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EFFECT OF PTEROSTILBENE COMPARED TO TETRAHYDROCURCUMIN ON ERYTHROCYTE MEMBRANE BOUND ENZYMES AND ANTIOXIDANT STATUS IN DIABETES

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Abstract

Pterocarpus marsupium has been used in the treatment of toothache, diarrhoea, heartburn, urinary tract infections, boils, sores and skin diseases. *P. marsupium* has been used for many years in the treatment of diabetes mellitus. Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium*. Pterostilbene, a phenolic compound derived from resveratrol, possesses greater bioavailability than its parent compound due to the presence of two methoxyl groups. In this review, the beneficial effects of pterostilbene on diabetes, liver steatosis and dyslipidemia are summarized. Pterostilbene is a useful bioactive compound in preventing type 1 diabetes, insulin resistance and type 2 diabetes in animal models. Hyperlipidemia is an associated complication of diabetes mellitus. Oral administration of pterostilbene (40 mg/kg body weight) THC (80 mg/kg body weight) to diabetic rats for 45 days. The effect of pterostilbene and THC on glucose, insulin, haemoglobin, glycosylated haemoglobin, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (Gpx), glutathione-S-transferase (GST), reduced glutathione (GSH) and membrane bound enzymes were studied. The effect of pterostilbene was compared with THC. The levels of blood glucose, glycosylated haemoglobin, erythrocyte TBARS, were increased significantly whereas the level of plasma insulin and haemoglobin, erythrocyte antioxidants (SOD, CAT, GPx, GST and GSH), membrane bound total ATPase, Na^+/K^+-ATPase, Ca^{2+}- ATPase, Mg^{2+}- ATPase were decreased significantly in diabetic rats. Administration of pterostilbene and THC to diabetic rats showed decreased level of blood glucose, glycosylated haemoglobin and erythrocyte TBARS. In addition the levels of plasma insulin, haemoglobin, erythrocyte antioxidants and the activities of membrane bound enzymes also were increased in pterostilbene and THC treated diabetic rats. The present study indicates that the pterostilbene and THC possesses a significant beneficial effect on erythrocyte membrane bound enzymes and antioxidants defense in addition to its antidiabetic effect.

Key words: Tetrahydrocurcumin, pterostilbene, Erythrocyte antioxidants, Lipid peroxidation, Ca^{2+}-ATPase, Na^+/K^+-ATPase, Mg^{2+}-ATPase, Streptozotocin, Nicotinamide.

Introduction

Diabetes mellitus is a non-communicable disease, which is considered as one of the five leading causes of death in the world (Ugochukwu and Babady, 2003). In the human population, a variety of diseases are associated with abnormalities in physicochemical and functional properties of erythrocytes. One important property of erythrocytes is their ability to change their shape depending on external forces exerted against them in the circulation. This ability of erythrocytes, also referred to as deformability, represents one determinant of whole blood viscosity affecting thus the blood flow in macrocirculation. Simultaneously, this property is very important for effective delivery of oxygen to target tissues—as erythrocytes are supposed to repeatedly pass through narrow capillaries in the microcirculation. It has been shown that skin blood flow after transfusion of packed erythrocytes is strongly dependent on their deformability (Pandey et al., 2010).

Hyperglycaemia causes tissue damage through multiple mechanisms including increased flux of glucose and other sugars through the polyol pathway, increased intracellular formation of advanced glycation end products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine pathway (Brownlee, 2005). Atherosclerosis and cardiomyopathy in type 2 diabetes are caused in part by pathway-selective insulin resistance, which increases mitochondrial ROS production from free fatty acids, and by inactivation of antiatherosclerosis enzymes by ROS. Diabetics differ significantly in their sensitivity to ROS. Inflammatory damage that characterizes type 1 diabetes is mediated at least in part through islet ROS, and in type 2 diabetes, the high nutrient flux and consequent ROS production appear to mediate loss of β-cell function. In insulin-sensitive tissues including muscle, liver, and heart, high fatty-acid flux leads to oxidative damage, whereas noninsulin-sensitive tissues including the eye, kidney, and nervous system are exposed to both high circulating glucose and fatty acid levels and, consequently, ROS-induced diabetic complications (Sivitz and Yorek, 2010).

