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ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF RHIZOME OF DRYOPTERIS COCHLEATA

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ABSTRACT

Objective: To evaluate the antidiabetic effects of ethanolic extracts of Dryopteris cochleata rhizome (EDC) in Wister rats. **Methods:** Alloxan induced diabetic test model were performed to evaluate antidiabetic activity of EDC at two different doses 100 and 200 mg/kg respectively. Ethanolic extract of rhizome of Dryopteris Cochleata (Dryopteridaceae) was tested for anti-diabetic activity for alloxan induced diabetics in wistar rats. After oral administration of the extract at two different doses (100 and 200mg/kg body weight) for 15 days to alloxan-induced diabetic rats, the blood glucose, level was assayed periodically on 0, 7th and 15th day. After 15 days treatment all biochemical parameters like total cholesterol, triglyceride, total protein levels were checked and compare with control and standard group and body weight was also determine which was compare with initial weight. **Results:** Ethanolic extract shown significant protection and lowered induced blood glucose level to normal in glucose tolerance test on the day 0,7th and 15th compare with diabetic control and standard groups. There was significant control of all biochemical parameters levels like total cholesterol, triglyceride, total protein in extracts of Dryopteris cochleata treated diabetic rats. Marked body weight loss was observed in diabetic rats. The data obtained from this study showed that the treatment of extracts Dryopteris cochleata protect the diabetic rats from loss of massive body. **Conclusions:** These results indicate that the rhizome of Dryopteris cochleata posses significant anti-diabetic activity.

KEYWORDS: Antidiabetic activity, Dryopteris cochleata, Dryopteridaceae, Alloxan- induced diabetis, biochemical parameters.

1. INTRODUCTION

Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders. Diabetes mellitus is ranked seventh among the leading causes of death in the world and is considered third when its fatal complications are taken into account. If not cured or controlled it may even lead to acute or chronic complications. By the year 2025, India shall have the maximum number of diabetes in the world making it, the "Diabetes capital of the world". It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic β cells and deficiency in insulin secretion, insulin activity or both. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be.

Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment.[1]

Dryopteris cochleata that belongs to the family Dryopteridaceae is considered to possess great medicinal value found at higher altitudes in semi-exposed, well shaded localities in forest and grasslands native to India, Pakistan, Srilanka and Southeast Asia east to Java. It has been reported to possess wide ethanomedical use, whose rhizome has antifungal property and is used as an antidote. It also has variety of applications against the disorders like epilepsy, leprosy, cuts, wounds, ulcers, swelling, pains and snake bites. The decoction of the dried rhizome, stem and stripe is used for blood purification[2]. The juice of fronds is used to treat muscular and rheumatic pain[3]. The leaves of the plant are used for the treatment of epilepsy.[4-5] The whole

plant is crushed in a bowl and their extract is given twice a day orally, in case of snake bite, besides a paste of the plant is also applied on the bite wound to prevent infection.[6] A small portion of the rhizome of the plant is powdered and taken with water twice a day in rheumatism, epilepsy and leprosy.[7]

2. MATERIALS AND METHODS

2.1. Plant materials and extraction

The rhizome part of Dryopteris cochleata was collected from Amarkantak forest district Bilaspur, Chattisgarh, in the month of July 2013.It was further identified and authenticated by the Botanical Department, Saifia Science College, Bhopal, Madhya Pradesh, Ref no. 476/Bot/Saf/13. The collected rhizomes were washed with tap water, prior to distilled water, shade dried and coarsely powdered using a cutter mill, extracted with petroleum ether for defatting followed by ethanolic extraction using hot continuous percolation for 16 hrs. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

2.2. Experimental animals

To evaluate the antidiabetic activity of ethanolic extracts of D. cochleata rhizome parts (EDC), Wistar rats weighting between (150-200) g were brought from animal house of TIT college, Bhopal, Madhya Pradesh. Standard laboratory conditions were maintained for the experiments. Drinking water ad libitum and standard diet were supplied for the animals. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), Reg No. TIT/IAEC/831/P'Col/2015/56 constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.3. Chemicals

Commercially available analytical grade chemicals and drugs like alloxan monohydrate (Thomas Baker Pvt.Ltd., India), Glibenclamide tablets (Daonil; Aventis Pharma. Ltd., India) were procured from the authorized distributor of the company.

