CHAPTER IV

Discussion
CHAPTER 4

DISCUSSION (EXPERIMENTAL DIABETES)

1. GENERAL
The experimental animal model of diabetes mellitus of the present study can be taken as equivalent to type I diabetes, in which insulin secretion is very low to \( \beta \)-cells destruction. Diabetic neuropathy is a frequent complication of diabetes mellitus, for which no adequate clinical treatment is currently available. One of the main reasons for the absence of effective treatment of this disease is that information on how metabolic, vascular, and other abnormalities involved in the pathogenesis of diabetic neuropathy lead to dysfunction of nerve cells and pathways remains insufficient.

Glucose is the main fuel for energy requirement in the body. Any variation in the metabolism of glucose through its normal metabolic pathways and some related pathways, may lead to impaired glucose metabolism, the onset of hyperglycemia and subsequently diabetes mellitus. It is projected that the worldwide prevalence of diabetes is likely to increase to more than 439 million by the year 2030 (Shaw et al., 2010). Insulin, since its discovery has been used as a life saving therapy for diabetes. Exogenous insulin, however, fails to produce a well-controlled hyperglycemia in association with variable dietary intake and variable physical activity. Insulin treatment does not effectively prevent the long-term complications associated with diabetes. In Type 2 diabetes insulin is relatively ineffective because of the increased insulin resistance of the responding tissue. Although sulphonylureas, biguanides, insulin sensitizers (thiazolidinediones) 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). Consequently there is a need to develop antidiabetic drugs that are effective, safe, produce a well-controlled normoglycemia and prevent long-term complications. In searching for effective drugs in diabetes, the insulin mimetic and the antidiabetogenic properties of various chemicals and natural agents have been studied.
The present study was undertaken to investigate the antidiabetic properties of *Trigonella* seeds on neuronal processes and the possible mechanisms of its action. *Trigonella foenum graecum* seed powder (TSP) has been earlier shown to have hypoglycemic and antihyperglycemic properties besides other medicinal properties (Genet et al., 2002, Mohammad et al., 2006). The present study explored the possibility of using *Trigonella* seed powder (TSP) and to evaluates its antidiabetic effects on physiological parameters of experimental animals, like body weight and tissue weight, blood glucose levels, insulin levels, key regulatory membrane linked enzymes like Na$^{+}$K$^{+}$ ATPase, MAO, Ca$^{2+}$ ATPase and antioxidant enzymes (SOD, GST), membrane lipid peroxidation (MDA and 4-HNE levels), calcium homeostasis, neurolipofuscin accumulation, peroxidation of membrane lipids and synaptosomal membrane fluidity in brain tissue of control, diabetic and diabetic treated rats with different antidiabetic compounds. Moreover, expression and distribution of glucose transporter (GLUT4) by RT-PCR, immunohistochemistry and Western blotting and oxidative degradation of genomic DNA in the brain has also been monitored in control and different experimental diabetic groups namely diabetic groups treated with insulin, 5% *Trigonella*. Three weeks treatment of the diabetic group with insulin, and TSP separately resulted in a marked reduction in hyperglycemia in the diabetic animals.

**Diabetic neuropathy** is characterized by segmental demyelination and axonal degeneration of peripheral neurons, together with functional abnormalities such as reduced nerve conduction and blood flow. The neurological consequences of diabetes mellitus (DM) in the central nervous system (CNS) are now receiving considerable attention (Gispen and Biessels, 2000; Biessels et al. 2002; Biessels and Gispen, 2005; Kamal et al. 2006; Edwards et al.2008; Fazeli , 2009 ; Baquer et al. 2009; Muresanu et al., 2010). Diabetes is characterized by hyperglycemia and metabolic abnormalities resulting from decreased insulin levels causing metabolic and other physiological changes in various organs including brain. Glucose utilization is decreased in the brain during diabetes, providing a potential mechanism for increased vulnerability to acute pathological events. Oxidative stress, leading to an increased production of reactive oxygen species (ROS), as well as lipid peroxidation, is increased in diabetes. Due to their high polyunsaturated lipid content, neuronal cells are particularly sensitive to oxygen free radical damage and lipid peroxidation may increase cell membrane rigidity and impair cell function. High oxidative stress can lead to microvascular
cerebral diseases, e.g., stroke, cerebral hemorrhage, and brain infarction (Vincent et al., 2004). The reason for high risk of microvascular cerebral diseases is that, despite the fact that brain consumes 20% of the oxygen in the body, it has a low content of antioxidants and antioxidant enzymes, and high content of unsaturated fatty acids and catecholamines that are easily oxidized, making it more vulnerable to oxidative damage than any other organ in the body (Hong et al., 2004; Nelson et al., 2009). 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 elderly. The emerging view is that the diabetic brain features many symptoms that are best described as accelerated brain aging.

Many plant extracts have also shown hypoglycemic effects. Some of these have been tested in experimental animal models and their hypoglycemic effect has been expounded. These plants include Alium sativum (garlic), Momordica charantia (bitter gourd) fruit extract, Ficus bengalagenesis (banyan) bark, Ficus carica (fig) leaves and Trigonella foenum graecum (fenugreek) whole seed powder. *Trigonella* seeds have been shown to have potent antihyperglycemic and hypoglycemic properties. Several studies have indicated that treatment with fenugreek seeds lower the elevated blood glucose in experimental diabetic rats and partially correct the disturbances in the metabolic pathways without causing any toxic effects. (Puri et al., 2002; Srinivasan, 2006; Mohammad et al., 2006). *Trigonella* seed powder like other antidiabetic plant extracts such as Momordica charantia and Ficus bengalensis are known to rejuvenate the β-cells in the islets of Langerhans, thus increasing the capacity of insulin secretion in Type 1 diabetes (Yadav et al., 2004). The insulinotropic property of 4-hydroxyisoleucine, an amino acid extracted from *Trigonella* is also suggestive of insulin secretion modulation in its therapeutic action (Broca et al., 1999). Therefore administration of *Trigonella* seeds would make the pancreatic beta cells to secrete insulin and help to normalize increased blood glucose levels in diabetic rats.

2. EFFECT OF ANTIDIABETIC COMPOUNDS ON GENERAL PARAMETERS
The present study employs albino Wistar rats made diabetic by alloxan administration. This experimentally induced diabetes is considered equivalent to Type-1 diabetes. Several physiological changes in body weight, brain weight and blood glucose levels were seen
which are characteristic of Type-1. The results obtained in the present study correlate well with those found in earlier studies (Mohammad et al., 2006; Siddiqui et al., 2006; Srichamroen et al., 2009).

After 21 days of insulin withdrawal there was a five-fold increase in blood glucose levels of alloxan-diabetic rats. Diabetic animals receiving three weeks of treatment with insulin showed a marked reduction in hyperglycemia. *Trigonella* treatment revived normoglycemia after three weeks of treatment (Table 1). Hyperglycemia during diabetes has been shown to generate free radicals from auto-oxidation of glucose, formation of AGEs and increased polyol pathway, with concomitant increase in cellular lipid peroxidation and damage to membranes (Kumar and Menon, 1993; Preet et al., 2005). Chronic hyperglycemia induced a number of changes in the neurochemical profile possibly linked to osmolarity regulation essential for the maintenance of cellular homeostasis (Kamboj et al., 2009). Present results show that three weeks treatment of the diabetic group with insulin, and TSP separately resulted in a marked reduction in hyperglycemia in the diabetic animals. TSP improved glucose homeostasis in diabetes by decreasing oxidative stress, probably by enhancing or mimicking insulin action (Mohammad et al., 2006).

Seeds of *Trigonella foenum-graecum* are therefore considered to be potentially useful for glucose control and for preventing hyperlipidaemia in diabetic subjects. The active component of *Trigonella foenum-graecum* seeds has been found to be associated with the defatted part of the seeds, rich in fiber containing steroidal saponins and proteins comparable to those of soybean (Madar and Shomer, 1990; Al-Habori et al., 2001). A possible mode of action of TSP suggested was an effect on intestinal carbohydrate digestion, it was found to decrease the digestion of starch and also decrease glucose absorption both *in vivo* and *in vitro*, may be as the result of a direct inhibitory effect on the digestive enzymes (Hannan et al., 2007).

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). Serum insulin levels decreased in DM as well as in experimental diabetes (Biessels et al., 2002). Present investigation leads us to conclude that TSP administration to diabetic rats significantly and effectively reversed the diabetic aberrations studied, in the
brain. TSP brought back the serum insulin levels, which were decreased in diabetic animals to almost that of the control levels probably by rejuvenating left over beta cells in the diabetic pancreas. TSP effectively controlled, plasma glucose levels and normalized alterations in the key functions of the cell membrane.

Therefore, after showing improvement in the glucose homeostasis of diabetic rats treated with antidiabetic compounds, the metabolic consequences of this treatment on key membrane linked parameters namely membrane bound enzymes, antioxidant enzymes, membrane fluidity, lipid peroxidation, neurolipofuscin deposition, calcium levels, DNA degradation and GLUT 4 levels were further examined in detail.

