CHAPTER I

Introduction and Review of literature
CHAPTER 1

INTRODUCTION

The body’s aging process might seem, at first glance, to be unrelated to the disease we call ‘diabetes’. After all, the root causes of diabetes have to do with how the hormone ‘insulin’ is produced or used in the body, whereas the root causes of aging appear to be free radicals, malfunctioning genes, and damaged proteins. Nevertheless, there is a connection between diabetes and aging — if not in their ultimate causes, then at least in the biochemical disruptions resulting from these causes, and in the symptoms that are produced by them. Because of these similarities, both diabetes and aging respond to some of the same treatments. This means that diabetes research is giving rise to anti-aging techniques, even though not a great deal of effort is going directly into anti-aging research itself. And it means that some of the supplements that are normally targeted at diabetic complications should also be used to fight aging in non-diabetics.

Close correlations have recently been shown among the late onset complications encountered in diabetes and aging linked to neurobiological disorders. Aging in females and males is considered as the end of natural protection against age related diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer’s disease (AD) and Parkinson’s disease (PD), dementia, cognitive dysfunction and hypernatremia. Besides the sex hormones other hormonal changes are also known to occur during aging and many common problems encountered in the aging process can be related to neuroendocrine phenomena. Diabetes mellitus is associated with moderate cognitive deficits and neurophysiologic and structural changes in the brain, a condition that may be referred to as diabetic encephalopathy, diabetes increases the risk of dementia especially in the elderly (Fazeli, 2009; Baquer et al., 2009).

The emerging view is that diabetic brain features many symptoms that are best described as accelerated brain aging. Diabetes is considered to be a kind of “accelerated aging” by increasing susceptibility to degenerative condition, including kidney disease, retinopathy, hypertension, coronary artery disease, stroke and atherosclerosis. Recently evidence has accumulated to suggest that diabetes also plays a role in accelerated brain aging. It is known that diabetes is associated with an increased risk of dementia; however, the exact mechanisms and mitigating factors remain unclear. New evidence describes the
connection between diabetes, dementia and Alzheimer’s related neurodegeneration (Biessels and Gispen, 2005; Li and Hölscer, 2007).

Diabetes mellitus, regardless of its type, is associated with cerebral alterations in both human and animal models of the disease (Biessels and Gispen, 2005; Alvarez et al., 2009). Diabetes mellitus is associated with cognitive deficits and an increased risk of dementia, particularly in the elderly. These deficits are paralleled by neurophysiological and structural changes in the brain. The multifactorial pathogenesis of diabetic encephalopathy is not yet completely understood, but clearly shares features with brain aging and the pathogenesis of diabetic neuropathy. It involves both metabolic and vascular changes, related to chronic hyperglycaemia, but probably also defects in insulin action in the brain.

ACCELERATED AGING OF THE BRAIN IN DIABETES
The observation that the effects of diabetes on the brain appear to be most pronounced in the elderly should be taken into consideration when the mechanisms that underlie diabetic encephalopathy are explored. The aging brain is possibly more sensitive to the effects of diabetes. Alternatively, the pathogenic processes of aging and diabetes might interact, leading to an accelerated cognitive decline in the elderly (Muresanu et al., 2010).

Like aging, diabetes is associated with an impairment of neuronal calcium (Ca²⁺) homeostasis (Murchison and Griffith, 2007; Lebeche et al., 2008; Baquer et al., 2009). Obviously, the relative contribution of ischaemia, oxidative stress, advanced glycation end-products (AGEs) formation and disturbances of neuronal Ca²⁺ homeostasis is different in brain aging and the development of diabetic encephalopathy; however, the similarities are evident and are likely to explain part of the increased susceptibility of elderly patients to the effects of diabetes on the brain. Experimental evidence for an interaction between diabetes and aging is provided by the aforementioned observation that streptozotocin (STZ)-diabetes produces more severe deficits in learning and synaptic plasticity in aged (24 months) than in young adult Wistar rats (Kamal et al., 2000).

DIABETES AND TRIGONELLA FOENUM GRAECUM TREATMENT
The term diabetes mellitus describes a metabolic disorder of multiple aetiology, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism,
resulting from defects in either insulin secretion or insulin action or both. A number of Indian herbal preparations have been used in the treatment of diabetes from ancient times. *Trigonella foenum graecum* (Fenugreek) appears to have potential anti-diabetic effects by stimulating or regenerating pancreatic beta cells, its extra pancreatic effects may be important. *Trigonella foenum graecum* seeds powder (TSP) administration for three weeks to alloxan diabetic rats stabilizes glucose homeostasis in brain by normalizing the glucose and membrane linked and antioxidant enzymes (Mohammad et al., 2006; Siddiqui et al., 2005; Tripathi and Chandra, 2009; Losso et al., 2009).

**AGING AND ESTRADIOL TREATMENT**

Estrogen acts through its receptor and regulates the development and the function of various human organ system including reproductive, skeletal and nervous system, it is also involved directly in numerous pathological and aging processes such as Alzheimer disease, osteoporosis, cardiovascular disease, mental disorder and carcinogenesis. Aging is associated with a decline in metabolic function, and one of the main illustrations of this metabolic decline is the development of insulin resistance. Although the mechanism underlying the development of insulin resistance with advanced age remains unclear, in the case of females, loss of gonadal function seems to determine the start of this period of metabolic decline. In addition, insulin resistance in aging is associated with metabolic syndrome, which is associated with increased incidences of depression, neurodegenerative diseases, and memory or cognitive dysfunction (Moorthy et al., 2004a; 2005a). Therefore, the increased incidences of neurodegenerative diseases in postmenopausal females seem to be clinically associated with aging, loss of gonadal function, and development of insulin resistance (Nelson, 2008).

**USE OF SYNAPTOSOMES AS NEURONAL CELLS TO STUDY VARIOUS PHYSIOLOGICAL PROCESS OF THE BRAIN**

Synaptosomes, the isolated terminal portions of axons that behave as metabolically autonomous mini-cells, provide a good experimental model to evaluate neural degenerative process and peroxidative events in the brain. Synaptosomal preparation preserve the functional activity of presynaptic terminal: thus they have proven very useful in the study of
various synaptic invitro events, including uptake, storage, synthesis and release of neurotransmitters. Synaptosomes are formed from the phospholipid layer of the cell membrane carrying out synaptic transmission, they contain the molecular machinery necessary for the uptake, storage, and release of neurotransmitters and are relatively easy to prepare in the laboratory (Mantha et al., 2006). These were used as invitro models to evaluate various effects on diabetic and glucose homeostasis and insulin signaling on brain, in diabetes and aging. A study was carried out to see in depth the modulation of metabolic functions during normal aging in animals together with their modulation and changes in diabetic animals. It has recently been suggested that diabetes a condition of insulin deficiency can be said to be a hastened process of aging. In the present work two compounds *Trigonella foenum graecum* and estradiol were tested for their antidiabetic and antiaging properties respectively.

**AIM OF THE STUDY**

*Membrane-Linked Functions and Glucose Transporter (GLUT4) in Diabetic and Aging Female Rats: Effect of Trigonella foenum graecum and Estradiol and compare membrane functions in diabetic and aging female rats. Correlation of metabolic changes associated with aging and diabetes.*

**OBJECTIVES**

1. The objective of the study was to correlate and study the biochemical, histochemical and molecular processes taking place in experimental diabetes and normal aging.

2. The two physiologically different condition, diabetes and aging are taken and various neuronal/ membrane markers, physiological, biochemical, histochemical, structural and molecular, were studied with their reversal using an antidiabetic compound, *Trigonella foenum graecum* and the hormone estradiol (E2).

3. The procedures used were that female animals of 3, 12, 24 months were taken together with estradiol groups of the same age, and diabetic animals treated with *Trigonella* and insulin.
4. General parameters and other neuronal/membrane parameters were studied, physiological (body weight, hormone levels, glucose and protein levels) biochemical (membrane bound (Na\textsuperscript{+}K\textsuperscript{+} ATPase, Ca\textsuperscript{2+} ATPase, Monoamine oxidase), antioxidant enzymes (Superoxide dismutase, Glutathione S-transferases), lipid peroxidation), structural changes (degree of membrane fluidity, intrasynaptosomal calcium levels), histochemical changes (neurolipofuscin accumulation, immunohistochemistry of Glucose transporter-4 (GLUT4), and molecular changes (GLUT4 mRNA expression, Western blotting, DNA laddering) including insulin levels in both diabetic and aging were measured.

5. Results from both physiologically induced conditions are discussed in relation to each other and separately to elucidate the similarities of the two conditions, diabetes and aging.

THE WORK CARRIED OUT IS COMPLIED IN THE FORM OF A DISSERTATION CONTAINING FIVE CHAPTERS.

Chapter 1. is review of literature related to the "Diabetes and aging", their damaging effects, neurological disorders and introduction to the modulators Trigonella and estradiol used and their pharmacological actions in present work. It also highlights the role of membrane functions, membrane bound and antioxidant enzymes, lipid peroxidation, degree of membrane fluidity, lipofuscin, intrasynaptosomal calcium and glucose transporter-4.

Chapter 2. is general methodology used during present work. Describing the details of the methods and techniques used.

Chapter 3. comprises results sections and presents the data obtained from the respective experiments including experimental diabetes and normal aging and its modulation with Trigonella and estradiol respectively.

Chapter 4. is the discussion and a critical analysis of the present work in the light of previous work addressing molecular and biochemical correlates in aging and diabetic neuronal functions.

Chapter 5. is the summary of the work done and some concluding remarks.
THE RELEVANCE AND EXPECTED OUTCOME OF THE PROPOSED STUDY

Diabetes (DM) and age related neurodegenerative disease like Alzheimer’s disease (AD) are conditions that affect a large number of people in most countries. Both conditions are on the increase and finding novel treatments to cure or prevent them are a major aim in research. DM and aging are becoming global problems with the world population becoming skewed towards the older age group. Both conditions lead to complications causing considerable morbidity, therefore identifying factors that predispose individuals with these two pathologies of old age would be of considerable assistance in prevention and management. The role of *Trigonella* and estradiol (E2) and their effects on glucose homeostasis and insulin signaling will provide alternative therapeutic options for diabetes mellitus and age related disorder respectively.

This study presents and compares biochemical, physiological, molecular and pathological data from neuronal tissue of aging and hormone treated control and diabetic animals to arrive at the similarities among the two naturally occurring physiological conditions. Antiaging strategies using hormone therapy, was carried out for reversal of aging effects. Neuronal markers have been presented in this study and similarities in changes were seen among the aging, diabetes and hormone treated (estrogen and insulin) brains from these animals. A close correlation was observed in parameters like oxidative stress, enzyme changes, and pathological changes like lipofuscin accumulation in aging and diabetic brain.

A study was carried out to see in depth the modulation of metabolic function during normal aging in animals, together with their modulation and changes in experimentally diabetic animals. The two physiologically different conditions were taken because it has been suggested that diabetes, a condition of insulin deficiency can be compared or referred to also as a hastened process of aging. Animal models can make a substantial contribution to our understanding of the pathogenesis, which shares many features with the mechanisms underlying brain aging. By unraveling the pathogenesis, targets for pharmacotherapy can be identified. This may allow treatments or prevention of diabetes and age related complication in the future.
1.1 Diabetes mellitus
The term diabetes mellitus describes a metabolic disorder of multiple aetiology, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in either insulin secretion or insulin action or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present itself in 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 hyperglycemia is sufficient to cause pathological and functional changes that may be present for a long time before the diagnosis is made.

1.2 Global prevalence of diabetes
Diabetes is possibly the world's fastest growing metabolic disease, and as knowledge of the heterogeneity of this disorder increases, so does the need for more appropriate therapies (Awdeh and Alper, 2005). It is projected that the worldwide prevalence of diabetes is likely to increase to more than 439 million by the year 2030 (Table 1). According to World Health Organization, the developing countries like India are expected to shoulder much of this burden. The world prevalence of diabetes among adults (aged 20–79 years) will be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries. India, China and the United States have and will have in 2030, the largest number of diabetes (Shaw et al. 2010).

