EFFECT OF PIMPINELLA TIRUPATIENSIS TUBEROUS ROOT EXTRACT IN RESTORATION OF ALTERATIONS IN RENAL CARBOHYDRATE METABOLIC PROFILE IN STZ INDUCED DIABETIC RATS

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KEY WORDS
Pimpinella tirupatiensis
Diabetes
Carbohydrate
Glibenclamide

INTRODUCTION
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2007). Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world (Gipsen and Biessels, 2000). The global prevalence of diabetes mellitus for all age groups was estimated to be 2.8% in 2000 and is projected to rise to 4.4% in 2030 (Wild et al., 2004). The pathological agents currently used for treatment of type 2 diabetes include sulfonylureas, biguanide, thiazolidinedione and α-glycosidase inhibitors. These agents, however, have restricted usage due to several undesirable side effects and fail to significantly alter the course of diabetic complications (Rang and Dale, 1991). Renewed attention to alternative medicines and natural therapies has stimulated new wave of research interest in traditional practices, and there is a need to look for more efficacious agents with lesser side effects. Presently, there is a growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus (Kim et al., 2006). Many traditional plant treatments for diabetes mellitus are used throughout the world. Few of the medicinal plant treatments for diabetes have received scientific scrutiny, for which World Health Organization (WHO) has also recommended attention.

Pimpinella tirupatiensis Bal. and Subr. (Family Apiaceae; local name, konda kothimera) is a rare and endemic medicinal plant and restricted to the Seshachalam hills of the Eastern Ghats, India (Balakrishnan and Subramanyam, 1960). Dried roots of Pt are administered along with few other ingredients to cure colic and rheumatic ailments in cattle (Sudarsananam et al., 1995). The local Yanadhi tribal community uses the tuberous roots of Pt to cure severe ulcers of stomach, throat and genital organs and also as aphrodisiac (Thammanna and Narayana Rao, 1990) and abortifacient agents (Vedavathi et al., 1997). Fruits are used to cure asthma and are considered as an effective remedy for ‘flatulent colic’ (Thammanna and Narayana Rao, 1990). The whole plant of P. tirupatiensis is used to treat cough, stomach, liver problems, asthma, ulcer and tooth ache (Madhava Chetty et al., 2008). The plant root extract is also used to treat skin disease (Jeevan Ram et al., 2004) and is used as an antimicrobial agent (Bakshu and Venkata Raju, 2002) it is even given in the treatment of venereal disease and peptic ulcers (Nagaraju and Rao, 1989).

Though, there is no scientific evidence to support P. tirupatiensis antidiabetic activity, Tribal’s of Tirumala region are continuing it’s use for the management of diabetes mellitus. Hence, the objective of this study was to ascertain the scientific basis for the use of Pimpinella tirupatiensis in the management of diabetes using STZ induced diabetic rats.

MATERIALS AND METHODS

Animals
Wistar strain male albino rats, aged 3 months (200-250 g)

ABSTRACT
Pimpinella tirupatiensis (Pt) is an endemic plant of Seshachalam hills of India. In the present study, the effect of Pimpinella tirupatiensis in diabetic rats has been investigated. An optimum dose of Pt ethyl alcohol extract (750 mg/kg body weight) was orally administered for 30 days to streptozotocin (STZ) induced diabetic rats for the assessment of total carbohydrate (TCH), total proteins (TP), free amino acids (FAs), pyruvate and lactate, in normal and STZ-diabetic rat renal tissue. A significant (p<0.01) reduction in the levels of TCH, TP, and pyruvate were observed in the kidney of diabetic rats. FAs and lactate levels were enhanced in diabetic control rats. Pt at 750mg/kg b.w dose enhanced the levels of TCH, TP, and pyruvate significantly. FAs and lactate levels were decreased significantly with the treatment of Pt. Pt produced similar beneficial effects on all biochemical parameters studied as that of glibenclamide (Glb), a standard anti diabetic drug. These results indicate that Pt possesses anti diabetic activity and other pharmacological properties.

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Received on : 07. 06. 2010
Accepted on : 15. 11. 2010

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were used for the present study. The rats were maintained on standard pellet diet and provided access to water ad libitum. They were housed in clean, dry polypropylene cages and maintained in a well ventilated animal house with 12 h light-12 h dark cycle. All the experiments were carried out between 8 am to 10 am in order to avoid circadian rhythm induced changes.