3. EFFECT OF ANTIDIABETIC COMPOUNDS ON MEMBRANE LINKED ENZYME ACTIVITIES

MONOAMINE OXIDASE

Monoamine oxidase is a mitochondrial enzyme responsible for the oxidative deamination of a variety of biogenic amines. MAO in brain is of physiological importance as the enzyme inactivates transmitter monoamines, namely dopamine, noradrenalin and serotonin (Soto-Otero et al., 2001). Neurodegenerative processes may preferentially affect the brain as a result of the production of free radicals associated with catecholamine metabolism. The present results show the marked increase in MAO activity during diabetes. It has been documented that H2O2 derived from MAO activity, represents another source of oxidative stress in brain (Soto-Otero et al., 2001; Mayanil et al., 1982b; Baquer et al., 2009). Since the activities of most membrane bound enzymes are regulated by the physiochemical state of their lipid environment, the reactive oxygen substances affect membrane linked enzyme activity through modification of the membrane fluidity and its structure. Despite the existence of two-enzymatic scavenging systems, Catalase and Glutathione peroxidase, to protect cells from the presence of H2O2, it is well known that the brain level of these two enzymes are low compared to other tissue (Marklund et al., 1984). In addition, the H2O2 generated by mitochondrial MAO does not easily reach the catalase compartment. The treatment of diabetic animals with insulin, and Trigonella reverts the increased activity of MAO to almost control levels in synaptosomal and supernatant fractions. TSP administration protected against diabetes-induced changes in MAO activity by attenuating oxidative stress.
and restoring membrane lipids to normal levels. TSP might also protect the enzyme activities by preventing hyperglycemia-induced.

**Na⁺K⁺ ATPase**

The results from the present study indicates that alloxan induced diabetes elicits alteration in synaptosomal Na⁺K⁺ ATPase enzyme activity. There was a significant decrease in Na⁺ K⁺ ATPase activity in the brain synaptosomal fractions of alloxan diabetic rats. These results are in agreement with those reported in earlier studies (Kjeldsen et al. 1987; Leong and Leung, 1991; Sennoune et al., 2000; Siddqui et al., 2005).

The decrease in Na⁺K⁺ ATPase activity observed in diabetic brain could be related to hyperglycemia or to insulinopenia. Earlier, it was shown that the decrease in Na⁺K⁺ ATPase observed in a small series of uncontrolled Type 1 diabetic patient was corrected in 24 hours after glycemic normalization, suggesting that hyperglycemia and/or insulinopenia could be involved in the regulation of erythrocytes Na⁺K⁺ ATPase (Jain and Lim, 2000). Hyperglycemia is the most important factor in the onset and progress of diabetic complications.

Since Na⁺ K⁺ ATPase is an essential enzyme of the plasma membrane of cells, it has been suggested to represent an important target of free radicals induced membrane damage (Mense et al., 1997). Nandhini and Anuradha (2003) showed that a reduction in the production of free radicals, lipid peroxidation and glycosylation could beneficially prevent reduction in the activity of Na⁺ K⁺ ATPase enzyme in RBC. Another possibility to explain the changes in diabetic Na⁺K⁺ ATPase functions is that through the enhanced synthesis of sorbitol, the myo-inositol turnover is damaged, resulting in the activation of Na⁺K⁺ ATPase enzyme in insulin independent tissues. Several reports showed that in rat brains of streptozotocin induced diabetes; there were higher myo-inositol and glucose levels (Greene et al., 1987).

Insulinopenia may be involved in decrease in Na⁺K⁺ ATPase activity in diabetes. Insulin is known to regulate Na⁺/K⁺ ATPase activity. However, the mechanism by which insulin activates the sodium pump is complex. Insulin increases intracellular sodium concentration and enhances sodium affinity of the pump. It could also induce a redistribution
of the different subunit of Na\(^+\)K\(^+\) ATPase from intracellular sites to the plasma membrane as observed in mammalian skeletal muscle (Ramlal et al., 1996).

Na\(^+\)K\(^+\) ATPase activity has been reported to be affected by alteration in membrane fluidity. This enzyme shows an increased activity in response to enhanced membrane fluidity. Polarization and anisotropy measurements on synaptosomes from diabetic animals indicate that the altered lipid structure correspond to a decrease in fluidity. The present results agree well with earlier published reports (Sennoune et al., 2000; Kamboj et al., 2009). The decrease in membrane fluidity of synaptosomes of diabetic tissues could be due to the peroxidation of membrane phospholipids through free radicals, which are generated by persistent hyperglycemia in uncontrolled diabetes. Associated with these changes in the membrane, several authors claim an increase in phospholipids bilayer rigidity after lipid peroxidation in relation to their numerous cellular functions. The changes in membrane fluidity may be attributed to the decrease in Na\(^+\)K\(^+\) ATPase activity in diabetic liver, brain and heart tissues (Sennoune et al., 2000; Ziegelhoffer-Mihalovicova et al., 2003; Kamboj et al., 2009).

Na\(^+\)K\(^+\)ATPase also showed a decrease in activity in the brain which was normalized after insulin administration (Lakhman and Kaur, 1997). Changes in brain Na\(^+\)K\(^+\)ATPase and MAO have been earlier reported from diabetic brain by Mayanil et al. (1982a,b) and the former i.e. Na\(^+\)K\(^+\)ATPase more recently by Siddiqui et al. (2006). TSP administration protected against diabetes-induced changes in Na\(^+\)K\(^+\)ATPase activity by attenuating oxidative stress and restoring membrane lipids to normal levels. The treatment of diabetic animals with insulin and TSP normalized the decreased activity of Na\(^+\)K\(^+\)ATPase to almost control levels with insulin and TSP administration. It is suggested that TSP protected the enzyme activities by normalizing hyperglycemia.

**Ca\(^{2+}\) ATPase**

Regulation of intracellular Ca\(^{2+}\) concentrations is vital for cells, especially for neurons; raised Ca\(^{2+}\) levels are associated with cytotoxicity and neuronal death. Present data suggest that the increase in synaptosomal Ca\(^{2+}\) due to oxidative stress may result from the inhibition of the plasma membrane Ca\(^{2+}\)ATPase activity, probably as a result of the alteration of the lipid environment required for its maximal activity (Pekiner et al., 2002 and Kamboj et al.,
The Ca\textsuperscript{2+} ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. Maintenance of the calcium gradient by Ca\textsuperscript{2+} ATPase is of fundamental importance in the control of hydration, volume, nutrient uptake and fluidity of cells, and is also essential for the contractibility and excitability properties of muscles (Lebeche et al., 2008). In the present study, synaptosomal Ca\textsuperscript{2+} ATPase activity was decreased in brain of alloxan diabetic rats. The present findings are in agreement with previous observations showing that the membrane abnormalities in Ca\textsuperscript{2+} ATPase activity led to the occurrence of intracellular calcium overload in experimental rat models of diabetes (Kamboj et al., 2009). Plasma membrane Ca\textsuperscript{2+} ATPase is regulated by a number of factors and various mechanisms. These include calcium, calmodulin, acidic phospholipids, fatty acids and also protein kinase-mediated phosphorylation (Pekiner et al., 2002). A decreased in Ca\textsuperscript{2+}ATPase 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). TSP and insulin treatment restored the altered synaptosomal Ca\textsuperscript{2+}ATPase activity to almost control levels. TSP treatment as mentioned earlier decreased the oxidative stress and lipid peroxidation levels. In earlier studies, Ca\textsuperscript{2+} dependent ATPase activity in liver homogenate of alloxan diabetic rats in the presence of Fe\textsuperscript{2+}/ ascorbate was protected by aqueous extract of Trigonella seed powder (Anuradha and Ravikumar, 2001). A reduction in the production of free radicals and lipid peroxides formation can beneficially prevent the decrease in the activity of Ca\textsuperscript{2+}ATPase (Siddiqui et al., 2005; Kamboj et al., 2009).

4. EFFECT OF ANTIDIABETIC COMPOUNDS ON ANTIOXIDANT ENZYMES

Increased levels of glucose is responsible not only for oxygen free radical generation due to non-enzymatic protein glycation, autooxidation of glycation products, ascorbic acid and glucose, but also due to changes in tissue content and activity of antioxidant defense systems (Oberley, 1988; Jain and Palmer, 1997). In the present study alterations in brain antioxidant enzyme activity was observed during diabetes. Endogenous and exogenous antioxidants, as well as the enzymes of antioxidative defense and phase 2 of detoxication metabolism are decreased in diabetic rat brain, these constitute an important defense system to clear up the detrimental ROS generated \textit{in vivo} (Cao and Li, 2004).
SUPEROXIDE DISMUTASE
The enzyme SODs are the cell’s first line of defense against the toxicity of superoxide radical and the subsequent radical derivatives (Fridovich, 1974). Although \( \text{O}_2^- \), 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 \( \text{O}_2^- \) 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 \( \text{O}_2^- \) but singlet oxygen and other reactive oxygen species (ROS) as well (Halliwell and Gutteridge, 1989; Marklund, 1984; Erlansson et al., 1990). The results from the present study indicate that alloxan induced diabetes showed significantly lowered levels of SOD activity in brain. The results are in agreement with earlier published studies (Oberley, 1988; Ceriello et al., 2000; Genet et al., 2002; Siddqui et al., 2005). The decrease in the activity of SOD in brain may be due to the inactivation or inhibition of SOD by increased production of ROS during diabetes (Van Dam et al., 1995; Kakkar et al., 1995; Genet et al., 2002). Treatment of diabetic rats with \textit{Trigonella foenum graecum} and insulin separately for three weeks reversed the lowered activity of SOD in brain, near to the activity in age-matched controls. The restoration of the altered SOD activities could primarily be due to the subsequent lowering of blood glucose levels. It has been postulated that excess free circulating glucose is toxic to the organism/cell and is the proximal source of increased oxidative stress in hyperglycemic condition. Glucose has been demonstrated to get oxidized generating \( \text{H}_2\text{O}_2 \). Autoxidation of glucose is directly linked to protein glycation, which is another source of free radicals (Wells-Knecht et al., 1995). Thus the lowering of elevated blood glucose levels would prevent the subsequent glucose induced toxic effects. Kumar and Menon (1993) also reported a decrease in the activity of SOD in diabetic brain (Kamalakkannan and Stanely, 2006). Administration of TSP to diabetic animals increased the activity of SOD in the diabetic brain.

