Therapeutic Effects of Sildenafil Citrate (Viagra) And/or Vitamin E on Some Brain Disorders of Alloxan Induced Diabetes Mellitus Rats

A.M. Mohamed and Laila M. Faddah

The objective of present study is to estimate whether the treatment with Viagra in compared to the antioxidative nonenzymatic antioxidant, vitamin E and their synergistic combination would reverse brain disorders of diabetic rats. Animals were divided into two classes, class 1 consists of four normal healthy groups, control (not received any medication) and Viagra, vitamin E and synergistic (Viagra and vitamin E)-treated groups (Gs 1, 2, 3 and 4, respectively). Class 2 consists of four diabetic groups injected intraperitoneally with a single dose of alloxan (150 mg kg⁻¹ body weight), diabetic control and diabetic-Viagra, vitamin E and synergistic treated Groups (Gs 5, 6, 7 and 8, respectively). The result revealed that diabetes causes an imbalance in the oxidative state of brain tissue which is confirmed by significant increase in Xanthine Oxidase activity (XO, an enzymes implicated in the production of reactive oxygen and nitrogen species, ROS, RNS, respectively) as well as in nitric oxide (NO, marker of vascular disorder) and malonaldehyde (MDA, index of polyunsaturated fatty acid oxidation) levels with concomitant decrease in the antioxidants, vitamin C and glutathione (GSH) contents. The present investigation also showed that diabetes induces impairment of Adenosine Triphosphatase (ATP) hydrolyzing enzymes, indicated by marked reduction in Na+K+ ATPase activity, an enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability and stimulation of ectonucleotidases, NTPDases and 5'-nucleotidase, enzymes have a key role in the control of purinergic neuromodulation and neurotransmission. Treatment of diabetic rats with either Viagra or vitamin E significantly improved the diabetic deviation in the above tested parameters. However, administration of the combination of both drugs restored most of these parameters to near their normal levels. The present data showed that Viagra has a beneficial effect against diabetic brain oxidative stress and related vascular and neuronal complications and its synergistic combination with vitamin E may render it to have an additional potential concern in ameliorating some brain disorders induced by diabetes.

Key words: Sildenafil citrate, vitamin E, Na+ K+ ATPase, ectonucleotidases, brain, diabetes

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INTRODUCTION

Diabetes mellitus is a syndrome characterized by hyperglycemia and metabolic abnormalities due to decreased insulin level, causing metabolic and physiological changes in various organs including brain (Genet et al., 2002). Hyperglycemia is the most important factor in the onset and progress of diabetic complications mainly by producing oxidative stress (Baynes, 1991; Swidan and Montgomery, 1998). Brain is the most sensitive organ susceptible to oxidative stress due to its great oxygen consumption, high lipid content and poor antioxidant defenses (Halliwell, 1996).

High oxidative stress and changes in antioxidant balance due to persistent and chronic hyperglycemia promote free radicals generation, evidence based mainly on increased Lipid Per Oxidation (LPO) (Hong et al., 2004) and contribute to alterations in membrane ion transport and permeability (Franzon et al., 2005; Siddiqui et al., 2005), neurochemical, neurophysiological and behavioural modifications as well as cerebrovascular disturbances in the brain, impairing its functional and structural integrity (Bissels et al., 2002).

Thus, the therapeutic potential of oxidative stress inhibition to prevent or reverse the diabetic brain disorders is promising strategy.

Sildenafil citrate (the active compound in Viagra) was initially developed to treat heart diseases, however the penile erection enhancing effect was noted (Chailin et al., 1999). Then it has become the first oral therapeutic agent used to treat sexual dysfunction associated with many diseases such as multiple sclerosis (Fowler et al., 1999), spinal cord injuries (Hulting et al., 2000), radical prostatectomy (Zagaja et al., 2000), cardiovascular diseases (Przyklenk and Klener, 2001) and diabetes (El-Sakka, 2004). The drug elicits smooth muscle relaxation and vasodilation by selective inhibition of guanosine 3',5'-cyclic monophosphate (cGMP)-specific phosphodiesterase 5, thereby amplifying cGMP-mediated signaling initiated by the release of nitric oxide (NO) (Chailin et al., 1999). However some studies showed that the drug has positive effects on some brain disorders related to oxidative stress (Prickearts et al., 2002; Nakamizo et al., 2003; Daven et al., 2004; Shukla et al., 2005).