The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CPCSEA/dt.17.07.2001) in its resolution number 09 (iii)/a/CPCSCA/IAEC/07-08/SVU/Zool/KSR-TL/dated 26/6/08.

Induction of diabetes
Diabetes was induced in healthy male Wistar Albino rats aged about 3 months, with body weights ranging from 200 – 250 g, by a single intra peritoneal injection of freshly prepared STZ (40 mg/kg b.w) dissolved in ice cold 0.1M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12-15 hr as per the method followed by Rakieten et al., (1963). 8 hr after STZ administration the rats were kept for next 24 hr on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of producing fatal hypoglycemia due to destruction of β cells which in turn results in to massive pancreatic insulin release. Diabetes was assessed by determining the fasting blood glucose after 48 hr of injection of STZ. The blood glucose levels in STZ rats were increased to markedly higher levels than normal. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (blood glucose level ≥ 250 mg/dl) were selected. Blood was collected from the tail vein.

Plant material and extraction
Tuberous roots of Pimpinella tirupatiensis (Pt) were collected from Shesachalam hills, (Chittoor district, Andhra Pradesh, India) and identified by the Taxonomist of the Herbarium, Department of Botany, S.V.University, Tirupati. Voucher specimen (AECBT-05/2007-2008) was deposited in S.V.University Tirupati, Andhra Pradesh, India. These roots were air dried and powdered. The powder was stored in airtight containers and was used for the extraction. To 500 g of root power, 1500 mL of ethyl alcohol was added. The clear filtrate was evaporated to dryness under vacuum using the rotavapor at 35-40ºC and further dried by freeze drying.

Experimental design
The rats were divided into 5 groups, six rats in each group and treated as follows:
1. Group I- Normal control (NC).
2. Group II -diabetic control (DC).
3. Group III -(D+Ea.e) diabetic animals were treated orally with 750 mg/kg b.w/day of Pt ethyl alcohol extract for 30 days,
4. Group IV -(N+Ea.e) normal animals were treated orally with 750 mg/kg b.w/day of Pt ethyl alcohol extract for 30 days
5. Group V (D+Glb) diabetic animals were treated with 20 mg/kg/day of glibenclamide for 30 days.

Analytical procedures
After completion of 30 days treatment the animals were sacrificed by cervical dislocation and the kidney tissue was excised at 4ºC. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at -80ºC for further biochemical analysis. The selected carbohydrate metabolic profiles such as Total carbohydrates, pyruvate, lactate, total proteins, free amino acids were ministered by the methods of Carroll et al., (1956), Friedmann and Hangen, (1942), Barker and Summerson (1941) as modified by Huckabee (1961). Lowry et al. (1951), Moore and Stein, (1954) respectively.

Statistical analysis
The data were expressed as mean values with their SE. In order to carry out statistical analysis, Ms Excel and SPSS 11.5 Version statistical packages was used. In our study the comparison is with respective groups, hence one way analysis of variance technique was applied to observe the significance between the groups. The post – Hoc test duncan’s multiple range test was also performed to know the significant difference among the groups. Entire statistical analysis was carried out at 0.01 levels.

RESULTS
Figs. 1 to 5 shows the levels of TCH, TP, FAs, pyruvate and lactate in the kidney of normal and experimental animals in each group. A significant reduction in the levels of TCH, TP, and pyruvate were observed in the kidney of diabetic rats. FAs and lactate levels were enhanced in diabetic control rats. The treatment of STZ induced diabetic rats with Pt caused an increase in the levels of TCH, TP, pyruvate and decrement in the content of lactate and FAs like that of Glb. No significant changes were observed in the normal rats treated with Pt.