1.3 Classification
Diabetes mellitus is classified primarily based on etiology. The new classification system as adopted by American Diabetic Association report in 1997 and World Health Organization report in 1999 identifies four types of diabetes mellitus: type 1, type 2, "other specific types"
Table 1. Top ten countries for estimated numbers of adults with diabetes, 2010 and 2030

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of adults with diabetes (millions)</th>
<th>Country</th>
<th>No. of adults with diabetes (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 India</td>
<td>50.8</td>
<td>1 India</td>
<td>87.0</td>
</tr>
<tr>
<td>2 China</td>
<td>43.2</td>
<td>2 China</td>
<td>62.6</td>
</tr>
<tr>
<td>3 USA</td>
<td>26.8</td>
<td>3 USA</td>
<td>36.0</td>
</tr>
<tr>
<td>4 Russian Federation</td>
<td>9.6</td>
<td>4 Pakistan</td>
<td>13.8</td>
</tr>
<tr>
<td>5 Brazil</td>
<td>7.6</td>
<td>5 Brazil</td>
<td>12.7</td>
</tr>
<tr>
<td>6 Germany</td>
<td>7.5</td>
<td>6 Indonesia</td>
<td>12.0</td>
</tr>
<tr>
<td>7 Pakistan</td>
<td>7.1</td>
<td>7 Mexico</td>
<td>11.9</td>
</tr>
<tr>
<td>8 Japan</td>
<td>7.1</td>
<td>8 Bangladesh</td>
<td>10.4</td>
</tr>
<tr>
<td>9 Indonesia</td>
<td>7.0</td>
<td>9 Russian Federation</td>
<td>10.3</td>
</tr>
<tr>
<td>10 Mexico</td>
<td>6.8</td>
<td>10 Egypt</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Prevalence of diabetes and estimated diabetes numbers by region among adults aged 20-79 years for the years 2010 and 2030.

<table>
<thead>
<tr>
<th>Region</th>
<th>Total adult population (000s)</th>
<th>No. of adults with diabetes (000s)</th>
<th>Diabetes prevalence (%)</th>
<th>Total adult population (000s)</th>
<th>No. of adults with diabetes (000s)</th>
<th>Diabetes prevalence (%)</th>
<th>Increase in the no. of adults with diabetes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>379</td>
<td>12.1</td>
<td>3.8</td>
<td>653</td>
<td>23.9</td>
<td>4.7</td>
<td>98.1</td>
</tr>
<tr>
<td>EME</td>
<td>344</td>
<td>26.6</td>
<td>9.3</td>
<td>533</td>
<td>51.7</td>
<td>10.8</td>
<td>93.9</td>
</tr>
<tr>
<td>Europe</td>
<td>646</td>
<td>55.4</td>
<td>6.9</td>
<td>659</td>
<td>66.5</td>
<td>8.1</td>
<td>20.0</td>
</tr>
<tr>
<td>N America</td>
<td>320</td>
<td>37.4</td>
<td>10.2</td>
<td>390</td>
<td>53.2</td>
<td>12.1</td>
<td>42.4</td>
</tr>
<tr>
<td>S &amp; C America</td>
<td>287</td>
<td>18.0</td>
<td>6.6</td>
<td>382</td>
<td>29.6</td>
<td>7.8</td>
<td>65.1</td>
</tr>
<tr>
<td>S Asia</td>
<td>838</td>
<td>58.7</td>
<td>7.6</td>
<td>1200</td>
<td>101.0</td>
<td>9.1</td>
<td>72.1</td>
</tr>
<tr>
<td>W Pacific</td>
<td>1531</td>
<td>76.7</td>
<td>4.7</td>
<td>1772</td>
<td>112.8</td>
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<tr>
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<td>6.4</td>
<td>5589</td>
<td>438.7</td>
<td>7.7</td>
<td>54.1</td>
</tr>
</tbody>
</table>

Modified by Shaw et al., (2010)
and gestational diabetes. The terms insulin-dependent (ID) state and non-insulin-dependent (NID) state can be used to describe the degree of deficiency of insulin effect (Table 2).

1.3.1 **TYPE-1 (BETA–CELL DESTRUCTION, USUALLY LEADING TO ABSOLUTE INSULIN DEFICIENCY)**

**(A) AUTOIMMUNE DIABETES MELLITUS**

This form of diabetes, previously encompassed by the term insulin-dependent diabetes (IDDM), Type 1 diabetes, or juvenile-onset diabetes, results from autoimmune mediated destruction of the beta cells of pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others. The rapidly progressive form is commonly observed in children, but also may occur in adults. The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Individuals with this form of Type 1 diabetes often become dependent on insulin for survival and eventually are at risk for ketoacidosis (Humphrey et al., 1998).

Markers of immune destruction, including islet cell autoantibodies, and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85–90% of individuals with Type 1 diabetes mellitus when fasting diabetic hyperglycaemia is initially detected (Verge et al., 1996). There is a genetic predisposition to autoimmune destruction of beta cells, and it is also related to environmental factors that are still poorly defined. Although patients are usually not obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients may also have other autoimmune disorders such as Graves’ disease, Hashimoto’s thyroiditis, and Addison’s disease (Betterle et al., 1983).

**(B) IDIOPATHIC DIABETES MELLITUS**

There are some forms of Type 1 diabetes, which have no known aetiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This form of diabetes is more common among individuals of African and
### Table 2. Aetiological Classification of Diabetes Mellitus and Related Disorders of Glycaemia (As per Recommendation of the ADA)

1. **Type 1** (beta-cell destruction, usually leading to absolute insulin deficiency)
   - *Autoimmune, B. Idiopathic*

2. **Type 2** (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance)

3. **Gestational diabetes**

4. **Other specific types**
   - **A. Genetic defects of beta-cell function**
     - Chromosome 20, HNF4-α (MODY1)
     - Chromosome 7, glucokinase (MODY2)
     - Chromosome 12, HNF1-α (MODY3)
     - Chromosome 13, IPF-1 (MODY4)
     - Mitochondrial DNA 3243 mutation
     - Others
   - **B. Genetic defects in insulin action**
     - Type A insulin resistance
     - Leprechaunism
     - Rabson–Mendenhall syndrome
     - Lipoatrophic diabetes
     - Others
   - **C. Diseases of the exocrine pancreas**
   - **D. Endocrinopathies**
   - **E. Drug- or chemical-induced**
   - **F. Infections**
   - **G. Uncommon forms of immune-mediated diabetes**
   - **H. Other genetic syndromes associated with diabetes**

(Modified from Alberti and Zimmet, 1998)
Asian origin. In another form found in Africans, an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis (McLarty et al., 1990).

1.3.2 TYPE-2 (PREDOMINANTLY INSULIN RESISTANCE WITH RELATIVE INSULIN DEFICIENCY OR PREDOMINANTLY AN INSULIN SECRETARY DEFECT WITH/WITHOUT INSULIN RESISTANCE)

Diabetes mellitus of this type previously encompassed non-insulin-dependent diabetes (NIDDM), or adult-onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency. Type-2 diabetes may account for about 90% to 95% of all diagnosed cases of diabetes and affects about 200 million people worldwide and has is predicted to increase to 485 million by 2030 (Shaw et al., 2010). It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce insulin. At least initially, and often throughout their lifetime, 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 et al., 1993).

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance. Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Campbell and Carlson, 1993). Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of another illness such as infection (Banerji et al., 1994). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the high blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their beta-cell function been normal (Polonsky et al., 1996). Thus, insulin secretion is defective and insufficient to compensate for the insulin resistance. On the other hand, some individuals have essentially normal insulin action, but markedly impaired insulin secretion. Insulin sensitivity may be increased by weight reduction, increased physical activity, and/or pharmacological treatment.
of hyperglycaemia, but is not restored to normal (Wing et al., 1994). The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity. Its frequency varies in different racial/ethnic subgroups. It is often associated with strong familial, likely genetic predisposition (Valle et al., 1997; Knowler, 1993). However, the genetics of this form of diabetes are complex and not clearly defined.

1.3.2.1 TYPE-2 DIABETES AND INSULIN RESISTANCE

When your muscle, fat, and liver cells cannot use insulin properly. There can be a lot of insulin circulating, but the cells are incapable of using it to allow glucose entering the cells and be converted into energy or stored as fat.

The pancreas keeps releasing more and more insulin until it cannot keep up with the body's need for insulin, and excess glucose builds up in the blood causing hyperglycemia, the main manifestation of diabetes. Before excess glucose builds up dangerously in the bloodstream, the person is in a state called "Pre-diabetes". Most people with pre-diabetes develop Type 2 diabetes within 10 years, unless they lose 10 or 15 pounds and increase their level of physical activity. Genes are part of the cause of the insulin resistance. Excess weight and especially too much fat in the abdomen contributes to insulin resistance and increases LDL blood cholesterol levels, lowers HDL cholesterol levels, raises tryglycerides levels, and increases high blood pressure, putting the heart at risk too. This combination of problems is called "metabolic syndrome" or "insulin resistance syndrome".

1.3.3 OTHER TYPES OF DIABETES DUE TO SPECIFIC CAUSES

These cases maybe classified into two major groups:

DIABETES IN WHICH SPECIFIC MUTATIONS HAVE BEEN IDENTIFIED AS A CAUSE OF GENETIC SUSCEPTIBILITY

Many genetic abnormalities leading to diabetes have been identified (Nanjo et al., 1998) and they can be related to (a) beta cell function (b) mechanism of insulin action. The first category includes abnormalities of the insulin gene, and MODY (maturity-onset diabetes of the young) cases. Mutations of genes for HNF4α, glucokinase, HNF1α, IPF-1 (PDX-1), HNF1β correspond to MODYs 1,2,3,4 and 5 respectively. This category also includes abnormality of mitochondrial DNA and the amylin gene (Sakagashira et al., 1996).
Maternally inherited diabetes and deafness (MIDD), a rare, monogenic diabetes occurs due to point mutation in mt DNA. The diabetes due to mitochondrial DNA mutations is called the mitochondrial diabetes. The second category includes a number of insulin receptor gene abnormalities (Kasuga and Kadowaki, 1994).

1.3.3.1 DIABETES ASSOCIATED WITH OTHER PATHOLOGIC CONDITION OR DISEASE
Diabetes and glucose intolerance may occur as a manifestation of various pathological conditions, diseases and syndromes. Some have been previously called ‘secondary diabetes’. These include pancreatic diseases, endocrinopathies, exposure to certain drugs and chemicals, viral infections, and various genetic syndromes.

1.3.3.2 GESTATIONAL DIABETES
Gestational diabetes mellitus (GDM) is a state of glucose intolerance occurring or detected for the first time during pregnancy. GDM patients share common genetic susceptibilities with type 1 or type 2 diabetes, and the deterioration of glucose tolerance is precipitated by the metabolic effect of pregnancy. A relative insulin deficiency is developed during the last half of the pregnancy in GDM. Glucose intolerance during pregnancy is often normalized after delivery, but such cases are at a higher risk of developing diabetes in the future.

1.4 COMPLICATIONS OF DIABETES MELLITUS
Diabetes mellitus is associated with numerous long-term clinical complications that contribute to the increased morbidity and mortality of the disease despite the available measures for metabolic control (DCCT Research Group, 1997, UKPDS Group, 1998). These complications may be divided into-

1.4.1 MACROVASCULAR COMPLICATIONS
Refers to changes in the medium to large-size blood vessels. The blood vessel walls thicken and become hard and non-elastic (arteriosclerosis). Blood vessels also become clogged with mounds of plaque (atherosclerosis). Eventually, the flow of blood may be blocked. Three types of this disease are: Peripheral vascular disease, Coronary artery disease and Cerebral vascular disease.
1.4.2 MICROVASCULAR COMPLICATIONS

(a) Diabetic Retinopathy
It is a deterioration of the small blood vessels that nourish the retina. Although diabetic retinopathy is a serious cause of blindness, only a small percentage of persons with diabetic retinopathy lose their sight.

(b) Diabetic Nephropathy
Diabetic nephropathy is a complication of long-term diabetes that results in damage to the bundles of capillaries that form the kidneys' filtering system. Diabetic nephropathy develops in stages over many years. Kidney filtering becomes less efficient, and certain proteins leak out. Proteins in the urine may be the first sign of nephropathy. Other signs include high blood pressure, weight gain from fluid retention, fatigue, and just feeling ill.

(c) Diabetic Neuropathy
Nearly 70% of persons with diabetes experience some degree of nerve damage or neuropathy. Neuropathy occurs in people with Type I and Type II diabetes, due to metabolic changes associated with diabetes. Constant high blood sugar destroys both nerve fiber (axon) and the fatty insulation that surrounds it (myelin). Damaged nerves do not transmit proper signals, resulting in a loss of sensation, hypersensation, or pain. Peripheral neuropathy is a frequent complication of diabetes mellitus. Kamal et al. (2000) studied the role of hippocampus in certain types of learning and memory where the function and synaptic plasticity has been studied as plastic changes in synaptic strength are assumed to be involved in learning and memory. Animal models have been used to examine the relation between memory deficits and changes in synaptic plasticity in diabetes (Kumagai, 1999). Like diabetic patients, STZ-diabetic rats develop end organ damage affecting the eyes, kidney, heart, blood vessels, and peripheral and central nervous system (CNS) (Figure 1).

Other long-term complications of diabetes include the diabetic foot, connective tissue and bone and joint diseases, ulceration, skin diseases and impotency in diabetic males. Effective control of hyperglycemia in diabetes may prevent or reverse the consequent metabolic derangements and resulting complications.
The multifactorial pathogenesis of diabetic encephalopathy is not yet completely understood but clearly shares features with mechanisms that underlie the development of peripheral diabetic neuropathy and brain aging. The pathogenesis can be divided into three main components: (1) direct neurotoxic effects of hyperglycaemia, including polyol pathways flux, oxidative stress, enhanced formation of advanced glycation end-products (AGEs), and disturbance of Ca\(^{2+}\) homeostasis; (2) vascular changes, including alteration in cerebral blood flow and angiopathy; and (3) alteration in neurotrophic support and neuromodulatory changes related to alterations in insulin and its receptor the brain.

