Study of the Possible Protective Effects of Certain Dietary Supplements in Experimental Diabetic Neuropathic Pain

Ph.D. Thesis Presented by

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ABSTRACT

Diabetic neuropathy is the most common chronic complication of diabetes. **Objective:** The aim was to evaluate the protective effects of curcumin and alpha lipoic acid against neuropathy in gliclazide-treated diabetic rats. **Methods:** Diabetes was induced by intraperitoneal injection of streptozotocin (45 mg/kg). Diabetic animals were given gliclazide alone (GZ; 10 mg/kg, p.o.) or combined with curcumin (CM; 100 mg/kg, p.o), alpha lipoic acid (ALA; 100 mg/kg, p.o) or gabapentin (GBP; 30 mg/kg, i.p. as positive control). Behavioural responses to thermal (hot plate and tail flick), mechanical (tail pinch) pain were estimated as well as some biochemical tests for monitoring carbohydrate metabolism (serum glucose and C-peptide), oxidative stress (serum peroxynitrite, serum lipid peroxide, serum glutathione) and inflammation (serum tumor necrosis factor alpha). All the parameters were measured after five consecutive weeks of daily treatment. **Results:** The combined treatment of CM or ALA with GZ significantly increased hot plate and tail flick latency time in comparison with the diabetic control group (DC) at p<0.05. Moreover, the threshold of mechanical hyperalgesia was significantly elevated (p<0.05). The serum glucose C-peptide and glutathione levels were improved in the combined treatment compared to DC (p<0.05). Peroxynitrite, lipid peroxide and TNF alpha production were significantly lowered in serum by the concurrent administration of CM or ALA with GZ as compared to DC (p<0.05). **Conclusion:** Data suggest that the combination of curcumin or alpha lipoic acid with a conventional antihyperglycemic may protect against the development of diabetic neuropathy, with favourable effects with respect to gliclazide/gabapentin combination.

**Keywords:** gliclazide, curcumin, alpha lipoic acid, gabapentin, streptozotocin, diabetic neuropathy.
Diabetes mellitus (DM) is a common and serious metabolic disorder of multiple etiology. It is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. (Alberti and Zimmet, 1998; Gispen and Biessels, 2000). The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs e.g. heart, kidney, brain, eyes). It has been reported that, by the year 2030, diabetes mellitus is expected to affect almost 5% of the world’s population (an estimate of 366 million people) (Wild et al. 2004).

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made (Alberti and Zimmet, 1998).

Normal fasting blood glucose level in humans is 4.4 to 6.1 mmol/l (82 to 110 mg/dl). The WHO diagnostic criteria for diabetes stated that fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or 2–h post-prandial plasma glucose ≥ 11.1 mmol/l (200 mg/dl).

Complications of diabetes may be microvascular or macro-vascular and may result in significant morbidity and mortality (Simpson et al., 2003). They include progressive development retinopathy with potential blindness, nephropathy that
may lead to renal failure. Painful peripheral diabetic neuropathy (PDN) is a common complication of diabetes (Vinik et al., 2000) with risk of foot ulcers, amputation and features of autonomic dysfunction causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction (American Diabetes Association, 2004). People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (Alberti and Zimmet, 1998).

Understanding of diabetes as a metabolic disease has evolved significantly since the discovery of insulin in the 1920s (Bliss, 1993). Insulin was identified as a potent hormonal regulator of both glucose appearance and disappearance in the circulation. Subsequently, diabetes was viewed as a mono-hormonal disorder characterized by absolute (type 1 DM/ insulin dependent DM/ juvenile onset DM) or relative insulin deficiency (type 2 DM/ non insulin dependent DM/ maturity onset DM). Since its discovery, insulin has been the only available pharmacological treatment for patients with type 1 diabetes.

Insulin is also a mainstay of therapy for a significant proportion of diabetic patients with insulin-deficient type 2 diabetes who will eventually require insulin to alleviate symptoms of poor control and improve glycaemia (Bolli et al., 1999).

