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EFFECT OF *SELAGINELLA BRYOPTERIS* ON DIABETIC SWISS ALBINO MICE CAUSED BY ALLOXAN

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ABSTRACT :

Diabetes mellitus is a disorder in which pancreas is unable to produce considerable amount of insulin leading to increase in blood glucose level. The International Diabetes Federation (IDF) estimated that around 15.8 million people currently in India have diabetes and will rise up to 87 million by 2030. Therefore, there is an urgent need to develop novel therapeutic agents for the treatment of diabetes, without the development and progression of complications. It is a group of disorders of carbohydrate metabolism in which the action of insulin is diminished or absent through altered secretion. It is characterized by hyperglycemia leading to severe complications. *Selaginella bryopteris* has various remedial properties *i.e.*, antimicrobial, anti-inflammatory, ant-allergic, hepatoprotective and anti-apoptotic activities. Alloxan (120mg/dl) was administered intra-peritoneal to Swiss albino mice and after establishment of diabetes, aquous extract of *Selaginella bryopteris* (150mg/kg b.w) was orally administered for 26 day. It was noticed that *Selaginella bryopteris* reduces the level of glucose, increases the decreased body weight of mice and release the shrunken pancreas.

KEY WORDS : *Selaginella bryopteris, International Diabetes Federation, Hepatoprotective and anti-apoptotic activities.*

INTRODUCTION:

Diabetes is a chronic disorder. It may be caused by either little or cease insulin production, or becomes progressively resistant to its action (Ranjan, *et al.*, 2002). Such types of disorder affects metabolism of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance (Barar, *et al.*, 2000; and Devlin, *et al.*, 1997). Now it becomes an epidemic with a worldwide incidence of 5% in the general population. The number of adult diabetes person in the world will rise from 135million in 1995 to 300 million in the year 2025 (Torban, *et al.*, 2002). The countries with the largest number of

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diabetic people in the year 2025 will be in India, China and United States (Ramchandran, *et al.*, 2002). There are more than 30 million people with diabetes mellitus in India and the incidence is increasing (Shankar, *et al.*, 2001) but many patients in the community are undiagnosed. Main disabler and killer in the next coming 25 years will be due to diabetes (Edvin, *et al.*, 2006). *Diabetes mellitus* is characterized by abnormal metabolic regulation of both carbohydrate and lipids resulting in hyperglycemia and hyperlipidemia. Due to altered and decrease in secretion of insulin by the β– cells of the islets of Langerhans in the pancreas. As the tissue damage leading to severe complications *i.e.*, Retinopathy, Neuropathy, Nephropathy, Cardiovascular disease and Foot ulceration. Patients with diabetes experience significant morbidity, mortality from micro vascular (Retinopathy, neuropathy, nephropathy) and macro vascular complications (heart attack, stroke and peripheral vascular disease). The complications are far less common and less severe in people who have well-controlled blood sugar levels (Andrew, *et al.*, 2000). Acute complications include diabetic ketoacidosis, nonketotic hyperosmolar coma and diabetic coma. In case of chronic complication, chronic elevation of blood glucose level leads to damage of blood vessels. In diabetes, the resultant problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macro vascular disease" (due to damage to the arteries) (Andrew, *et al.*, 2000). Microvascular disease leads to retinopathy, neuropathy and nephropathy (Nephropathy leads to anaemia) (Halder, *et al.*, 2003, Merlin, *et al.*, 2005). Macro vascular disease leads to cardiovascular disease, mainly by accelerating atherosclerosis. These disorders include: (1) Coronary artery disease, leading to myocardial infarction (heart attack) or angina, (2) Stroke (mainly ischemic type), (3) Peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot (Andrew, *et al.*, 2000).