(Adapted from Gispen and Biessels, 2000)
1.5 MECHANISMS BY WHICH HYPERGLYCEMIA CAUSES DIABETIC COMPLICATIONS

Three major hypotheses at the cellular and molecular level, have each generated a great deal of supporting data regarding the mechanisms by which hyperglycemia causes diabetic complications. The three mechanisms include:

1) Increased flux through the polyol pathway with resultant increased consumption of NADPH, which is necessary to reduce oxidized glutathione as glucose is reduced to sorbitol by the enzyme aldose reductase.

2) Formation of advanced glycation end-products (AGEs) from intracellular glucose-derived dicarbonyl precursors, creating a damaging positive feedback loop by modifying plasma proteins, which might then activate the receptor for AGEs, the central signal transducing receptor for these modified adducts (Schmidt et al., 1999).

3) Activation of protein kinase C isoforms by de novo synthesis of diacylglycerol, which results in pathological gene expression and protein modification in organs targeted for dysfunction in diabetes (Greene et al., 1987; Koya and King, 1998).

1.6 EXPERIMENTAL DIABETES

Diabetes mellitus can be induced experimentally in an intact animal by selectively destroying the islet cells in order to study uncontrolled diabetes and the sequence of events following administration of insulin. Several diabetogenic chemicals used for this purpose are streptozotocin, alloxan, chlorozotocin, vacor and diazoxide. The diabetes induced is typically analogous to the clinical conditions of Type I DM characterized by hyperglycemia, glucosuria, polydipsia, polysuria, body weight loss, ketosis, acidosis and hyperlipidemia (Emudianughe et al., 1988).

The present study employs alloxan monohydrate to induce experimental diabetes. Pancreatic islet cells treated with alloxan exhibit multiple cellular necrosis, marked degranulation and extensive vesiculation of the endoplasmic reticulum and golgi complex as well as enlarged mitochondria with disrupted cristae and ruptures (Abdel-Rehman et al., 1992). Brugnatelli in 1818 first described alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil). However, its diabetogenic properties were reported many years later by Dunn, Sheehan and McLethic (1943), who studied the effect of its administration in rabbits and
reported a specific necrosis of pancreatic islets. Alloxan is administered parenterally (intravenously, intraperitoneally or subcutaneously) to fasted animals (Katsumata et al., 1992) as increased blood glucose in a fed state is known to provide partial protection of pancreatic beta cells from alloxan toxicity (Bansal et al. 1980; Szkudelski, 2001). The cytotoxic action of alloxan is mediated by generation of reactive oxygen species (Heikkila et al., 1976). Alloxan is reduced to dialuric acid in the cell where it establishes a redox cycle leading to the formation of superoxide radicals (Szkudelski, 2001). These radicals dismutate to hydrogen peroxide, which in turn form highly reactive hydroxyl radicals by Fenton reaction (Grankvist, 1981; Kurahashi et al., 1993). Alloxan also causes a simultaneous massive increase in cytosolic calcium concentration (Kim et al., 1994). The toxic action of alloxan causing rapid destruction of beta cells are then the sum of several processes such as oxidation of essential –SH groups, inhibition of glucokinase (Lanzen and Mirzaie-Petri, 1991), generation of free radicals and disturbances in intracellular calcium homeostasis (Kim et al., 1994).

2. ANTIDIABETIC COMPOUNDS

2.1 INSULIN AND SIGNALING

Insulin and its receptors are known to be present in the brain (Havrankova et al., 1978; Azam et al., 1990b). The physiological function of insulin in brain region is still a controversy, and glucose uptake by brain is considered to be mainly insulin insensitive (Kumagai, 1999; Gispen and Biessels, 2000). Insulin does affect cerebral glucose utilization to some extent (Schulingkamp et al., 2000), analogous to its role in the periphery. In addition brain insulin does seem to play a role in the regulation of food intake and body weight (Schwartz et al., 1999), and it may act as a neuromodulator influencing the release and reuptake of neurotransmitters, and probably also learning and memory (Zhao et al., 1999).

Impairment of insulin signaling pathway in the periphery and brain have been implicated in AD, diabetes and aging (Li and Hölscher, 2007). Moreover, the brain insulin/IR signal transduction, beside the regulation of glucose metabolism, delivers signals necessary for synaptic activity and plasticity, of memory formation and for memory storage as well as for cell differentiation (Kremerskothen et al., 2002). Insulin levels are decreased in diabetes mellitus as well as experimental diabetes in animal models (Biessels et al., 2002;
Fulop et al., 2003). Insulin degrading enzyme (IDE) has been measured in diabetic brain and diabetic treated with insulin (Azam et al., 1990a,b). IDE is a metalloendopeptidase also involved in the degradation of amyloid beta protein the latter is involved in the pathology of AD and has been shown to induce apoptosis invitro. It has been reported that invitro insulin, inhibits IDE activity and proteosomal function competitively (Hamel et al., 1998) impairing protein turnover, thereby facilitating the gradual accumulation of oxidized protein. Diabetes and its treatments with insulin are likely to affect cerebral insulin levels and insulin signaling. It is different , however to separate the direct effect of alterations in insulin homeostasis on the brain from the consequence of the accompanying alteration in peripheral and control glucose homeostasis , which in themselves can affect the brain.

Insulin is a small protein containing 51 amino acids and consists of 2 polypeptide chains, A (21 amino acids) and B (30 amino acids) fragments. Insulin is synthesized as a preprohormone in the β cells of the islets of Langerhans. Its signal peptide is removed from the cisternae of the endoplasmic reticulum and it is packaged into secretory vesicles in the Golgi, folded to its native structure, and locked in this conformation by the formation of 2 disulfide bonds. Specific protease activity cleave the center third of the molecule, which dissociates as C peptide, leaving the amino terminal B peptide disulfide bonded to the carboxy terminal A peptide. Insulin secretion from β cells is principally regulated by plasma glucose levels, but the precise mechanism by which the glucose signal is transduced remains unclear.

Insulin acts by initiating a cascade of intracellular signals by binding to its cell surface transmembrane receptors (Figure 2). The insulin receptors are tetrameric proteins containing two alpha and two beta subunits and belonging to the class of receptor tyrosine kinase. Insulin binds to the alpha subunit stimulating autophosphorylation in the beta subunit and activation of the intrinsic tyrosine kinase activity (Patti and Kahn, 1998), which phosphorylates several downstream signal proteins. These proteins include- Insulin Receptor Substrate (IRS) protein, Phosphatidyl inositol-3-kinase (PI-3-K), Protein kinase B and AKT complex (PKB), Protein kinase C (PKC) and other accessories proteins. PI-3-kinase plays a crucial role in insulin signaling mechanism to transport glucose, translocation of GLUT4 protein from intracellular storage vesicles to plasma membrane. Insulin stimulated glucose uptake by PI-3-K independent pathway, which involves the
FIGURE 2. SIGNAL TRANSDUCTION BY INSULIN

(Adapted from Saltiel and Kahn, 2001)
tyrosine-mediated phosphorylation of Cb1 (a protooncogene). Cb1 interact with its adaptor protein CAP (Cb1 adaptor protein). After phosphorylation this Cb1-CAP complex recruits another adaptor protein Crk11 to the lipid raft, which interacts with guanyl nucleotide-exchange protein C3G. C3G activates the G protein TC 10 which provides a second signal to the GLUT4 protein (Elmendorf, 2002).

Insulin receptors phosphorylation also activates the Ras/MAP kinase cascade, which plays an important role in the mitogenic effects of insulin (Lazar et al., 1995). Insulin action is attenuated by rapid dephosphorylation of the receptor and its substrates by protein tyrosine phosphatases. Insulin regulates blood glucose homeostasis by decreasing the release of glucose from liver by inhibiting the hepatic gluconeogenesis and increasing the transport of glucose into the peripheral tissues like skeletal muscle and adipose tissues. Insulin also regulates cell growth and differentiation, promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogenesis and protein synthesis and inhibiting lipolysis, glucogenesis and protein breakdown. Insulin induces the translocation of GLUT4 protein and Na⁺K⁺ATPase subunits from cellular storage vesicles to cell membrane.

2.2 OTHER ANTIDIABETIC COMPOUNDS/ DRUGS

The impediment of diabetes mellitus is an urgent necessity for medicine and the human society at the beginning of this millennium. Epidemic growth, devastating complications, huge costs and other arguments support the above assertion. Prevention projects for diabetes have a strong experimental basis. There is an impressive array of methods for prevention of Type-1 diabetes in animal models. Although sulphonylureas, biguanides, insulin sensitizers (thiazolidinediones) (Table 3) and other current drugs are valuable in the treatment of type 2 diabetes mellitus, their use is restricted by cost, limited pharmacokinetic properties, secondary failure rates and accompanying side-effects (Krentz and Bailey, 2005; Derosa and Maffioli, 2010).

2.2 MEDICINAL PLANTS

From the dawn of civilization, medicinal plants have been a part and parcel of human society to combat diseases (Middleton et al., 2000; Srinivasan, 2006). Traditional medicines like Ayurveda and Unani in India and other countries have used medicinal plants for diseases
### TABLE 3. CURRENT THERAPEUTIC AGENTS FOR DIABETES

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Molecular Target</th>
<th>Site(s) of action</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Insulin receptor</td>
<td>Liver, Fat, Muscle</td>
<td>Hypoglycemia, weight gain</td>
</tr>
<tr>
<td>Sulphonylureas:</td>
<td>SU receptors</td>
<td>Pancreatic beta cell</td>
<td>Hypoglycemia, weight gain</td>
</tr>
<tr>
<td></td>
<td>K+/ATP channel</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>First generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolbutamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpropamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Second generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyburide, Glipizide,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide, Glimepiride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanides:</td>
<td>Unknown</td>
<td>Liver, Muscle</td>
<td>Gastrointestinal disturbances, lactic acidosis</td>
</tr>
<tr>
<td>Metformin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-glucosides</td>
<td>Alpha glucosidase</td>
<td>Intestine</td>
<td>Gastrointestinal disturbances</td>
</tr>
<tr>
<td>inhibitors:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiazolidinediones:</td>
<td>PPAR gamma</td>
<td>Fat, Liver, Muscle</td>
<td>Weight gain, oedema, anemia</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pioglitazone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Modified from Moller, 2001*
including diabetes mellitus and are considered to be effective, non-toxic and without serious side effects. Many plants and their products (active natural principles and crude extracts) have been mentioned and used in the Indian traditional system of medicine and have shown experimental or clinical antidiabetic activity (Grover et al., 2002). *Allium cepa, Allium sativum, Aloe vera, Cajanus cajan, Coccinia indica, Caesalpinia bonduc, Ficus bengalensis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzygium cumine, Tinospora cordifolia* and *Trigonella foenum graecum* are the most effective and most commonly studied Indian plants in relation to diabetes and their complications (Grover et al., 2002) (Table 4).

2.3.1 **TRIGONELLA FOENUM-GRAECUM (FENUGREEK)**

*Trigonella foenum-graecum* Linn. is an annual herb belonging to the family Leguminosae (Alarcon-Aguilara et al., 1998), widely grown in India, Egypt, and Middle Eastern countries (Srinivasan, 2006). It is commonly known as Fenugreek or Methi. It is a 2 to 3 foot tall annual herb with light green leaves and small white flowers. The seedpods contain 10 to 20 small, flat, yellow-brown, pungent, aromatic seeds to a pod. The seeds have a strong aroma and somewhat bitter taste, variously described as similar to maple syrup, or burnt sugar (Figure 3).

