallichii* towards health promotion and dietary application.

Conclusion

A. wallichii extract exhibited significant antihyperglycemic activities against type-2 diabetic rat.

Financial Support

Self-funded

Ethical Issue

The protocols and procedures were approved by the Institutional Animal Experimentation Ethics Committee, Faculty of Pharmacy, DIT University, Dehradun, Uttarakhand, India (Reg. No. DITU/IAEC/21-22/07-09; dated 10th July 2021). The toxicity level of the *A. wallichii* extract was evaluated according to the guidelines of OECD 420.

Conflict of Interest

Authors declare no conflict of interest

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