2.4. Experiments

The overnight fasted rats were made diabetic by induced a single intraperitoneal injection of 120 mg/kg body weight of alloxan monohydrate in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. These animals were tested for diabetes and only those animals with blood glucose (fasting) level above 250mg/dl were selected for experimentation[8]. Diabetic induced rats were randomly divided in to five experimental groups and each with six rats marked as Group I to V. Group II indicated as diabetic control supplied only distilled water. Reference antidiabetic drug glibenclamide (600µg/kg p.o) was provided for Group III marked as positive control. Group IV and V were treated with EDC at two different doses of 100 and 200 mg/kg respectively for 15 days. Previously selected Group I marked as control which had no diabetes. BGL of all groups of rats was measured at 0,7th and 15th day during the experiment. Total cholesterol, Triglyceride, Total protein levels were measured after 15 days of treatment and compare to diabetic control group. After 15 day, body weight of all groups of rats was determined and compare with initial weight.

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 15 days.
Group IV: Rats (diabetic) were administered extract of Dryopteris cochleata (EDC) (100 mg/kg p.o.) for 15 days.
Group V: Rats (diabetic) were administered extract of Dryopteris cochleata (EDC) (200 mg/kg p.o.) for 15 days.

2.5. Statistical analysis

The data are expressed as mean ± SEM (n=6). Statistical significance was determined by two way ANOVA followed by Bonferroni post-tests with $P < 0.001$ and $P < 0.05$ and one way ANOVA followed by Tukeys post-tests with $P < 0.001$ and $P < 0.05$ considered significant.

3. RESULTS

Ethanolic extract of rhizome of Dryopteris cochleata was subjected to anti-diabetic activity in rats where Alloxan monohydrate (120 mg/kg b.w., i.p.) was used as the diabetogenic agent. A marked rise in blood glucose level was observed in diabetic control compared to normal control rats. Ethanolic extract of Dryopteris cochleata (100 and 200 mg/kg) exhibited a dose dependent significant anti-hyperglycaemic activity on 0, 7th, 15th day post treatment as compare with reference standard, Glibenclamide. The result is depicted in Table 1 and Fig. 1. Total cholesterol, Triglyceride, Total protein, levels were decreased significantly in a dose related manner by ethanolic extract of Dryopteris cochleata (100 and 200 mg/kg) after 15 days of treatment, compare to diabetic control group (Table 2, 3, 4 and Fig.2, 3, 4). Changes in initial and final body weight of normal control and experimental groups are shown in Table 5 and Fig 5. Marked body weight loss was observed in diabetic rats.

Table 1 Effect of EEDC on Blood Glucose Level (BGL) in Alloxan induced diabetic rats

Group	Treatment	Blood Glucose (mg/dl)		
		Day 0	Day 7	Day 15
I	Normal	90.00 ± 5.00	98.00 ± 5.10	105.00 ± 5.12
II	Diabetic Control	270.15 ± 11.15	281.10± 12.10$^{\#}$	297.00 ± 12.16$^{\#}$
III	Diabetic + Glibenclamide (600µg/kg)	245.00 ± 3.00	147.00 ± 2.30***	110.04 ± 2.00***
IV	Diabetic + EDC (100 mg/kg)	252.00 ± 3.50	150.00 ± 2.16**	129.00 ± 2.16**
V	Diabetic + EDC (200 mg/kg)	249.00 ± 3.00	148.00 ± 2.17**	119.98 ± 2.52***

Values are expressed as mean ±S.E.M (n = 6).Values are statistically significant at $^{\#}$ P <0.001 vs. normal group; *P < 0.05 vs. diabetic control group (Two-way ANOVA followed by Bonferroni post-tests).

Table: 2 Antidiabetic effect of Dryopteris cochleata treatment on body weight

Group	Treatment	Initial weight(gm)	Final weight(gm)
I	Normal	150.00 ± 8.00	207.10 ± 9.00
II	Diabetic Control	170.00 ± 8.00	153.00 ± 8.00
III	Diabetic + Glibenclamide (600µg/kg)	160.00 ± 7.80	190.00 ± 7.00*
IV	Diabetic + EDC (100 mg/kg)	161.00 ± 9.00	200.00 ± 8.00*
V	Diabetic + EDC (200 mg/kg)	170.00 ± 7.00	205.00 ± 8.00*

Values are expressed as mean ± SEM of six samples from each group. (Two-way ANOVA followed by Bonferroni post-tests).