Vitamin E is one of the major lipid soluble antioxidant vitamins. It is found in membranes and lipoproteins, so it is considered membrane antioxidant (Sies and Stahl, 1995). Also, vitamin E represents the principal chain breaking antioxidant in the lipo-soluble phase and its antioxidant ability is augmented by serum ascorbate and urate (Niki et al., 1995). It protects against membrane permeability caused by peroxidation, attenuates ischemic and postischemic brain swelling, prevents neuronal damage and normalizes endothelial function (Abe et al., 1998; Siman and Erikson, 1997).

The present study aims to evaluate the beneficial effects of oral administration of Viagra in comparison to vitamin E and their synergistic combination against the oxidative stress and associated brain disorders in response to diabetes. This can be achieved by measuring some markers of oxidative stress, as XO activity, NO (as indices of brain vascular disorder) MDA (index of lipid peroxidation), GSH and vitamin C levels (non enzymatic antioxidants) as well as some markers of neuronal disorder, namely Na+/K+ ATPase, NTPDase and 5' nucleotidase.

MATERIALS AND METHODS

Chemicals: All chemicals used were of high analytical grade, product of Sigma and Merck companies. Sildenafil citrate (Viagra) and vitamin E were purchased from Fyzer and Farco companies, respectively.

Animals: Eighty male albino rats (150-200 g) were obtained from animal house of National Research Centre, Dokki, Giza, Egypt. Rats were fed a standard diet and free access to tap water. They were kept for two weeks to acclimatize to the environmental conditions. The animals were divided into two categories:

Category 1, consists of four normal healthy groups (groups 1, 2, 3 and 4), each of ten rats.

Group 1: Normal control rats (not received any medication).
Group 2: Viagra-treated group.
Group 3: vitamin E-treated group.
Group 4: synergistic group, treated with both Viagra and vitamin E. Viagra and vitamin E were given orally in doses of 3 and 300 mg kg⁻¹ day⁻¹, respectively for two weeks.

Category 2, consists of four diabetic groups (groups 5, 6, 7, 8, each of ten rats), diabetes was induced by alloxan, each rat was injected intraperitoneally with a single dose of alloxan monohydrate (150 mg kg⁻¹ body weight) dissolved in sterile normal saline (Saraswathi and Swaminathan, 2002). After injection, they had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock.
(Bhandari et al., 2005). After two weeks hyperglycemic rats (200-300 mg dL⁻¹) (Pari and Satheesh, 2004) were used for the experiment.

Group 5: Diabetic control group.
Group 6: Diabetic group treated with Viagra
Group 7: Diabetic group treated with vitamin E.
Group 8: Diabetic group treated with synergistic combination of both Viagra and vitamin E. The two drugs were given as the same manner of healthy groups.

After two weeks of drugs treatment, the animals were fasted overnight and sacrificed. The brains from different animal groups were immediately removed weighed and washed using chilled saline solution. Brains were minced and homogenized in either 10% trichloroacetic acid (for metabolites analysis) or in ice cold bidistilled water (for enzymes determination) to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 min at 10000 g at 4°C and the supernatants were used for biochemical analysis.

Biochemical analysis
Metabolites analysis: Nitrite concentration in brain (an indirect measurement of NO synthesis) was assayed spectrophotometrically using Griess reagent (sulfanilamide and N-1-naphthylethylene diamine dihydrochloride) in acidic medium (Moshag et al., 1995). Nitrite concentration is evaluated as µmol g⁻¹ tissue. Lipid peroxidation was assayed by measuring the formed Malon Dihaldehyde (MDA) (an end product of fatty acid peroxidation) by using Thiobarbituric Acid Reactive Substances (TBARS) method. MDA concentration was calculated using extinction coefficient value (ε) of 1.56×10⁵/M/cm (Buege and Aust, 1978). Results are expressed as µmol MDA formed/g tissue. Vitamin C was estimated by the method of Jagota and Dani (1982) using Folin-Ciocalteu reagent. The colour developed was read at 760 nm. The vitamin C content is expressed as µg g⁻¹ tissue. The total glutathione (GSH) content in brain tissue was determined using the method of Bentler et al. (1963) based on its reaction with 5,5'-dithiobis (2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm. GSH level is expressed as µmol g⁻¹ tissue.