Oxidative stress in diabetes mellitus causes several adverse effects on the cellular physiology. This is particularly relevant and dangerous for the islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defences. Multiple biochemical pathways and mechanisms of action have been implicated in the deleterious effects of chronic hyperglycemia and oxidative stress on the function of vascular, retinal, and renal tissues (Fiorentino et al., 2013).

THC, produced from curcumin by hydrogenation, are colorless which render these products useful in non-colored food and cosmetic applications that currently employ synthetic antioxidants (Murugan and Pari, 2006). THC is one of the major metabolites of curcumin, with potential bioactivity. This metabolite was identified in intestinal and hepatic cytosol from humans and rats (Murugan and Pari, 2007; Murugan and Pari, 2008). The reduction of curcumin to THC seems to occur primarily in a cytosolic compartment (Ireson, 2002). Final reduction of THC to hexahydrocurcuminol may occur in microsomes (possibly by cytochrome P450 reductase) (Ireson, 2002). Recently, attention has focused on THC, as one of the major metabolites of curcumin, because this compound appears to exert greater antioxidant activity in both in vitro and in vivo systems (Pari and Murugan, 2004). In our previous study, we have demonstrated the antidiabetic effect of THC in streptozotocin (STZ) induced diabetic rats ((Pari and Murugan, 2005).

Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. Pterocarpus marsupium has been used for many years in the treatment of diabetes mellitus (Warrier, 1995). Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of Pterocarpus marsupium (Maurya et al. 2004). It is suggested that pterostilbene might be one of the principal anti-diabetic constituents of Pterocarpus marsupium (Maurya et al. 2004). An aqueous extract of heartwood of P. marsupium has been tested clinically and found to be effective in non-insulin dependent diabetes (NIDDM) patients. When administered to STZ-induced hyperglycemic rats, pterostilbene and marsupin, two of the major phenolic constituents in aqueous decoction of the heartwood of P. marsupium, significantly decreased plasma glucose (Pari and Amarnath Satheesh, 2006).

To our knowledge, so far no other biochemical investigations have been carried out on the effect of pterostilbene compared THC in erythrocyte antioxidants and the activities of membrane bound enzymes of experimental diabetic rats. The present investigation was carried out to study the effect of pterostilbene compared THC on erythrocyte antioxidants and the activities of membrane bound enzymes in rats with STZ and nicotinamide induced diabetes.

Materials and methods

Drugs and Chemicals

THC and pterostilbene was a gift provided by Sabinsa Corporation, USA. All other chemicals and biochemicals were of analytical grade.

Induction of diabetes

Type 2 diabetes mellitus was induced (Masiello et al., 1998) in overnight fasted rats by a single intraperitonial injection (i.p) of 65 mg/kg body weight STZ, 15 min after the i.p administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design

In this experiment, rats were divided into 4 groups of 6 rats each.
Group 1: Normal rats.
Group 2: Diabetic control rats.
Group 3: Diabetic rats given aqueous extract of THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Pari and Murugan, 2005).
Group 4: Diabetic rats given Pterostilbene (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Pari and Amarnath Satheesh, 2006).

At the end of experimental period, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose, haemoglobin and glycosylated haemoglobin. Plasma was separated for the assay of insulin.

Analytical procedure

Determination of blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner, 1975). Plasma insulin was assayed using an enzyme linked immunosorbent assay (ELISA) kit (Boeheringer – Mannheim, Germany).

Determination of haemoglobin and glycosylated haemoglobin levels

Haemoglobin was estimated by using the cyanmethaemoglobin method described by Drabkin and Austin (1932). Glycosylated haemoglobin was estimated according to the method of Sudhakar Nayak and Pattabiraman (1981) with modifications according to Bannon (1982).

Preparation of haemolysate

From 2 ml of blood, erythrocytes were separated by centrifugation at 1000×*g* for 10 min at 4 ºC. The erythrocyte layer was washed three times with 10 volumes of 10 mmol/L PBS. The washed erythrocytes were suspended in phosphate buffer saline (PBS) and adjusted to a hematocrit (HCT) of 5 or 10%. An aliquot of 0.5 ml washed RBC was lysed with 4.5 ml of ice cooled distilled water to prepare haemolysate.