Although the most commonly identified disease categories would include type 1 and 2, it also includes gestational diabetes, malnutrition diabetes as well as drug or chemically induced diabetes (American Diabetes Association, 2001 and 2004). The following table illustrates the etiologic classification of diabetes mellitus:
Table (i): Etiologic classification of diabetes mellitus (American Diabetes Association, 2004)

<table>
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<th>Classification</th>
<th>Etiology</th>
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| **I. Type 1 diabetes**  
(beta cell destruction, usually leading to absolute insulin deficiency) | A. Immune mediated  
B. Idiopathic |
| **II. Type 2 diabetes** | It may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance |
| **III. Other specific types** | • Genetic defects of beta-cell function  
• Genetic defects in insulin action  
• Diseases of the exocrine pancreas  
• Endocrinopathies (Acromegaly, Cushing’s syndrome, Hyperthyroidism  
• Drug or chemically-induced (Nicotinic acid, Glucocorticoids, Thyroid hormone, Diazoxide, Thiazides, Dilantin)  
• Infections (Congenital rubella, Cyto-megalovirus, Coxsackie virus)  
• Uncommon forms of immune-mediated diabetes (“Stiff-man” syndrome, Anti–insulin receptor antibodies)  
• Other genetic syndromes sometimes associated with diabetes (Down’s syndrome) |
| **IV. Gestational DM**  
(GDM) | |


**Pathophysiological considerations:**

The natural history of type 1 diabetes is progression to complete loss of insulin secretory capacity and dependence on exogenous insulin for survival. Lack of or severe reduction in insulin secretion due to autoimmune destruction of β - cell (Palmer *et al.*, 2004) is responsible for type 1 diabetes mellitus, as assessed by measurements of islet cell autoantibodies (Rabinovitch, 1998; Krischer *et al.*, 2003; Franke *et al.*, 2005) and insulitis (inflammation of the islets). Hyperglycemia occurs in type 1 diabetes when insulin secretion becomes inadequate or absent as manifested by low or undetectable levels of plasma C- peptide (Hother–Nielsen *et al.*, 1988). Other initiating factors include viruses such as Coxsackie, rubella or cytomegalovirus (Forrest *et al.*, 1971; Yoon *et al.*, 1979; Pak *et al.*, 1988). Less common causes of type 1 DM are conditions that result in reduction in the mass of islet β-cell tissue, such as pancreatitis, pancreatic carcinoma and pancreatectomy (Rodrigues *et al.*, 1999).

Type 1 disease occurs at any age but it is typified by rapid onset diabetes in non-obese juveniles where acute ketoacidosis is the presenting symptom (Adams *et al.*, 2005). These patients may also have other autoimmune disorders such as Graves’ disease, Hashimoto’s thyroiditis, and Addison’s disease (Betterle *et al.*, 1983).

However patients with type 2 diabetes display a relative or absolute loss of insulin production as well (Bernard-Kargar and Ktorza., 2001). The pathogenesis of type 2 diabetes is complex, involving progressive development of insulin resistance and a relative deficiency in insulin secretion, leading to overt hyperglycemia (Bergman *et al.*, 2002). These individuals do not need insulin treatment to survive. This form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable
symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Harris, 1993; Mooy et al., 1995).

The new millennium has witnessed the emergence of a modern epidemic; the metabolic syndrome, also referred to as "Diabesity" (Astrup and Finer, 2000). This term describes the increasing incidence of diabetes in combination with obesity as a result of changes in human behaviour and available nutrition. Obesity and type 2 diabetes are occurring at epidemic rates. This epidemic (metabolic syndrome) of type 2 diabetes is complicated by the fact that it is a multifactorial disease, frequently associated with a cluster of pathologies including obesity, impaired glucose tolerance, insulin resistance (Larsson and Ahre´n, 1996 and 2000) hypertriglyceridemia, low levels of high density lipoproteins, hypertension and coronary heart disease (Reaven, 2004). This constellation of abnormalities is collectively referred to as the metabolic syndrome and formerly known as syndrome X or insulin resistance syndrome (Davidson, 1995; Reaven, 2004; Basciano et al., 2005). Insulin sensitivity may be increased by weight reduction, increased physical activity, and/or pharmacological treatment of hyperglycaemia but is not restored to normal (Simonson et al., 1984; Wing et al., 1994).