GLUTATHIONE S-TRANSFERASES
Glutathione S-transferases belong to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemicals, metabolites and oxidative stress (Singh et al., 2002). Regulation of GST expression both by induction and inhibition is a crucial factor in determining the sensitivity or resistance of cells
to a wide variety of foreign chemicals, carcinogens, drugs and metabolites under normal and pathophysiological conditions. A number of GST inducers and inhibitors occur naturally in vegetables and fruits (Hayes and Pullford, 1995). Biological mechanisms responsible for controlling the selective expression of GSTs are complex owing to the multiplicity of these enzymes and their tissue specific and subcellular distribution. In addition, GSTs also exhibit differential response to a wide variety of chemicals and oxidative stress in the normal and pathophysiological conditions. It appears that GSTs are also regulated both in vivo and in vitro by reactive oxygen species (ROS) such as superoxides, \( \text{H}_2\text{O}_2 \) and by the products of membrane lipid peroxidation (Mari and Cederbaum, 2001). The controlled expression of the different GST isoenzymes has presumably evolved as an adaptive response to chemical stress within cells. It was suggested that GST distribution in tissues of diabetic rats may be crucial in the etiology, pathology and prevention of diabetes (Raza et al., 2004). A reduction in the GST activity was observed in the present study showing that diabetic brain can lead to an excess availability of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) in the biological systems, which in turn generate hydroxyl radicals resulting in initiation and propagation of lipid peroxidation. Administration of TSP increased the activity of GST in diabetic brain. These observations are an evidence that TSP include natural products exhibiting a range of antidiabetic effects (Puri et al., 1995) and antioxidant properties (Genet et al., 2002; Vats et al., 2003; Raju et al., 2001; Mohammad et al., 2006).

5. EFFECT OF ANTIDIABETIC COMPOUNDS ON MEMBRANE LIPID PEROXIDATION

In this study, parameters for the membrane lipid damage were measured in the form of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). The formation of MDA and 4-HNE were significantly increased in diabetic cerebral cortex, as reported earlier (El-Missiry et al., 2004; Kamboj et al., 2008). These increased lipid peroxide formation disturbs the anatomical integrity of the membrane leading to changes in several membrane functions, including the membrane bound enzyme. It is generally agreed that lipid peroxidation is elevated in diabetes (Slatter et al., 2000; Genet et al., 2002; Chang et al., 2005; Siddiqui et al., 2005; Kamboj et al., 2009). Among lipid peroxidation products, plasma MDA
concentrations are the most frequently used biomarker for assessing in vivo oxidative stress in human subjects (Nielsen et al., 1997).

A significant increase in the level of lipid peroxidation in diabetic rats could be due to several mechanism occurring in the diabetic condition like increased generation of free radicals by several mechanisms, including direct glucose auto-oxidation, non-enzymatic protein glycation, activation of NAD(P)H oxidases, nitric oxide synthase and xanthine oxidase (Inoguchi et al., 2000; Desco et al., 2002). As recently reviewed (Baquer et al., 2009), enhanced glucose flux through the polyol pathway leads to depletion of the NADPH available for glutathione reductase, thereby provoking changes in the glutathione redox status, hyperglycaemia has also been shown to disrupt intracellular antioxidant defense mechanisms and the increased production of hydroxyl radicals, which are stimulators of lipid peroxidation (Edwards et al., 2008). Kaviarasan et al. (2004) reported that a polyphenol-rich extract from the seeds of TSP protects normal and diabetic human erythrocytes (RBCs) against hydrogen peroxide induced oxidative stress. Trigonella has been shown to have an antioxidant action against hydroxyl radical formation (Annida and Prince, 2005).

Present results showed increased lipid peroxidation in the brains of the diabetic rats and the treatment of the diabetic rats with insulin and TSP, decreased the level of lipid peroxidation. It may, therefore, be presumed that free radicals mediated lipid peroxidative injury, and neuronal damage which plays a crucial role in the pathophysiology of diabetes, can be prevented and controlled in the brain by administration of TSP and insulin. TSP supplementation to diabetic rats was effective in bringing the lipid peroxides levels almost to control values. Inhibition of these lipid peroxides by TSP administration strongly suggests its anti-lipidperoxidative abilities.

6. EFFECT OF ANTIDIABETIC COMPOUNDS ON MEMBRANE FLUIDITY

Plasma membranes are fluid structures and the maintenance of fluidity is a prerequisite for function, viability, growth and reproduction of cells. Membrane fluidity is dependent on the freedom of mobility of the membrane constituents. Increased release of free radicals and
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reactive oxygen species (ROS) affect membrane fluidity, cellular Ca\(^{2+}\) homeostasis, induce lipid peroxidation and finally cell death (Waczulikova et al., 2002; Kamboj et al., 2009).

In the present study, polarization and anisotropy measurement on synaptosomes from brain tissue of alloxan diabetic animals showed a significant decrease in their fluidity. The results of present study are in agreement with the previous reports, which also reported a decreased in the fluidity of brain fractions. (Ziegelhoffer-Mihalovicova et al., 2003; Garcia et al., 1997; Hong et al., 2004; Sennoune et al., 2000; Siddiqui et al., 2005).

Results show that diabetes, induces a significant decrease in the membrane fluidity and the bilayer lipid membrane becomes more rigid than normal. The decrease in membrane fluidity in synaptosomes from the diabetic brain could be due to the peroxidation of membrane phospholipids through free radicals, generated by persistent hyperglycemia. Diabetes could therefore affect the overall physiological responses in the central nervous system. Garcia et al. (1997) reported the membrane fluidity of liver microsomes decreased when lipid peroxidation was induced by incubating the membranes with FeCl\(_3\) and melatonin treatment, which stabilizes microsomal membrane rigidity induced by free radical attack. Further, cross-linking of protein by the end products of lipid peroxidation, malonaldehyde (MDA), resulting in lipoxidation products, can influence membrane fluidity.

The decreased membrane fluidity could be alter membrane permeability and thus affect basic physiological phenomenon as well as functioning of a neuron. Diabetes could therefore affect overall physiological responses in the central nervous system. Since the cell membrane requires good fluidity to maintain homeostasis and metabolism in the body, fluidity is an effective index of adult disease. Membrane fluidity has a strong influence on important membrane functions such as the conformation, thus affecting the activity of membrane-associated enzymes. In order to see if the structural changes in synaptosomal membrane can result in functional changes in membrane-associated enzymes, Na\(^{+}\)K\(^{-}\)ATPase, Ca\(^{2+}\)ATPase and MAO were assayed. Insulin and TSP treatment brought the membrane fluidity values close to controls. TSP treatment decreased the oxidative stress and lipid peroxidation levels.
7. EFFECT OF ANTIDIABETIC COMPOUNDS ON NEUROLIPOFUSCIN ACCUMULATION

Lipofuscin is a morphological structural entity and is mainly accumulated in post-mitotic cells of brain. The accumulation of lipofuscin in cells occurs because it is undegradable and cannot be removed from the cells via exocytosis (Moorthy et al., 2005b). Diabetes mellitus (DM) is one of the diseased model for aging (Cerami, 1987) and its complications include peripheral neuropathies that exhibit axonal degeneration, demyelination and impaired axonal transport that would affect cell body functions.

It has been shown that DM significantly increased the fluorescent pigments in the neurons of experimentally induced DM, with the possibility that the induction of DM makes some functional changes due to the accumulation of the fluorescence materials in neurons (Sugaya et al., 2004). The authors therefore concluded that experimentally induced DM by alloxan increases the production of autofluorescent pigments “lipofuscin” in the neurons; that this change is thought to correlate with aging. This result suggests the possibility that the diabetes mellitus could make the functional change in several types of neurons. Neurolipofuscin deposition was increased with experimental diabetes as reported earlier (Hellweg et al., 1994; Sugaya et al., 2004; Alvarez et al., 2009), in the three brain regions studied, suggesting that diabetes could have functional change in the neurons. The cells in the cerebral hemispheres were found to be more vulnerable in diabetes where as the brain stem seems to be more resistant to the diabetic changes. Insulin and TSP treatment to diabetic animals, resulted in a decrease in lipofuscin deposition in neurons with an increase in the number of neurons without lipofuscin, when compared with the control. TSP treatment decreased the oxidative stress and lipid peroxidation levels. Results show that three weeks treatment of the diabetic group with insulin, and TSP separately resulted in a marked reduction in hyperglycemia in the diabetic animals.