<table>
<thead>
<tr>
<th>Scientific classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom:</td>
</tr>
<tr>
<td>Plantae</td>
</tr>
<tr>
<td>Division:</td>
</tr>
<tr>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class:</td>
</tr>
<tr>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order:</td>
</tr>
<tr>
<td>Fabales</td>
</tr>
<tr>
<td>Family:</td>
</tr>
<tr>
<td>Fabaceae</td>
</tr>
<tr>
<td>Genus:</td>
</tr>
<tr>
<td>Trigonella</td>
</tr>
<tr>
<td>Species:</td>
</tr>
<tr>
<td><em>T. foenum-graecum</em></td>
</tr>
</tbody>
</table>
**Table 4. Antidiabetic Effects of *Trigonella foenum graecum* Seeds in Different Model Systems**

<table>
<thead>
<tr>
<th>Antidiabetic Effects of <em>Trigonella</em> Seeds</th>
<th>Model Systems</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypoglycemic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed extract</td>
<td>Alloxan diabetic rats</td>
<td>Shani et al 1974</td>
</tr>
<tr>
<td>Seed</td>
<td>Alloxan diabetic rats</td>
<td>Ghafghazi et al 1977</td>
</tr>
<tr>
<td>Defatted seeds</td>
<td>Alloxan diabetic dogs</td>
<td>Ribes et al 1986</td>
</tr>
<tr>
<td>Seeds</td>
<td>STZ diabetic mice</td>
<td>Swanston et al 1989</td>
</tr>
<tr>
<td>Whole seed powder</td>
<td>Alloxan diabetic rats</td>
<td>Khosla et al 1995</td>
</tr>
<tr>
<td>Whole seed powder</td>
<td>Type 1 diabetic patients</td>
<td>Sharma 1986</td>
</tr>
<tr>
<td>Debatterized seed powder</td>
<td>Type 1 diabetic patients</td>
<td>Sharma et al 1990</td>
</tr>
<tr>
<td>Seed powder</td>
<td>Type 2 diabetic patients</td>
<td>Madar et al 1988</td>
</tr>
<tr>
<td>Whole seed powder</td>
<td>Type 2 diabetic patients</td>
<td>Sharma &amp; Raghuram 1990</td>
</tr>
<tr>
<td>Whole seed powder</td>
<td>Type 2 diabetic patients</td>
<td>Sharma et al 1996</td>
</tr>
<tr>
<td><strong>Hypocholesterolemic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>Alloxan diabetic dogs</td>
<td>Valette et al 1984</td>
</tr>
<tr>
<td>Defatted seed/lipid extract</td>
<td>Alloxan diabetic dogs</td>
<td>Sharma 1984</td>
</tr>
<tr>
<td>Defatted seeds</td>
<td>Alloxan diabetic rats</td>
<td>Sharma 1986b</td>
</tr>
<tr>
<td>Ethanol/water extract</td>
<td>Normal rats</td>
<td>Petit et al 1993, 1995</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>Hypocholesterolemic rats</td>
<td>Stark &amp; Madar 1993</td>
</tr>
<tr>
<td>Purified principle</td>
<td>Hypocholesterolemic rabbits</td>
<td>Puri et al 1994</td>
</tr>
<tr>
<td>Whole seed powder</td>
<td>Type 1 diabetic patients</td>
<td>Sharma 1986a</td>
</tr>
<tr>
<td>Whole seed powder</td>
<td>Type 1 diabetic patients</td>
<td>Sharma &amp; Raghuram 1990</td>
</tr>
<tr>
<td><strong>Hyperinsulinomic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defatted seeds</td>
<td>Alloxan diabetic dogs</td>
<td>Ribe et al 1984</td>
</tr>
<tr>
<td>Ethanol/water extracts</td>
<td>Normal rats</td>
<td>Petit et al 1993</td>
</tr>
</tbody>
</table>

*Modified from Raju et al., (2001)*
Seed of *Trigonella foenum graecum* showed antidiabetic properties.

**FIGURE 3: TRIGONELLA FOENUM GRAECUM AND ITS ACTIVE COMPONENTS**

Adapted from Srinivasan, (2006)
Traditional medicines in India and other countries since ancient days, have employed hypoglycemic plants such as *Trigonella foenum-graecum* to protect against diabetic pathogenesis (Puri et al., 1995). Various reports have demonstrated that the TSP seeds have hypoglycemic, hypocholesterolemic and hyperinsulinemic effects on type 1 and type 2 DM patients and experimental diabetic animals (Sharma et al., 1996; Sur et al., 2001; Grover et al., 2002; Mohammad et al., 2004; Hannan et al., 2007; Srichamroen et al., 2009). The chemical constituents of TSP seed include volatile oils, alkaloids, saponins, sapogenins, flavonoids and mucilage (Duke et al., 1992). Furostanol saponins increase food consumption and induce hypocholesterolemia in streptozotocin diabetic rats (Petit et al., 1995). Diasgenin (saponin) and trigonelline (alkaloid) are found to inhibit glucose uptake *in vitro* (Al-Habori et al., 2001). The hypoglycemic property of fenugreek is not destroyed by cooking or roasting.

Fenugreek seed supplementation in the diet also normalizes the free radical metabolism in alloxan diabetic rats (Anuradha and Ravikumar, 2001; Genet et al., 2002). Lipid peroxidation was lowered, content of antioxidants like glutathione and beta carotene were increased (Anuradha and Ravikumar, 2001), altered antioxidant enzymes like Catalase, SOD and GPx were normalized (Genet et al., 2002) in alloxan diabetic rats after feeding fenugreek seeds. Ca$^{2+}$ dependent ATPase activity in liver homogenate of alloxan diabetic rats in the presence of Fe$^{2+}$/ ascorbate was protected by aqueous extract of *Trigonella* seed powder (Anuradha and Ravikumar, 2001). Human clinical studies have confirmed that fenugreek seed decreases blood glucose levels and blood lipids (total cholesterol, LDL and VLDL cholesterol and triglycerides) without affecting the HDL in Type 1 DM (Sharma et al., 1996) and Type 2 DM patients (Gupta et al., 2001). Whole powder of *Trigonella* also reduces the rate of gastric emptying in Type 2 DM patients.

The composition of the *Trigonella foenum-graecum* seed and the antidiabetic and hypoglycemic effect of certain components have been mentioned in the (Table 5). Petit et al. (1995) and Yoshikawa et al. (1997) reported the isolation of furostanol saponins called trigoneosides Ia, Ib, IIa, IIb, IIIa, IIIb; glycoside D and trigofoenoside A. These steroid saponins showed increased food consumption and induced hypocholesterolemia in streptozotocin diabetic rats (Petit et al. 1995; Srichamroen et al.2009). Saponin compounds diasgenin and alkaloids, trigonelline inhibits intestinal glucose uptake in-vitro (Al Habori et
TABLE 5. COMPOSITION OF *TRIGONELLA FOENUM-GRAECUM* (FENUGREEK)

<table>
<thead>
<tr>
<th>Component</th>
<th>Fresh fenugreek leaves</th>
<th>Fenugreek seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>86.0 g</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>4.4 g</td>
<td>30 g</td>
</tr>
<tr>
<td>Fat</td>
<td>1.0 g</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.0 g</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sapogenins</td>
<td></td>
<td>2 g</td>
</tr>
<tr>
<td>Trigonelline</td>
<td></td>
<td>380 mg</td>
</tr>
<tr>
<td>Ca</td>
<td>395 mg</td>
<td>160 mg</td>
</tr>
<tr>
<td>Mg</td>
<td>67 mg</td>
<td>160 mg</td>
</tr>
<tr>
<td>P</td>
<td>51 mg</td>
<td>370 mg</td>
</tr>
<tr>
<td>Fe</td>
<td>16.5 mg</td>
<td>14 mg</td>
</tr>
<tr>
<td>Na</td>
<td>76 mg</td>
<td>19 mg</td>
</tr>
<tr>
<td>K</td>
<td>31 mg</td>
<td>530 mg</td>
</tr>
<tr>
<td>Cu</td>
<td>0.26 mg</td>
<td>33 mg</td>
</tr>
<tr>
<td>S</td>
<td>167 mg</td>
<td>16 mg</td>
</tr>
<tr>
<td>Cl</td>
<td>165 mg</td>
<td>165 mg</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td>1.5 g</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td>7.0 mg</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>0.1 mg</td>
</tr>
<tr>
<td>Choline</td>
<td>1.35 g</td>
<td>50 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>52 mg</td>
<td>43 mg</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>2.3 mg</td>
<td>96 μg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>40 μg</td>
<td>340 μg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>310 μg</td>
<td>290 μg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>800 μg</td>
<td>1.1 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
<td>84 μg</td>
</tr>
</tbody>
</table>

Values expressed per 100g.

Adapted from Srinivasan, (2006)
Soluble fiber, mostly galactomannans sequester fat and appears to bind directly to dietary fat and reduces its absorption into the body.

The seeds are rich in protein and contain a unique major free amino acid 4-hydroxyisoleucine (4-OH-Ile), which has been characterized as one of the active ingredients in *Trigonella* seeds. Specific for plants not present in mammalian system. The effect of 4-OH-Ile has been shown to stimulate insulin secretion and improve glucose tolerance in normal and diabetic animals as the result of direct beta-cell stimulation. 4-OH-Ile, an amino acid extracted and purified from fenugreek seeds displayed an insulinotropic property in-vitro. It also stimulates insulin secretion in-vivo and improved glucose tolerance in normal rats and dogs and in rat model of Type 2 DM. Basal hyperglycemia was also reduced by subchronic administration of 4-OH-Ile to Type 2 DM rat model (Broca et al., 1999; Sauvaire et al., 1998). Besides 4-OH-Ile, arginine and tryptophan are the other amino acid having antidiabetic and hypoglycemic affect. Ascorbic acid, niacin, nicotinic acid, chromium, copper, magnesium, manganese, zinc, alkaloids like choline and gentianine and flavonoids, quercitin are some of the other components of *Trigonella* seeds, which have antidiabetic and hypoglycemic effects (Srichamroen et al., 2009; Losso et al., 2009).

3. AGING
Aging is “a genetic physiological process associated with morphological and functional changes in cellular and extracellular components aggravated by injury throughout life and resulting in a progressive imbalance of the control regulatory systems of the organism, including the hormonal, autocrine, neuroendocrine, and immune homeostatic mechanisms” (Table 6) (Yu, 1996). Differentiating the biological aging process from age-related disease processes may be the first step towards understanding the basic mechanisms involved in biological aging. Aging contributes to the susceptibility to disease and one dies from a distinct pathological event, not the aging process (Timiras, 1994; Harman, 1992).

In accordance with the present state of scientific knowledge, the excessive production of free radicals in the organism, and the imbalance between the concentrations of these and the antioxidant defenses may be related to processes such as aging and several diseases. The aging process has been described by various theories. In particular, the free radical theory of aging has received widespread attention which proposes that deleterious actions of free
1. Genomically programmed mechanisms of aging

2. Failure of coordinating systems
   a. Neural
   b. Neuroendocrine
   c. Endocrine

3. Informational failure
   a. Base substitution and deletion
   b. Loss of DNA and RNA
   c. Single strand breaks
   d. Transcriptional and translational impairment

4. Structural damage or modification
   a. Cell loss and membrane damage
   b. Protein alteration, cross-linking, and glycation
   c. Oxidative modification of lipids and carbohydrates

5. Accumulation of deleterious substances
   a. Loss of repair capacity
   b. Loss of detoxification process

*Modified form Yu BP (1996)*
radicals are responsible for the functional deterioration associated with aging. The CNS is especially vulnerable to oxidative stress for various reasons: non-replicating cells, high metabolic rates in comparison to other tissues, relatively high levels of polyunsaturated fatty acids and relatively low levels of natural antioxidants and of protective enzymes. Consequently, a relatively uniform functional decline of CNS cells takes place with age. Aging is a multifactorial process involving neurodegenerative changes in cell morphology and biochemistry. The free radical or "oxidative stress" theory holds that oxidative reactions are the factors underlying these changes (Harman, 1992; Babusikova et al., 2007; Calabrese et al., 2008). Highly reactive oxygen species (ROS) cause a wide spectrum of cell damage, including lipid peroxidation, inactivation of enzymes, alteration of intracellular oxidation-reduction state, and DNA damage.

Aging is associated with a decline in metabolic function, affects the endocrine system by altering endocrine cells, the hormones produced by these cells and hormone receptors or post receptor processes in the target cells, in the case of females, loss of gonadal function seems to determine the start of this period of metabolic decline. There are significant gender differences in human brain disease. For example, females are significantly more likely to suffer from AD than men (even after correcting for differences in life expectancy), and females on hormone replacement therapy (HRT) are significantly less likely to suffer from AD than women who do not take HRT (Compton et al., 2001; Guevara et al., 2009).

3.1 BRAIN AGING

The brain is exposed to chronic oxidative stress during aging (Wong et al., 2006; Ian and Grotewiel, 2006). The brain is especially vulnerable to oxidative damage as a result of its high oxygen consumption rate, its abundant lipid content, and the relative paucity of antioxidant enzymes compared to other tissues (Leutner et al., 2001). As a result, there is a gradual accumulation of damaged proteins and a functional decline in the brain’s endogenous defense system (Hou et al., 2010; Min et al., 2008). Oxidative damage can lead to several events such as loss in specific protein function, abnormal protein clearance, depletion of cellular redox—balance and interference with the cell cycle and ultimately to neuronal death. (Halliwell, 2001; Butterfield et al., 2006; Mantha et al., 2006; Kamboj et al., 2009). There is
increasing evidence, that the level of activity of the cerebral immune response system is
dysregulated in the aged animal. In many ways, microglia acts as the brain’s macrophages. The number of activated microglia is substantially increased in the CNS white matter of aged rats and primates. In fact, the reactivity of the intrinsic immune system within the CNS to such a material as lipopolysaccharide is considerably decreased in the elderly mouse (Sharman et al., 2002).