DISCUSSION
The current study investigates the protective effect of Pt against diabetes induced alterations in carbohydrate metabolic profiles of kidney. Carbohydrates play not only a structural role in the cell, but may also serve as reservoirs of chemical energy. These polysaccharides are usually stored in the liver as glycogen. The major function of carbohydrates in metabolism is, as a fuel to be oxidized and provide energy for other metabolic processes (Martin and peters, 1985). In the current investigation TCH levels were decreased in diabetic rats. The abnormal regulation of glucose and impaired carbohydrate utilization that results from this defective and/or deficient insulin secretory response are the key pathogenic events in diabetes mellitus leading to the development and progression of micro and macro vascular complications which include neuropathy, nephropathy, cardiovascular and cerebrovascular disease (Adisakkwattana et al., 2005). The significant decrease in TCH levels in the kidney of diabetic rats suggest possible utilization of carbohydrates to meet the energy demand during STZ toxicity. Similar pattern of changes in carbohydrate levels has been reported in the brain and other tissues of rats during STZ induced diabetic condition. Where as with Pt extract supplementation to the diabetic rats TCH were increased in the kidney, this may due to the pharmacological and antioxidant compounds in Pt. These
compounds of Pt may elevated the total carbohydrate levels in STZ induced diabetic rats.

Proteins are an important class of biological macromolecules, which occupy an unique position in the cellular metabolism and are highly specific to each tissue. The protein profiles in tissue can be considered as a diagnostic tool in assessing the physiological status of a tissue or animal as a whole (Murray, 1971). The protein profiles in tissue are highly specific to each tissue. The protein profiles in tissue can be considered as a diagnostic tool in assessing the physiological status of a tissue or animal as a whole (Murray et al., 2000). In diabetes a variety of protein are subjected to nonenzymatic glycation and this is thought to contribute to the long term complications of disease (Vlassara et al., 1981). The content of total protein was found to be decreased in this study. This decrease in TP content may be ascribed to 1. decreased amino acid uptake; 2. greatly decreased concentration of variety of essential amino acids, 3. increased conversion rate of glycogenic amino acids to carbon dioxide and water, 4. reduction in protein synthesis secondary to a decreased amount and availability of mRNA (Ahmed, 2005). The decrease in protein may be due to microproteinuria, which are important clinical markers of diabetic nephropathy (Mauer et al., 1981) and/or may be due to increased protein catabolism (Almdal and Vilstrup, 1987). The results of the present study demonstrated that the treatment of diabetic rats with the ethanolic extract of Pt caused a noticeable elevation in the total protein levels as compared with their normal levels.

In the present investigation FAs levels were increased in diabetic control kidney than normal control. Treating with Pt decreased the levels of FAs like that of GLB. Enhanced levels of FAs in diabetic rat kidney could be due to the degradation of proteins in diabetic condition.

Pyruvate is at the centre of metabolic disposition of substrates from the utilisation of proteins and carbohydrates and pyruvic dehydrogenase (PD) is crucial for the complete oxidation of glucose and for lipid biosynthesis from glucose (Halperin, 1970; Jungas, 1970). The levels of pyruvate indicate the efficiency of oxidative metabolism. In this study pyruvate level was decreased in kidney tissue of diabetic rats. Lipid synthesis is decreased in diabetes (Holcomb, 2006) ensuing fat catabolism leads to an increased accumulation of acetyl CoA and fatty acids which in turn reduce the amount of pyruvate (Lebkova, 2000). So pyruvate level was decreased in diabetic rats. These results have tended to support the suggestion of Moorhouse (Moorhouse, 1964) that diabetics have a metabolic defect in the handling of pyruvate. Where as with Pt treatment in diabetic rats these pyruvate levels were increased this may be due to the compounds of Pt. Which may corrects the pyruvate metabolism and so in diabetic rats with Pt treatment pyruvate level was increased. The content of lactate was increased in diabetic rat kidney than control rats. After treatment with Pt the levels of Lactate were decreased like that of glibenclamide (D+Glb). Each vertical bar represents the Mean±SE. (n=6). Top of the vertical bars having the same letter do not differ significantly at p < 0.01.

Figures 1 to 5: Total carbohydrate, Lactate, Pyruvate, Total proteins and Free amino acids level in the Kidney tissue of Normal control rats (NC), Diabetic control rats (DC), Diabetic rats treated with Pt Aqueous extract (D+Ea.e), Control rats treated with Pt Aqueous extract (C+Ea.e), Diabetic rats treated with glibenclamide (D+Glb).
From the present study we conclude that Pt alters the carbohydrate metabolism and so these carbohydrate profiles were came to normalcy.

REFERENCES