8. EFFECT OF ANTIDIABETIC COMPOUNDS ON CALCIUM HOMEOSTASIS

Recent studies demonstrated that substantial abnormalities of calcium homeostasis in synaptosomal system are associated with many symptoms of diabetic neuropathy. Although proof of the causal linkage between calcium abnormalities and neuropathic complications is not conclusive, current research in neuroscience mostly indicates that such a linkage exists.
Practically all known modifications of synaptic transmission in both central and peripheral nervous systems result from calcium-dependent modifications of the molecular players involved in this transmission. Regulation of intracellular $\text{Ca}^{2+}$ concentrations is vital for cells, especially for neurons; raised levels are associated with cytotoxicity and neuronal death. Present data suggest that the increase in synaptosomal $\text{Ca}^{2+}$ due to oxidative stress may result from the inhibition of the plasma membrane $\text{Ca}^{2+}$ATPase activity, probably as a result of the alteration of the lipid environment required for the maximal activity. In the present study the synaptosomal $\text{Ca}^{2+}$ which plays a role in fine tuning the regulation of neurons was shown to be increased in diabetes (Pekiner et al., 2002; Kamboj et al., 2009) due to oxidative stress and membrane damages. An increased in synaptosomal $\text{Ca}^{2+}$ in any diabetic tissue could be due to excessive lipid peroxidation and decrease in membrane fluidity (González Flecha et al., 1990). Hyperglycemia during diabetes has been shown to generate free radicals from auto-oxidation of glucose, formation of AGEs and increased polyol pathway, with concomitant increase in cellular lipid peroxidation and damage to membranes (Kumar and Menon, 1993; Preet et al., 2005). Chronic hyperglycemia induced a number of changes in the neurochemical profile possibly linked to osmolarity regulation essential for the maintenance of cellular homeostasis (Kamboj et al., 2009). TSP and insulin treatment restored the altered synaptosomal $\text{Ca}^{2+}$ levels to controls. TSP treatment decreased the oxidative stress and lipid peroxidation levels. Results show that three weeks treatment of the diabetic group with insulin, and TSP separately resulted in a marked reduction in hyperglycemia in the diabetic animals. TSP improved calcium homeostasis in diabetes by decreasing oxidative stress, probably by enhancing or mimicking insulin action.

9. EFFECT OF ANTIDIABETIC COMPOUNDS ON DNA DEGRADATION
Oxidative stress is known to play an important role in the pathogenesis and complications of diabetes and as a result increased risks of oxidative DNA degradation/fragmentation have been observed in diabetic patients (Li et al. 2004). In the present results, the DNA laddering method has been used to evaluate genomic DNA degradation of control, alloxan induced diabetic animals and diabetic animals treated with antidiabetic compounds. Oxidative DNA degradation was increased in the DNA sample isolated from brain of diabetic animals as compared to the normal controls as has been shown earlier (Farhangkhoee et al., 2003).
The decrease of antioxidant enzymes leads to the accumulation of ROS during diabetes. These ROS cause damage to the vital processes of the cell like DNA degradation and induce apoptosis (Morel and Barouki, 1999). Murata et al. (2004) showed that the concentration of many endogenous aldehydes such as 3-deoxyglucosone and glyceraldehyde increase under hyperglycemic conditions and glyceraldehydes has the strongest ability to damage DNA, and the addition of low concentrations of H$_2$O$_2$ markedly enhanced the DNA damage. Pathological condition such as diabetes, which increases the rate of H$_2$O$_2$ production with decrease antioxidant system will lead to the accumulation of H$_2$O$_2$ in tissues and cause DNA degradation (Burdon, 1995). It can, therefore be concluded that oxidative DNA damage by hyperglycemia generated aldehydes, and marked enhancement of DNA damage by H$_2$O$_2$ may participate in diabetes-associated long-term pathogenesis. Thus, the measurement of oxidative DNA degradation in a diabetic state by means of laddering can be a suitable marker for the evaluation of systemic oxidative stress in diabetic patients. Administration of TSP and insulin restored the DNA degradation in diabetic groups near to controls.

10. EFFECT OF ANTIDIABETIC COMPOUNDS ON GLUT4 TRANSLOCATION

Insulin receptors, and perhaps insulin-containing neurons, have long been known to be present in the brain (Havrankova et al., 1978; Gupta et al., 1992). Whether or not glucose utilization in the brain is insulin-dependent is still a controversial issue. The presence of the insulin-sensitive glucose transporter (GLUT4) in rat brain was assessed using a reverse transcription-polymerase chain reaction (RT-PCR) on RNA from the rat brain by GLUT4 transcripts. Immunocytochemistry, using a polyclonal antibody to GLUT4, revealed a specific immunostaining pattern, indicating a neuronal localization, elucidating a putative neuromodulator role of insulin and TSP via glucose transporter, in brain.

Sankar et al. (2002) and El Messari et al. (2002) reported that GLUT4 is highly expressed in specific regions of the rat brain. The analysis of the ultra structural localizations of GLUT4 could indicate if this transporter is likely to be translocated from intracellular storage vesicles to the plasma membrane. This might be related to the frequent or sustained electrophysiological activity of these neurons resulting in a high requirement for energy substrate. Hence, this important glucose supply might be assumed by GLUT4 since the
translocation mechanism described in peripheral cells leads to a strong and rapid amplification of glucose transport (Bakirtzi et al., 2009). The observation that, in the brain, GLUT4 is often localized in the same areas as insulin receptors or insulin receptor-related receptors, supports the possibility that blood insulin regulates the transport of glucose by means of GLUT4, at least in certain neurons of the CNS. (El Messari et al., 1998; Fernando et al., 2008).

The presence of the GLUT4 was assessed using a semiquantitative RT-PCR from the rat brain by GLUT4 transcripts. Earlier GLUT4 mRNA was found to be highly colocalized with its protein, coexisting in the cerebral cortex, striatum, hippocampus, and cerebellum (Vannucci et al., 1998). Our immunohistochemical data showed that GLUT4 protein was present in rat brain, confirming previous results of Leloup et al. (1996), indicating a neuronal localization, elucidating a putative neuromodulator role of insulin and TSP via glucose transporter, in brain.

Collectively, many studies have demonstrated that 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).

Leloup et al. (1996), had shown that GLUT4 mRNA exists in a single form in brain of the rat. Indeed, the levels of GLUT4 are probably low in brain since 35 cycles of amplification were required for producing a significant signal (El-Messari et al., 2002). GLUT4, a 45-kDa protein, was also found in neuronal cell bodies and in neuronal processes, specially at the synaptic thickening (Apelt et al., 1999; El Messari et al., 1998). As in myocytes and adipocytes, GLUT4 in neurons seems to be localized in the endoplasmic reticulum and in the cisterns of the Golgi apparatus (El Messari et al., 1998; Leloup et al., 1996). GLUT4 mRNA was found to be highly colocalized with its protein, coexisting in the following cerebral areas: olfactory bulb, cerebral cortex, striatum, hypothalamus, thalamus, hippocampus, midbrain, medulla oblongata and cerebellum (Rayner et al., 1994; Vannucci et al., 1998).
GLUT 4 is mainly located at the plasma membrane and is considered to be responsible for basal glucose transport. GLUT4 expression is down-regulated when there is relative insulin deficiency, such as in STZ-induced diabetes and chronic fasting (Charron et al., 1999). Studies have also shown that the impaired utilization of glucose by the diabetic myocardium is linked to a reduction in glucose transporter activity, GLUT4.

In the present research, GLUT4 protein levels have been measured by immunoblot analysis in the membrane fractions of cerebral cortex of control diabetic and diabetic animals treated with different antidiabetic compounds. In agreement with the previous studies GLUT4 protein significantly decreased in the total membrane fraction of cortex of alloxan-diabetic animals (Sankar et al., 2002; Vannucci et al., 1998). The reduction in the GLUT4 levels result in decreased uptake of glucose and, therefore, contributes to the increased blood glucose levels in diabetic condition. Treatment of the diabetic rats with insulin, *Trigonella* alone restored the GLUT4 levels close to the normal values.

Similar results as those obtained from cortex membrane fractions, were obtained by the immunohistochemical analysis of GLUT4 in the brain of control and the experimental diabetic groups. In alloxan diabetic condition GLUT4 content decreased drastically in the cortex membranes as compared to the control rats. Treatment of diabetic animals with insulin and with 5% *Trigonella* restored the GLUT4 content of the cortex cell membrane.