Increased oxidative stress in the aged brain has been attributed to reduced efficiency of mitochondrial energy production (Liu and Ames, 2005; Cocco et al., 2005). Such compromised respiratory function can lead to oxidative stress and impaired antioxidant enzyme systems. Primary targets of oxidative damage include mitochondrial membranes, mitochondrial proteins, and the mitochondrial DNA (mtDNA). Mitochondria and mitochondrial dysfunction also play key parts in the regulated processes of cellular self-destruction, apoptosis, autophagy and necrosis (Skulachev, 2006). When functioning properly, regulatory control of these processes of cell death is exquisitely sensitive to imbalances in cellular homeostasis as gauged by such measures as Ca$^{2+}$ levels and cellular redox status (Skulachev, 2006).

3.2 AGING RATS AND ESTROUS CYCLE

The circulating level of gonadotropins and ovarian steroids throughout the reproductive cycle is needed in each species as a basis for a clear understanding of the physiological events during cycle. Many species have been reported to have a decline in reproduction performance with advancing age. Associated with the decline in reproduction during aging, there is an increase in the incidence or irregular menstrual cycle and a decrease in the number of total oocytes and growing follicles. Vaginal smear are widely used to identify the phase of the estrous cycle (Talbert, 1968). The female rat shows 4 or 5 day estrous cycle. A cycle consists of proestrus, estrous, diestrous and metestrous. A 4-day cycle has a 2-day diestrous (I and II) and 5-day cycle has a 3-day diestrous (I, II and III). Diestrous I is often called metestrous. Previous studies showed that irregular estrous cycle emerged in female rats beginning at about 12 months of age and these were followed in sequence by constant estrous (CE), repetitive pseudopregnancy (PSP), Persistent diestrous (PD) and anestrous (Hung et al., 1978).
Puberty in the female rat is associated with the vaginal opening and first proestrus stage where the level of estradiol is high. The level is then declined rapidly at early morning of estrous stage and a small increase at day metestrous stage. There is a decline to near the base level in estradiol level at diestrous stage. Smith et al. (1974) showed that E$_2$ concentration declined after proestrous stage in relation to the peaks in LH, prolactin and progesterone secretion and had reached baseline values by estrous and progesterone value remained low until the afternoon of proestrous. In aged rats, changing serum levels in steroid hormones provide functional modulations for pituitary, prolactin and gonadotropin release under different reproductive stage. A hallmark of reproductive aging in the female rats is the attenuation of the proestrous that serves as a marker for neuroendocrine aging and the imminent loss of regular estrous cycle (Hung et al., 1978). Around 12 months of age the rat shows the constant estrous stage and the estradiol and progesterone level is reduced as compared to proestrous and metaestrous, respectively as found in normal cyclic rats.

3.3 MENOPAUSE

Menopause is defined as the cessation of menstruation for one year in a woman over age 45 and occurs on average at the age of 51. This is a result of a decline in ovarian function - estrogen and progesterone are no longer being produced because the ovary has completely depleted its follicular pool. There is no relationship between a woman's age at menarche and menopause. However, a woman's age at menopause is reflective of her mother's age at menopause. Menopause is known to occur approximately one and a half years earlier in tobacco users. (Nelson, 2008; Henderson, 2009). During menopause a woman's body slowly makes less of the hormones estrogen and progesterone. This often happens between the ages of 45 and 55 years old. A woman reaches the menopause condition when she has not had a period for 12 months in a row (and there are no other causes for this change).

3.4 AGING AND HORMONAL REPLACEMENT THERAPY

Hormone Replacement Therapy (HRT) or ovarian hormone therapy (OHT), also called Hormone therapy (HT), refers to the use of estrogen and progesterone, often prescribed to supplement the declining levels of these hormones, which occur during menopause. Using the term "hormone replacement therapy" implies that menopause is a disease of hormone
deficiency and a normal process. Economical and medical developments have caused an increase in human life span. As a result, number of postmenopausal women and research related to their problem has also increased. The physiological hallmark of menopause is the decrease in the production of endogenous sex hormones by the ovaries. (Myers et al., 1990; Nelson, 2008). The use of hormone replacement therapy is highly effective for improving the quality of life of women suffering from acute symptoms of menopause, such as hot flashes, night sweats, insomnia, increased fatigue and irritability, depression, skin changes, vaginal dryness and incontinence. There was significant evidence that HRT provides some long-term protection against cardiovascular disease, osteoporosis and colon cancer.

4. ANTIAGING COMPOUND
There is tremendous public interest in the development of antiaging therapy and the idea that the aging process can be reversed by approaches such as antiaging medicine is emerging (De Grey, 2003) although limitations (Henderson, 2009) are there. Pharmacological intervention, besides many other types of interventions, to reduce oxidative stress, and to prevent lipofuscin formation is an interesting approach to slow the aging process (Terman and Brunk, 2006). A variety of substances and drugs have been investigated for their antiaging influences.

4.1 ROLE OF ESTRADIOL (E2) IN BRAIN AGING
Veiga et al. (2004) and Baquer et al. (2009) have reviewed the changes in the sex hormones and brain aging, and the authors have discussed the role of these hormones in modulating neurotransmitter synthesis, neurotransmitter receptor expression and synaptic transmission and remodeling, emphasizing that the nervous system is a target for sex hormones and a source of sex steroids (Koric' anac et al., 2004). Saravia et al. (2007) have reported that estradiol plays a neuroprotective role in the hippocampal neurons and glia of middle age mice and data suggest, as reported by Moorthy et al. (2004b) also, that older women might also benefit from the protective effects of HRT that uses a relatively lower concentration of hormones.

The sex hormones, androgen, estrogen and progestins, are among the peripheral signals that modulate neural function and development. These cholesterol derivatives act on
neurons and glial cells, regulating differentiation, survival and neuronal connectivity, both in
the brain and spinal cord. During neural development, a number of sex hormone effects are
permanent, such as the sexual specification of the brain. Sex hormones act also in adulthood,
modulating neurotransmitter synthesis, neurotransmitter receptor expression, and synaptic
transmission and remodeling (McEwen et al., 2001). The nervous system is not only a target
for sex hormones, but also a source of sex steroids. The brain, the spinal cord and the
peripheral nerves can synthesize these steroids which then act as neuromodulators in a
paracrine or autocrine mode (Shibuya et al., 2003).

Estrogen has been one of the most studied hormones in the scientific arena. The area
of estrogen research has seen a relative explosion in the last decade. Recently, there has been
a growing interest in the actions and functions of the ovarian steroid hormone 17β-estradiol
(E2), particularly in whether they are neuroprotective for such age related disease,
neurodegenerative conditions as stroke, AD, and PD (Henderson, 2009). Much of the interest
has been sparked by a desire to understand the actions and mechanisms of estrogen
throughout the body, and to formulate better diagnoses and treatment for breast cancer,
osteoporosis, cardiovascular and neurological disease and a multitude of postmenopausal
symptoms. Classically, estrogen is considered a “reproductive” hormone, due to its well-
known role in feedback signaling in the hypothalamic-pituitary-ovarian axis. Estradiol has
important regulatory roles in a wide variety of biological processes including reproduction,
differentiation, development, cell proliferation, apoptosis, inflammation, metabolism,
homeostasis, and brain function (Tsai and O’Malley, 1994; Craig and Murphy, 2007; Baquer
et al., 2009).

Estrogens are important in the development and maintenance of the female
reproductive system and secondary sex characteristics. By direct action, they cause growth
and development of the uterus, fallopian tubes and vagina. With other hormones, such as
pituitary hormones and progesterone, they cause enlargement of breasts through promotion
of ductal growth, stromal development, and the accretion of fat. Estrogens are intricately
involved with other hormones, especially progesterone, in the process of the ovulatory
menstrual cycle and pregnancy, and affect the release of pituitary gonadotropins. They also
contribute to the shaping of skeletal, maintenance of the tone and elasticity of urogenital
structures, changes in the epiphysis of the long bones that allow for the pubertal growth spur
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and its termination, and pigmentation of the nipples and genitals. Estrogens combined with other hormones regulates carbohydrate and lipid metabolism, enhance arterial reactivity, promotes vasodilatation, and inhibits the migration and proliferation of vascular smooth muscle cells. Estrogen acts through its receptor and regulates the development and the function of various human organ system including reproductive, skeletal and nervous system. It is also involved directly in numerous pathological processes such as AD, osteoporosis, cardiovascular disease, mental disorder and carcinogenesis (Sherwin and Henry, 2008; Henderson, 2009).

Estradiol is commonly recognized as playing a pivotal role in female reproductive physiology. However, it is also involved in bone and lipid metabolism, in maintenance of the cardiovascular and neuronal systems, and in male reproductive development and physiology. In particular, recent studies analyzed the involvement of E2 and several of its metabolites in the CNS and considered to be a neuromodulatory and neuroprotective hormone that regulates transcription of various gene families (Pozzi et al., 2006; Craig and Murphy, 2007; Spencer et al., 2008). Two subtypes of estrogen receptors (ERα and ERβ) are expressed throughout the body, but primarily in uterine, heart, breast and brain tissue (Thakur and Sharma, 2008). Via activation of ERα and ERβ, estrogen modulates multiple functions of the brain, including development, cognition and memory, highlighting its protective effects against neuronal damage (García-Segura et al., 2001).

The sharp decline of estrogen after menopause may lead to impaired cognition and memory and increase the incidence of neurodegenerative diseases like AD and PD. Estrogen deficiency may accelerate brain aging in postmenopausal animals (Sherwin and Henry, 2008). E2 regulates carbohydrate and lipid metabolism (Moorthy et al., 2004b).

4.2 MECHANISM OF ACTION OF E2

4.2.1 HORMONAL RECEPTORS

Receptors for estrogen are members of a super family of nuclear receptors that function as ligand or hormone-dependent transcription factors. The main feature of nuclear receptors is to control expression of specific sets of target genes in response to an activating ligand or other signals. In most vertebrates there are two forms of estrogen receptors, ERα and ERβ. The two ER subtypes arise from different genes, whereas the PR isoforms are produced from
a single gene through alternate use of two promoters that give rise to two different PR mRNAs. The precise physiological role of multiple forms of ER and PR is not well understood. Nuclear receptors are sequence-specific DNA-binding proteins that recognize cis acting hormone response elements (HREs) typically located in the promoter regions of target genes (Khorasanizadeh and Rastinejad, 2001; Brann et al., 2007).

4.2.2 GENE TRANSCRIPTION BY ESTROGEN
The basic pathway that show how steroid hormones regulate gene transcription has been examined extensively (Figure 4).

(A) GENOMIC ACTION
Intracellular estrogen receptors (ER) are widespread, and in the brain they are found in the cerebral cortex, midbrain, hippocampus, brain stem, hypothalamus and pituitary gland. In the absence of ligand, the classical intranuclear ER exists as a monomer (inactive state) and forms a multiprotein complex and heat-shock protein (Hsp-90). Activation of ER by E2 causes phosphorylation of several distinct serine/threonine residues of the ER inducing dissociation from Hsp-90. Activated ERs then homo- or heterodimerize and interact with specific DNA sequences (estrogen responsive element, ERE) within the promoter regions of target genes to alter gene transcription. Estrogen binding to the ligand-binding domain induces a conformational change producing a hydrophobic cleft recognized by transcriptional coactivators such as cyclic adenosine monophosphate response element binding protein (CREB). ER-coactivator complexes facilitate transcription of estrogen-responsive genes by induction of a change in chromatin structure in target promoters and by recruitment of components of the transcriptional machinery to DNA (Norbury et al., 2003).

(B) NONGENOMIC ACTION
In parallel, Estrogen also exert their physiological effects through a nongenomic activity, which is characterized by very fast responses and mediated by activation of second messenger (Ca$^{2+}$, cGMP, protein kinase, MAP kinases) pathways via a direct interaction of estrogen. Plasma membrane-associated ER and ER exist in a variety of neural and extraneural targets to mediate steroid action.
Fig. 4. Putative mechanisms of estrogen action. Indirect genomic activity involves binding to cell surface receptors and activation of G-proteins. Subsequent phosphorylation of substrate proteins leads to a change in gene transcription. Direct genomic activity involves oestrogen receptor dimerization and subsequent migration to the cell nucleus to alter gene transcription.

*Adapted from Norbury et al., (2003)*
In addition to a genomic effect, nongenomic effects of estrogen may also play a role in mediating its neuroprotective effects in the brain. For instance, estrogen can rapidly activate the extracellular signal-regulated kinases (ERK) and phosphoinositol-3-kinase (PI3K)-Akt pathways in cortical and hippocampal cells \textit{in vitro}, effects implicated in estrogen neuroprotection action. Estrogen has also been shown to enhance Akt activation in the cerebral cortex and CA1 of the hippocampus following focal or global cerebral ischemia (Choi et al., 2004). Akt can phosphorylate two downstream death kinases to inactivate them, BAD and GSK-3\(\beta\) (glycogen synthase kinase-3\(\beta\)), which could be another important mechanism of estrogen neuroprotection (Goodenough et al., 2005). Additionally, work by Wang et al. (2006) revealed that E2 benzoate treatment induced phosphorylation of Akt in the CA1 following cerebral ischemia is associated with inhibition of the proapoptotic MLK3-MKK4/7-JNK1/2 (mixed lineage kinase-3/MAP kinase kinase-4-7/c-jun-N-terminal kinase) pathway.

