

The Impact of Ginger on Kidney Carbohydrate Metabolic Profiles in STZ Induced Diabetic Rats



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Abstract : Ginger has been mentioned in Indian system of traditional medicine to be of value in the treatment of many diseases. The present study was undertaken to assess the effect of ginger 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 glycogen, total carbohydrates, total proteins and pyruvate. These metabolic profiles were decreased in diabetic rats except glycogen. Where as, with ginger treatment in diabetic rats these carbohydrate metabolic profiles were upregulated and glycogen downregulated. The blood glucose levels were also came to normalcy in ginger treated diabetic rats. The observed reductions in carbohydrate metabolic profiles during diabetic condition in renal tissue may be due to the alterations in the carbohydrate metabolism. From the results, its is concluded that ginger posses hypoglycemic property and other pharmacological properties so in diabetic rats, all these carbohydrate metabolic profiles were came to normalcy.

Key words : Diabetes, Ginger, Carbohydrate metabolic profiles, kidney.

Introduction

Diabetes 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 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). Oxidative stress is currently suggested as one of the mechanism underlying diabetes mellitus, which affects carbohydrate, lipid and protein metabolism. Several alterations in diabetic individuals are oxidative in nature or may depend on increased oxidative stress (Baynes, 1991). In diabetes, major impairments in

carbohydrate, fat and protein metabolisms occur (May and Mikulecky, 1983). 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).

Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments. Ginger is an essential

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ingredient in many traditional Chinese medicines and has been used since the 4th century BC. Africans and West Indians also use ginger medicinally and the Greeks and Romans use it as spice (Tyler *et al.*, 1981). Ginger contains Mg, Ca and P, which play important roles in bone formation, and curbing muscle spasm, depression hypertension, convulsion, nausea, gastrointestinal disorders, paralysis, kidney damage, and a host of other biodysfunctions (Kikuzaki and Nakatani, 1993; Kikuzaki *et al.*, 1994; Meyer *et al.*, 1995).

In Ayurveda, ginger has been recommended for use as carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic, digestive aid, antiasthmatic and stimulant to the gastrointestinal tract (Warrier, 1989; Mascolo *et al.*, 1989). Western herbal medical practices for the treatment of arthritis, Rheumatic disorders and muscular discomfort (Dedov *et al.*, 2002; Jiang *et al.*, 2006). The main antioxidant active principles in ginger are the gingerols and shogaols and some related phenolic ketone derivatives. Hence, compounds especially from natural sources capable of protecting free radicals mediated damage may have potential application in prevention and / or curing of diseases.

There were reports on diabetes and carbohydrate metabolic profiles, and ginger on carbohydrate metabolic profiles. But there were no reports on the ginger treatment in diabetic rats with reference to carbohydrate profiles. Hence the present study was carried out. The purpose of this investigation was to evaluate the effect of ginger ethanolic extract on streptozotocin (STZ) induced diabetes by measuring blood glucose levels and assaying the carbohydrate metabolic profiles in kidney.

Materials And 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

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fischer (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

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 for 30 days.

Ginger ethanolic Extraction

The fresh rhizomes of *Zingiber officinale* was locally and authenticated by botanist in the department of Botany, S.V. University, Tirupati. Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted in cold percolation with 95% ethanol for 24h. The extract was

recovered and 95% ethanol was further added to the ginger powder and the extraction was continued. This process was repeated three times. The three extracts were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanolic extract was air-dried, finally give 80 grams of dark brown, gelatinous extract of ginger dried rhizomes. Without any further purification, the crude ethanolic extract was used for the experiments. Dose equivalent to 200 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).

Grouping of Animals

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

I). Normal Control (NC) : This group of rats received vehicle solution (2% of tween 80).

II). Ginger treatment (Gt): This group of rats received ginger ethanolic extract via orogastric tube for a period of 30 days at the dose of 200 mg/kg body weight.

III). Diabetic control (STZ 50 mg/kg body weight), (DC) : Streptozotocin is given intraperitoneally for the induction of diabetes to this group.

IV). Diabetic plus Ginger treatment, (D+Gt) : Diabetic rats received ginger ethanolic extract described in group II for a period of 30 days.

