Available online on 30.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Antihyperglycemic Effect of Methanolic Extract of *Aphanamixis polystachya* Leaves on Streptozotocin-Induced Diabetic Rats

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ABSTRACT

This study aimed to investigate the antidiabetic effect of *methanolic extract of Aphanamixis polystachya leaves* in streptozotocin (STZ) induced diabetic rats. Wistar rats in each were divided into six groups and these animals were used for the study. In our study methanolic extract of *Aphanamixis polystachya* leaves was screened for antidiabetic activity in streptozotocin (STZ)-induced diabetic rats. The anti-diabetic activity was assessed using blood glucose level, body weight and various biochemical parameters like serum total cholesterol level (TC), triglyceride (TG) level, high-density lipoproteins (HDL), total protein (TP), serum alanine transaminase (SGPT) and aspartate aminotransferase (SGOT), respectively. The methanolic extract of *Aphanamixis polystachya* leaves exhibited an antidiabetic effect by significantly decreased the level of blood glucose, body weight, TC, TG, TP and increase HDL. The results of the study demonstrated that the treatment with methanolic extract of *Aphanamixis polystachya* leaves significantly ($P<0.05$) and dose-dependently prevented STZ-induced diabetic rats. The antioxidant property of plant phenolic and flavonoid contents present in methanolic extract of *Aphanamixis polystachya* leaves might be responsible for the antidiabetic activity.

Keywords: Aphanamixis polystachya leaves; diabetes; streptozotocin

Article Info: Received 29 May 2019; Review Completed 12 July 2019; Accepted 19 July 2019; Available online 30 July 2019

Cite this article as:

Tiwari P, Loksh KR, Yadav R, Antihyperglycemic Effect of Methanolic Extract of *Aphanamixis polystachya* Leaves on Streptozotocin-Induced Diabetic Rats, Journal of Drug Delivery and Therapeutics. 2019; 9(4):783-787
DOI: http://dx.doi.org/10.22270/jddt.v9i4.4221

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INTRODUCTION

Diabetes mellitus (DM) is considered as an important health confounder in our world, which necessitates its better management by new methods. DM consists of a number of endocrine impairments, which are mostly associated with uncontrolled blood glucose levels. Pancreatic damage may be due to inflammatory processes such as pancreatitis, or may be induced mainly by autoimmune diseases, drugs or toxins, infections, starvation, low protein diet and high fat diet. In order to perform the studies over DM, several animal models were designed. Among them, Streptozotocin (STZ) induced diabetes can be named as one of the most popular. STZ is a compound which has preferable toxicity for pancreatic beta cells[1]. DM consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease[2]. Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes[3]. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated[4].

Aphanamixis polystachya is widely used in analgesic, CNS depressant activity, cytotoxicity, antimicrobial activity, anti-hepatotoxic activity and antioxidant. The present study was undertaken to evaluate the antidiabetic effect of methanolic extract of *Aphanamixis polystachya* leaves in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHOD

Experimental

The *A. polystachya methanolic* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity Study

In this study, acute toxicity study was carried according to Organization for Economic Cooperation and Development, guideline 423.Alimit dose of 2,000 mg/ kg body weight/oral was used. The signs of toxic effects and/or mortality were observed 3 h after administration

then for the next 48 h. The body weight was recorded for consecutive 14 days. Since the both extracts were found safe up to the dose level of 2,000 mg/kg body weight, a dose of 100 and

200 mg/kg body weight of the two extracts was selected for screening of the antidiabetic activity.

Induction of Experimental Diabetes in Rats

Streptozotocin was dissolved in 100 mM citrate buffer (pH 4.5) and calculated amount of the dose (60 mg/kg) of the fresh solution was injected intraperitoneally to overnight fasted rats. Blood glucose was checked 48 h later and animals showing blood glucose value more than 250 mg/dl were included in the experiments and termed as diabetic1.

Experimental Protocol

Animals were divided into five groups of 6 rats each.

Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)

Group II: Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat)

Group III: Rats (diabetic) were administered Glibenclamide (600µg/kg p.o.) for 21 days.