In of great efforts that have been made in the understanding and management of diabetes and disease related complications are increasing unabated. *Diabetes mellitus* represents a heterogeneous group of disorders causing hyperglycemia, which is due to impaired carbohydrate "glucose" utilization resulting from a defective or deficient insulin secretary response. Along with hyperglycemia, there are also abnormalities in serum lipids (Reaven, *et al.*, 1988). The disease causes morbidity and long-term complications and an important risk factor for cardiovascular diseases (Yeh, *et al.*, 2003). Now a days, there are different groups of oral hypoglycemic drugs and insulin for clinical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the modern medical system. This leads to increasing the demand for complementary and alternative medicine with antidiabetic activity to overcome the side effects and toxicity of synthetic

drugs. There are many reports of herbal extracts being used in Ayurvedic literature as antidiabetic which are directly or indirectly used for the preparation of many modern drugs. However, these plants have not gained much importance as medicines and one of the factors is lack of specific standards being prescribed for herbal medicines and supportive animal/clinical trials. The problem becomes complicated when several active compounds are involved in the medicinal action. Alloxan (2, 4, 5, 6-Pyrimidinetetrone) is a toxic compound, glucose analogue which selectively destroys insulin producing β-cells in the pancreas when administered to the rodents. It accumulates in β-cells through uptake via the GLU2 transporter and glucose level drastic increased. *Selaginella bryopteris* is pteridophyte. Its common name is "Sanjeevani". It has medicinal properties, like relief from heat stroke and the burning sensation during urination, in treatment of jaundice, restoring menstruation irregularities, anti-cancer, anti-apoptotic, anti-fungal, anti-bacterial, and anti-viral, activity but procurement of diabetes is not reported. Therefore, in the study "effect of *Sellaginella bryopteris* against diabetes" is proposed.

MATERIALS AND METHODS:

Preparation of plant extracts:

Aqueous extract of *Selaginella bryopteris* was prepared by shocking 40gms of air dry clean *S. bryopteris* in 200ml distilled water for 12hr at room temperature. Aqueous substance was filtered and finally dried in a vacuum desiccator. Different concentration stock solution was prepared and LD_{50} was calculated.

Animals:

6 weeks aged in bred Swiss albino mice with average body weight 22 ± 2gm were obtained from animal house of University Department of Zoology, T. M. B. U., Bhagalpur (Bihar) India and approval of the ethical committee was sought prior the commencement of the experiment, L. N. M. U., Darbhanga (Bihar). Food and water to mice were provided *Ad libitum* (prepared mixed formulated feed by the laboratory itself). Mice were housed in colony rooms with 12 hrs light/dark cycle at 22 ± 2° C and were grouped in four different groups.

EXPERIMENTAL PROTOCOL:

Mice were grouped into four different groups. Group-I Normal Control, Group-II Alloxan Control, Group-III Alloxan + Insulin, Group-IV Alloxan + Selaginella. Alloxan (120mg/dl) was prepared in distilled water and administered intra-peritoneal at the interval of 48 hrs, diabetes was confirmed by blood sugar test, with the help of glucometer (Lever check Pvt. Ltd.) and bio-chemical method. Animals have more than 200mg/dl blood sugar level was selected for the further study and maintained for next 4 days in diabetic condition for well establishment of diabetes. Insulin (0.05U/Mouse) (Brezar, *et al.*, 2012) at the interval of 72 hrs was administered intra-peritoneal and *S. bryopteris* (150mg/kg bw) for next 22 days.

HISTOLOGICAL EXAMINATION :

Histological examination was done by fixing the organs of the rabbits in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris Haematoxylin and Eosin (Luna, 1968). However, to demonstrate pancreatic islet cells, Gomoris modified stain (Halami, 1952) was used with a modification of substituting the Lugol's iodine by equal parts of 0.5% $KMNO_4$ and 0.5% sulphuric acid, and sodium thiosulphate by 2% sodium bisulphite respectively.

RESULT AND DISCUSSION:

Table 1 presents the data of various biochemical parameters of peripheral blood serum of different groups of Swiss albino mice. Group - I Healthy Normal animals, Group - II Untreated diabetes induced animals, Group - III Diabetes animals treated with aqueous extraction of *S. bryopteris*, Group - IV Diabetes animal treated with aqueous extract of S. bryopteris. Experimental results showed a significant increase in blood glucose level in the alloxan treated diabetic group by compared to that of normal control. While the oral administration of aqueous extract of S. bryopteris showed decreased glucose concentration (Table-1) but as compared to insulin treated group glucose concentration was decreased slowly. Histological results also shown that S. bryopteris help in cure the shrinkage of pancreas damaged due to alloxan (fig. 1a-d).

Table 1: Different groups of mice showing changes in glucose leavel (mg/dl) during the treatment (data represent mean value of six replicates).

