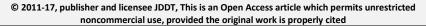


Available online on 15.07.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research







Research Article

PHARMACOLOGICAL INVESTIGATION OF LEAVES OF *POLYPODIUM DECUMANUM* FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

This research was performed to characterize the hypoglycemic effect of ethanolic extract of *Polypodium decumanum* leaves in alloxanized diabetic rats. The ethanolic extract of *Polypodium decumanum* leaves was administered orally at different doses (250 and 500 mg kg-1) to normal rats. The dose of 500 mg kg-1 was found to be more effective dose in oral route and it decreases Blood Glucose Level (BGL) by 22.2 % in normal healthy rats after 14 days of administration. After daily treatment with the both dose (250 and 500 mg kg-1) of ethanolic *Polypodium decumanum* extract for 21 days to severely Diabetic (FBG 300-350 mg dL-1) rats, the BGL reduced to 128.2 mg dL-1 by 250 mg kg-1 and 111 mg dL-1 by 500 mg kg-1. The findings of our study indicate the hypoglycemic and potential antihyperglycemic nature of the extract. It is not similar effective when compared with the standard drug Glibenclamide, but quite effective in reducing BGL. It reduces blood glucose level up to 194.1, 119.4, 111 mg dL-1 at successive days of 7, 14, 21, at the dose of 500 mg kg-1 in rats compare with the standard drug which reduces blood glucose level up to 256.6, 140.7, 101.2 mg dL-1. We found that the ethanolic extract of plant *Polypodium decumanum* leaves is only 10 % less effective in reducing the BGL compare to standard drug (Glibenclamide).

Keywords: Polypodium decumanum, hypoglycemic effect, Glibenclamide

Article Info: Received 28 May, 2017; Review Completed 10 July, 2017; Accepted 11 July, 2017; Available online 15 July, 2017



Cite this article as:

Niranjan PS, Kaushal C, Jain SK, Pharmacological investigation of leaves of polypodium decumanum for antidiabetic activity, Journal of Drug Delivery and Therapeutics. 2017; 7(4):69-72

DOI: http://dx.doi.org/10.22270/jddt.v7i4.1468

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INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder as old as mankind and its incidence is considered to be high (4-5%) all over the world¹. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and post-paradinal blood sugar levels. The synthetic oral hypoglycemic agents can produce serious side effects^{2,3}. In addition they are not considered safe for use during pregnancy⁴. Furthermore, after the recommendation made by⁵ on diabetes mellitus investigation on hypoglycemic agents from medicinal plants have

become important. Plants have played a major role in the introduction to new therapeutic agents. A multitude of herbs, spices and other plant materials have been described for the treatment of diabetes throughout the world^{6,7,8,9}. The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies¹⁰. Few of the plants used for the treatment of diabetes have received scientific or medical scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention⁵. Despite the presence of known antidiabetic medicines in the pharmaceutical

ISSN: 2250-1177 [69] CODEN (USA): JDDTAO

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market, screening for new antidiabetic sources from natural plants is still attractive because they contain substances that have an alternative and safe effect on diabetes mellitus.

Polypodium Decumanum is terrestrial or epiphytic ferns with a creeping. The creeping rhizomes are 8-15 mm in diameter with the golden brown scales. They are densely hairy or scaly rhizome bearing fronds at intervals along its length. The species differ in size, general appearance and in the character of the fronds, which are evergreen, persisting for 1-2 years, pinnate (rarely simple) and about 10-80 cm long and 10-50 cm broad. It is having up to 35 pinnae with multiple colors from bright green to glaucous green with having undulate margins. Several round sori or groups of spore-cases (sporangia) are borne on the back of the frond and run along each side of the pinna midrib and the minute spores are wind dispersed. it has the ability to regulate and support the growth of various cells in the body including the skin and brain. It is most commonly used in traditional medical system to treat skin disorders and upper respiratory infection; however newer research discovered an even more useful ability. That is to protect the brain cells from damage.

Other uses of Polypodium Decumanum include treatment for vitiligo, kidney disease, cleansing the blood, an addition to detox formulation, improving and modulating the immune system, arthritis, and cancer. Polypodium Decumanum contains flavonoids, alkaloids and lipids. It is a rich source of lipids and fatty acids. The main plant chemicals identified in Samambaia adenosine, alkaloids, arachidonic arabinopyranosides, calagualine, ecdysone, ecdysterone, eicosapentaenoic acid, elaidic acid, juglanin, kaempferols, linoleic acids, linolenic acids, melilotoside, oleic acid, ferulic acid, polypodaureine, ricinoleic acid, rutin, selligueain, and sulphoquinovosyl diacylglycerols.

MATERIAL AND METHOD

The *Polypodium decumanum* used in this study was procured from local market in indore and authenticated by Dr. D.K Srivastava, Scientist, College of Agriculture, ICAR division.