Table 3: Effect of Dryopteris cochleata treatment total cholesterol in normal and diabetic rats

Group	Treatment	TC(mg/dl)
I	Normal	90.00 ± 9.5
II	Diabetic Control	186.00 ± 5.5
III	Diabetic + Glibenclamide (600µg/kg)	99.00 ± 3.00**
IV	Diabetic + EDC (100 mg/kg)	105.00 ± 5.00**
V	Diabetic + EDC (200 mg/kg)	103.00 ± 4.60**

Values are expressed as mean ±S.E.M (n = 6).Values are statistically significant at $^{\#}$ P <0.001 vs. normal group; *P < 0.05 vs. diabetic control group (one-way ANOVA followed by Tukeys post-tests).

Table 4: Effect of Dryopteris cochleata treatment on triglyceride level in normal and diabetic rats

Group	Treatment	TG (mg/dL)
I	Normal	76.50 ± 3.50
II	Diabetic Control	122.0 ± 8.00
III	Diabetic + Glibenclamide (600µg/kg)	81.00 ± 6.00*
IV	Diabetic + EDC (100 mg/kg)	88.50 ± 4.50*
V	Diabetic + EDC (200 mg/kg)	83.00 ± 5.00*

Values are expressed as mean ±S.E.M (n = 6).Values are statistically significant at $^{\#}$ P <0.001 vs. normal group; *P < 0.05 vs. diabetic control group (one-way ANOVA followed by Tukeys post-tests).

Table 5: Effect of Dryopteris cochleata treatment on total protein level in normal and diabetic rats

Group	Treatment	Total protein(g/dl)
I	Normal	8.5 ± 0.5
II	Diabetic Control	4.7 ± 0.7
III	Diabetic + Glibenclamide (600µg/kg)	8.7 ± 0.2**
IV	Diabetic + EDC (100 mg/kg)	8.4 ± 0.4**
V	Diabetic + EDC (200 mg/kg)	8.5 ± 0.5**

Values are expressed as mean ±S.E.M (n = 6).Values are statistically significant at $^{\#}$ P <0.001 vs. normal group; *P < 0.05 vs. diabetic control group (one-way ANOVA followed by Tukeys post-tests.

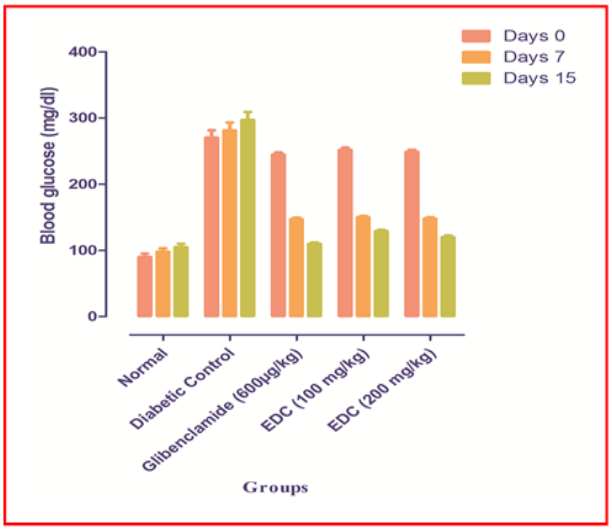

Fig: 1. Antidiabetic effect of Dryopteris cochleata treatment on blood glucose (mg/dl) in normal and diabetic rats.