It is known that three major metabolic abnormalities contribute to the development of hyperglycemia in type 2 diabetes mellitus: impaired insulin secretion in response to glucose, increased hepatic gluconeogenesis and glycogenolysis. The high glucose output results in fasting hyperglycemia (DeFronzo, 1988). These hepatic effects are in part attributed to hyper-glucagonemia resulting from failure of the normal paracrine effect of insulin to suppress α-cell function (Reaven et al., 1987). In addition, there is evidence suggesting that hyperglycemia perpetuates the insulin secretion defect and the state
of insulin resistance (Rossetti et al., 1990; DeFronzo, 1992) and decreased insulin-stimulated glucose uptake in peripheral tissues.

The increased hepatic gluconeogenesis and glycogenolysis that contribute to hyperglycemia in type 2 diabetes are due primarily to insulin resistance (Kahn and Porte, 1990) i.e. decreased binding of insulin to cell membranes accompanied by decreased numbers of receptors, the highly specific sites of insulin-cell interaction responsible for activating transmembrane glucose transport and use (Olefsky, 1990). This type is associated with an increased risk of premature death and substantial disability, largely mediated through its adverse effects on the vasculature (Varma et al., 1997).

Insulin resistance of peripheral organs, i.e. muscle and adipocytes, as well as liver may contribute to fasting hyperglycemia. Peripheral insulin-resistance reduces the ability of peripheral organs to clear glucose from the circulation. Hepatic insulin-resistance develops in two stages. During the early stage in the development of type 2 diabetes, characterized by hyperinsulinemia and normoglycemia, hepatic glucose production is still normal under fasting conditions. However, hepatic glucose production remains inappropriately high during absorptive phases when insulin concentrations are elevated. At the later stage in the development of type 2 diabetes in humans, hepatic glucose production is increased also under fasting conditions (DeFronzo, 1988 and 1992). Both gluconeogenesis and glycogenolysis may contribute to an elevated hepatic glucose production (Kecskemeti et al., 2002). Furthermore, data indicates that cycling of glucose, i.e. the process of sequential glucose phosphorylation by glucokinase (GK) and dephosphorylation by Glucose-6-phosphatase, occurs at increased rates in humans with type 2 diabetes (Efendic et al., 1985; Rooney et al., 1993).
The persistent hepatic production of glucose, despite normal or elevated levels of insulin, has been demonstrated in rodent, canine, and human models of pancreatogenic diabetes (Sun et al., 1986; Seymour et al., 1988), although the mechanism of this loss of insulin regulation of hepatic glucose output has remained unclear. This may be attributed to the fact that various factors including insulin inhibitors and defects at the receptor or post-receptor level are involved, possibly down-regulation of insulin receptors or genetic defects in the receptors or may involve abnormalities in glucose transporter function (Wheatcroft et al., 2003), ultimately leading to insulin resistance (Ciaraldi et al., 1982; Wilcox, 2005).

Complications of Diabetes Mellitus:

The main etiology for morbidity in diabetic patients is vascular disease. (Stern, 1995; Standl et al., 1996). Diabetic vascular complications are primarily categorized into macroangiopathy (large vessels) and microangiopathy (small vessels). Macroangiopathy is manifested by accelerated atherosclerosis that affects vital organs (heart and brain) leading to hypertension, myocardial infarction, cardiomyopathy and stroke as reported by Laakso (1999); Dube and Peiris (2002). However, microangiopathy includes retinopathy (Gottsater et al., 2004), nephropathy (Ende et al., 2004) and neuropathy (Auslander et al., 2002).