The normalization of GLUT4 levels is an important parameter to evaluate the antidiabetic properties of *Trigonella* because one of the main reasons of hyperglycemia in the diabetes mellitus is the decreased uptake of glucose. Restoration of GLUT4 levels would, therefore, enhance the uptake of glucose in the brain, thus helps in regulating the hyperglycemic condition, thereby controlling all other related complications. Administration of TSP and insulin restored the GLUT4 protein expression in diabetic groups. Earlier from our laboratory Mohammad et al. (2006) showed that TSP treatment restored the GLUT 4 in skeletal muscle of alloxan diabetic rats. Our data showed that GLUT4 protein was present in rat brain, confirming previous results of Leloup et al. (1996) and Sankar et al (2002), indicating a neuronal localization, elucidating a putative neuromodulator role of insulin and TSP via glucose transporter, in brain. The observation that, in the brain, GLUT4 is often localized in the same areas as insulin receptors or insulin receptor-related receptors, supports the possibility that blood insulin regulates the transport of glucose by means of GLUT4, at least in certain neurons of the CNS (El Messari et al., 1998; Fernando et al., 2008; Bakirtzi et al., 2009).
Trigonella foenum-graecum has been documented as a traditional natural treatment for diabetes, it is shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism in various animal model systems (Vats et al., 2003; Ali et al., 1995; Mohammad et al., 2006). The components responsible and the mechanism by which TSP exerts these effects is not clearly understood. However, as mentioned earlier several studies have shown the presence of steroid saponins in TSP seeds. 4-Hydroxyisoleucine, a modified amino acid extracted and purified from Trigonella seeds, stimulated insulin secretion. Trigonella is also known to rejuvenate the beta cells of pancreas (Sauvaire et al., 1998; Basch et al., 2003; Al-Habori et al., 2001). The active component of Trigonella foenum-graecum seeds has been found to be associated with the defatted part of the seeds, rich in fibre containing steroidal saponins and proteins comparable to those of soybean (Madar and Shomer, 1990; Al-Habori et al., 2001; Losso et al., 2009). A possible mode of action of TSP suggested was an effect on intestinal carbohydrate digestion, it was found to decrease digestion of starch and also glucose absorption both in vivo and in vitro, may be as a the result of a direct inhibitory effect on the digestive enzymes (Hannan et al., 2007).

TSP exerts, its hypoglycaemic effect by delaying glucose absorption and enhancing its utilization. Seeds of Trigonella foenum-graecum are therefore considered to be potentially useful for glucose control and for preventing hyperlipidaemia and atherosclerosis in diabetic subjects. The hypolipidemic action of the TSP could be the result of retardation of carbohydrate and fat absorption due to the presence of bioactive fibre in the agent. (Hannan et al. 2007; Losso et al., 2009). Raju et al. (2001) showed that there was complete normalization of blood glucose with feeding of 5% TSP in the diet.

Our data showed that TSP administration to diabetic rats significantly and effectively reversed the diabetic aberrations in the rat brain. TSP improved glucose homeostasis in diabetes by decreasing oxidative stress, probably by enhancing or mimicking insulin action. TSP also brought back the serum insulin levels, decreased in diabetic animals to nearly that of the control levels. TSP effectively controlled, plasma glucose levels and normalized alterations in the key functions of cell membrane. Hence TSP can be considered as an alternative to be explored further for clinical trials as a means of amelioration of diabetes in human subjects. Trigonella therefore represents a potentially useful dietary adjunct in the treatment of diabetes and a potential source of a new orally active antidiabetic dietary supplement in the treatment of diabetes.
DISCUSSION (AGING)

1. GENERAL
Brain aging has become an area of intense research and a subject of much speculation fueled largely from the widely recognized fact that age is the biggest risk factor in most neurodegenerative diseases and age-related increase of reactive oxygen species is particularly detrimental to postmitotic tissues. Sex steroids exert pleiotropic effects in the nervous system, preserving neural function and promoting neuronal survival. Therefore, the age-related decrease in sex steroids may have a negative impact on neural function (Henderson, 2009).

The brain is exposed to chronic oxidative stress during aging. Free radical production and oxidative stress are known to increase during aging, and may contribute to the oxidative damage, which plays an important role in the aging process (Harman, 1992). 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 (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 (Butterfield et al., 2006).

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. Saravia et al. (2007) have reported that E2 plays a neuroprotective role in the hippocampal neurons and glia of middle age mice and data suggest, that older women might also benefit from the protective effects of E2 and progesterone using a relatively lower concentration of hormones.

In postmenopausal women the levels of estradiol and progesterone are reduced because the ovary has completely depleted its follicular pool. The postmenopausal period has long been considered to be a time of various undesirable symptoms including hot flushes,
insomnia, depression and anxiety, moreover there are increased risk of various health problems such as diabetes, cardiovascular complications, osteoporosis, Alzheimer’s and other diseases (Grodstein and Stamfer, 1995; Alka et al, 2003; Nelson, 2008).

Earlier work and ample evidence has shown that peroxidative damage to lipid and protein occurs with the aging process and the products of these reactions accumulate in the brain with age (Bondereff, 1964; Davison and Wright 1980; Baquer et al., 1990; Moorthy et al., 2005a,b; Bala et al., 2006; Sinha et al., 2005; 2008; Kumar et al., 2008). The changes observed in the activity of enzymes in isolated synaptosomes seemed to be more severely affected with aging in brain. These changes (decrease) could be due to the extreme complexity of brain structure and the compartmentalization of various modulators for enzyme activities. As lipid and protein are essential components of membranes, a change in the enzymes linked to membrane may occur during the aging process. Estradiol levels of aging animals have been measured earlier (Moorthy et al., 2005a). Authors showed decreased levels of E2 at 12 months as compared to the 3 months cyclic rats. At 24 months, the rats showed an increase when compared with 12 months, still showing a decrease when compared with the 3 months young rats.

The present study investigated the anti-aging and neuroprotective potential of E2 treatment on body weight and tissue weight, blood glucose levels, insulin levels, activities of membrane linked ATPases (Na\(^+\)K\(^+\) ATPase, Ca\(^{2+}\)ATPase), MAO, antioxidant enzymes (SOD, GST), membrane lipid peroxidation (MDA and 4-HNE levels), intrasynaptosomal calcium levels, neurulipofuscin accumulation, synaptosomal membrane fluidity, expression and distribution of glucose transporter (GLUT4) by RT-PCR, immunohistochemistry and Western blotting and oxidative degradation of genomic DNA in the brain has also been monitored in female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, to assess whether these changes are restored to normal levels after exogenous administration of estradiol.

2. EFFECT OF E2 ON GENERAL PHYSIOLOGICAL PARAMETERS IN AGING
In the present study the body weight of the female rats increased with age. The increased body weights were primarily due to the increased amount of adipose tissue in the aged rats. A significant decrease in body weight was found with E2 treatment which could be due to
the anti-obesity action of E2 (Moorthy et al., 2004a). Although adipose tissue is considered a non-classical target of estrogen stimulation, in vivo and in vitro studies have shown that estrogen receptors (ER) are involved in the modulation and distribution of body fat mass, and this receptor, expressed in adipose tissue, appears to mediate the lipolytic effect of estrogen (Mueller and Korach, 2001).

In the present study brain weights were increased with age when compared to young control (3 months old) animals. This increase was due to the increase in the size of the above-mentioned tissue and protein content of the tissue also increased with age. After the hormone treatment the tissue weight was increased when compared to age matched control rats. This increase may be due to increased level of protein content in the tissues.

In the present work the protein content of the hormone treated rats increased significantly in brain may be due to decreased protein catabolism and increased protein synthesis and also due to decreased gluconeogenesis. This indirectly suggests that the treatment with E2 increases the glucose transporter in the brain of treated rats. In the present results the protein level was increased with age. The present results are concurrent with the results of Kristofikova et al (1991) also reported that the soluble proteins are increasing with age. Mukherjee et al. (1999) who showed that the total protein synthesis is significantly higher in adult males than females and decreases in old males but not in females, he also reported that estradiol treatment enhances the synthesis of proteins remarkably in both sexes, 9.7-fold in adults, 4.1-fold in old males, 4.0-fold in adults and 3.4-fold in old females. On the other hand, the intracellular dry mass content increases i.e. the water content decreases with aging. Therefore, the slow increase in the total protein content may be due to the decrease in the water content because the protein content is expressed with respect to the tissue wet weight.

The physiological variations in the concentration of sex hormones during this period could affect the insulin receptor’s action and their interaction with glucose, thereby altering the carbohydrate metabolism (Valdes and Elkind-Hirsch, 1991). The present results show that treatment with estradiol increased the random sugar level in serum in the normal range. Both peptide and steroid hormones including estrogen regulate the rate of glucose transport into cells. In the present study also the increased blood sugar level was measured after the treatment with E2. Reduced glucose uptake has been shown to occur early in Alzheimer’s
disease prior to neuronal degeneration (Jay et al., 1994). The decreased rate of both glucose and ketone body utilization have been observed in aged brain, but energy requirement remains the same when compared with young animals (Smith et al., 1980).

In the present study there was an increased in insulin levels with aging and E2 treatment restored the insulin levels in aging rats. Experimental studies (Ordóñez et al., 2008) and clinical observations (Karjalainen et al. 2001) have demonstrated the importance of small estrogen in the maintenance of insulin sensitivity in postmenopausal women. Recently insulin levels have been measured in aging and shown to increase with age (Escriva et al., 2007). Alonso et al. (2008) had shown that E2 effects rat neuronal homeostasis and decreases the insulin resistance in aging female rats.

3. EFFECT OF E2 ON MEMBRANE BOUND ENZYMES IN AGING
Steroid hormones are known as potent inducers of many enzymes. The activities of the enzymes in a tissue may depend on the rate of their synthesis and/or degradation and modulation by various effectors. The hormones exert their control either at transcriptional or posttranscriptional levels. In the present study, several enzymes have been studied which are involved in calcium regulation, neurotransmitter signals, monoamine regulation / metabolism.