Days	Group 1	Group 2	Group 3	Group 4
0	112	116	113	115
2	112	128	129	130
4	112	168	171	175
6	112	175	165	170
8	112	179	155	165
10	112	188	145	160
12	112	221	135	155
14	112	237	120	150
16	112	250	117	145
18	112	255	118	140
20	112	277	117	135
22	118	350	116	130
24		kill		125
26				120
				117

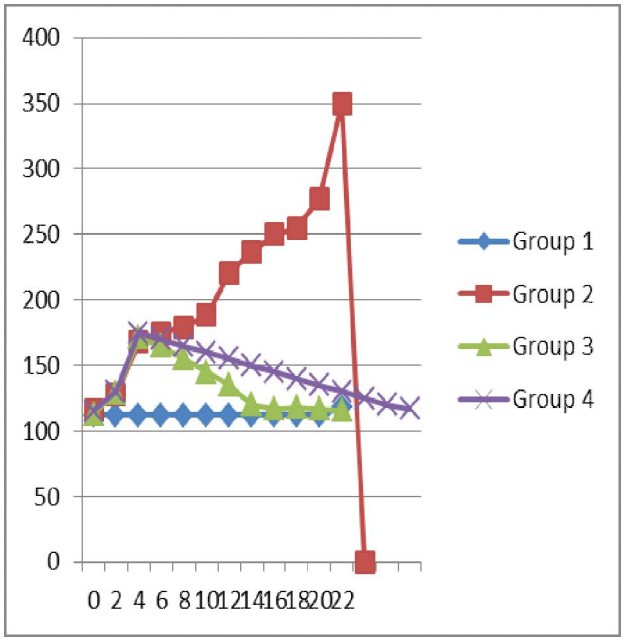

Table 2: Different groups of mice showing changes in weight (gms) during the treatment (data represent mean value of six replicates)

	Body weight (gm)			
Days	Group 1	Group 2	Group 3	Group 4
0	23.4	22.1	23	24.3
2	23.4	22	21	21.5
4	23.5	21	20.5	20
6	23.4	21.2	20	20.5
8	23.2	21	20.5	20.7
10	23.4	20	20.7	20.9
12	23.4	19	21	21
14	23.5	18	21.5	21.5
16	23.4	17	21.5	22
18	23.4	16.5	22	22.5
20	23.5	16	22.5	23.5
22	23.2	15.5	22.5	23.8
		kill		