In type 2 insulin resistant states, where residual insulin is present, hyperglycemia develops to a severe degree prompting diuresis and consequent dehydration and electrolyte derangement. This may impair renal flow which further raises blood glucose finally provoking hyperosmolar non-ketotic crisis (Veech, 2004). The hyperglycemia also fosters chemical interactions leading to the formation of glycosylated products. A sugar molecule in its aldehydic form reacts
non-enzymatically with protein (Valencia et al., 2004). These are reversible at first but eventually undergo irreversible rearrangement and reduction and may disrupt the protein activity (Tsukushi et al. 1999). A variety of structural and functional proteins can be glycosylated, such as haemoglobin, and membrane proteins, particularly in vasculature, nervous tissue, lens and renal tubules (Varma et al., 1997).

There is a close correlation between oxidative stress in diabetes and the development of complications. In type 1 diabetic patients, oxidative stress is evident within a few years of diagnosis before the onset of complications. As the disease progresses, antioxidant potential decreases, and plasma lipid peroxidation products increase depending upon the level of glycemic control (Tsai et al., 1994). Type 2 diabetic patients have increased lipid peroxidation compared with age matched control subjects, as well as decreased plasma glutathione (GSH) and GSH-metabolizing enzymes and antioxidant potential, all of which relate directly to the rate of development of complications (Altomare et al., 1992; Sundaram et al., 1996). Similarly, oxidative stress is linked to preclinical features of disease, such as vascular endothelial activation that can lead to atherosclerosis (Elhadd et al., 1999). The early increase of oxidative stress in diabetes is more pronounced in women and may account for increased cardiovascular disease in female patients (Marra et al., 2002).

**Diabetic Neuropathy**

Diabetes mellitus targets the peripheral nervous system in a unique but disabling way. Although several mechanisms may target peripheral neurons, they render a degenerative pattern of damage that begins in distal terminals. The
pathology of diabetic neuropathy involves oxidative stress in the peripheral and central nervous system as stated by Oberdley (1988) and plays a basic role in the genesis of endothelial dysfunction (Giugliano et al., 1996).

Oxidative stress resulting from enhanced free radical formation and/or a defect in antioxidant defenses has been implicated in the pathogenesis of experimental diabetic neuropathy and is well correlated with chronic hyperglycemia (Sayyed et al., 2006). Reactive oxygen species (superoxide radical, hydrogen peroxide and hydroxyl radical) and reactive nitrogen species (peroxynitrite) contribute to pathophysiological changes in diabetic neuropathy (Vincent et al., 2004). It also involves advanced glycation end products, polyol pathway flux, and protein kinase C activation which all contribute to microvascular disease and nerve dysfunction (Van Dam 2002; Feldman 2003; Duby et al., 2004; Vincent, et al., 2004).

A study by Zochodne et al. (2008) reported that sensory neurons are involved early but motor neurons later. Approximately 50% of diabetic patients develop peripheral neuropathy, or damage to the peripheral nervous system, which is an irreversible complication. Spontaneous pain, hyperalgesia, allodynia, parasthesia, dysthesia are common in 10-20% of diabetic neuropathy cases (Boulton and Ward, 1986; Partenen et al., 1995).

Patients with diabetic peripheral neuropathy develop insomnia, depression and anxiety, decreased mobility, psychomotor impairment and inability to work (Dworkin et al., 2005; Jensen-MP et al., 2006; Jensen-TS et al., 2006; Dworkin et al., 2007a, b). Over a period of time that could last several years, PDN subsides and the disabling pain is replaced by a complete loss of sensation, leading to the numb, insensate diabetic foot (Feldman et al., 1997, 2005). An estimated 15% of all patients with diabetes will develop foot ulcers (Gordois et al., 2003), and
diabetic neuropathy was reported to be the leading cause of non-traumatic limb amputation (Thomas, 1999).