**MONOAmine OxIDase**
Monoamine Oxidase (MAO) is predominantly a mitochondrial enzyme responsible for the oxidative deamination of a variety of biogenic amines. The enzyme has a crucial role in neurophysiology since it inactivates transmitter monoamines like dopamine, noradrenaline and serotonin. Therefore, any change in the activity of this enzyme would alter the neurotransmitter function (Consolo and Valzelli, 1970).

Changes in MAO activity has also been observed during aging (Kumar et al., 2008 ). 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 PD and AD without any change in MAO A (Benedetti and Dostert, 1989; Saura et al., 1994). There is also evidence that MAO B inhibitors improve the quality of life in the elderly (Naoi and
Maruyama, 2010). In nerve cells, a source of H$_2$O$_2$ results from the metabolism of catecholamines and indoleamines by MAO (Soto-Otero et al., 2001). It is likely that MAO activity could predispose certain types of neuronal cells to glutamate toxicity. The MAO activity measured in different age group synaptosomes showed a significant increase in enzyme activity as an age dependent phenomenon in support of observations made by Benedetti and Dostert, (1989). The activity of MAO increased with age in the brains of 12 and 24 months rats as compared to the 3 months control rats, agreeing well with earlier studies (Saura et al., 1994; Kumar et al., 2008). Oreland and Gottries (1986) explained this age-related increase in brain MAO activity by the increase in extrasynaptosomal astroglia.

E2 administration to older rats significantly decreases MAO activity, showing that E2 plays an important role in the regulation of MAO activity in the neural tissues. The present and earlier results indicate a possible role of estrogen in the regulation of MAO in the brain of young, adult and aged rats (Holschneider et al., 1998; Nicotra et al., 2004). High levels of MAO have been linked to depression and Parkinson’s disease. E2 treatment reverses the MAO activity in both synaptosomes and supernatant fractions of different age groups of rats when compared to respective controls. E2 has antioxidant properties which decreased the free radicals formation during increase MAO activity in aging rat synaptosomes. Present results elicited that administration of estrogen decreases MAO in aging brain, the results are consistent with those reported earlier (Holschneider et al., 1998; Meyers et al., 2010).

Na\textsuperscript+K\textsuperscript+ ATPase

The enzyme Na\textsuperscript+K\textsuperscript+ ATPase utilizes the energy derived from ATP hydrolysis to pump out Na\textsuperscript+ from inside the cell and to transfer K\textsuperscript+ from outside to cytosol. The pump functions as an antiport and is instrumental in restoring ion-gradients in nerve cells following periods of electrical activity like nerve impulses and synaptic potentials (Murali et al., 2008). In the present study, the Na\textsuperscript+K\textsuperscript+ ATPase activity was decreased significantly in brain synaptosomes of aged animals when compared to 3 months control animals. This is in agreement with earlier studies showing decreases in the enzyme activity during aging (Kaur et al., 2001; 2003; Arivazhagan and Panneerselvam, 2004; Mantha et al., 2006; Tanaka and Ando, 1990; Gorini et al., 2002; Murali et al., 2008). With aging the decreased in Na\textsuperscript+K\textsuperscript+ ATPase could
be due to increased oxidative stress and ROS formation with aging, that leads to increased lipid peroxidation and further damage membrane structure and membrane bound enzymes. These observations agree with those of Chakraborty et al. (2003). A decreased activity of Na\(^+\)K\(^+\) ATPase with aging has been reported in female rat brain synaptosomes and its significance discussed (Fraser and Arief, 2001). A decreased Na\(^+\)K\(^+\) ATPase activity with age could result in neurotoxicity resulting in neuronal vulnerability to excitotoxicty insults to the neuronal cells.

E2 treatment to aging animals restored the Na\(^+\)K\(^+\) ATPase activity to almost control levels. E2 acts as a neuroprotectant and decreased ROS in aged rats and also helps to maintain the synaptosomal fluidity (Henderson, 2009). The effect of aging on brain enzymes has been studied by many investigators (Kaur et al., 1998; 2001; 2003; Bala et al., 2006; Baquer et al. 2009). Baquer et al. (1990) had shown a clear decrease in myoinositol incorporation into phospholipids with aging. The result was important since it is known that phospholipids are the most rapidly renewed components of the nerve sheath, that they bind Ca\(^2+\), one of the source of fatty acid precursors of the prostaglandin and are associated with transport phenomenon. This fall in the myoinositol incorporation would be reflected in changes in the structure and fluidity of the membranes (Mantha et al., 2006) and hence would produce wide spread changes in aspects of nerve behavior. Dena and Phyllis (2001) have reported that estradiol plays a neuroprotective role in the injured brain of both young and middle aged rats and data suggest, as reported by Moorothy et al. (2004b) also, that older women might also benefit from the protective effects of HRT (hormone replacement therapy) that uses a relatively lower concentration of hormones. Keller et al. (1997) have demonstrated that estradiol can directly act on the synapse and protect critical membrane transport systems from oxidative impairment particularly Na\(^+\)K\(^+\) ATPase.

Ca\(^{2+}\)ATPase

Ca\(^{2+}\)ATPase enzyme is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. In the present study, Ca\(^{2+}\)ATPase activity was decrease in synaptosomes of aging rats when compared with
3 month young controls. There was a strong correlation between the decreased synaptosomal membrane fluidity with the decreased Ca$^{2+}$ATPase activity. The decreased Ca$^{2+}$ATPase activity may lead to elevation in intracellular Ca$^{2+}$ levels, which has been reported as a definite event in the etiopathogenesis of the neurodegenerative diseases (Choi, 1992; Murali et al., 2008). The cross-linking of membrane proteins by MDA and 4-HNE may lead to the inactivation of important membrane-spanning proteins including the ion transport ATPases, especially the Ca$^{2+}$ATPase (Tappel et al., 1973). Ca$^{2+}$ATPase activity is quite sensitive to exposure to oxidative stress and are inhibited by a variety of oxygen radicals (Viner et al., 1997; Kukreja et al., 1988). Inhibition of Ca$^{2+}$ATPase activity can intern increase intracellular concentration of Ca$^{2+}$ and alter the signal transduction pathways and cellular fluidity and eventually results in cell death (Aubier and Viires, 1998). E2 treatment to aging rats prevented the decreased Ca$^{2+}$ATPase activity and helps to transport in brain. E2 administration decreased oxidative stress and ROS in aged rats, which helps to decrease lipid peroxidation and restored the membrane fluidity in synaptosomes (Moorthy et al., 2005b).

4. EFFECT OF E2 ON ANTIOXIDANT STATUS IN AGING

Antioxidant enzymes play an important role in brain aging. The nervous system of both humans and animals are especially vulnerable to oxidative damage caused by free radicals due to low levels of the protective antioxidant enzymes. Antioxidative defense is critically important in nervous tissue protection. Menopausal condition is considered as the end of natural protection against aging diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer's disease, and Parkinson's disease. Free radical production and oxidative stress, which are known to increase during aging, may contribute to the oxidative damage proposed to play a role in the aging process. The changes in the physiological concentration of estradiol, progesterone and testosterone significantly alter the production of O$_2^-$ and H$_2$O$_2$ in macrophages of rats (Chao et al., 1994).

The present study reports age-related decline in activities of antioxidant enzymes in the brains of aging rats. Decreased activities of SOD and GST enzymes were the prominent
alterations observed in the present investigation with aging. The destruction of reactive oxygen intermediates and of free radicals involves the activities of SOD and GST.

**Superoxide Dismutase**

In the present study, the Cu/Zn SOD activity decreased significantly in the brains of aged animals. These observations agree with earlier reports (Reiss and Gershon, 1976; Sandhu and Kaur, 2002; Mantha et al., 2006). SOD is involved in the protection against superoxide anions and free radical damage in aging, the antioxidant enzyme, SOD activity decreased significantly in cytosol of aging brain in the present data and has been shown by Vertechy et al. (1993). These changes may be due to the increased oxidative stress in neuronal cells.

High level of Cu/Zn SOD activity protects neurons and other neuronal cell types against oxidative stress and nerve growth factor deprivation. The down regulation of Cu/Zn SOD has been shown to accelerate spontaneous cell death (Troy and Shelanski, 1994), whereas in transgenic mice with higher than normal brain Cu/Zn SOD activity, neurons were significantly protected against the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Przeborski et al. 1996). Another study reported that the overproduction of Cu/Zn SOD prevents the brain mitochondrial respiratory dysfunction induced by glutathione depletion (Merad-Saisoune et al. 1999). It can be reiterated that oxidative stress is one of the most important mechanisms behind age related changes in anti-oxidative enzymes and increased apoptosis in aged rats (Kokoszka et al., 2001). Most of the evidences indicate that SOD contributes in the protection of cells from oxygen toxicity by catalyzing the oxygen and it can be modified by sex hormones in various tissues. Markides et al. (1998) showed that estrogen might affect free radical production, which depends on the structure of the estrogen concerned, the administration dose and the organ being studied.