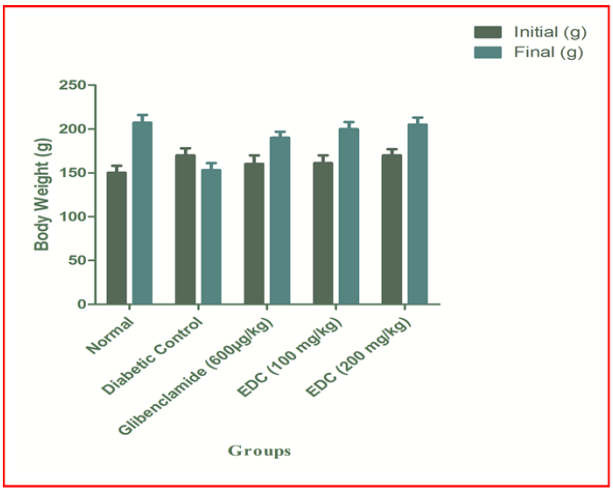

Fig 2. Antidiabetic effect of Dryopteris cochleata treatment on body weight

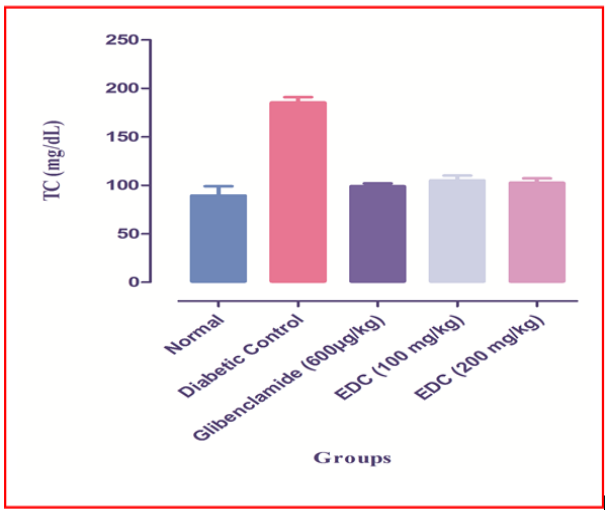

Fig. 3: Effect of Dryopteris cochleata treatment total cholesterol in normal and diabetic rats

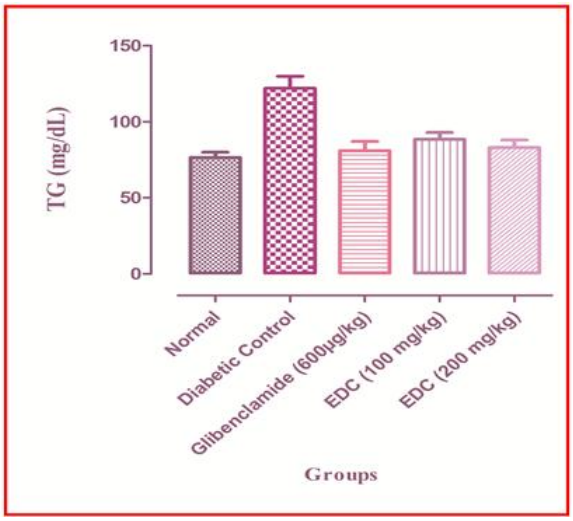

Fig.4: Effect of Dryopteris cochleata treatment on triglyceride level in normal and diabetic rats

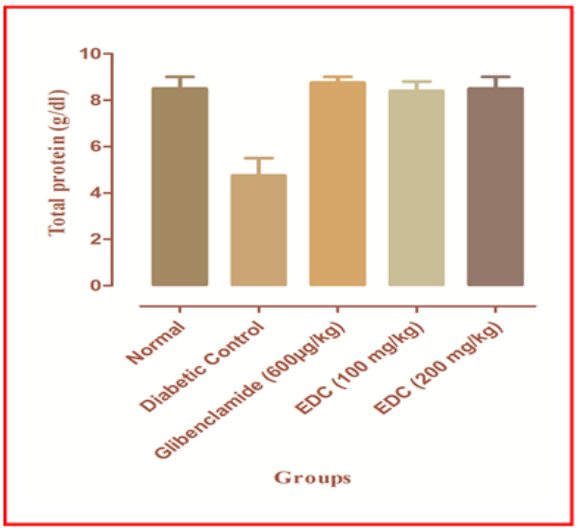

Fig .5: Effect of Dryopteris cochleata treatment on total protein level in normal and diabetic rats

4. DISCUSSION

Current study focused the effect of different doses of Dryopteris cochleata extract and comparison of the effects with those of a single dose standard antidiabetic drug in induced diabetic condition. Presence of phytochemical metabolites in extract was also assessed to assume their role in antihyperglycemic activity. The possible mechanism of action of extracts could be correlated with the suggestive effect of the reference antidiabetic drug glibenclamide that promotes insulin secretion by closure of K^{+}-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion[9]. Other possible mechanisms by which the plant extracts lowered blood glucose may be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption glucose from the intestine. It is noted that the induction with alloxan of same dose to different groups of rat is also varied. The same object may be implied to the administration and effect of extracts and glibenclamide.

A marked increase in total cholesterol and triglyceride level has been observed in diabetic control rats. Insulin deficiency results in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia[10-11]. There was a significant control of the levels of serum lipids in extracts of Dryopteris cochleata treated diabetic rats. A marked decrease in total protein level has been observed in diabetic control rats. There was a significant increase of the levels of protein in extracts of Dryopteris cochleata treated diabetic rats.

Marked body weight loss was observed in diabetic rats. The data obtained from this study showed that the treatment of extracts Dryopteris cochleata and glibenclamide protects the diabetic rats from massive body.

5. ACKNOWLEDGMENT

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