Group IV: Rats (diabetic) were administered methanolic extract of Aphanamixis polystachya leaves (100 mg/kg p.o.) for 21 days.

Group V: Rats (diabetic) were administered methanolic extract of Aphanamixis polystachya leaves (200 mg/kg p.o.) for 21 days.

Biochemical Evaluation in Serum

Serum total cholesterol level (TC), triglyceride (TG) level, high-density lipoproteins (HDL), total protein (TP), serum alanine transaminase (SGPT) and aspartate aminotransferase (SGOT) was determined by using standard kits from Transasia BioMedical Limited, Mumbai, India. The estimation procedure is obtained in detail from leaflets provided by the commercially available kits are as follows.

Statistical Analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The values were expressed as mean ± SD and $P<0.05$ was considered significant.

RESULTS

Phytochemical screening

The results of qualitative phytochemical analysis of the crude powder of leaves, stems and root bark of *A. polystachya*. Methanolic leaves of *A. polystachya* showed the presence of alkaloids, glycosides, flavonoids, saponins, phenols, proteins and amino acids and carbohydrate.

Antidiabetic activity

Blood glucose level of animals in all groups was recorded at 0,8th and 21th day and change in % blood glucose was also mentioned as shown in Table 2. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment, all treatment groups, with 100 mg/kg and 200 mg/kg of methanolic extract of *Aphanamixis polystachya leaves* was decrease significantly ($p < 0.001$) serum glucose level, as represented in Table 1.

Table 1: Antidiabetic effect of Aphanamixis polystachya extract on blood glucose (mg/dl) in in STZ induced diabetic rats

Groups	Treatment	Blood glucose (mg/dl)		
		Days 0	Days 8	Days 21
I	Normal	71.00 ± 3.60	90.00 ± 5.00	109.10 ± 7.42
II	Diabetic Control	293.00 ± 7.70	390.00 ± 10.00#	408.15± 11.10#
III	Diabetic + Glibenclamide (600µg/kg)	260.10± 7.40	132.80 ± 7.00*	120.00 ± 6.20*
IV	Diabetic + *Aphanamixis polystachya* leaves (100 mg/kg)	270.00 ± 6.78	162.00± 8.10	132.15 ± 7.10*
V	Diabetic + *Aphanamixis polystachya* leaves (200 mg/kg)	246.00 ± 5.00	142.00 ± 3.10*	118.34± 4.80*

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # $P<0.01$ vs. normal group;**$P<0.01$, *$P<0.05$ vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Body weight of animals in all groups was recorded at initial and final day weight was mentioned. In all treatment groups, with 100 mg/kg and 200 mg/kg of methanolic extract of *Aphanamixis polystachya leaves* was not significant in body weight during study period as represented in Table 2.

Table 2: Antidiabetic effect of *methanolic extract of Aphanamixis polystachya leaves* on body weight in STZ induced diabetic rats.

Groups	Treatment	Initial weight (g)	Final weight (g)
I	Normal	200.00 ± 9.90	208.00 ± 11.18
II	Diabetic Control	198.00 ± 9.70	188.00 ± 9.64
III	Diabetic + Glibenclamide (600µg/kg)	198.60 ± 9.90	216.00 ± 10.98*
IV	Diabetic + *Aphanamixis polystachya* leaves (100 mg/kg)	194.00 ± 9.75	192 .00 ± 6.00*
V	Diabetic + *Aphanamixis polystachya* leaves (200 mg/kg)	193.10 ± 9.59	206.00 ± 09.90*

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # P<0.01 vs. normal group;**P<0.01, *P<0.05 vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

The effect of methanolic extract of *Aphanamixis polystachya leaves* on lipid profile of diabetic rats is displayed in Table 3. In diabetic rats, the levels of triglycerides (TG), total cholesterol (TC) and low density lipoprotein (LDL) were significantly increased and high density lipoprotein (HDL) level was significantly decreased. In methanolic extract of *Aphanamixis polystachya leaves* (100 and 200 mg/kg) treated groups, the TG, TC and LDL levels activities were significantly decreased and high density lipoprotein (HDL) level was significantly increased.