In peripheral diabetic neuropathy, sensory deficits usually overshadow motor nerve dysfunction and appear first in the distal portions of the extremities and progress proximally in a “stocking-glove” distribution with increasing duration or severity of diabetes as illustrated in the following figure (i):

**Figure** (i): Stocking-glove configuration of diabetic neuropathy. It is dependent on axon length, initiating in the toes and progressing upward until reaching the calf. Neuropathy presents at the fingertips at this point. (adopted from Edwards et al., 2008).
Pathogenesis of Diabetic Neuropathy and the Involved Mediators:

There may be multiple etiologies which account for the various neuropathic syndromes shown in patients with diabetes. Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy as well as the other microvascular complications of diabetes. Accordingly, investigations into the molecular and biochemical pathophysiology of diabetic neuropathy have focused on glucose metabolic pathways (Edwards et al., 2008). Each pathway follows a mechanism that contributes to diabetic neuropathy as shown in the following diagram (fig ii).
Figure (ii): Schematic Diagram of hyperglycemic effects on biochemical pathways in diabetic neuropathy (Duby et al., 2004).
1. Polyol pathway

The enzyme aldose reductase (AR) reduces glucose to sorbitol and sorbitol dehydrogenase oxidizes sorbitol to fructose (fig ii). Both of these enzymes are abundantly expressed in tissues prone to diabetic complications. Hyperglycemia activates the aldose reductase pathway whereby increased flux through the AR pathway causes increased intracellular sorbitol (Nakamura et al., 1999; Vincent et al., 2004). Accumulation of sorbitol within the nerve and lens tissues causes cellular swelling or death (Chandrasoma and Tailor, 1995). Since NADPH is consumed by aldose reductase-mediated reduction of glucose to sorbitol (Jermendy et al., 1991; Brownlee, 2005) and NADPH is required for regeneration of reduced glutathione (GSH), this in turn contributes to oxidative stress. The second step in the polyol pathway oxidizes sorbitol to fructose via sorbitol dehydrogenase (Feldman et al., 1997). Formation of fructose promotes glycation as well as depletes NADPH, further augmenting redox imbalance. Activation of aldose reductase may also increase formation of diacylglycerol, which activates the deleterious protein kinase C (PKC) pathway (Yamagishi et al., 2003; Uehara et al., 2004).

2. Protein Kinase C (PKC) Pathway

The protein kinase C (PKC) pathway is an additional mechanism by which hyperglycemia causes injury in complications-prone tissues. Elevated glucose levels stimulate diacylglycerol (DAG), which in turn activates PKC. Increased PKC pathway flux plays a role in neuropathy as well (Arikawa et al., 2007; Das Evcimen and King, 2007). PKC pathway activation alters vasoconstriction and capillary permeability, and can cause hypoxia, angiogenesis, basement membrane
thickening, and endothelial proliferation (Williams et al., 1997; Edwards et al., 1999). PKC activation also alters the function of the Na⁺/K⁺ ATPase pump and other enzymes crucial to proper nerve conduction.

Activation of different PKC isoforms has been shown to decrease Na⁺/K⁺ ATPase activity in smooth muscle cells and normalize activity in peripheral nerves (Greene et al., 1987; Xia et al., 1995). The link of PKC to diabetic neuropathy is supported by studies in streptozotocin (STZ) induced diabetic rats, where PKC inhibition normalizes both sciatic nerve blood flow and nerve conduction velocity (Nakamura et al., 1999). Overexpression of PKC isoforms can also directly induce insulin resistance (Cortright et al., 2000; Naruse et al., 2006).

3. Advanced glycation endproducts (AGE) pathway

Non-enzymatic reactions between reducing sugars or oxaldehydes and proteins/lipids result in advanced glycation endproducts (AGEs) (Ahmed, 2005; Toth et al., 2008). AGEs are heterogeneous modified intracellular and extracellular biomolecules. Inside cells, both protein and DNA adducts alter function and cellular transport. Extracellular protein AGEs include plasma and matrix proteins that disrupt cellular adhesion and activate the receptor for AGEs (RAGE) (Ramasamy et al., 2007). AGE–RAGE interaction activates the transcription factor nuclear factor kappa B (NF-κB) (fig ii). NF-κB regulates a number of activities including inflammation and apoptosis (Ramasamy et al., 2005). Activation of neuronal RAGE induces oxidative stress (Vincent et al., 2007). Increased levels of AGE and RAGE were found in human diabetic tissue (Tanji et al., 2000). Collectively, the biochemical damage induced by AGEs results in impaired nerve blood flow and diminished neurotrophic support (Wada and Yagihashi, 2005).
4. Inflammation

