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Hypolipidemic activity of *Trichopus zeylanicus* leaf extract against alloxan induced diabetes mellitus in male albino rats.

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Abstract

Trichopus zeylanicus is a plant with adaptogenic properties. This experiment with three groups of rats was designed to evaluate the beneficial properties of T. zeylanicus consumption on LPO, AO, blood glucose regulation and lipid profile in albino rats in alloxan induced stage. For the study, the male albino rats were divided into three groups normal, alloxan treated and alloxan along with the plant treated group. The Intraperitonial injection of alloxan with two days caused diabetes mellitus and the third group had plant treatment up to 5 days. Effects of T. zeylanicus consumption on LPO, antioxidant, GSH, glucose and lipid profile were also evaluated. The plant treatment had remarkable effects on LPO, GSH and glucose level in the male albino rats. An improvement in lipid profile was observed with lower plasma LDL and total- cholesterol levels as well as higher HDL cholesterol after 5 days of T. zeylanicus leaf extract treatment. Thus the leaf extract of T. zeylanicus was found to have hypolipidemic activity.

Keywords*: *Trichopus zeylanicus* Gaertn; *Diabetes Mellitus ; lipid profile; male albino rats; antidiabetic effect.

Introduction

In recent times, focus on plant research has been intensified all over the world and a large amount of evidence has been collected to show immense potential of medicinal plants used in various traditional systems .In Indian system of medicine, a large number of herbal drugs have been advocated for various types of diseases/ stress related disorders. Herbal and especially herbal extracts are very attractive not only in modern phytotherapy but also for adaptogens. *Trichopus Zeylinicus* is a adaptogenic plant.

Trichopus zeylanicus Gaerten (*Trichopodaceae)* is a wild plant, a rare genus ,small glabrous herd growing in the Agasthyar hilly forests of kerala, India. The tribal inhabitants (kani tribe) of this area call this plant Arogyappacha meaning the greener of health and use this plant as a health tonic and rejuvenator. This information is based on ethno-medico-botanical investigations (Pushpangadan et al., 1988; Evans et al.,2002) Earlier studies on *T.zeylanicus* in experimental animals have shown several pharmacological activities such as hepatoproetection, anti-ulcer activity anti- fatigue ,anti-inflammatory activity, antioxidant activity, aphrodisiac activity, anti-stress enhancement of swimming performance and immunomodulation (Sharma et al. 1989; Subramoniam et al., 1997; Singh *et al.*, 2001; Singh *et al*.,2005).Therefore this plant was taken for treatment analysis on diabetes induced hyperlipidmic condition in the present study.

Diabetes mellitus is a serious health problem characterized by deficient plasma glucose regulation due to tissue insulin resistance and /or β-cell failure which causes high morbidity and mortality rates. Type-2 diabetes accounts for the majority cases of diabetes (90%) and is becoming more prevalent due to the increasing rates of obesity in youth and adulthood and sedentary lifestyles.Dyslipidemia is also common among diabetic patients and plays a critical role in the development of cardiovascular complications. These imbalances in the internal metabolic environment, combined with the characterstic low anti-oxidant defences of diabetics can lead to oxidative stress and cellular damage. Oxidative strers has been demonstrated to be a contributor to the propression of the disease, accerating both β-ceel failure and cardiovascular compliations.

Recent decades have seen a resurgent interest in traditional plant treatments for diabetes. This has pervaded nutrition,the pharmaceutical industry and academic research, fuelled by a growing public interest and awareness of so-called complementary and natural types of medicine. Before the advent of insulin therapy in 1922, starvation diets andtraditional plant treatments were the cornerstone of antidiabetictherapies. Traditional herbal preparations continueto form the predominant therapeutic approach in manydeprived regions of the globe, but in occidental societiesinsulin was soon recognized as the miracle life-saver andtraditional plant treatments were forgotten (Day & Bailey,1988).Plants are not a known source of insulin, and aside from some unsubstantiated anecdotal claims there is no known herbal insulin substitute.

Traditional antidiabetic plant treatments provide anobject lesson in the functionality of foods (Swanston-Flatt et al. 1991). Enriching the diet with natural fibre,complex carbohydrate, vegetable protein, antioxidants andminerals is encouraged. Added value occurs by achieving this with plants that have antidiabetic properties in their own right. Many traditional antidiabetic plants probably act at leastin part through their fibre, vitamin or mineral content. Mineral deficiencies are common in diabetes and canexacerbate insulin resistance. Several of these minerals are co-factors for signalling intermediaries of insulin action and key enzymes of glucose metabolism. Mineral supplements can benefit patients with mineral deficiencies, as demonstrated with magnesium and zinc. Plants rich in minerals have also been shown to benefit glycaemic control indiabetic patients, for example manganese in lucerne, chromium in brewer's yeast, and a cocktail of minerals in Atriplex halimus (saltbush) (Day, 1990).Several plants have provided entirely new hypoglycaemic compounds such as castanospermine in Castanospermum australe and neomyrtillin in bilberry (Day, 1990).