Preparation of extract

In the continuous hot extraction method the plant leaves extracted in Ethanol in 3 regular days at the temperature of 78-80 °C. The mixture was subsequently filtered and concentrated under reduced pressure at 40 °C. The extract yield was 26% w/w.

Animal

Albino mice 200-250 g obtained from Institute of Pharmacy, Bundelkhand University, Jhansi, India were housed in animal house (App. No. 716/02/a/CPCSEA) with 12/12 h light/dark cycle at 21±20C and fed with laborator pellet chow and given water ad libitum. Animals were acclimatized to their environment for one week prior to experimentation. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United

States Guidelines (United States National Institutes for Health Publication No. 85-23, revised in 1985) and our ethical committee on animal care approved the protocol.

Induction of Diabetes in Mice

After 15 h fasting mice were injected in intraperitoneally with alloxan monohydrate (Sigma chemical, USA) dissolved in sterile normal saline at a dose of 120 kg⁻¹ body weight. To confirm diabetes, glycemia was daily determined after the administration of the last alloxan dose. Depending on their fasting blood glucose (FBG) level, the animals were divided arbitrarily in to two groups⁹, mild diabetic (MD) animals with FBG of 120-250mg dl⁻¹, severaly diabetic (SD) animals showing FBG 250-300mg dl⁻¹.

Estimation

Blood glucose level was estimated by using one touch Glucometer (Accu-chek censor) of Roche Diagnostic, Germany for regular check-up. Blood sample were collected from tail veins.

Biological Assays

Oral glucose tolerance test (OGTT)

Animals were fasted 18 hrs before the day of experiment with free access to water and were separated in 5 groups of 10 rats each. Animals of all groups were treated with an oral D-glucose load of 2 gm kg⁻¹ by means of cannula, group third and fourth were treated orally with ethanolic extract at a dose of 250 mg kg⁻¹ b.w. and 500 mg kg⁻¹ b.w each for 30 min before the oral administration of oral glucose load. Control animals were treated with vehicle, blood sample were withdrawn from the cordal(tail) vein of each animals just after oral glucose administration 0 min, 30 min, 90 min and 120 min after glucose challenge and the fifth group received Glibenclamide (200 mg kg⁻¹) as positive control.

Statistical analysis

All biological results expressed as mean \pm SEM. Significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett-t test. Statistical significance was considered at P< 0.05.

RESULT

The plant extract of *Polypodium decumanum* leaves (ethanolic extract) showed antidiabetic activity by reducing blood glucose level significantly. It is also much effective when compare with the standard drug Glibenclamide. It reduce blood glucose level up to 194.1, 119.4 and 111 mg dl⁻¹ at successive days of 7,14,21 at the dose of 500 mg kg⁻¹ in rats compare with the standard drug which reduce blood glucose level up to 256.6,140.7 and 101.2. We found that the ethanolic extract of plant *Polypodium decumanum* leaves is 9-10 % less effective in reducing the blood glucose level compare to the standard drug (Glibenclamide).

LD_{50}

The extract of the test substance (*Polypodium decumanum*) was found to be safe for further biological

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studies as no toxic effect and lethality was observed up to 3000 mg kg⁻¹ per oral in mice. Only the consumption of food was increased by 20 % in the dose of 2000 and

3000 mg kg⁻¹·during 4h, but remaining normal afterwards.

Table 1: Effect of Polypodium decumanum leaves (Ethanolic extract) on body weight

Treatment	Body weight in gm					
	1 day	7 day	14 day	21 day		
Normal mice	169 <u>+</u> 4.5	170 <u>+</u> 4.9	173 <u>+</u> 5.5	178 <u>+</u> 5.4		
Control Diabetic Mice	171 <u>+</u> 7.5	156 <u>+</u> 8.5	145 <u>+</u> 7.5	139 <u>+</u> 7.3		
Diabetic Mice + PD I	168 <u>+</u> 5.5*	161 <u>+</u> 5.5*	175 <u>+</u> 5.7*	180 <u>+</u> 5.6*		
Diabetic Mice + PD II	140 <u>+</u> 5.6*	133 <u>+</u> 7.5*	146 <u>+</u> 7.7*	159 <u>+</u> 7.8*		
Diabetic Mice + SD (Glibenclamide)	135 ± 7.4*	142 <u>+</u> 8.6*	150 ± 7.6*	165 <u>+</u> 6.5*		