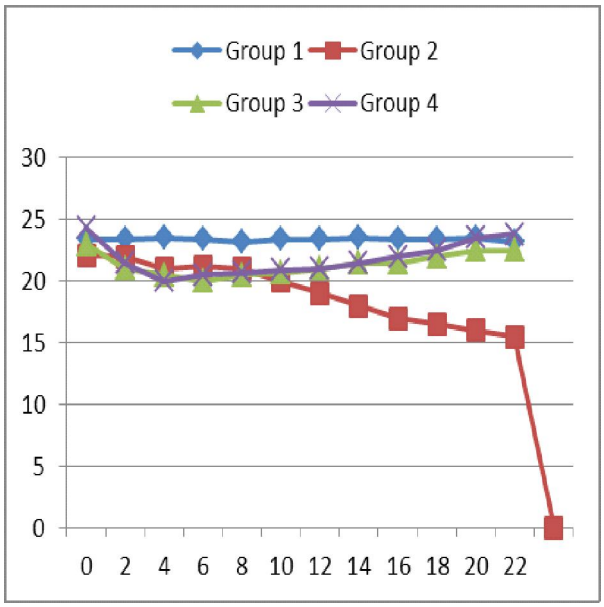

Fig. 1 Histological slide of Pancreas of different groups of diabetic mice

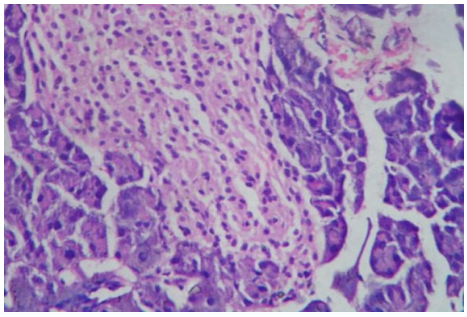

Fig. a: Normal control

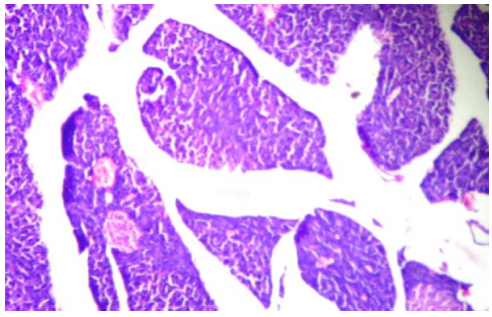

Fig. b Alloxan control

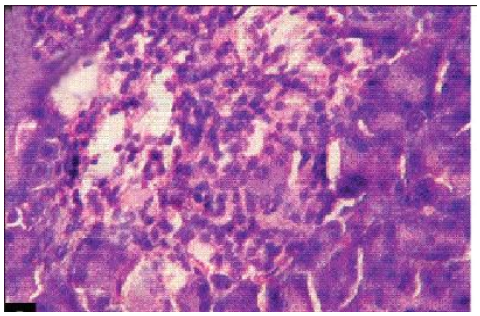

Fig. c Effect of Insulin in Alloxan treated control group

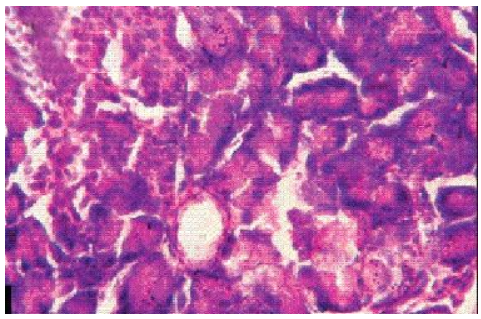

Fig. d Effect of *S. bryopteris* in Alloxan treated group

CONCLUSIONS:

Selaginella bryopteris has potential to cure diabetes. It controls the glucose level and may be recovered the damage of β – cells of pancreas induced by Alloxan. β-cells are revived and produce insulin in spite of stopping the dose of *S. bryopteris*. These results show *S. bryopteris* may be effective in the treatment of the *Diabetes mellitus.*

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REFERENCES:

1. Brezar V., Culina S., Gagnerault M. C. and Mallone R., Short-term subcutaneous insulin treatment delays but does not prevent diabetes in NOD mice. *Eur J. Immunol.* 2012; 42 (6): 1553 - 61.
2. Sethi J., Sood S., Seth S. and Talwar A., Evaluation of Hypoglycemic and Antioxidant Effect of *Ocimum sanctum*. *Indian Journal of Clinical Biochemistry,* 2004; 19 (2): 152 - 155.
3. Lenzen S., The mechanisms of alloxan- and streptozotocin-induced diabetes, Diabetologia, 2008; 51(2): 216 - 26.
4. Rai V., Mani U. V. and Iyer U. M., Effect of *Ocimum sanctum* Leaf Powder on Blood Lipoproteins, Glycated Proteins and Total Amino Acids in Patients with Noninsulin-dependent Diabetes Mellitus. *Journal of Nutritional & Environmental Medicine*. 1997; 7(2): 113 -118.
5. Pankaj S., Does the Magical Himalayan Herb "Sanjeevani Booti" really exist in Nature? The *Journal of American Science,* 2008; 4(3).
6. Sah N. K., Singh S. N. P., Sahdev S., Banerji S., Jha V., Khan Z. and Hasnain S. E., Indian herb 'Sanjeevani' (*Selaginella bryopteris*) can promote growth and protect against heat shock and apoptotic activities of ultra violet and oxidative stress; *J. Biosci*. 2005; 30: 499-505.
7. Sah N. K., Singh S. N. P., Sahdev S., Banerji S., Jha V., Khan Z. and Hasnain S. E. Indian herb 'Sanjeevani' (*Selaginella bryopteris*) can promote growth and protect against heat shock and apoptotic activities of ultra violet and oxidative stress, *J. Biosci*. 2005; 30: 499 - 505.
8. Chikmawati T., Setyawan D. A., and Makara M., Phytochemical Composition of *Selaginella spp.* from Java Island Indonesia. *Journal of Science*. 2012; 16(2)129 - 133.

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