\section*{4.3 ESTROGENS AND NEURODEGENERATIVE DISORDERS

CLINICAL STUDIES

(A) CEREBRAL ISCHEMIA

A potential estrogen receptor is the recently discovered GPR30. GPR30 is a G-protein-coupled receptor which has been reported to bind estrogen with high affinity, and has been shown to be expressed in breast cancer cells and various tissues in the body, including the brain. The role, if any, of GPR30 in estrogen actions in the brain is unknown. However, preliminary research work have shown that GPR30 is expressed in various regions of the brain including the hippocampus, cortex and striatum, and may thus have a role in mediating estrogen actions (Funakoshi et al., 2006). With respect to genes regulated by estrogen that may facilitate its neuroprotection, estrogen has been shown to increase the expression of the anti-apoptotic gene, bcl-2, in the ischemic penumbra and global ischemia. Estrogen also increases bcl-2 \textit{in vitro} in rat hippocampal neurons and human neurons, while it inhibits expression of pro-apoptotic BAD (bcl-2-antagonist of cell death) (Wu et al., 2005). Additionally, estrogen has also been demonstrated to reduce cytochrome c translocation, as well as caspase 3 activation and DNA fragmentation (Choi et al., 2004), further implicating an anti-apoptotic action of estrogen in cerebral ischemia.
(B) PARKINSON'S DISEASE (PD)

Animal and human studies suggest a beneficial regulatory effect of estrogen on the nigrostriatal dopaminergic system, which may underlie putative protective effects of estrogen in PD. A corollary and exciting finding was the recent discovery that estrogen treatment induces differentiation of human neural stem cells (NSCs), giving rise to tyrosine hydroxylase (dopaminergic) neurons, an effect blocked by use of an estrogen receptor antagonist. Estrogen also increased the number of dopaminergic neurons derived from human NSCs in vivo when the cells were grafted into mouse brains. This finding revealed another layer to the beneficial regulation by estrogen of the dopaminergic neuronal system, and raises the possibility of a potential role for estrogen in transplantation of NSCs for PD. Finally, oxidative stress is suggested to play an important role in neuronal degeneration in PD (Przedborski et al., 2005). It is thus intriguing that estrogen has been shown to suppress free radical production and protect striatal neurons against oxidative stress (Sawada et al., 1998), potentially providing an additional mechanism for estrogen neuroprotection in PD.

(C) ALZHEIMER'S DISEASE (AD)

Recent studies in both animals and humans have provided additional evidence supporting a potential beneficial protective role for estrogen in AD (Yue et al., 2005). The mechanisms of how estrogen may exert protection in AD are not clear. However, a number of studies have shown that estrogen can protect neurons against β-amyloid toxicity (Chen et al., 2006), oxidative stress and excitotoxicity, events suggested to participate in the pathology of AD. Additionally, estrogen has been shown to induce dephosphorylation of the tau protein and prevent its hyperphosphorylation in neurons. At the molecular level, estrogen has been shown to enhance activation of the survival factor, Akt, while inducing phosphorylation and deactivation of GSK-3β and BAD, known death signals in neurons. Collectively, these effects could explain the reported protective actions of estrogen in AD. It should be mentioned that the SERM, raloxifene has also been shown to protect against β-amyloid-induced cell death, and it has been reported to decrease risk of AD and cognitive impairment in postmenopausal women (Zhang et al., 2001).

Taken together, these results suggest that E2 is a preventative but probably not a curative factor in neurodegenerative diseases (Garcia-Segura et al., 2001). Indeed, we have to
consider the possibility that the neuritogenic effects of E2, promoting the formation and remodeling of synaptic contacts (McEwen et al., 2001), may have a homeostatic role under physiological conditions, but may be detrimental if exerted in a pathologically altered neuronal circuit.

5. MODULATION OF MEMBRANE LINKED FUNCTIONS IN DIABETES AND AGING

5.1. PROPERTIES AND FUNCTIONS OF MEMBRANE

The physiological importance of modifications in the physical properties of membranes resides in their relation to numerous cellular functions, including the activity of membrane-associated enzymes, membrane solute transport, hormone-induced signal transduction and membrane fluidity. The structure and function of cell membranes have been shown to play a crucial role in maintaining intracellular homeostasis through the activity of membrane-bound enzymes and receptors as well as of the trans-membrane transport system, all of which are influenced by membrane fluidity. Free radicals oxidize polyunsaturated fatty acid, a main component of the membrane, thereby destroying the spatial arrangement of the membrane and affecting biological activities. As a result, the functions of the membranes deteriorate and fluidity decreases, impairing the crucial membrane functions of transport and permeability and also hindering homeostasis (Kamboj et al., 2009).

The metabolic disturbances and their consequences in diabetes mellitus and aging are well known. However, our knowledge on the diabetic and age related disorders in membrane functions is limited. These damages are connected mostly with the disregulation of the membrane protein synthesis due to deficiency of insulin. Early glycation of membrane protein could also give rise to alterations of the membrane structure (Jain and Lim, 2000; Kamboj et al., 2009; Mantha et al., 2006). Changes in membrane lipid composition (increased cholesterol and lipid peroxidation and decreased polyunsaturated fatty acids (PUFA) content) have also been reported during diabetes and aging process (Moorthy et al., 2004a; Kamboj et al., 2009). The physiological importance of modifications in the physical properties of membranes resides in their relation to numerous cellular functions, including the activity of membrane-associated enzymes, calcium homeostasis, membrane solute transport, hormone-induced signal transduction and membrane fluidity (Garcia et al., 1997).
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In the present study the impairments of the membrane linked enzymes (MAO, \( \text{Na}^+\text{K}^+ \) ATPase, \( \text{Ca}^{2+} \) ATPase), membrane fluidity, antioxidant enzymes (SOD, GST), neurolipofuscin, DNA degradation, calcium homeostasis as well as the insulin-dependent glucose transporter GLUT4 has been characterized in control and different experimental groups. The present study elucidates that beside this the severe metabolic disorders, general membrane damage and extended disturbances in membrane functions are also very characteristic for diabetes and aging process. The acknowledgements of these alterations are very important for the exact planning of the control of age related disorders and treatment of diabetes.

6. PARAMETERS STUDIED IN DIABETES AND AGING CONDITIONS

6.1. ENZYMES

(I) MEMBRANE BOUND ENZYMES

Membrane bound enzymes are enzymes in a membrane that are responsible for the maintenance of cellular functions such as ion transport, secretion and uptake of a variety of substances, as well as cell to cell interactions.

(A) MONOAMINE OXIDASE (MONOAMINE; OXIDOREDUCTASE, DEAMINATING, E.C. 1.4.3.4)

Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD) dependent enzyme localized in the outer membrane of the mitochondria, which plays an essential role in the turnover of monoamine neurotransmitters such as dopamine, serotonin and noradrenalin (Soto-Otero et al., 2001; Girgin Sagin et al., 2004). The role of MAO has been implicated in several processes, such as affective disorder, aggressive behavior and regulatory effect of adrenocortical studies (Consolo and Valzelli, 1970). MAO catalyzes the oxidative deamination of biogenic amines to their corresponding aldehydes, which is accompanied by the reduction of molecular oxygen to hydrogen peroxide \( (\text{H}_2\text{O}_2) \) (Soto-Otero et al., 2001), which is normally cleaned up and causes no problem.

MAO A and MAO B may either be colocalized in the same cellular type, or else one of the two forms may be predominant. MAO A normally occurs in adrenergic, noradrenergic and, in most cases, in dopaminergic neurons, while MAO B is unexpectedly predominant in serotoninergic neurons (Jahng et al., 1997). The B form is also expressed in histaminergic neurons, astrocytes and ependymal cells. MAO A and MAO B localization thus does not
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always correspond to the localization of the preferential substrate. The two isoenzymes are functionally distinct owing to their different affinity for the various substrates. MAO A preferentially degrades serotonin (5-HT), adrenaline and noradrenaline (NE), while MAO B displays greater affinity for phenylethylamine (PEA) and benzylamine (Fowler and Tipton, 1984). Dopamine and tyramine are considered a substrate for both MAO forms. The selective inhibitor of MAO-A is clorgyline. In contrast, the selective inhibitor of MAO-B is deprenyl (Knoll and Magyar, 1972).

Disturbances in catecholamines metabolism and MAO activity play a role in the pathogenesis of the acute and chronic complications of diabetes (Gupta et al., 1992; Lakhman and Kaur, 1997; Baquer et al., 2009). Regulation of this enzyme is very important without enough of this enzyme brain would not function normally and possibly lead to mental retardation. High level of MAO has been linked to depression and to PD (Hauptmann, 1996).

Studies on brain MAO activity during aging were stimulated by the relationships that exist between MAO changes and some age-related neuropsychiatric and neurological diseases. Several authors report high brain MAO B in neurodegenerative diseases such as Parkinson’s and Alzheimer’s diseases without any change in MAO A. There is also evidence that MAO B inhibitors improve the quality of life in the elderly (Naoi and Maruyama, 2010). In a neuronal cell line, glutamate toxicity via the oxidative pathway requires monoamine metabolism as a source of free radicals. MAO B was predominantly found to be located in glial cells (Shih, 1979; Westlund et al., 1985). The large increase in MAO B with aging may be attributable to the proliferation of these cells.

(B) Na⁺K⁺ ATPase (Na⁺ K⁺ EXCHANGING ATPase, E.C. 3.6.3.9)

Na⁺K⁺ ATPase, a membrane bound enzyme, maintains neuronal and brain excitability. The ATPases are ion-pumps which actively exchange various ions across the cell membrane and expressed in virtually all cells of higher organisms. The Na⁺K⁺ ATPase, the first ion-pump to be identified, is an integral membrane protein (Esmann et al., 2008) that simultaneously extrudes 3Na⁺ from the intracellular compartment in exchange of transporting into the cell 2K⁺ from the extra-cellular space. The energy required for the exchange of these ions is derived from hydrolysis of one molecule of ATP (Lubarski et al., 2007).
Several types of ATPases have been identified and they have been classified as P-, V-, F-types and ABC-superfamily. V-type ATPase is present in vacuoles and possesses multimeric subunit complex; the F-type possesses ATP synthesizing activity, is found in mitochondrial membrane and it shows structural similarity with that of V-type; while those grouped under ABC-superfamily help in the transport of multiple substances. The Na\(^{+}\)K\(^{+}\) ATPase belongs to P-type ATPase and is present in almost all metazoa cells (Horisberger, 2004). It is characterized primarily having a phosphorylated intermediate during the reaction cycle (Axelsen and Palmgren, 1998; Kuhlbrandt, 2004).

Thus, the enzyme plays a significant role in maintaining the Na\(^{+}\) and K\(^{+}\) Concentration gradients across neuronal membrane which is essential for generation of action potential in the neurons. By modulating the ionic concentrations across cell membrane, the enzyme helps maintaining the osmotic pressure in cells, which has been implicated with several cellular processes including apoptosis (Wang et al., 2003). Re-uptake of neurotransmitters and efflux of Ca\(^{2+}\) from neurons are also coupled to the activity of the enzyme (Das et al., 2008). Its activity has been reported to be involved in water as well as glucose transport across the cell membranes of various tissues and with secretion of gastric juice in the stomach. In kidney the enzyme is associated with renal reabsorption of ions and maintenance of osmotic balance.

In experimental diabetes, changes in Na\(^{+}\)K\(^{+}\) ATPase activity have been reported in the heart, peripheral nerve, kidney and intestine (Kjeldsen et al., 1987; Sennoune et al., 2000). Decreased Na\(^{+}\)K\(^{+}\) ATPase activity in brain of diabetic and aging animals affects the intracellular concentrations of Na\(^{+}\) and K\(^{+}\), alter the signal transduction pathway, and affect the contractibility and excitability and cellular functions, suggested as a contributing factor in the development of diabetic neuropathy and neurological disorders (Raccah et al., 1992; Siddiqui et al., 2006; Mayanil et al., 1982a; Murali et al., 2008).

