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PIMPINELLA TIRUPATIENSIS IMPROVES THE LEVELS OF BRAIN CARBOHYDRATE METABOLIC PROFILES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract:

The present study was designed to investigate the protective role of Pimpinella tirupatiensis on the carbohydrate metabolic profiles in STZ-induced diabetic rats. Wistar strain male albino rats were divided in to 5 groups as stated in the experimental protocol. The parameters studied are total carbohydrates, total proteins, glycogen and free amino acids. These metabolic profiles were decreased in diabetic rats except glycogen. Whereas, with Pimpinella tirupatiensis treatment in diabetic rats these carbohydrate metabolic profiles were up regulated and glycogen down regulated. The blood glucose levels were also came to normalcy in Pimpinella tirupatiensis treated diabetic rats. The observed reductions in carbohydrate metabolic profiles during diabetic condition in brain tissue may be due to the alterations in the carbohydrate metabolism. From the results, it is concluded that Pimpinella tirupatiensis posse's hypoglycemic property and other pharmacological properties so in diabetic rats, all these carbohydrate metabolic profiles were came to normalcy.

Key words: *Pimpinella tirupatiensis, diabetes, total carbohydrates, blood glucose, brain*

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INTRODUCTION:

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). Diabetes mellitus is a syndrome characterized by the loss of glucose homeostasis as a result of defects in insulin secretion and functionality. Chronic hyperglycemia leads to the glycation of cellular proteins and may lead to complications affecting the eyes, nerves, kidneys, and arteries [Afolayan and Sunmonu 2010). The deficiency in insulin causes impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins (Scheen., 2003). The carbohydrate homeostasis depends on the balance between their formation and their utilization by major peripheral tissues and is significantly altered during diabetes (Sochor et al., 1985).

Plants have played a major role in the introduction of new therapeutic agents (Yakabu et al., 2010) and have gained attention as a source of biologically active substances including antioxidants and hypoglycemic and hypolipidemic agents (Shafa et al., 2009). Few of the medicinal plant treatments for diabetes have received scientific scrutiny, for which World Health Organization (WHO) has also recommended attention. pimpinella tirupatiensis (Balakrishnan and Subramanyam, 1960) is an herbaceous medicinal plant, distributed on Tirumala hills (1000m above MSL) of chittoor district, Andhra Pradesh (Mahadeva Chetty and Rao, 1990). Dried roots of Pimpinella tirupatiensis are administered along with few other ingredients to cure colic and rheumatic ailments in cattle (Sudarsanam et al.,1995). The whole plant of P. tirupatiensis is used to treat cough, stomach, liver problems, asthma, ulcer and tooth ache (Madhava Chetty et al., 2008). In this study, we investigated the effects of Pimpinella tirupatiensis on the blood glucose levels, body weight changes, carbohydrate metabolic profiles (total carbohydrate, glycogen, and protein) in experimentally induced diabetic rats to determine if this herb has the potential to be used in the treatment of diabetes.

MATERIAL METHODS:

Animals:

Wistar strain albino rats of male sex weighing 180±20gms were obtained from Indian Institute of Science, Bangalore. The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature-controlled room (27 ± 20C) with a photoperiod of 12 h light and 12 h dark cycle. The rats were fed with a standard rat pellet diet and water *ad libitum.*

Chemicals:

Streptozotocin (STZ) was purchased from Sigma (USA). All other chemicals and reagents used in this study were of analytical grade. Glibenclamide (Sugatrol, Hyderabad, India) was purchased from a local drug store.

Induction of diabetes:

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (50 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5) (Pepato et al., 1996). The animals were allowed to drink 5% glucose solution overnight to overcome the drug- induced hypoglycemia. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The treatment of ginger was started on the eighth day after STZ injection and this was considered as first day of treatment. The treatment was continued for30 days.

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. Dose equivalent to 750 mg of the crude drug per kg body weight, was calculated and suspended in 2%, v/v Tween 80 solution for the experiment (Bhandari et. al., 2005).

Experimental design:

The rats were divided into 5 groups, six rats in each group and treated as follows:

1. Group I- Normal control (NC). This group of rats received vehicle solution (2% of tween 80).
2. Group II -diabetic control (DC). Streptozotocin (50 mg / kg body weight) is given intraperitonially for the induction of diabetes to this group.
3. Group III - (DC+Pt) diabetic animals were treated orally with 750 mg/kg b.w/day of Pt ethyl alcohol extract for 30 days,

4. Group IV - (NC+Pt) normal animals were treated orally with 750 mg/kg b.w/day of Pt ethyl alcohol extract for 30 days
5. Group V - (DC+Glb) diabetic animals were treated with 20 mg/kg/day of glibenclamide for 30 days.