V). Diabetic + Glibenclamide treatment (D+Gli) : Diabetic rats treated with glibenclamide 600 µg/kg body weight in aqueous solution.

After completion of one month treatment the animals were sacrificed by cervical dislocation and the kidney tissue was excised at 4°C. 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, Pyruvate levels were monitored by the methods of Carroll *et al.*, 1956, Kemp and Van Hejnigen, 1954, Lowry *et al.*, 1951 and Friedmann and Hangen, 1942 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/SVU/2001/dt. 4.03.2002).

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 ginger ethanolic extract for 30 days period exhibited upregulation of Total carbohydrates, Total proteins and Pyruvate levels and down regulation fo glycogen in kidney. A significant reduction in Total carbohydrates, Total proteins and Pyruvate levels and elevation of Glycogen levels was observed in the diabetic control rats when compared to the normal control rats. The diabetic rats with ginger treatment, we observed a significant increase in Total carbohydrates, Total proteins and Pyruvate levels, where as, Glycogen level was decreased which reflects restoration of the levels of carbphydrate metabolic profiles to the near-normal values.

With ginger treatment in diabetic rats blood glucose levels were also come to control levels.

Discussion

The present study investigates the effects of ginger 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 ginger supplementation to rats was able to considerably reduce the toxic effects of STZ suggesting renal protective potential.

The elevated blood glucose levels in diabetes are thought to lead to cell death through oxidative stress induction that occur as a common sequel of diabetes-induced modification of sugar moieties on proteins and lipids (Donnini *et al.*, 1996). One of the consequences of hyperglycemia is increased metabolism of glucose by sorbitol pathway. Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species, which results in an imbalance between free radicals and the antioxidant defense system of the cells. The out come of the present study showed that ethanolic extract of ginger

lowered the blood glucose level in ginger treated animals, as well as in the diabetic animals which are treated with ginger ($p < 0.001$). Flavanoids, terpenoids and a host of the secondary metabolites of many plants posses hypoglycemic effects in various experimental animal models (Ross, 2001; Ojewole, 2002). Many investigators reported that compounds of ginger such as 6-gingerol, tannins, polyphenolic compounds, flavonoids, and triterpenoids of possess hypoglycemic and other pharmacological properties (Young *et al.*, 2005; Jiang *et al.*, 2006). (Table 1).

Dehydration and loss of body weight have been associated with diabetes (Pupim *et al.*, 2005). In diabetic rats, decreased body weight was observed. 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 ginger 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).

Table 1 : Changes in blood glucose, body weight and kidney weights, in Normal Control (NC), Ginger treated (Gt) (200mg/body weight), Diabetic Control (50 mg/kg body weight) (DC), Diabetic+Ginger treated (D+Gt), Diabetic + Glibenclamide treatment (600g/kg) (D+Gli) rats.

Groups	Blood Glucose (mg/dl)		Body weight (gms.)		Kidney wieght (gms.)
	0 Day	30 Days	0 Day	30 Days	
Group I (NC)	81±1.41	94±2.8 (+16.049)	195±9.66	215±14.28 (10.256)	1.55±0.06
Group II (Gt)	83±1.47 (+2.469)	78±1.87 (-3.703)	78±1.87 (+2.564)	190±8.01 (-2.564)	1.50±0.06 (-3.225)
Group III (DC)	253±3.53 (+212.345)	269±15.6* (+232.098)	269±15.6* (-4.102)	150±6.83* (-23.076)	1.70±0.04 (+9.677)
Group IV (D+Gt)	259±4.09 (+219.753)	138±5.84* (+70.370)	138±5.84* (-5.128)	190±4.08* (-2.564)	1.49±0.04 (-3.870)
Group V (D+Gli)	260±1.79 (0222.987)	94±3.71* (+16.049)	94±3.71* (-2.564)	205±0.07 (+5.128)	1.52±0.02 (-1.935)

Carbohydrates are the major sources of energy fuels for metabolic processes readily assimilable, though fats yield more energy. The carbohydrates serve as energy fuels for metabolic processes (Martin and Peters, 1985). In the current 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 carbohydrate 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 brain and other tissues of rats

during STZ induce diabetic condition. Toxic compounds inhibit the formation of glucose from other compounds such as aminoacids etc. (Patel, 1981) Where as with ginger treatment in diabetic rats total carbohydrate levels were increased this may be due to the pharmacological and antioxidant compounds in ginger. These compounds of ginger may elevated the total carbohydrate levels in STZ induced diabetic rats. (Table 2).