(C) Ca\(^{2+}\) ATPase (Ca\(^{2+}\) TRANSPORTING ATPase, E.C. 3.6.3.8)
Calcium ATPases are members of the P-type family of ion pumps, which are responsible for the ATP-dependent active transport of calcium ions across a wide variety of cellular membranes (MacLennan et al., 1997). Calcium ions play a crucial role in the metabolism and physiology of eukaryotes. Regulation of intracellular calcium (Ca\(^{2+}\)) concentrations is vital
for cells, especially for neurons; raised levels are associated with cytotoxicity and neuronal death. The plasma membrane Ca$^{2+}$ ATPase (PMCA) pumps Ca$^{2+}$ from the cytosol to the extracellular environment and plays a critical role in Ca$^{2+}$ homeostasis. Together with the sarcoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) and the sodium calcium exchanger (NCX), the PMCA maintains precise levels of intracellular Ca$^{2+}$ under resting conditions and following neuronal excitation (Miller, 1991). PMCA activity is regulated by calmodulin (CaM), a 16-kDa protein known to serve as a universal Ca$^{2+}$ sensor in all cells. In the absence of CaM, the C-terminal autoinhibitory domain interacts with the catalytic site of PMCA, thus inhibiting ATP hydrolysis and Ca$^{2+}$ transport. As Ca$^{2+}$ levels rise, binding of Ca$^{2+}$/CaM to PMCA relieves autoinhibition and the pump is maximally activated (Pottorf and Thayer, 2000; Paszty et al., 2005). Another possibility involves the modification of enzyme molecules either by direct oxidation or by modification mediated by products of lipid peroxidation. A decrease in ATPase enzyme activity in any diabetic tissue could be due to excessive nonenzymatic glycation of the enzyme itself and/or of calmodulin (González Flecha et al., 1990). Ca$^{2+}$ ATPase was shown to be particularly sensitive to cross-linking and the concomitant decrease in protein rotational mobility due to protein aggregation (Viner et al., 1997).

Plasma membrane Ca$^{2+}$ ATPase is a high affinity low capacity Calcium pump. The basic functions of the plasma membrane calcium pumps are to maintain the 10,000-fold calcium gradient across the plasma membrane via the highly regulated active expulsion of calcium from the cell. In addition, they are involved in calcium signalling and the modulation of calcium spikes. Dysregulation of Ca$^{2+}$ leads to oxidative stress and cell death (Ihara et al., 2005). Sustained levels of intracellular Ca$^{2+}$ leads to stimulation of many different enzymes: endonucleases, proteases, kinases and phosphatases and phospholipases, all of which may lead to a rapid loss of cell function and integrity and trigger a process known as apoptosis (programmed cell death) (Evcimen et al., 1999).

(II) ANTIOXIDANT ENZYMES
Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically this means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a
non-free-radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. The antioxidant molecule may be very large (allowing it to "dilute" the unpaired electron), it may be readily neutralized by another antioxidant and/or it may have another mechanism for terminating its free radical condition.

(A) Superoxide Dismutase (SOD, E.C. 1.15.1.1)

Superoxide dismutase is a beta barrel protein with 152 amino acids that catalyzes the dismutation of the superoxide radical to hydrogen peroxide and molecular oxygen. SOD is categorized as an oxidoreductase class of enzyme.

Superoxide dismutase catalyzes the dismutation of superoxide radical as shown below:

\[
2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2
\]

The superoxide anion has been implicated in inflammation, hyperoxic cell damage and is involved in a wide range of diseases (Bulkley, 1983). The enzyme SODs are the cell's first line of defense against the toxicity of superoxide radical and the radical derivatives (Fridovich, 1974). Although \(O_2^-\), once formed, undergoes spontaneous dismutation to peroxide and oxygen, the presence of SOD increases the reaction rate by \(10^9\) fold. The nonenzymatic dismutation of \(O_2^-\) results in the production of singlet oxygen, and it has, therefore, been proposed that the function of SOD is to protect the aerobic cell from the toxic effects of not only \(O_2^-\) but singlet oxygen and other reactive oxygen species (ROS) as well (Halliwell and Gutteridge, 1989).

There are at least three SOD isozymes in the mammalian body; copper zinc-superoxide dismutase (CuZn-SOD) in the cytosol of cells, manganese-superoxide dismutase (Mn-SOD) in the mitochondrial matrix, and extracellular superoxide dismutase in the extracellular space (Marklund, 1984; Erlanson et al., 1990). Copper zinc containing SODs are highly stable enzymes and thus can easily be isolated. CuZn-SODs are also quite resistant to heating, to attack by proteases and to denaturation by reagents like SDS or urea. The Cu ion functions in the dismutation reaction by undergoing alternative oxidation and reduction whereas the zinc ion stabilizes the enzyme (Halliwell and Gutteridge, 1989). Copper/Zinc Superoxide Dismutase (CuZnSOD) is an enzyme that appears to play a very vital role in health, both at the tissue level and at the whole-body level.
Oxidative stress is postulated to be one of the most important mechanisms behind age-related changes in SOD level and increased apoptosis in aged rats (Kokoszka et al., 2001). Valls et al. (2005) estimated the activity of SOD and other antioxidant enzymes in 6, 12 and 22 months old rats, their results suggested a decrease in the antioxidant enzymes SOD and catalase during aging and for this older rats are more susceptible to oxidative stress (Ames et al., 1993; Kokoszka et al., 2001).

(B) GLUTATHIONE S-TRANSFERASES (GST, E.C. 2.5.1.1.8)
Glutathione S-transferases are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. The enzyme protects cells against toxicants by conjugating them to glutathione, thereby neutralizing their electrophilic sites, and rendering the products more water-soluble. Glutathione conjugates are further metabolized by the cleavage of glutamate and glycine residues, followed by acetylation of the resultant free amino group of the cysteiny1 residue to their final product, a mercapturic acid (Habig et al., 1974). The mercapturic acids, i.e. S-alakylated derivatives of N-acetylcysteine, are than excreted. GSTs also show glutathione peroxidase activity and can thus protect against oxidative damages (Mosialou et al., 1993; Zimniak et al., 1997). Based on their sequence homology, substrate specificity, and immunological cross-reactivity, GSTs have been grouped into species-independent classes of isozymes.

In the rat brain the role of GST has been suggested in the conjunction of certain drugs and their metabolites and their subsequent detoxification (Singh et al., 2002). The relatively high catalytic efficiency of GST towards lipid aldehydes and its ability to detoxify peroxidized DNA suggests an important antioxidative role of this enzyme. In addition to this, GST with the help of glutathione (GSH) also protects the cells against 4-HNE (An aldehyde product of lipid peroxidation) toxicity. The decrease in the GST activity with increasing age may be a contributing factor to the rise in oxidative stress including lipid peroxidation levels. GST subserves a variety of functions in brain besides antioxidative action and therefore its deficiency during aging will contribute to the generation of variety of deleterious effects (Jensson et al., 1986). GST enzyme activity and tumor incidence in the mucosa along the human gastrointestinal tract, suggesting the importance of GSTs in cancer prevention (Wang
et al., 2010). GSTs are also engaged in the intracellular transport of variety of hormones, endogenous metabolites and drugs (Pickett et al., 1987).

6.2 LIPID PEROXIDATION

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds. The process of lipid peroxidation leads to the formation of endo-peroxides and hydro-peroxides. Once formed, unstable peroxides are able to propagate a chain reaction and form lipidperoxides, which decompose double bonds of unsaturated fatty acids and destruct membrane lipids (Shaheen et al., 1996).

Two broad outcomes to lipid peroxidation are 1) structural damage to membranes generation of fragmented fatty acyl chains, lipid-lipid cross-links, endocyclization to produce novel fatty acid esters, and lipid-protein cross-links (Catalá, 2006). 2) secondary products of lipid peroxidation for example: include chemically reactive aldehydes such as malondialdehyde (MDA), acrolein and 4-hydroxy-2-nonenal (4-HNE) (Esterbauer et al., 1991). Malonaldialdehyde is one of the end products in the lipid peroxidation process (Hagihara et al., 1984). Associated with these changes in the membrane, several authors claim an increase in phospholipids rigidity after lipid peroxidation (Garcia et al., 1997; Kamboj et al., 2008).

4-hydroxynonenal (4-HNE) an amphiphilic compound, an aldehyde product of lipid peroxidation is water-soluble with much stronger lipophilic properties (Esterbauer et al., 1991; Hagihara et al., 1984; Poli et al., 2008). 4-HNE has a considerably longer half-life than free radical species and is thereby capable of inhibiting the function of most key enzymes, impair function of membrane transporters and promote neuronal death in a variety of experimental models (Keller et al., 1997). Secondary products were found to further enhance the deterioration process inside the cell. For example: 4-HNE impairs Na⁺ K⁺ ATPase activity, induced an increase of neuronal intracellular free Ca²⁺ concentration in a time- and concentration dependent manner (Keller et al., 1997). It may damage proteins by interacting with lysine, histidine, serine, and cysteine residues (Uchida and Stadtman, 1992).

This process of lipid peroxidation is under the control of the efficient endogenous cellular defense system to insure the maintenance of cell integrity as well as optimum metabolic and functional performance. In diabetic condition the function of endogenous
protective systems like antioxidant system is depressed and contributes to diabetic tissue pathology by elevating the cellular and membrane lipid peroxidation. Jain and Lim (2000) have reported that the high glucose treated human erythrocytes showed increased lipid peroxides formation, decreased Na$^+$ K$^+$ ATPase and Ca$^{2+}$ ATPase activity and these alterations were effectively controlled by lipoic acid treatment. Lipid peroxidation of the neural membrane causes loss of membrane fluidity and of the membrane potential, inhibition of membrane bound enzyme (Halliwell and Gutteridge, 1989; Kukreja et al., 1988; Kamboj et al., 2009) increase calcium and diminished neurotransmitter uptake and loss of membrane assymetry. Increasing evidence indicates that oxygen radical production in the cell increases with age in mammals. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. The age related increase in production of peroxidants might be derived from the membrane damage by O$_2^-$ and H$_2$O$_2$. It is one of the major outcomes of free radical-mediated injury to tissue. This may cause peroxidative damage in inflammation, cancer, toxicity of xenobiotics and aging.

6.3 MEMBRANE FLUIDITY

The term fluidity is applied to anisotropic bilayer membrane, used to denote the relative motional freedom of the lipid molecules or substituents. The physiological importance of modifications in the physical properties of membranes resides in their relation to numerous cellular functions, including the activity of membrane-associated enzymes, solute transport, hormone induced signal transduction process, impairment of cell permeability and also hindering homoeostasis. Jones et al. (1998) showed the abnormal regulation i.e. decreased of cell membrane fluidity in diabetic nephropathy. Changes in membrane structure and function have also been reported during aging process. Hashimoto et al. (2005) determined the effect of aging on membrane cholesterol content and fluidity of cultured endothelial cells from thoracis aorta of aging rats, these authors reported that age related increase in cholesterol and lipid peroxide occurs in vascular endothelial cells plasma membrane which reduces membrane fluidity. Therefore, the membrane fluidity can be used as an index for the complications of several diseases like diabetes and AD.

An abnormality of the physical properties of the membrane may underlie the defect that unites the clinical and biochemical abnormalities found in subjects with diabetes. The
physical state of the lipid bilayer of the cell membrane can be assessed by the measurement of the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Jones et al., 1998). DPH is a relatively hydrophobic molecule and positions itself in the deep hydrophobic regions of the lipid bilayer. The fluidity of the regions in which the dyes are situated affects their rate of rotation and hence the degree of depolarization of fluorescent light. The anisotropy value is a quantitative expression of this relationship with the fluidity of the lipid bilayer.

Prolonged hyperglycemia and enhanced oxidative stress are considered to be the major causes responsible for the onset of diabetic complications mainly due to glycation and oxidation of protein and by increasing the formation of free radicals (Giugliano et al., 1996; Baynes, 2003). One of the targets for the glycation (no-enzymatic glycosylation) and free radicals in diabetic condition are the membrane proteins. The non-enzymatic glycosylation is interrelated with the enhanced oxidation of protein, glycoxidation that produces reactive carbonyl intermediates. These intermediate products may be involved in creation of advanced glycation end products (AGEs) and formation of cross-links between membrane proteins, thus altering the properties of membrane. Hong et al. (2004) reported that free radicals oxidized polyunsaturated fatty acids, the main component of membrane, thereby destroying the spatial arrangement of the membrane and removing biological activities. As a result, the functions of the membrane deteriorate and fluidity decreases.

6.4 INTRASYNAPTOSMAL CALCIUM
Calcium is an ubiquitous and universal intracellular secondary messenger in different cells, and changes in the intracellular cytosolic Ca\(^{2+}\) concentration triggers a wide range of various cellular responses, including growth, survival, death and long-term modifications of synaptic transmission in the nervous system (Rose and Konnerth, 2001).

The intracellular calcium signalling system is involved in the regulation of a wide range of physiological reactions (Toescu et al., 2004). This ancient system, which developed very early in evolution, utilises the tremendous transmembrane gradient for Ca\(^{2+}\) ions which by far exceeds transmembrane gradients for all the other physiologically relevant ions. Therefore, even tiny changes of membrane Ca\(^{2+}\) permeability result in huge changes in
cytoplasmic Ca\textsuperscript{2+} concentration, providing a signaling system with an intrinsic high signal-to-
noise ratio.