Preparation of erythrocyte membrane

The erythrocyte membrane (haemoglobin free ghost erythrocyte) was isolated according to the procedure of Dodge et al., (1963) with a change in buffer according to Quist (1980). The packed

cells were washed three times with isotonic 310 mM Tris–HCl buffer, pH 7.4. Haemolysis was prepared from red blood cells into 20mM hypotonic Tris–HCl buffer, pH 7.2. Ghosts were sedimented by using a high-speed refrigerated centrifuge at 20,000×g for 40 min. The haemolysate was decanted carefully and the ghost button was resuspended and the buffer of the same strength was added to reconstitute the original volume. The ratio of cells to the washing solution was approximately 1:3 by volume. The procedure was repeated thrice, till the membrane became colourless. The pellets were resuspended in 100mM Tris–HCl buffers, pH 7.2. Aliquots from this were used for the estimations.

Determination of erythrocyte membrane bound enzymes

Total ATPase was assayed by the method of Evans (1986) with modification, the activity of Na^+/K^+-dependent ATPase and Mg^{2+}-ATPase were assayed according to the procedure of Bonting (1970) and Ohinishi et al., (1982) respectively. The incubation mixture in a total volume of 2.0 ml contained 0.1ml buffer 1.5 ml KCl, 0.1 ml NaCl, 0.1 ml $MgCl_2$, 0.1 ml ATP and 0.1 ml RBC membrane. The mixture was incubated at 370 for 20 min. the reaction was arrested by 1.ml 10% TCA and then centrifuged. The phasphorus liberated was estimated by the method Fiske and Subbarow (1925) supernatants with 1.0 ml ammonium molybdate and 0.4 ml of ANSA were added the blue colure developed was read at 640 nm after 30 minutes. ATPases activity expressed as μ mole of phosphorus liberated/min/mg protein at 37°C.

The incubation mixture after incubation at 37^0 C for 10 min. the reaction was initiated by 0.2 ml of RBC membrane. The contents were incubated at 37^0 C for 15 minuted.1.0 ml of 10% TCA was added at the end of 15 min to arrest the reaction. The incubation mixture contained 1.0 ml of buffer 0.2 ml of magnesium sulphate, 0.2 ml of NaCl, 0.2ml of EDTA and 0.2 ml of ATP. After incubation at 37° C for 10 min, the 0.2ml RBC membrane initiated the reaction. The contents were incubated at 37^0C for 15 minuted.1.0 ml of 10% TCA was added at the end of 15 min to arrest the reaction. The phasphorus liberated. Mg^{2+}- ATPase activity expressed as μg of phosphorus liberated/min/mg protein.

The activity of Ca^{2+}- ATPase was assayed according to the method of Hjerken and Pan (1983). The reaction mixture contained 0.1 ml of buffer 0.1ml of $CaCl_2$, o.1 ml of ATP and 0.1 ml of RBC membrane, the contents were incubated at 37^0 C for 15 min. the reaction was then arrested by 0.5 ml 10% TCA. The phasphorus was liberated. Ca^{2+}- ATPase activity expressed as μg of phosphorus liberated/min/mg protein.

Determination of lipid peroxidation and antioxidants

The activity of TBARS was estimated by the method of Donnan (1950). Superoxide dismutase (SOD) was assayed by the method of Kakkar et al. (1984). Catalase (CAT) was estimated by the method of Sinha (1972). Glutathione peroxides (GPx) activity was measured by the method described by Rotruck et al. (1973). Glutathione-S-transferase (GST) activity was determined spectrophotometrically by the method of Habig et al. (1974). Erythrocyte reduced glutathione (GSH) was determined by the method of Beutler et al. (1963).

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p < 0.05$ (Duncan, 1957).

Table 1. Effect of THC and Pterostilbene on the levels of blood glucose, plasma insulin, haemoglobin and glycosylated haemoglobin in normal and experimental rats

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)
Normal	100.23 ± 5.22^{a}	12.58 ± 0.48^{a}	12.25 ± 0.45^{a}	0.30 ± 0.03^{a}
Diabetic control	295.35 ± 5.32^{b}	3.90 ± 0.30^{b}	8.32 ± 0.30^{b}	0.76 ± 0.04^{b}
Diabetic+THC (80 mg/kg)	115.20 ± 6.35^{c}	9.90 ± 0.60^{c}	11.58 ± 0.55^{c}	0.40 ± 0.04^{c}
Diabetic+Pterostilbene (40 mg/kg)	137.45 ± 6.52^{d}	8.02 ± 0.28^{d}	10.89 ± 0.50^{d}	0.45 ± 0.04^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 2. Changes in the levels of total ATPase, Na^{+} /K^{+}ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase normal and experimental rats