The plant infusions is used to cure different types of disease is very common in Indian folk medicine. Large number of plant species are a great source of biologically active compounds whose effect on human health or diabetes is mostly unknown. They frequently substitute for modern medicine. In recent years there has been greater interest in investigating compounds originating from plant and their effect on diabetes. Based on the view ,this study speculate effect of *Trichopus zeylanicus Gaertn* on diabetes.The aim of the present study was to examine the antioxidant / antidiabetic / hypolipedemic activity of the extracts of *Trichopus zeylanicus Gaertn* in male albino rats in alloxan induced stage.

Material and Methods

Eighteen male rats of albino species(150-250g) were randomly divided equally into 3 groups.The first group was fed on normal diet while the second of third groups were got intraperetonial injection of alloxan and the third group had additional ingestion of the plant Trichpuszeylanicus extract upto 5 days. The animals were housed in stainlers steel cages at room temperature of $27+2^{o}$ and were standardized for one week prior to introduction of the experiment.

Sample collection

After the treatment period all the groups of rats were euthanized by anaesthesia using chloroform vapour, and the rats were sacrificed by decapitation .The blood was collected in a Tube for serum separation .The serum thus obtained was used for biochemical analysis.

Determination of Biochemical parameters

Serum was used for the following analysis Serum LPO, plasma glucose, serum totalchostaol and HDL cholestad were estimated by Nichans and samuelson(1968), Hultmann.E (1959), Zlatkis-zak(1953) and Allin(1974) methods respectively. GSH level was determined by Ellman (1959) method And the LDL, VLDL levels were calculated by using Friedeward et al., (1972) Formula. Finally Serum triglyceride was estimated by the method of Jacolos and Vandenmark(1960).

Statistical analysis:

The results were presented as mean±SD. students 't' test used to analyzed statistical significant. P -values lower than 0.001 considered as statistical significance.

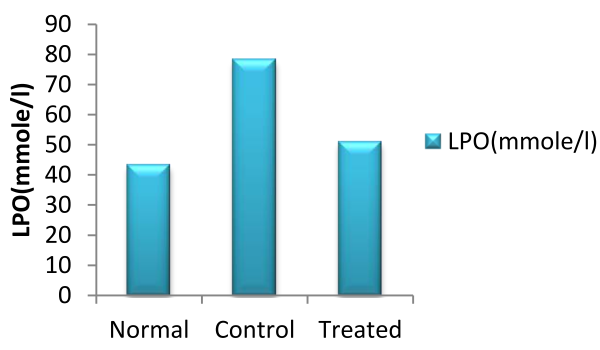
RESULTS

Table:1 Effects of *Trichopus* on LPO, GSH and Glucose in experimental animals

parameter	**Normal**	**Diabetic control**	***Trichopus* treated**
LPO(mmole/l)	43.2±1.6	78.1±2.8*	50.8±1.8#
GSH(µg/l)	36.3±6.1	22.8±4.2*	32.4±5.8#
Glucose(mg/dl)	86.2±8.2	162±10.6	88.4±9.2#

significantly different from controll(p<0.001)

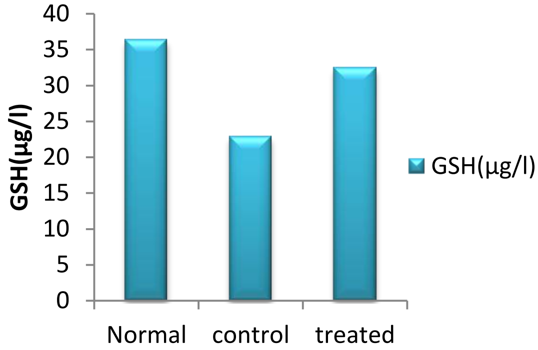
* significantly different from normal(p<0.001)



significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)

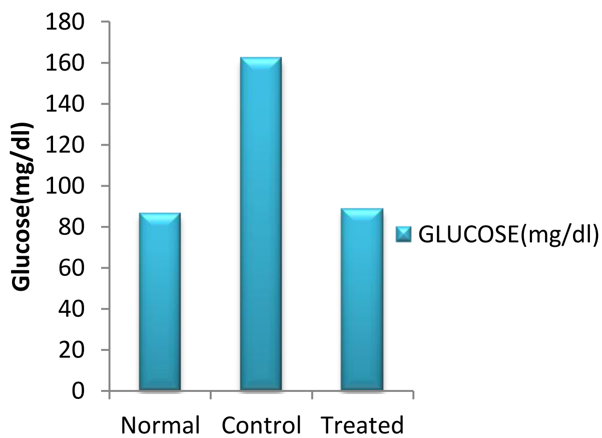
Fig:1 Effects of *Trichopus* on LPO in experimental animals



significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)