Results of Pajovic et al. (1993) and D'Almeida et al. (1995) showed that the activity of SOD is increased in the brains of proestrous rats, indicating that estradiol and progesterone can modulates the activity of SOD in tissues through its receptors. In aging the levels of estradiol was reduced as compared to the normal cyclic rats, therefore, aging rats treated with estradiol can reduce or delay the burden of free radical induced aging disease as shown by Moorthy et al., (2004a;2005a).
The present study reports age related decline in activity of SOD in brain when compared to young three months control rats. These changes may be increasing the oxidative stress. E2 treatment significant increased the SOD activity in aging rats. Taking into consideration that free radicals have an important role in the menopausal condition, it may be concluded that estradiol may delay or retard these aging processes in the brain by modulating the SOD activity. The results suggest that ovarian steroid hormones may modulate the activity of cytosolic SOD in the brains of aged female animals.

**GLUTATHIONE S-TRANSFERASES**

It has been shown that in aging tissues from female and male animals the oxidative stress increases due to decreased activity of antioxidant enzymes and proteolysis increases due to decreased activity of aminotransferase (Moorthy et al., 2005a; Sinha et al., 2008). Oxidative stress as well as gene expression profile has been identified as causal factors in the aging process (Harman, 1992; Ames et al., 1993; Sohal et al., 2000). Endogenous reactive oxygen species (ROS) that are generated from aerobic metabolism are probably the most important cause of age related neuronal damage. Reactive oxygen species are continually produced within the body from normal oxidative metabolism. Sources of ROS are mainly the mitochondrial electron transport chain, and also the arachidonic acid cascade or nitric oxide (Miquel, 1992).

Glutathione itself plays a major role as a reductant in detoxification (Carlberg and Manneuik, 1985). Present results depict a parallel decline in GST activity with aging. A major factor that affects glutathione homeostasis is its utilization by conjugation primarily via GST (Hayes and McLellan, 1999). The ability of GST to alter levels of cellular glutathione in response to production of reactive oxygen species has been implicated in protection of cells from reactive oxygen species inducing agent (Tew and Ronai, 1999). Decreased GST activity in brain was also observed in AD patients (Xie et al., 2001) which further leads to the pathogenesis of AD and thus supports our present findings. With the E2 treatment to aging rats, the GST activity was restored, which can be due to the decrease in free radicals generation by E2 and its antioxidant properties. The mechanism of action of the neuroprotective antioxidant activity of estrogens is dependent on the presence of the
hydroxyl group in the C3 position on the A ring of the steroid molecule under clinical conditions (Behl, 2002). Such data suggest a fundamental capacity for one of the basis of E2’s neuroprotective actions (Brann et al., 2007; Henderson, 2009). This increased activity may be due to the antioxidant property of the E2, thereby reducing the free radical production as well as may be increasing GST synthesis in the brain. Taking into consideration that free radicals have an important role in the aging process, it may be concluded that E2 treatment may delay or retard these aging processes in the brain tissue by modulating the antioxidant enzymes activities.

5. EFFECT OF E2 ON MEMBRANE LIPID PEROXIDATION IN AGING

Free radical production and oxidative stress are known to increase during aging, and may contribute to the oxidative damage, which plays an important role in the aging process. Lipid peroxidation is one of the determinants of oxidative stress induced by ROS and the lipid peroxidation parameters determine the pathological alterations in the brain and other tissues. Lipid peroxides have been shown to produce an irreversible impairment of membrane fluidity and elasticity, which can lead to rupture of the cell and these changes are likely to be particularly significant in long-lived and predominately post-mitotic cells such as neurons. In the present study the MDA and 4-HNE levels were measured in brain in rats, which were given one-month E2 treatment together with age matched controls.

With aging there is significant increase in lipid peroxidation (formation of MDA and 4-HNE) at 12 and 24 months old rats as compared to 3 months. Ample evidence showed that peroxidative damage to lipid and protein occurs with the aging process and that the products of these reactions accumulate in the brain with age (Kaur et al., 2001; Kumar et al., 2008; Baquer et al., 2009). Aging rats treated with E2 reversed the MDA and 4-HNE levels to almost normal levels in the brain, the reversal was higher at 12 than at 24 months, suggesting its anti-lipidperoxidative abilities.

Present results showed a significant decrease in lipid peroxidation levels in E2 treated rats of all the age groups as compared to age matched control groups. Estrogen may be used for the treatment of neurodegenerative diseases because of its neuroprotective effects, and the
antioxidant actions of estrogen may play a role in these effects (Garcia-Segura et al., 2001; Henderson, 2009, Brann et al., 2007).

Estradiol also has an antioxidant action against hydroxyl radical formation and protects dopaminergic neurons against cell damage induced by neurotoxins in Parkinson's disease. Estrogen function as a radical scavengers and inhibits lipid peroxidation in vivo and in vitro (Miura et al., 1996; Knoll, 1988; Garcia-Segura et al., 2001; Moorthy et al. 2005b).

The present findings show that the activity of the antioxidant enzyme SOD is affected along with increased lipid peroxidation in the brain of the aging rat. Treatment with E2 to the aging animals decreased the level of lipid peroxidation and increased antioxidant enzyme activities in the brain of aging animals studied. It may therefore, be presumed that free radicals mediated lipid peroxidative injury, which plays a crucial role in the pathophysiology of aging, can be prevented and controlled by administering a lower dose of HRT to aging rats.

6. EFFECT OF E2 ON MEMBRANE FLUIDITY IN AGING
Membrane fluidity is an important physical property of cell membranes responsible for its permeability, thereby influencing membrane embedded enzyme activities. Lipid peroxides have been shown to produce an irreversible impairment of membrane fluidity and elasticity, leading to rupture of the cell and these changes are likely to be particularly significant in long-lived and predominately post-mitotic cells such as neurons. Fukaya et al. (2007) showed an age-related decrease of membrane fluidity in rat hippocampal neurons. The lipid dynamics of the biological membranes modulates essential cell functions including cell growth, solute transport, signal transduction and membrane-associated enzyme activities. Changes in the fluidity of membrane lipids are known to occur during aging and during lipid peroxidation. It was evidenced that the fluidity state of the lipid phase in a membrane is important for the activity of intrinsic membrane proteins.

The fluorescence (polarization) anisotropy of membrane-bound DPH, which is inversely correlated with membrane fluidity, was measured in isolated rat brain synaptosomes at different ages showed significant increases in anisotropy as a function of age in support with the data obtained by Muller et al. (2001). An interesting possible explanation for the age-dependent reduction of membrane fluidity is the oxidation of lipid
components of the membrane (Choi and Yu, 1995; Mantha et al., 2006). Polarization and anisotropy measurement on membranes from different age groups indicate decreased fluidity in the lipid structure. The decrease in membrane fluidity of aging brain is due to increased oxidative stress and the treatment of aging animals with E2 reverted the membrane fluidity to normal level as compared to untreated age matched controls. E2 treatment decreased free radicals formation and decreased lipid peroxidation levels, which further helps to maintains proper membrane fluidity. Estrogen functions as a radical scavengers and inhibits lipid peroxidation in vivo and in vitro (Moorthy et al., 2004a;2005b). E2 administration could thereby decrease oxidative stress and ROS in aged rats.

7. EFFECT OF E2 ON NEUROLIPOFUSCIN ACCUMULATION IN AGING

Lipofuscin is a morphological structural entity and is mainly accumulated in postmitotic cells of brain. It is brown yellow autofluorescent, electron dense materials and accumulates in the cytoplasm. This accumulation of lipofuscin in cells occurs because it is undegradable and cannot be removed from the cells via exocytosis. Lipofuscin formed by reaction of lipids with either an aldehyde or a ketone in the presence of peroxide yielded stable products fluorescing at the appropriate wavelength 360-420nm. As age progress the lipofuscin content per neuron as well as the number of pigmented neurons have been shown to increase in a linear fashion in many regions of the brain. (Sharma et al., 1993; Bala et al., 2006; Kumar et al., 2008).

Intraneuronal accumulation of lipofuscin is considered to be the marker of neuronal aging. It is formed mostly from peroxidation of protein and lipid material autophagocytosed from various cellular constituents and fragmented within the lysosomes (Mesulam, 1987). Lipofuscin is a peroxidation products and its formation appears to be integrative and proportional to the occurrence of lipid peroxidation. An age-related increase in lipid peroxidation has been shown to correlate with the gross level of lipofuscin (Sharma et al., 1993). Aging increases accumulation of fluorescent "lipofuscin" or "wear and tear" pigments in post-mitotic cells such as neurons and muscle cells (Terman and Brunk, 2006). After E2 treatment to aging animals, a decrease in lipofuscin in all brain regions was observed. Decrease in lipofuscin indicates that cells are relieved from this age-associated pathology, perhaps due to the anti-oxidant and neuroprotective properties of estrogens (Behl,
E2 treatment decreased free radical formation and also decreased lipid peroxidation levels, which further decrease lipofuscin accumulation in aging brain. Estrogen has been shown to function as a radical scavengers and inhibit lipid peroxidation *in vivo* and *in vitro* (Moorthy et al., 2004a; 2005b).