Various intracellular enzymes bind Ca\textsuperscript{2+} ions with different affinities, determining the
amplitude coding of the signaling system. These enzymes act as effectors of the signaling
system, as on Ca\textsuperscript{2+} binding they change their catalytic activity. In the nervous system the best
example for the role of Ca\textsuperscript{2+} ions is synaptic transmission. Calcium, entering presynaptic
terminals following the depolarisation caused by the arrival of the action potential, triggers
the fusion of the neurotransmitter vesicles with plasmalemma, thus allowing the chemical
transduction of nervous impulse. Nevertheless, this calcium entry could be toxic and, in
certain conditions, neurons overloaded with Ca\textsuperscript{2+} die, a process referred to as excitotoxicity
(Burke and Barnes, 2006). Obviously these are not the only roles of Ca\textsuperscript{2+} in nerve cells, and
changes in Ca\textsuperscript{2+} regulate many other neuronal functions (e.g. excitability, metabolism and
gene expression, but these two faces of Ca\textsuperscript{2+} action are important for our further discussion.
Thus, a better understanding of how neurons handle Ca\textsuperscript{2+} with advancing age would allow us
to ascertain their increased vulnerability to various insults which lead to the development and
progression of aging related deficits.

Changes in calcium homeostasis are prominent components of brain aging and
contribute to impaired cognition and increased vulnerability to excitotoxicity and
neurodegenerative diseases (Thibault et al., 1998; Burke and Barnes, 2006; Murchison and
Griffith, 2007; Raza et al., 2007). Most studies examining age-related changes in Ca\textsuperscript{2+}
regulation have been conducted in male animals, although sexual dimorphism is evident in
many brain regions not directly associated with reproduction, including the hippocampus
(Cahill, 2006; Murali et al., 2008).

6.5 NEUROLIPOFUSCIN

Lipofuscin is an autofluorescent pigment that accumulates inside neurons due to increased
oxidative stress represents an end-product of oxidative degradation of lipids by free radical
mechanism (Moorthy et al., 2005b; Riga et al., 2006). This so-called age-pigment, contains
derivatives of lipid peroxidation e.g., MDA (Jung et al. 2007; Terman and Brunk, 2006).
There are good evidences that lipofuscin is formed within secondary lysosomes, being
strongly accelerated by iron-catalyzed oxidative reactions of autophagocytosed cellular
material. Critical parameters seem to be the amount of intralysosomal low-molecular-weight iron in redox-active form, degree of autophagocytosis, and influx of hydrogen peroxide to the lysosomal apparatus (Terman and Brunk, 2006).

Recent (Kumar et al., 2008) and earlier studies have shown that lipofuscin is a peroxidation products and its formation appears to be proportional to the occurrence of lipid peroxidation (Riga and Riga, 1995; Bala et al., 2006). It has been shown that DM significantly increased the fluorescent pigments in the neurons of experimentally induced DM, with the possibility that DM makes some functional changes due to the accumulation of the fluorescence materials in neurons (Sugaya et al., 2004). Aging is associated with accumulation of neuronal lipofuscin, which is formed mostly from protein and peroxidized lipid material autophagocytosed from various cellular constituents and fragmented within the lysosomes (Mesulam, 1987). An age-related increase has been shown in both lipid peroxidation and in the gross level of lipofuscin (Sharma et al., 1993). Although all the available evidence indicates that lipofuscin which accumulates in lysosomes and related organdies is the end product of the physiological decay of the cells own constituents, the mechanisms involved in its formation are not totally understood. Since this pigment is properly considered the hallmark of neuronal aging, it is evident that the unraveling of these mechanisms will provide important information to the still uncertain underlying causes of the aging process. The rate of lipofuscin accumulation varies from cell to cell and species to species and this accumulation is relatively slow, and probably for this reason, the pigment is not present in the cells of young subjects or in short-lived labile cells (Porta, 1991). The main chemical constituents of lipofuscin granules isolated from all these different sources are presented in (Table 7).

6.6 DEGRADATION OF GENOMIC DNA

Programmed cell death has been well described in a number of organs of the body during various developmental, physiological, as well as pathological states (King and Cidlowski, 1995; Majno and Joris, 1995). It is characterized morphologically by cellular shrinkage, membrane blebbing, and, in most cases, by the fragmentation of nuclear DNA into multiple segments of approximately 200 bp in length. One hallmark of programmed cell death is a lack of inflammatory response. Moreover, it is a form of cellular death which in most, but not
TABLE 7. MAIN CHEMICAL CONSTITUENTS OF ISOLATED LIPOFUSCIN

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage</th>
<th>Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>30-70%</td>
<td>Mainly glycine, valine, alanine and proline, several hydrolytic enzymes, and still uncharacterized low molecular weight proteins</td>
</tr>
<tr>
<td>Lipids</td>
<td>20-50%</td>
<td>Cholesterol, phospholipids, triglycerides, free fatty acids, bis (monoacylglycero) phosphate, ubiquinone, dolichol</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4-7%</td>
<td>Mainly mannose, N-acetylglucosamine, glycine, glucose and galactose</td>
</tr>
<tr>
<td>Metals</td>
<td>Traces</td>
<td>Iron, copper aluminum, zinc</td>
</tr>
</tbody>
</table>

Table adapted from Porta, (1991)
all, instances requires new protein synthesis that is followed by an orderly sequence of signal
transduction events resulting in death of the cell (Majno and Joris, 1995; Bossenmeyer et al.,
1998). Among the variety of proteins that are produced in response to cellular injury are
those mediating DNA fragmentation, such as bax, fas, bcl-xS, and bak, as well as the anti-
apoptotic ones, such as the bcl-2, bcl-xL and bag-1 (Misao et al., 1996). Although
programmed cell death has been well documented in post mitotic tissues such as the heart
and the brain, there has been relatively less characterization of it in the normal heart and
brain in response to stress during aging (Misao et al., 1996; Sutherland et al., 1996; Schmidt-
Kastner et al., 1997; Rao, 2007).

Elevated oxidative stress is considered to be a likely cause of atherosclerosis, the
most significant complication in diabetes and the most common cause of premature death by
affecting biomolecules other than protein, lipids - notably DNA. Oxidative stress can also
cause proliferation or apoptosis in vascular smooth muscle (Sandberg et al., 2004), which
again contributes to the progression of the diabetic condition. Neither superoxides anions nor
hydrogen peroxide reacts directly with DNA in-vivo, transition metal ions such as \( \text{Fe}^{2+} \) or
\( \text{Cu}^{2+} \) catalyze their conversion into the highly reactive hydroxyl radicals, which in turn
provokes a broad spectrum DNA damage (Dincer et al., 2003). Delaney and Eizirik in 1996
have shown that the generation of Nitric Oxide had increased during Type 1 diabetes and
caused beta cell damage. Nitric Oxide has also been shown to break the DNA strand in
mammalian cells. DNA oxidation may therefore be of considerable value in following the
progress of the disease and its metabolic control.

6.7 GLUCOSE TRANSPORTER-4 (GLUT4)
Glucose is the major source of energy in the mammalian brain. The transport of glucose into
most mammalian cells occurs by facilitated diffusion, mediated by a family of glucose
transporter proteins. This family of highly homologous, integral membrane proteins now
comprises 7 distinct genes, encoding 6 proteins, named GLUT 1-7 (Gould and Holman,
1993; Ibberson et al., 2000) (Figure 5). Molecular and morphological studies have shown
that most GLUT isoforms reside constitutively on the plasma membrane of peripheral organ
and brain cells in order to optimize the uptake of extracellular glucose. In contrast, GLUT4,
also referred to as the insulin-sensitive transporter, is primarily expressed in the periphery
Fig. 5 Localization of GLUT isoforms in the CNS. Shown in green is the 55 kDa isoform of GLUT1 expressed in endothelial cells. Shown in light blue is the 45 kDa isoform of GLUT1 expressed in astrocytes. Neuropil expression of GLUT3 is depicted in yellow. Illustrated in dark blue is the somatodendritic labeling of GLUT4. Potential overlap of GLUT3 and GLUT4 distribution in some neurons of the brain is shown as yellow and dark blue-hatched areas. Shown in brown is GLUT5 expressed in microglia. Illustrated as red dots is the intracellular and somatodendritic localization of GLUT8.

Adapted from McEwen and Reagan, (2004)
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i.e., heart, skeletal muscle, and adipose tissue, which respond to insulin by acutely increasing their rate of glucose uptake. Insulin stimulates this increased glucose uptake by promoting a translocation of GLUT4 from their basal, intracellular location to the plasma membrane (Simpson and Cushman, 1986; Vannucci et al. 1998).

In the last few years, however, there have been several reports of GLUT4 mRNA and protein expression in regions of rat brain, including cerebellum and several cortical regions, ependymal lining of ventricles and microvessels (Leloup et al., 1996). El Messari et al. (2002) showed that in the central nervous system, GLUT4 protein is consistently expressed by neurons of specific areas (Leloup et al., 1996; Apelt et al., 1999). In addition, GLUT4-immunoreactive cells are widely distributed throughout the rat CNS where they are preferentially localized in insulin receptor-rich areas and/or in motor regions of the brain and spinal cord (El Messari et al., 1998; Apelt et al., 1999; Bakirtzi et al., 2009). GLUT4 proteins are highly regulated in physiological as well as pathophysiological states. Levels of regulation include gene transcription, protein synthesis, and degradation. Indeed, noninsulin-dependent diabetes mellitus and streptozotocin (STZ) diabetic rats are associated with a decrease in cellular number and activity of GLUT4 (Kahn and Cushman, 1987).

In the diabetic state due to the deficiency of insulin GLUT4 translocation does not take place efficiently, GLUT4 glucose transporters are therefore, present in cytoplasmic vesicles, where they are not used for transporting glucose. This results in a decreased uptake of glucose by the muscle cells, which contributes significantly to the elevated glucose levels. Therefore, regulation of alterations in the expression and translocation of GLUT4 could be an important parameter for the evaluation of an antidiabetic compounds. The insulin receptor is also expressed in discrete neuronal populations in the CNS, including the cerebellum, hypothalamus and the hippocampus (Marks et al., 1991). Interestingly, the insulin receptor and GLUT4 often exhibit overlapping distributions in the rat brain. In this regard, GLUT4 mRNA and protein were localized in neurons in the hypothalamus (Leloup et al., 1996) and cerebellum (Kobayashi et al., 1996), neuronal populations that also express the insulin receptor (Figure 6).

Subsequent studies confirmed and extended these observations and demonstrated that GLUT4 was also expressed in other regions in the brain, including the cortex and hippocampus (El Messari et al., 1998). Collectively, these studies have demonstrated that
FIGURE 6. SIMPLIFIED REPRESENTATION OF MOLECULAR MECHANISM INVOLVED IN INSULIN SIGNALING PATHWAY THAT REGULATES GLUCOSE TRANSPORTER (GLUT4) TRANSLOCATION TO CELL MEMBRANE.

**Nomenclature**: GLUT4: Glucose-transporter isoform 4; IRS-1: Insulin receptor substrate-1; PI3K: Phosphatidylinositol-3-kinase; PI (3, 4, 5) P3: Phosphatidylinositol (PI)-3, 4, 5-tiphosphate; PDK1: phosphosinositide-dependent kinase 1; Akt: Protein kinase Akt or protein kinase B (PKB); PKC: Protein kinase C-c; PTP1B: Protein tyrosine phosphatase 1B; PTEN: 3' lipid phosphatase; SHIP2: 5' lipid phosphatise.
GLUT4 expression in the brain is limited to neuronal cell bodies and dendrites and often exhibits overlapping distribution with the insulin receptor, suggesting that insulin stimulates the translocation of GLUT4 to the plasma membrane in the brain. Indeed, plasma membrane association of GLUT4 is modulated in the cortex and cerebellum in experimental models of type 1 and type 2 diabetes (Vannucci et al., 1998). GLUT4 trafficking in the rat hippocampus is also impaired in experimental models of type 1 diabetes (Reagan, 2002). These results indicate that neuronal GLUT4 trafficking is sensitive to changes in insulin levels, as described for peripheral tissues such as muscle and fat (Watson and Pessin, 2001) and provide evidence that insulin-stimulated trafficking of GLUTs may participate in the centrally mediated actions of insulin and glucose. In the aging state due to insulin resistance GLUT4 translocation does not take place efficiently, GLUT4 glucose transporters are hence, present in cytoplasmic vesicles, where they cannot transport glucose, resulting in a decreased uptake of glucose by the cells, which can contribute to the elevated glucose levels in aging. Hence a reversal of alterations in the expression and translocation of GLUT4 could be an important parameter for the evaluation of an antiaging compound. As has been reported earlier there was a decrease in GLUT4 levels in the brain of aging rats, E2 treatment of aging rats resulted in normalization of GLUT4 levels in the brains of aging female rats thus improving homeostasis during aging (Alonso et al., 2008; Fernando et al., 2008; Moreno et al., 2010).