Fig:2 Effects of *Trichopus* on GSH in experimental animals



significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)

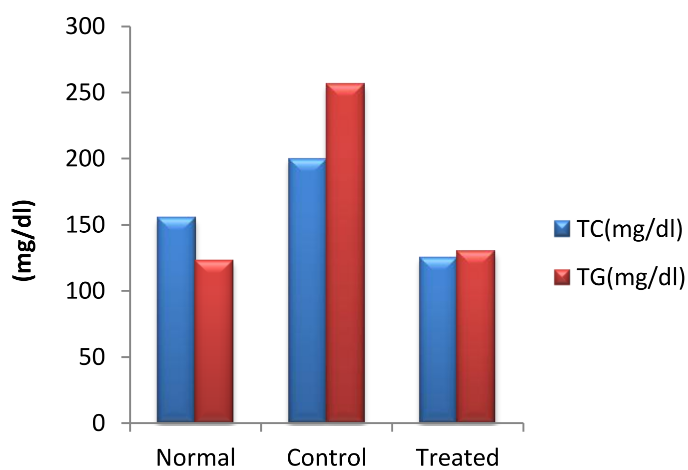
Fig:3 Effects of *Trichopus* on glucose in experimental animals

Table:2 Effects of *Trichopus* on TC and TG in experimental animals

parameter	Normal	Diabetic control	*Trichopus* treated
TC(mg/dl)	155±6.2	200±6.5*	125±9.5#
TG(mg/dl)	123±0.2	256±1.6*	130±1.5#

significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)



significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)

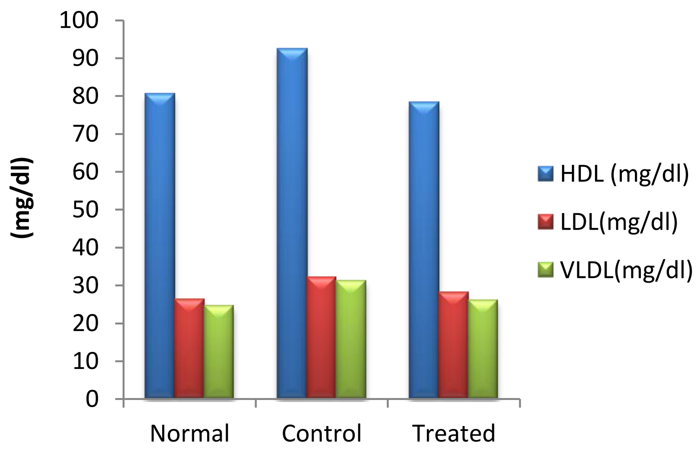
Fig:4 Effects of *Trichopus* on TC and TG in experimental animals

Table:3 Effects of *Trichopus* on HDL,LDL and VLDL in experimental animals

parameter	Normal	Diabetic control	*Trichopus* treated
HDL (mg/dl)	80.4±2.5	92.3±2.7 *	78.2±1.8 #
LDL (mg/dl)	26.4±.18	32.2 ±0.21*	28±.14#
VLDL (mg/dl)	24.6±1.8	31.2±.2*	26±.28#

significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)



significantly different from controll(p<0.001)

* significantly different from normal(p<0.001)

Fig:5 Effects of *Trichopus* on HDL,LDL and VLDL in experimental animals

There was a significant increase in serum MDA in the groups treated with alloxan compared to normal and plant treated groups.The serum GSH was decreased in alloxan groups than other two groups.

The Serum TG,TC,LDL,VLDL and glucose was significantly increased in diabetic group than normal. Likewise HDL level was decreased in the same group. But the administration of the plant extract changed these altered level to nearnormal level.

Discussion

Diabetes mellitus is a metabolic disease associated with impaired glucose metabolism which in effect adversely alters intermediary metabolism of lipid and proteins. Formation of protein glycation products releases free radicals, subsequently causing oxidative-stress (ceriello and motz, 2004) Most of the complications of the diabetic state are initiated by the generation of free radicals(Felmenden et al., 2003)

Free radical induced oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders and great importance has been attributed to antioxidants in the prevention and the treatment of diseases. Increasing evidence in both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both type -1 and type -2 diabetes mellitus (sundaram et al.,1996)

Alloxan has the ability to destroy the insulin- producing β –cells damage, leading- necrosis(Jorns et al., 1997). The cytotoxic action of alloxan is mediated by reactive oxygen species and simultaneous massive- increase in cytosolic calcium concentration, leading to damage of β –cells (szkudelski, 2007). Decreased insulin secretion leads to impairment of carbohydrate and fat metabolism .(Altamer et al., 1991).