Table 3: Antidiabetic effect of *methanolic extract of Aphanamixis polystachya leaves* on serum lipid profile in STZ induced diabetic rats

Group	Treatment	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I	Normal	81.50 ± 6.50	79.00 ± 8.90	60.18±2.00	90.90±6.70
II	Diabetic Control	194.50 ± 6.70	142.5 ± 7.50	30.20±2.10	191.30±6.50
III	Diabetic + Glibenclamide (600µg/kg)	118.00 ± 5.95***	81.00 ± 9.30**	57.60±2.55***	93.60±5.61***
IV	Diabetic + *Aphanamixis polystachya* leaves (100 mg/kg)	130.00 ± 6.10**	85.10 ± 6.60*	55.00±2.10**	119.20±7.10**
V	Diabetic + *Aphanamixis polystachya* leaves (200 mg/kg)	121.00 ± 6.00***	83.20 ± 6.00*	56.20±2.16***	100.30±6.50***

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # P<0.01 vs. normal group;**P<0.001, *P<0.05 vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

There was a significant increase in activities of SGOT and SGPT in diabetic rats. After treatment with methanolic extract of *Aphanamixis polystachya leaves* (100 and 200 mg/kg) the activities of SGOT and SGPT activities were significantly ($p < 0.05$) reduced as compared to diabetic control rats. A significant decrease in serum total protein (TP) level was observed in diabetic rats. After treatment with methanolic extract of Aphanamixis polystachya leaves (100 and 200 mg/kg) for 21 days the TP level was significantly compared to diabetic control rats. Glibenclamide (600µg/kg) treated rats also showed significant effects on blood levels of SGOT, SGPT, SALP and TP in diabetic rats (Table 4).

Table 4: Antidiabetic effect of *methanolic extract of Aphanamixis polystachya leaves* on SGOT and SGPT in STZ induced diabetic rats

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	TP (g/dL)
I	Normal	50. 90±4.88	60.00±4.00	7.00 ± 2.0
II	Diabetic Control	138.47±4.00	130.66±6.30	7.60 ± 1.5
III	Diabetic + Glibenclamide (600µg/kg)	59.50±4.20***	68.90±4.95***	9.1 ± 2.0**
IV	Diabetic + *Aphanamixis polystachya* leaves (100 mg/kg)	86.88±4.10*	85.70±5.50**	10.0 ± 2.3**
V	Diabetic + *Aphanamixis polystachya* leaves (200 mg/kg)	77.79±3.90**	77.76±6.10**	9.9 ± 1.8**

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # P<0.01 vs. normal group; **P<0.01, *P<0.05 vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test)

DISCUSSION

In accordance, findings from the current investigation showed that STZ administration (i.p) effectively induced diabetes mellitus in physiologically normal rats as reflected by hyperglycemia, pancreatic beta cells degeneration, decreased insulin and glycogen levels in diabetic control animals[5]. In contrast, Aphanamixis polystachya leaf extract at the administered doses (100 and 200 mg/kg BW) significantly lowered STZ-induced hyperglycemia, improved insulin and glycogen contents in extract-treated rats compared to diabetic control animals. The above finding is in agreement with previous study where oral administration of extracts significantly reduces blood glucose in STZ-induced diabetes in rats[6]. Improvement in oral glucose tolerance capacity is one of the several mechanisms by which diabetes is controlled and managed. In this study, the extract appeared to have significantly suppressed the degree of hyperglycemia induced by glucose loading in treated rats compared to control animals. This connotes that Aphanamixis polystachya extract may be capable of providing some degree of delay in the onset of diabetes. Improved glucose tolerance is associated with the availability and functionality of plasma insulin. This is evident in the present study in which treatment of rats with the extract significantly improved the level of insulin both in the serum and pancreas. This is may have arguably play a critical role in the hypoglycemic ability exhibited by the extract in lowering STZ-induced hyperglycemia in the treated animals relative to the diabetic control animals. The observed increase in both serum and pancreatic insulin concentrations is obviously connected to the ability of the extract to protect the animals against STZ- induced beta cell degeneration, and consequent activation of insulin production and release[5].