(Values are mean \pm SEM, (n=5) *p< 0.05 when compare with control

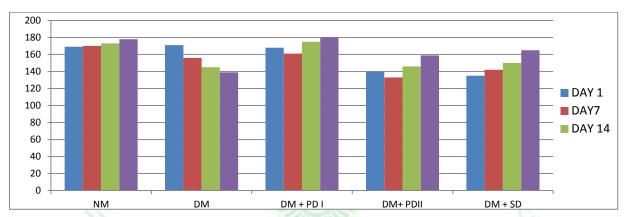


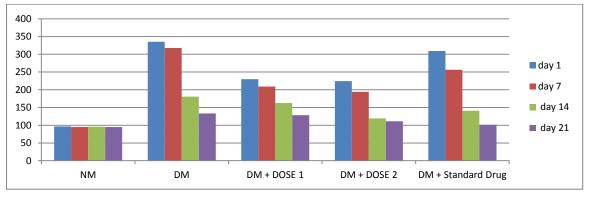
Figure 1: showing the effect of Polypodium decumanum plant extrat on body weight

Fig. Bar showing body weight of treated (extract of Polypodium decumanum) and non treated, normal and diabetic mice with the standard drug (Glibenclamide) at day 1,7,14 and 21 day of growth.(NM= normal mice, DM= diabetic mice, PD=Polypodium decumanum plant extract)

Table 2: Effect of Polypodium decumanum (Ethanolic extract) on Blood Glucose Level (mg/dl)

Treatment	Blood glucose level			
	1 day	7 day	14 day	21 day
Normal mice	96.5 <u>+</u> 1.77	95.0 <u>+</u> 2.56	95.8 <u>+</u> 1.97	94.7 <u>+</u> 2.01
Control Diabetic Mice	335 .5 <u>+</u> 2.59	317.6 <u>+</u> 2.78	180.7 <u>+</u> 3.21	133.3 <u>+</u> 2.10
Diabetic Mice + PD I(250mg/kgBW)	229 .8 <u>+</u> 3.45*	209 <u>+</u> 3.90*	162.4 <u>+</u> 3.76*	128.2 <u>+</u> 2.87*
Diabetic Mice + PD II(500mg/kg BW	224 .2 <u>+</u> 2.53*	194.1 <u>+</u> 2.09*	139.4 <u>+</u> 3.21*	111 <u>+</u> 2.32*
Diabetic Mice + SD (Glibenclamide)	309 <u>+</u> 7.4*	256.6 <u>+</u> 8.6*	140.7 <u>+</u> 7.6*	101.2 <u>+</u> 6.5*

(values are mean \pm 7), (n=5) *p<0.05 when compared with control



(NM= normal mice, DM= diabetic mice, dose 1= 200mg/kg b.w plant extract, dose 2= 500 mg/ kg b.wplant extract)

Figure 2: showing the effect of plant extract of Polypodium decumanum plant extract on blood glucose level

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DISCUSSION

The result showed important reduction of blood glucose level in healthy mice when administered the ethanolic extract of Polypodium decumanum extract by oral route. Ethanolic extract showed a dose-dependent effect on FBG up to a dose of 500 mg kg⁻¹. After daily treatment with the dose (250 and 500 mg kg⁻¹) of ethanolic Polypodium decumanum extract for 21 days to severely diabetic (FBG 300-350 mg dl⁻¹) mice, the BGL reduced to 128.2 mg dl⁻¹ and 111 mg dl⁻¹ by 250 and 500 mg kg⁻¹ respectively. The findings of our study indicate the hypoglycemic and potential antihyperglycemic nature of the extract. It is not as much effective when compare to standard drug but its effect quite significant because it reduce the BGL 194.1 mg dl⁻¹, 139 mg dl⁻¹and 111 mg dl⁻¹ with the dose of 500 mg kg⁻¹ at successive days of 7.14 and 21 of treatment with ethanolic extract of Polypodium decumanum. In the study on the effect of body weight the present study also showed that the body weight os the mice increased by 133, 146 and 159 gm at the successive days of 7.14 and 21 when given the treatment of ethanolic extract of Polypodium decumanum at the dose of 500 gm kg⁻¹ compare to standard drug Glibenclamide which increase the body weight by 142, 150 and 165 gm at the successive days of 7,14 and 21. So we found that the plant extract increases the body weight significantly when given the plant extract dose 500 mg kg⁻¹ when compare to 250 mg kg⁻¹. Recently it is reported that extract of Polypodium decumanum leaves exhibit significantly radical scavenging activity and thus antioxidant activity and the present finding indicates that administration of extract of Polypodium decumanum leaves confirms the possibility that the major function of the extract is on the protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in these animals. So protective effect of extract of Polypodium decumanum on alloxan induced diabetes mice could be attributed directly to scavenging activity and for more extent to the regeneration properties of the extract.

CONCLUSION

In conclusion our study indicates that extract of *Polypodium decumanum* produced antihyperglycemic effect in experimental diabetes by providing a regenerative modification against damage caused by alloxan to endocrine cells of the pancreas. The use of this plant in diabetes id the supported but the active substances and cellular and molecular mechanism(s) of this pharmacological effect are still to be determined.

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