Groups	Total ATPase	Na^{+} /K^{+} ATPase	Ca^{2+}ATPase	Mg^{2+}ATPase
Normal	1.85 ± 0.10^{a}	0.79 ± 0.06^{a}	0.40 ± 0.04^{a}	2.45 ± 0.03^{a}
Diabetic control	0.85 ± 0.06^{b}	0.41 ± 0.03^{b}	0.25 ± 0.01^{b}	1.75 ± 0.04^{b}
Diabetic + THC (80 mg/kg)	1.55 ± 0.07^{c}	0.75 ± 0.04^{c}	0.35 ± 0.02^{c}	2.35 ± 0.03^{c}
Diabetic + Pterostilbene (40 mg/kg)	1.33 ± 0.05^{d}	0.59 ± 0.02^{d}	0.31 ± 0.01^{d}	1.43 ± 0.06^{d}

Values are given as mean ± SD from 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 3. Changes in activities of TBARS, catalase, superoxide dismutase, glutathione peroxidase, glutathione–S–transferase and reduced glutathione in erythrocytes of normal and experimental rats

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)
TBARS (pM/mgHb)	1.75 ± 0.13^{a}	3.15 ± 0.20^{b}	2.15 ± 0.19^{c}	2.39 ± 0.15^{d}
CAT (Units^{A} / mg Hb)	165.45 ± 7.32^{a}	89.36 ± 6.12^{b}	149.54± 7.15^{c}	129.01 ± 6.28^{d}
SOD (Units^{B} / mg Hb)	6.98 ± 0.35^{a}	3.47 ± 0.27^{b}	5.87 ± 0.30^{c}	5.68 ± 0.25^{d}
GPx (Units^{C} / mg Hb)	14.55± 0.68^{a}	9.65 ± 0.54^{b}	13.25± 0.55^{c}	12.55 ± 0.58^{d}
GST(Units^{D} / mg Hb)	6.54 ± 0.35^{a}	4.25 ± 0.29^{b}	5.67± 0.37^{c}	5.21 ± 0.36^{d}
GSH (mM/g Hb)	14.25 ± 0.54^{a}	9.89 ± 0.68^{b}	11.99 ± 0.75^{c}	11.05 ± 0.25^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$.
A - μ mole of H_2O_2 consumed / minute.
B – One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in one minute.
C - μg of GSH consumed / min
D - μ moles of CDNB – GSH conjugate formed / min.

Results

Table 1 shows the level of blood glucose, total haemoglobin, glycosylated haemoglobin and plasma insulin of different experimental groups. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in diabetic rats, compared with normal rats. Administration of pterostilbene and THC tended to bring blood glucose and plasma insulin towards normal. The diabetic control rats showed a significant decrease in the level of total haemoglobin and

significant increase in the level of glycosylated haemoglobin. Oral administration of pterostilbene and THC to diabetic rats significantly restored total haemoglobin and glycosylated haemoglobin levels. The effect of THC was more prominent when compared with pterostilbene.
The effect of pterostilbene and THC on changes in the activities of erythrocyte membrane bound total ATPase, Na^+/K^+ ATPase, Ca^{2+}-ATPase and Mg^{2+}- ATPase of normal and experimental rats are shown in table 2. In diabetic rats, the activities of total ATPase, Na^+/K^+ ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase were significantly lowered when compared to normal rats. Administration of pterostilbene and THC to diabetic rats significantly elevated the activities of total ATPase, Na^+/k^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase as compared to diabetic control rats. The effect of THC was more potent than pterostilbene.
Table 3 shows the activities of TBARS, CAT, SOD, GPx, GST, and GSH in the erythrocytes of diabetic and normal rats. Rats treated with pterostilbene and THC, however, had significantly lower erythrocytes TBARS levels as compared with the untreated diabetic rats. Diabetic rats had decreased activities of CAT, SOD, GPx, GST and GSH in the erythrocytes as compared with normal rats. Diabetic rats treated with pterostilbene and THC showed reversal of these parameters to near normal. The effect of THC was better than pterostilbene.