After completion of one month treatment the animals were sacrificed by cervical dislocation and the brain tissue was excised at 40C. The tissue was washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at -80°C for further biochemical analysis. The blood glucose levels were measured by using Accucheck glucometer. The selected carbohydrate metabolic profiles such as Total Carbohydrates, Glycogen, Total proteins, free amino acids levels were monitored by the methods of Carroll et al., 1956, Kemp and Van Hejnigen, 1954, Lowry et al., 1951 and Moore and Stein, (1954) respectively. 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 9 / IAEC /07-08/SVU/Zool/KSR-DVNK/dated 26/6/08.

Statistical analysis:

The data has been analyzed by using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects (factors), and treatments along with their interactions. The data has been compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at $p < 0.001$.

RESULTS:

Oral administration of Pimpinella tirupatiensis ethanolic extract for 30 days period exhibited up regulation of Total carbohydrates, Total proteins and free amino acids levels and down regulation of glycogen in brain. A significant reduction in Total carbohydrates, Total proteins and free amino acids levels and elevation of Glycogen levels was observed in the diabetic control rats when compared to the normal control rats. The diabetic rats with Pimpinella tirupatiensis treatment, we observed a significant increase in Total carbohydrates, Total proteins and free amino acids levels, whereas, Glycogen level was decreased which reflects restoration of the levels of carbohydrate metabolic profiles to the near-normal values. With Pimpinella tirupatiensis treatment in diabetic rats blood glucose levels were also come to control levels.

DISCUSSION:

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). The present study investigates the effects of Pimpinella tirupatiensis on the carbohydrate metabolic profiles in STZ induce diabetic rats. Here we demonstrate that STZ induction resulted in a wide variety of alterations in the carbohydrate metabolic profiles. Interestingly Pimpinella tirupatienses supplementation to rats was able to considerably reduce the toxic effects of STZ suggesting neural protective potential.

In the current study, we observed significant increase in blood glucose levels in diabetic rats (Table 1). This may be due to the destruction of pancreatic beta cells by STZ, reinforcing the fact that,STZ induces diabetes, probably through the generation of oxygen free radicals (Gupta, Kataria, Gupta, Murganandan, & Yashroy,2004).). The elevation of glucose in STZ-treated rats was due to anoxidative stress produced in the pancreas, due to a single strandbreak in pancreatic islets DNA (Yamamoto, Uchigata, & Okamoto, 1981). The outcome of the present study showed that ethanolic extract of Pimpinella tirupatiensis lowered the blood glucose level in Pimpinella tirupatiensis treated animals, as well as in the diabetic animals which are treated with Pimpinella tirupatiensis ($p<0.001$). In the present study, the blood glucose data clearly showed that pimpinella tirupatiensis restrained the level of hyperglycaemia resulting from the experimental destruction of beta pancreatic cells induced by STZ. The hypoglycaemic effect of ginger increased gradually and was observed to be maximum at the end of the study period, i.e. 30 days. Our findings are similar to those reported previously for Azadiracta indica (Khosla, Sangeeta, Singh, Seth, & Srivastava, 2000).

We have registered a decrease in body weight in STZ diabetic rats (Table 1). The characteristic loss of body weight associatedwith STZ-induced diabetes is due to increased muscle wasting in diabetes (Ravi, Ramachandran, & Subramanian, 2004). This indicates the polyphagic condition and loss of weight due to excessive break down of tissue protein (Kamalakkannam and Prince, 2006) and protein wasting due to unavailability of carbohydrate as an energy source (Chen and Ianuzzo, 1982), dehydration and catabolism of fats and proteins (Hakim et al., 1997). Oral administration of Pimpinella tirupatiensis

for 30days to diabetic rats improved the body weight. This could be due to a better control of the hyperglycemic state in diabetic rats. (Table 1).

In the present investigation total carbohydrates 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 (Adisakwattana et al., 2005). The significant decrease in total carbohydrates levels in the brain of diabetic rats suggests possible utilization of carbohydrates to meet the energy demand during STZ toxicity. Similar pattern of changes in carbohydrate levels has been reported in the kidney and other tissues of rats during STZ induced diabetic condition. Whereas with Pimpinella tirupatiensis treatment in diabetic rat's total carbohydrate levels were increased this may be due to the pharmacological and antioxidant compounds in Pimpinella tirupatiensis. These compounds of Pimpinella tirupatiensis may elevated the total carbohydrate levels in STZ induced diabetic rats. (Table 2).

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 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 total protein 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 ret tissues 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 pimpinella tirupatiensis caused a noticeable elevation in the total protein levels as compared with their normal levels.