Inflammatory agents including C-reactive protein and TNF-α are present in the blood of both T1DM and T2DM (Gomes et al., 2003; Gonzalez-Clemente et al., 2005). Higher levels of these proteins correlate with the incidence of neuropathy (Gonzalez-Clemente et al., 2005). The glucose-induced pathways previously mentioned produce the initiating inflammatory mediator TNF-α which in turn induces other inflammatory mediators; cyclooxygenase-2 (COX-2), IL-6, and IL-8 (Vincent and Feldman, 2004; Brownlee, 2005). As illustrated in figure (ii), excess glucose shunted through alternative metabolic pathways lead to increases in NF-κB (Brownlee, 2001). Extracellular AGEs that activate RAGE also lead to intracellular inflammatory signaling to upregulate NF-κB (Toth et al., 2008), and consequently upregulate COX-2 enzyme (Lee et al., 2004). COX-2 has been previously implicated in diabetes-induced changes of peripheral nerves including depletion of GSH, increases in TNF-α, and blood flow and nerve conduction deficits (Kellogg et al., 2007; Matsunaga et al., 2007).

Another inflammatory enzyme regulated by NF-κB is inducible nitric oxide synthase (iNOS) (Kim et al., 2008). Like COX-2, iNOS induces and is induced by NF-κB, leading to a vicious cycle of inflammation (Hasnis et al., 2007; Kim et al., 2008). Nitric oxide (NO) was found to play direct roles in axon and myelin breakdown following an injury and also contributes to the development of neuropathic pain (Levy and Zochodne, 2004; McDonald et al., 2007). Excessive local levels of NO during inflammation may damage axons and growth cones (Zochodne and Levy, 2005). The cytokines induced by NF-κB in endothelial cells, Schwann cells and neurons also lead to macrophage recruitment in diabetic nerves (Yamagishi et al., 2008). Macrophages promote diabetic neuropathy through a variety of mechanisms, including production of ROS, cytokines and
proteases, which result in myelin breakdown and cellular oxidative damage (Conti et al., 2002a,b; Tesch, 2007; Kawamura et al., 2008). Excessive macrophage recruitment likely impairs nerve regeneration in diabetic neuropathy (Conti et al., 2002a,b; McDonald et al., 2007).

5. Poly(ADP-ribose) polymerase (PARP) pathway

Poly(ADP-ribose) polymerase (PARP) found in Schwann, endothelial cells, and sensory neurons is also implicated in glucotoxicity. PARP is a nuclear enzyme closely associated with oxidative–nitrosative stress, whereby its activation is stimulated by free radicals and oxidants (fig ii). PARP causes and is activated by oxidative stress (Obrosova et al., 2005a). It acts by cleaving nicotinamide adenine dinucleotide (NAD+) to nicotinamide and ADP-ribose residues attached to nuclear proteins (Southan and Szabo, 2003), resulting in NAD+ depletion and increased free radical and oxidant concentration, with further diversion to other pathogenic pathways such as PKC and AGE formation (Garcia Soriano et al., 2001; Ha et al., 2002; Du et al., 2003; Obrosova et al., 2005a). Such PARP-implicated abnormalities manifest clinically as decreased nerve conduction velocity (NCV), small fiber neuropathy, neurovascular abnormalities, retinopathy, thermal and mechanical hyperalgesia, and tactile allodynia (Pacher et al., 2002; Obrosova et al., 2004; Li et al., 2005a,b; Obrosova et al., 2005a; Ilnytska et al., 2006).

6. Oxidative stress and apoptosis

The polyol, AGE, PKC, and PARP pathways all contribute to neuronal damage. Glycation and oxidative stress are closely linked, and both phenomena are referred to as ‘‘glyoxidation’’. All steps of glyoxidation generate oxygen-free radical production, some of them being common with lipid peroxidation pathways.
Glycated proteins activate membrane receptors such as RAGE through AGEs, and induce an intracellular oxidative stress and a pro-inflammatory status. Some oxidative products (reactive aldehydes such as methylglyoxal) or lipid peroxidation products (malondialdehyde) may bind to proteins and amplify glycoxidation generated lesions (Hunt et al., 1988; Hicks et al., 1989; Ravelojaona et al., 2007). As illustrated in figure ii, the AGE and polyol pathways directly alter the redox capacity of the cell either through direct formation of ROS or by depletion of necessary components of glutathione recycling. The PKC and PARP pathways exhibit damage through expression of inflammatory proteins.