In the present study, all treatment groups, with 100 mg/kg and 200 mg/kg of methanolic extract of Aphanamixis polystachya leaves 100 mg/kg and 200 mg/kg was not significant in body weight during study period. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins[7].

Hyperlipidemia is a recognized complication of diabetes mellitus, characterized by elevated levels of cholesterol, triglycerides, and phospholipids, as well as changes in lipoprotein composition. In the present study, serum triglycerides, cholesterol and LDL cholesterol were significantly elevated while HDL cholesterol was significantly decreased in diabetic rats. Interestingly, the results further indicated that all these lipid and lipoprotein abnormalities were countered by mulberry leaves in diabetic rats. The most characteristic lipid abnormality is hypertriglyceridemia with associated increase in plasma cholesterol. Elevated plasma triglyceride concentration is seen in type1 DM and type 2 DM either due to triglyceride over-production and /or underutilization. Lipoprotein lipase activity is markedly impaired, besides, a significant improvement in LDL internalization and degradation suggesting that chemical modification of LDL particle like nonenzymatic glycation of LDL itself might result in its increased incorporation in the arterial wall via a receptor independent pathway. Previous studies have demonstrated that multidirectional lipid-lowering effects on the rat metabonome, including limitation of the absorption of cholesterol, inactivation of HMG-CoA, and favorable regulation of profiles of essential polyunsaturated fatty acid. In diabetes, hyperglycemia is accompanied with dyslipidemia characterized by increase in TC, LDL and TG and fall in HDL[8]. A dose of 100 mg/kg and 200 mg/kg of methanolic extract of Aphanamixis polystachya leaves was significantly decreased TC, TG, LDL level and increased HDL level as compare to control group. The present study in line with Komeili et al.[9] which revealed that the hydroalcoholic extract for 4 weeks significantly decreased the levels of glucose, triglyceride, cholesterol, and LDL-c and increased the level of HDL-c in diabetic rats. In the present study, the significant increase in serum SGOT and SGPT levels that was observed in STZ-induced diabetic rats represents liver damage compared to control rats. Liver necrosis in STZ-induced diabetic rats increased the activities of SGPT and SGOT in plasma by leakage of these enzymes from liver cytosol into the blood stream. Oral administration of Aphanamixis polystachya showed its protective nature on liver tissue by reducing the elevated levels of SGOT and SGPT[10]. In this study, a dose of 100 mg/kg and 200 mg/kg of methanolic extract of Aphanamixis polystachya leaves was significantly ($p < 0.05$) normalized the content of protein total protein. Kondeti et al have reported that STZ induced diabetic rats account for the observed decrease in the total protein content. Increased urea production in diabetes might be due to enhanced catabolism of both liver and plasma proteins. The plant extract treatment has appreciably normalized the content of protein and urea[11]. The methanolic extract of Aphanamixis polystachya extracts have been chosen because of its expected flavonoid contents that were reported to have antidiabetic activity. The activity of phenolics having antioxidant activity further confirms this view. The phytochemical analysis of methanolic extract of Aphanamixis polystachya leaves showed the presence of tannins, flavonoids, saponins, and sterols. Their antidiabetic ability to regenerate the pancreatic β-cell has already been proved. Sterols can decrease blood sugar in experimental animal models. Our current investigation supports the traditional use of Aphanamixis polystachya in the treatment of diabetes.

CONCLUSION

As a conclusion, it could be speculated that the observed hypoglycemic and antioxidant activities of methanolic extract of *Aphanamixis polystachya leaves* might be related to the tannins, terpenoids, and flavonoids contents. The extracts significantly reduced fasting glucose levels in diabetic rats and also reduced the lipid profile parameters in diabetic rats. The extracts were found significantly decreasing the activities of SGPT and SGOT in diabetic rats

Conflict of interest statement

No conflict of interest.

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