In the present investigation free amino acid levels were increased in diabetic control kidney than normal control. Treating with Pimpinella tirupatienses decreased the levels of free amino acid levels like that of glibanclamide. Enhanced levels of free amino acid levels in diabetic rat brain could be due to the degradation of proteins in diabetic condition.

Glycogen is the major reserve carbohydrate stored in muscle liver and kidney for biological functions and maintenance of normal metabolism. The amount of glycogen present in tissues varied widely with diet and physiological status (Nelson and Cox, 2001). The glycogen of liver, muscles, kidney and other tissues are formed primarily from glucose and serves as an immediate source of reserve energy. Glycogen is the major storage form of carbohydrate in animals for biological function and the maintenance of the glycogen reserves is an important feature of the normal metabolism (Turner and Manchester, 1972). In the present study glycogen content was increased in the brain tissue of diabetic rats. Earlier reports also stated that glycogen level was increased in kidney tissue of diabetic induced rats (Shanmugham et al., 2009). But with pimpinella treatment in diabetic rat's glycogen content was decreased and also it came to normalcy. It shows that Pimpinella modulates the blood glucose levels which in turn alter the carbohydrate metabolic profiles. (Table 2).

CONCLUSION:

From the above results it is concluded that Pimpinella tirupatienses possess the hypoglycemic and other pharmacological properties. Pimpinella tirupatienses also alters the carbohydrate metabolism and so these carbohydrate profiles were come to normalcy. Further studies are needed to know the effects of Pimpinella tirupatienses on carbohydrate metabolism in STZ induced diabetic rats.

Table 1: Changes in blood glucose and body weight in Normal Control (NC), Diabetic Control (50 mg/kg body weight) (DC), Diabetic+ Pimpinella tirupatiensis treated (DC+Pt), Pimpinella tirupatiensis treated (Pt) (750mg/body weight), Diabetic + Glibenclamide treatment (20mg/kg) (DC+Gli) rats.

Groups	Blood Glucose (mg/dl)		Body weight (gms.)	
	0 Day	30 Days	0 Day	30 Days
Group I (NC)	79±1.56	91±2.654 (+15.059)	189±8.26	205±13.26 (10.256)
Group II (DC)	248±3.39 (+206.345)	224±15.3* (+214.098)	71±1.82 (+2.235)	189±7.95 (-2.954)
Group III (DC+Pt)	224±3.98 (+209.624)	137±5.84* (+69.370)	133±5.84* (-5.114)	189±3.02* (-2.236)
Group (NC+Pt)	83±1.47 (+2.469)	78±1.87 (-3.703)	78±1.87 (+2.564)	190±8.01 (-2.564)
Group (DC+Glb)	259±1.25 (0201.845)	93±3.65* (+165.49)	94±3.69* (-2.564)	203±0.08 (+4.125)

Table 2 : Changes in Total Carbohydrates (TC), Pyruvate (Py), Glycogen (Gly) and Total protein (TP) levels in the brain tissue of Normal Control (NC), Diabetic Control (50 mg/kg body weight) (DC), Diabetic+ Pimpinella tirupatiensis treated (DC+Pt), Pimpinella tirupatiensis treated (Pt) (750mg/body weight), Diabetic + Glibenclamide treatment (20mg/kg) (DC+Gli) rats.

Parameter	Group I (NC)	Group II (DC)	Group II (DC+Pt)	Group IV (NC+Pt)	Group V (DC+Glb)
[1]Total Carbohydrate	1.232± 0.068	0.987 ±0.052* (-17.591)	1.214±0.089* (+1.195)	1.362±0.059* (+23.996	1.065±0.017 (-6.831)
[2]Total proteins	12.973±0.443	8.235±1.019* (-22.452)	12.368±0.643* (+3.326)	2.598±1.156* (+4.255)	11.886±0.042* (+0.1898)
[3]Free Amino acids	3.854±0.649	1.986±0.649 (-17.362)	2.54±0.649 (+9.265)	3.989±0.649 (+5.384)	2.45±0.649 (+4.465)
[4]Glycogen	1.783±0.0448	2.14±0.023* (-16.564)	1.845±0.078* (+0.658)	1.897±0.056* (+4.141)	1.875±0.045* (+0.546)

All the values are mean, ± SD of six individual observations,
[1] value are expressed mg of glucose/gram wet weight of the tissue.
[2] expressed in mg of protein /gram wet weight of the tissue.
[3] mg free amino acid/g wet weight of the tissue
[4] expressed as mg of glycogen/gram wet weight of the tissue.
*significant at p< 0.001 with normal control.

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