Mounting evidence suggests that the hyperglycemic environment coupled with a compromised blood supply overloads the metabolic capacity of the mitochondria, producing oxidative stress (Brownlee, 2001). This oxidative stress leads to mitochondria damage followed by axonal degeneration and death. Mitochondrial damage occurs due to excess formation of ROS and reactive nitrogen species (RNS) (Nishikawa et al., 2000; Obrosova et al., 2005b,c and 2007). ROS, such as superoxide and hydrogen peroxide, are produced under normal conditions through the mitochondria electron transport chain and are normally removed by cellular detoxification agents such as superoxide dismutase, catalase, and glutathione (Leininger et al., 2006).

Hyperglycemia leads to increased mitochondria activity, raising ROS production in the mitochondria. Peroxynitrite, the primary RNS, is formed by the reaction of superoxide and nitric oxide (NO). RNS induces a number of cytotoxic effects including protein nitrosylation and activation of PARP (Obrosova et al., 2005a,c; Obrosova and Julius, 2005). Excessive ROS/RNS formation eventually overloads the natural antioxidant capacity of the cell, resulting in injuries to lipids, proteins and DNA. This damage ultimately compromises cellular function and
integrity. As the mitochondria is the origin of ROS/RNS generation, it is most susceptible to damage. Cellular oxidative stress is further enhanced when excessive glucose leads to overproduction of superoxide. Thus, all glucose-activated metabolic pathways converge to produce cellular oxidative stress which leads to decreased nerve blood flow/ischemia and exacerbate tissue injury (Edwards et al., 2008).

Figure (iii): Schematic diagram showing the role of oxidative stress in pathophysiology of diabetic neuropathy. ETC: electron transport chain; GSH: reduced glutathione; PARP: poly ADP-ribose polymerase; SOD: superoxide dismutase (Negi et al., 2011).
Induction of Neuropathy

1) Streptozotocin-Induced Diabetic Neuropathy

Streptozotocin (STZ) is an antibiotic extracted from *Streptomyces acromogenes*. It is diabetogenic due to a selective cytotoxic action upon pancreatic beta cells (Rakieten et al., 1963). The model of STZ-induced diabetes in rat has been increasingly used in an attempt to provide information on underlying processes, and to evaluate potential therapies for neuropathy. Experimental diabetes and neuropathy can be induced in overnight food-deprived rats by a single dose of STZ (45 mg/kg, i.p.), dissolved in cold citrate buffer (0.1 M, pH 4.5) (Stevens et al., 2007). This dose was selected to cause incomplete destruction of pancreatic beta cells as it has been reported that STZ is capable of producing mild to severe types of diabetes according to the dosage used, when it was administered to adult rats by either single i.v. or i.p. injection (Junod et al., 1969; Sima and Sugimoto, 1999; Aybar et al., 2001; Cameron, 2003). Junod et al. (1969) and Ledoux et al. (1986) included the triphasic pattern of changes in blood glucose and insulin during the 24 h period following STZ injection to male Wistar rats. There is an initial hyperglycaemia that lasts for 1 h followed by a critical period of hypoglycaemia (lasting around 6h) due to massive β-cell degranulation and an enormous release of pancreatic insulin (hence the need to add 5% glucose in drinking water). Finally, stable hyperglycaemia develops within 48 h and remains 3 to 4 times higher than the normal value accompanied by almost 50% reduction in insulin level (Junod et al., 1969). A single intraperitoneal STZ injection was reported to induce long lasting thermal and mechanical hyperalgesia as well as cold and thermal allodynia (Wuarin-Bierman et al., 1987; Courteix et al., 1994). It also caused 50% blood flow deficit and neurovascular dysfunction (Cameron et