Discussion

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Oxidative stress is currently suggested as a mechanism underlying diabetes and diabetic complications (Baynes and Thrope, 1999). Oxidative stress may be defined as a measure of the steady state level of reactive oxygen or oxygen radicals in a biological system (Halliwell and Chirico, 1993). Increased oxidative stress may result from over production of precursor to reactive oxygen radicals and/or decreased efficiency of inhibitory and scavenger systems. The stress then may be amplified and propagated by an autocatalytic cycle of metabolic stress, tissue damage and cell death leading to a simultaneous increase in free radical production and compromised inhibitory and scavenger mechanisms, which further exacerbate the oxidative stress.
A free radical is any species capable of independent existence that contains one or more unpaired electron. Free radicals and its intermediates are produced in the biological system under physiological and pathological conditions (Halliwell and Gutteridge, 1993). These free radicals react with biomembrane causing oxidative destruction of poly unsaturated fatty acids (PUFA) forming cytotoxic aldehydes by a process known as lipid peroxidation (Glugliano et al. 1996).
Glycosylated haemoglobin was significantly increased in diabetic control rats, and this increase is directly proportional to fasting blood glucose (Koenig et al., 1976). Anemia is much more common disease in type 2 diabetic patients, contributing to the pathogenesis of diabetic complications. In the present study, the decreased concentration of haemoglobin indicates the anemia in STZ diabetic rats, in as much as during diabetes, the excess glucose transport in the blood reacts with haemoglobin to form glycosylated haemoglobin.
Lipid peroxidation is defined as the oxidative deterioration of PUFA to form free radical intermediate and peroxides, which damage cellular constituents. Lipid peroxidation does not require an enzyme but usually takes place by a free radical mechanism and catalyzed by traces of metals such as iron and copper (Glugliano et al. 1996). End products of lipid peroxidation are toxic, mutagenic and carcinogenic. Lipid peroxidation has been associated with several types of diseases including atherosclerosis, cancer and diabetes (Kurimura et al. 1988). Increased oxidative stress may contribute to the development of complications of diabetes mellitus.
Lipid peroxidation of cellular structures is thought to play a key role in atherosclerosis. Significantly higher values of thiobarbituric acid-reactive substances (TBARS) in the red blood cells as well as in serum and decreased erythrocyte antioxidant enzyme activities have been reported in diabetic condition (Singh and. Shin, 2009). In diabetic rats increased lipid peroxidation was associated with hypertriglyceridemia (Stringer et al. 1989). Modifications of long lived proteins (eg. collagen, elastin etc.) and structural changes in tissues rich in these proteins are associated with the

development of complications in diabetes such as cataract, microangiopathy, atherosclerosis and nephropathy (Jakus, 2000). Increased glycation of collagen and plasma proteins in diabetes may inturn stimulate autoxidative reaction of sugars, enhancing damage to both lipids and proteins in the circulation and the vascular wall, continuing and reinforcing the cycle of oxidative stress and damage (Baynes, 1999).

Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. Associated with the changes in lipid peroxidation the diabetic tissues showed decreased activities of key antioxidants SOD, CAT, GPx, GST and GSH which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals *in vivo*. Previous studies have reported that the activity of SOD is low in diabetes mellitus (Murugan and Pari, 2007). A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion $O_2^{\bullet-}$ and hydrogen peroxide in biological systems, which inturn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation (Murugan and Pari, 2006). The result of increased activities of SOD and CAT suggest that pterostilbene and THC contains a free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by the presence of $O_2^{\bullet-}$ and $OH^{\bullet}$. The increased activity of SOD accelerates dismutaion of $O_2^{\bullet-}$ to hydrogen peroxide, which is removed by CAT (Aebi, 1984). This action could involve mechanisms related to scavenging activity of pterostilbene and THC.

GSH can maintain SH groups of proteins in a reduced state, participate in amino acid transport, detoxify foreign radicals, act as coenzyme in several enzymatic reactions, and also prevent tissue damage (Tsai et al., 2012). It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level (Rizvi et al., 2011). There are several reports that claim reduced level of GSH in diabetes. Decreased GSH level may be one of the factors in the oxidative DNA damage in type 2 diabetics.