Normally free radicals produced in metabolism are effectively scavenged. Oxidative stress occurs when there is an imbalance between production and scavenging. Increased lipid-peroxidation in diabetes mellitus is due to excess formation of free radicals. (Maritm et al., 2003)

In the diabetesmellitus abnormal increased level of lipidperoxide in serum may be due to the alteration of functions of erythrocyte membrane (Freitas et al., 1997) and abnormal lipid- metabolisam (Suckling et al., 1993).Abnormally high level of free radical lipidperiodation and decline in antioxidant defense mechanisms promote the development of – complications in diabetes mellitus .(Harris,1992). Antioxidant enzyme dependent defences play an important role in scavenging free radicals produced under oxidative stress (Harris,1992).One of the most prominent indicators of the presence of oxidative stress is the increased level of lipidperoxidation products (Malanodialdehyde) MDA. Plasma lipid-peroxidation was a good indicator for glucose induced oxidative stress (Catherwood *et al.*, 2002).

Glutathione(GSH) a tripeptide, present in all the cells, is an important antioxidant. Decreased glutathione level in diabetic has been considered to be an indicator of increased oxidative stress (Lenna *et al*., 1997). GSH also functions as free radical scavenger and in the repair of free radicals caused biological damage (Nicotera and orrenius, 1986).Shilaja *et al.*, (2003) reported that diabetic have shown increased lipid peroxidation and decreased level of reduced glutathione.In the study, elevated level of TBARS and decreased level of GSH were observed in alloxan treated rats it was a clear manifestation of excessive formation of free radicals and activation of lipidperoxidation. The significant decline of LPO in serum and nearnormal level of GSH of alloxan and plant treated animals indicates antioxidant and antidiabetic effect of Trichopus zeylanicus.

Alloxan induce hyperglycemic condition which is an initial signal in diabetes leading to altered metabolism, free radical generation and subsequent complications. Both acute and chronic hyperglycemia increase the production of reactive oxygen species(Hont *et al*., 1998).The insulin deficiency, results in improved glucose utilization, causing hyperglycemia. In diabetes, blood glucose is not utilized by tissue resulting in hyperglycemia (Shin et al.,1997).

The increase in blood glucose might be due to stress produced by the stress procedure (Feldhahn et al., 1999). Alloxan induced diabetes has been observed to cause a massive reduction of the β –cells of the islets of the pancreas leading to hyperglycemia (Chattoppadhyay *et al*., 1997). Hyperglycemia is due to glycogenolysis in liver. Hyperglycemia is a key causative factor in oxidative stress in tissue site for diabetic complications (Obrosova,2002).

In our study, increased level of glucose was observed in diabetic rats, that may be due to insulin deficiency. Administration of *Trichopus zeylanicus* significantly decreased the level of glucose in diabetic rats. It indicates that the plant *Trichopus zeylanicus* have good hypoglycemic effects.

In the present result, plasma triglyceride, total cholesterol, LDL- cholesterol, HDL – cholesterol and VLDL – Cholesterol were significantly higher in untreated diabetic rats compared with diabetic rats administered with *Trichopus zeylanicus* ,this values were significantly higher than the values of the normal control rats.

Lipid profile, which is altered in diabetes state (Betteridge,1994) is one of the significant factors in the development of cardiovascular disease.In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipidperoxide in serum may be due to the abnormal lipid metabolism. Maximum increase in lipid peroxide in serum may be found in the group diabetes mellitus with complications. Elevated level of lipidperoxide in diabetes mellitus inhibit the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes (Freitas e*t al.*, 1997).

Peroxidation of apolipoproteins may affect the lipoprotein metabolism. Higher concentration of serum cholesterol in diabetes may be attributed to inhibition of cholesterol catabolism. It has been suggested that the increase in triglyceride may be due to insulin deficiency which results in faulty glucose utilization, causing hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by tissue resulting in hyperglycemia. The fatty acid from adipose tissue are mobilized for energy purpose and excess fatty acid are accumulated in the liver, which are converted to triglyceride (Shin *et al*., 1997). The present study indicate that insulin increases the number of LDL receptor so chronic insulin deficiency might be associated with a diminished level of LDL-receptor. This causes the increase in LDL particles and result in the increased in LDL – cholesterol value in diabetes mellitus. In this study, HDL cholesterol level is increased in control group than other groups. It may be due to abnormal metabolism of Apo – c (or) Apo-A-I. The changes in lipid profile are thought to be a consequence of decreased biosynthesis or defective catabolism of either Apo-A-I (or) HDL. (Breslow, 1995). Accumulation of partially metabolized TG rich – LP causes storage of VLDL in blood due to Apo- B defect have been linked to the pathogenic process (Attman, 1997).

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