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

Table 2 : Changes in Total Carbohydrates (TC), Pyruvate (Py), Glycogen (Gly) and Total protein (TP) levels in the Kidney tissue of Normal control (NC), Ginger treatment (Gt) (200 mg/body weight), Diabetic (DC) (50 mg/kg body weight), Diabetic + Ginger treatment (D+Gt), and Diabetic + Glibenclamide treatment (600 µg/kg) (D+Gli) rats.

Parameter	Group I (NC)	Group II (Gt)	Group III (DC)	Group IV (D+Gt)	Group V (D+Gli)
TC ^a	1.171± 0.071	1.452±0.067* (+23.996)	0.965 ±0.044* (-17.591)	1.185±0.099* (+1.195)	1.091±0.012 (-6.831)
Py ^b	11.447 ±0.388	13.929±0.567* (+21.682)	9.470±0.389* (-17.270)	11.669±0.386* (+1.939)	10.942±0.491* (-4.411)
Gly ^c	1.851±0.0571	1.931±0.038* (+4.321)	2.20±0.053* (-18.854)	1.863±0.043* (+0.648)	1.901±0.091* (+0.648)
TP ^d	11.973±0.649	12.51±1.156* (+4.485)	8.924±1.019* (-25.465)	12.491±0.643* (+4.326)	11.992±0.086* (+0.158)

All the values are mean, ± SD of six individual observations,

^avalues are expressed mg of glucose/gram wet weight of the tissue.

^b as mmoles of pyruvate formed/gm wet wt of the tissue.

^c expressed as mg of glycogen/gram wet weight of the tissue.

^d expressed in mg of protein /gram wet weight of the tissue.

values in the parenthesis denote percent change over normal control,

*significant at $p < 0.001$ with normal control.

is an important feature of the normal metabolism (Turner and Manchester, 1972). In the present study glycogen content was increased in the kidney tissue of diabetic rats. STZ induced diabetic animals tend to show renal hypertrophy. The entry of glucose in renal tissue is not dependent on action of insulin and , therefore, in the event of hyperglycemia there is an increase in the entry of glucose (Belfiore *et al.*, 1986). This has been posutulated to cause increased intrarenal glycogen deposition, which leads to glycosylation of basement membrane collagen in the kidney (Anderson and Stowring, 1973). This reflected in present finding as diabetic rats showed a 18% increase in diabetic kidneys. Rise in renal glycogen content has been purported earlier studies (Rasch, 1980; Nielsen *et al.*, 1999). But with ginger treatment in diabetic rats glycogen content was decreased and also it came to normalcy. It shows that ginger modulates the blood glucose levels which in turn alters the carbohydrate metabolic profiles. (Table 2).

Proteins are an important class of biological macromolecules, which occupy a 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 levels of total protein were found to be decreased in this study. This decrease in total protein may be ascribed to 1. decreased aminoacid uptake; 2 greatly decreased concentration of variety of essential aminoacids, 3. increased conversion rate of glycogenic aminoacids to carbon dioxide and water, 4. reduction in protein synthesis secondary to a decreased amount and

availability of m RNA (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 ginger caused a noticeable elevation in the total protein levels as compared with their normal levels.

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 (Jungas, 1970; Halperin, 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 ginger treatment in diabetic rats these pyruvate levels were increased this may be due to the compounds of ginger. Which may corrects the pyruvate metabolism and so in diabetic rats with ginger treatment pyruvate level was increased. (Table 2).

Form the above results it is concluded that ginger posses the hypoglycemic and other pharmacological properties. Ginger also alters the carbohydrate metabolism and so these carbohydrate profiles were came to normalcy. Further studies are needed to know the effects of ginger on carbohydrate metabolism in STZ-induced diabetic rats.

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