Changes in membrane lipid composition and enzymatic properties of membrane bound enzymes are shown to occur in diabetes. In this investigation the activities of erythrocyte membrane bound enzymes such as Na^+/ K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase were significantly inhibited in STZ diabetic rats. Similarly, the reduced activities of ATPase in erythrocytes and other tissues of STZ induced diabetic rats have been already reported (Murugan and Pari, 2007). STZ induced diabetes is characterized by a severe rearrangement of sub cellular metabolism and structural alterations of cell membrane. Thus the observed significant aberrations in the activity of erythrocyte membrane bound ATPases indicates the structure and function of the cell membranes are severely altered in diabetes, which may in turn, play an important role in the development of diabetic vascular complications.

The activity of Na^+/K^+-ATPase was found to be diminished in diabetic erythrocytes (Mamta and Surendra, 1992). The membrane Na^+/K^+-ATPase is concerned with the maintenance of a low intracellular concentration of Na^+. Decreases activity of Na^+/K^+-ATPase can lead to a decrease in Na^+ efflux and thereby alter the membrane permeability. The functional state of red blood cells depends on their active Na^+/K^+ sequestering property. The alter membrane structure and function of erythrocytes in diabetes may in turn affect the membrane microenvironment responsible for variations in the activity of membrane bound enzymes. *In vivo* insulin treatment restoring all the altered membrane bound ATPases activities to near normal and insulin also is responsible for the regulations of membrane bound ATPases activities. The lack of insulin reduces the units of Na^+/K^+-ATPase in the membrane. Insufficient Na^+/K^+-ATPase activity in diabetes leads to a consequently increases the amount of activator Ca^{2+} accessible for contraction the Na^+/K^+-ATPase in several tissues (Ver et al.,1999). These finding were accompanied by raise in intracellular Ca^{2+} concentrations, a fact that occurs when the Na^+/K^+-ATPase activity is reduced.

The Ca^{2+}-ATPase is the major active Ca^{2+} transport protein responsible for maintenance of normal intracellular calcium level in variety of cell types. The homeostasis of intracellular calcium is mainly regulated by a balance between membrane influx and efflux, the influx is due both to

voltage-dependent and receptor operated channels, while the main mechanisms of efflux is the active transport by the Ca^{2+} -ATPase. Mg^{2+}-ATPase is involved in energy requiring processes in the cell whereas Ca^{2+}-ATPase is responsible for the signal transduction pathways and membrane fluidity. Moreover Ca^{2+} transport is strictly integrated with the regulation of sodium transport as the Na^{+}-Ca^{2+} exchange pathway is driven by the electrochemical potential of sodium and maintained by the activity of the Na^{+}-pump. The intracellular concentration of calcium regulates the activities of Mg^{2+}- ATPase (involved in energy requiring process in the cell) and Na^{+}/K^{+}-ATPase and therefore, Ca^{2+} may play role in the regulations of sodium reabsorption. The inhibition of these transport systems in the cell may result in a sustained increase in cytosolic Ca^{2+} concentrations producing over stimulation of cellular processes leading ultimately to cell death (Fairhurst et al., 1982).

Defective increase of cytoplasmic concentration of Ca^{2+} is assumed to account for the impaired glucose tolerance in various peripheral and central diabetic patients. The activity of Ca^{2+}-ATPase has been shown to be decrease in erythrocyte membrane of diabetic rats, which results in the increased erythrocyte total Ca^{2+} levels as a result of the permeability alterations (Ramana devi et al., 1997).

In conclusion lipid peroxidation and glycosylation of proteins can cause reduction in the activities of enzymes and alteration in the structure and function of membranes (Flecha et al., 1990). A reduction in the lipid peroxidation and glycosylation of proteins can prevent diminution in the activities of ATPases, which is beneficial because any reduction in ATPases activity can affect the intracellular concentrations of Na^{+}, K^{+} and Ca^{2+}, alter the signal transduction pathway, and affect contractility, which in turn leads to cellular dysfunction. Administration of pterostilbene and THC to diabetic rats showed significant elevation in the activities of total ATPase, Na^{+}/K^{+}-ATPase, Ca^{2+}- ATPase and Mg^{2+}- ATPase in erythrocyte membrane when compared with diabetic control rats. The reversal of erythrocyte membrane bound ATPases activity in diabetic rats by pterostilbene and THC could be due to increase in metabolism of glucose, and thus the lowering of the glucose concentration in diabetic rats, would result in the activate antioxidant defense, reduction of free radical production, lipid peroxidation and the glycosylation of haemoglobin observed in this study.

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