### 8. EFFECT OF E2 ON CALCIUM HOMEOSTASIS IN AGING

In the present study, the levels of intracellular Ca\(^{2+}\) were significantly increased in aged rats. This increase in intracellular calcium may be due to increased production of free radicals and loss of mitochondrial membrane integrity (Maciel et al., 2001; Murali et al., 2008). The calcium hypothesis of brain aging and dementia has been put forward to account for a number of the phenomena in the pathogenesis of dementia. A cellular mechanism that regulates the homeostasis of calcium plays a critical role in brain aging. Small changes in free calcium (Ca\(^{2+}\)) sustained over a period of time results in cellular damage. A close relation between Ca\(^{2+}\) homeostasis, the production of ROS, ischemia and brain cell death has been reported earlier (Finkel and Holbrook, 2000; Kristian and Siesjo, 1996).

In aging brain as Ca\(^{2+}\) levels rise, binding of Ca\(^{2+}\)/Calmodulin to Ca\(^{2+}\) ATPase relieves autoinhibition and the Ca\(^{2+}\) pump is maximally activated (Pottorf and Thayer, 2000). Autoinhibition can also be relieved by the proteolytic cleavage of the CaM binding domain by proteases such as calpain and caspase, resulting in an irreversibly activated Ca\(^{2+}\) ATPase (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. 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). These data suggest that the increase in synaptosomal Ca\(^{2+}\) due to oxidative stress may result from the inhibition of the plasma membrane and the endoplasmic reticulum membrane Ca\(^{2+}\) ATPase activities, probably as a result of the alteration of the lipid environment required for the maximal activity of these membrane enzymes. The consequent increase in Ca\(^{2+}\) may be responsible for the injury of the nervous tissue observed during several pathological conditions in which free radical generation seems to be involved.

The present results show that the prevention of dysregulation of intracellular Ca\(^{2+}\) homeostasis by E2 is advantageous in aged rats. Possibly, E2 may prevent the inhibition of
the Ca\textsuperscript{2+} ATPase pump and selective Ca\textsuperscript{2+} permeability, by scavenging reactive oxygen radicals. E2 increased membrane linked ATPases, perhaps E2 acts as an endogenous modulator of ATPases and attenuates the impact of age-related Ca\textsuperscript{2+} dyshomeostasis, cognitive decline, and vulnerability to neuropathologies (Toescu et al., 2004). Thus, some of the beneficial effects of E2 and hormone replacement therapy (HRT) on the brain (Wise et al., 2006; Spencer et al., 2008) may be attributed to modulation of membrane bound ATPases by E2.

Present results demonstrate E2 modulation of several Ca\textsuperscript{2+} dependent processes, particularly those associated with cognition (Liu et al., 2009; Brewer et al., 2009) and neuroprotection (Zhao and Brinton, 2007). E2 exerts its neuroprotective and nootropic actions by various mechanisms (Nilsen and Brinton, 2003; Scharfman and Maclusky, 2005; Wu et al., 2005; Simpkins and Singh, 2008) and its effect on calcium homeostasis represents only one component of its potentially beneficial effects (Sherwin and Henry, 2008). Estrogen thereby may help control/delay the onset or progression of age related disorders.

9. EFFECT OF E2 ON DNA DEGRADATION IN AGING

Programmed cell death has been well described in a number of organs of the body during various developmental, physiological, as well as pathological states (Collins et al., 1998; Johnson et al. 1995; King and Cidlowski, 1995; Majno and Joris, 1995; Rao, 2007). Although programmed cell death has been well documented in post mitotic tissues such as the heart and the brain (Misao et al. 1996; Sutherland et al. 1996; Schmidt-Kastner et al. 1997), there has been relatively less characterization of it in the normal heart and brain in response to stress during aging (Li et al., 1997; Sen et al., 2007).

The progressive loss of organ mass in aging could be due to either decreased cell proliferation or to increased apoptotic cell death. It may as well be due to a dysregulation of both processes, resulting in an imbalance between the two. It is however not yet established whether apoptosis plays a role in the process of aging. Literature addressing this issue is scarce (Zhang and Herman, 2002) and conflicting data have been in fact reported (Rao, 2007). Although the majority of these studies found an increased programmed cell death with age, opposite results, namely loss of apoptotic control with age, have also been reported. It is
therefore important to examine whether cell loss by apoptosis is a potential mechanism of the aging process. This may eventually lead to the finding of modalities to counteract the process. Ladder type DNA degradation, typical for apoptosis, was much more pronounced in DNA derived from brain of old as compared to young mice (Sen et al., 2007).

Aging is an important event in the life of mammals, including human beings, during which a number of metabolic and hormonal changes take place. Earlier results showed (Moorthy et al. 2005a) that the hormone levels used were at lower doses, than that given for HRT treatments. The amount of estrogen levels taken were equivalent to those found in three months old, adult rat. E2 treatment to aging rats decreased in DNA degradation. Estrogen and combined, estrogen and progesterone given to aging rats caused a series of growth related responses, such as increase uptake of glucose, increased protein levels, increased glucose oxidation and decreased gluconeogenesis (Birge, 2003; Veiga et al., 2004). Additionally, estrogen has also been demonstrated to reduce cytochrome c translocation (Bagetta et al., 2004), as well as caspase 3 activation and DNA fragmentation (Choi et al., 2004), further implicating an anti-apoptotic action of estrogen during cerebral ischemia.

10. EFFECT OF E2 ON GLUT4 EXPRESSION IN AGING

Glucose transporters play a critical role in mammalian brain energy metabolism because glucose is the principal brain energy source and these transporters promote glucose movement into neural cells. GLUT4, a 45-kDa protein, is found in neuronal cell bodies (Apelt et al., 1999; El Messari et al., 1998) and in neuronal processes, specially at the synaptic thickening (Leloup et al., 1996). As in myocytes and adipocytes, GLUT4 in neurons seems to be localized in the endoplasmic reticulum and in the cisterns of the Golgi apparatus (El Messari et al., 1998; Leloup et al., 1996).

GLUT4, the insulin-sensitive glucose transporter, was originally identified and characterized in peripheral tissues such as muscle, adipose and heart (Charron et al., 1989). In peripheral tissues such as muscle and adipose cells, insulin receptor activation stimulates GLUT4 translocation to the plasma membrane to increase glucose uptake and utilization. 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 to neurons in the hypothalamus (Leloup et al., 1996) and cerebellum, neuronal populations that also express the insulin receptor (Kobayashi et al., 1996). 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).

Present studies revealed that GLUT4 was expressed in neuronal cell bodies and neuronal processes in the rat cerebral cortex. Earlier studies have characterized GLUT4-positive neurons in the brain and based upon their neuronal phenotypes (Apelt et al., 1999). 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. Results of Moorthy et al. (2004b), showed that treatment of older rats with estrogen/progesterone alleviate the glucose levels in the tissues of older rats treated with the hormone.

Interestingly, in brain cortex, the aging process reduced the GLUT4 levels at 12 months. This inflection point coincides with the time at which all intact animals showed irregular cycling, mainly a persistent diestrous phase. Therefore, it could be considered that this point marks the onset of a gradual decline in regular estrous cyclicity, which is associated with a gradual impairment in ovulatory function and altered patterns of steroid secretion (Bakirtzi et al., 2009). It is also probably associated with a gradual impairment in brain homeostasis, as shown by data that demonstrate changes in the glucose transporters.

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). GLUT4 distribution in the brain tissue of aging and E2 treated rats was also monitored by immunohistochemistry and analyzed by light microscopy. The results
are in agreement with those obtained with RT PCR and Western blotting. GLUT4 was localized predominantly in the membrane in control rat brain. With aging there was a marked decrease in the GLUT4 distribution in the membranes. Treatment with E2 corrected the alterations in the distribution of GLUT4 expression and localization of GLUT4 mRNA and protein in the rat brain.

In agreement with earlier findings, present results showed that the GLUT4 diminished from 3 months, at 12 and 24 months. A possible role for E2 in GLUT4 translocation during aging, and a decrease in E2 levels could result in an impairment of GLUT4 translocation to the plasma membrane, thus leading to insulin resistance, with increased circulating plasma insulin levels during aging, also found in the present results, suggesting a possible role for E2 in GLUT4 translocation (Alonso et al., 2008; Moreno et al., 2010). The results suggest that low doses of E2 could have beneficial effects on neuronal homeostasis and may slow the progressive loss of glucose transporters that are associated with aging.

The brain regulatory effects of estrogen have been of significant interest due to the evidence that estrogen may delay onset or ameliorate the severity of several neurological disorders, such as Alzheimer’s disease, Parkinson’s disease, and stroke (Brann et al., 2007). Raz et al. (2008) discussed that the rapid nongenomic effects of estrogen have a physiologically important role in estrogen-induced neuroprotection, regulation of homeostasis and modulation of synaptic plasticity in the brain. E2 functions as radical scavengers and inhibits lipid peroxidation in vivo and in vitro (Moorthy et al., 2005b). Multiple cellular mechanisms may be involved in mediating the beneficial effects of estrogen on inflammatory and immune responses in aged individuals who sustain an injury and this effect may play a role in the neuroprotective effects of E2 in brain (Gomez et al., 2007; Brann et al., 2007). E2’s beneficial effects seemed to arise from its antilipofuscin, antioxidant, antilipidperoxidative, and anti-aging action. It can therefore be concluded that the long-term hormone treatment at much lower doses than HRT levels, used in the present experiments contribute to the decreased oxidative stress, and other neurodegenerative factors, which increased with aging. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders including metabolic syndrome.