Enzymes determination: All the following enzymes were assayed spectrophotometrically. XO (EC 1.1.3.22) activity was determined by the reduction of Nitro Blue Tetrazolium (NBT) in the presence of xanthine, forming formazan. The enzyme activity was calculated using the extinction coefficient of reduced NBT (7.5 cm² µmol⁻¹ at 540 nm) (Fried and Fried, 1974). The activity of the enzyme is expressed as nmol uric acid/min/mg protein. Na⁺/K⁺ ATPase (EC 3.6.1.37) (Tsakiris and Delicostantios, 1984), NTPDase-like activity (EC 3.6.1.35) apyrase, ATP diphosphohydrolase, ectoCD39 (Battistri et al., 1991) and 5'-Nucleotidase (EC 3.1.3.5, CD37) (Heymann et al., 1984) activities were assayed through measuring the inorganic phosphate (Pi) (Chan et al., 1986). Enzyme activities are expressed as µmol Pi released/min/mg protein.

Statistical analysis: Data were analyzed by comparing values for different treatment groups with the values for individual controls. Result are expressed as mean±SD. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) coupled with post-hoc (LSD).

RESULTS

The pattern of different biochemical profiles of rat brains of various experimental groups were shown in Table 1 and 2.

Diabetic group (G5) showed significant increase in XO activity as well as in nitrite (index of NO) and MDA (marker of lipid peroxidation) levels accompanied by marked decrease in the levels of non-enzymatic antioxidants, GSH and vitamin C compared to normal non-diabetic groups (G 1, 2, 3, 4). Supplementation of either Viagra or vitamin E or their synergistic combination significantly ameliorated the diabetic alteration in the above studied parameters (Table 1). The best results were obtained with the combination of both drugs.

The data revealed that diabetes induced inhibition in brain Na⁺/K⁺ ATPase activity and stimulation of ectonucleotidases, 5'-nucleotidase, NTPDase (using ADP as substrate) and NTPDase (using ATP as substrate) activities in compared to normal healthy groups (Table 2).

Administration of either Viagra or vitamin E, each alone, up regulates the level of Na⁺/K⁺ ATPase and down modulates the levels of ectonucleotidases, however the combination of both drugs more or less regulates the deviation of the enzyme activities to near normal levels. No significant changes were seen in the studied parameters on treating the normal rats with the used drugs either alone or in combination (G6, 2, 3 and 4) in compared to normal untreated group (G1) (Table 3).
Table 1: Levels of some oxidative stress markers in rat brains of different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal healthy groups</th>
<th>Diabetic groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>XO</td>
<td>12.2±2.49</td>
<td>13.5±1.3</td>
<td>12.6±1.4</td>
</tr>
<tr>
<td>LSD</td>
<td>(5.6)</td>
<td>(5.6)</td>
<td>(5.6)</td>
</tr>
<tr>
<td>NO</td>
<td>0.61±0.03</td>
<td>0.69±0.042</td>
<td>0.56±0.31</td>
</tr>
<tr>
<td>LSD</td>
<td>(5.6)</td>
<td>(5.6)</td>
<td>(5.6)</td>
</tr>
<tr>
<td>LPO</td>
<td>1.48±0.16</td>
<td>1.7±0.16</td>
<td>1.27±0.10</td>
</tr>
<tr>
<td>GSH</td>
<td>2.8±0.13</td>
<td>2.75±0.64</td>
<td>3.02±0.27</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.47±0.045</td>
<td>1.48±0.04</td>
<td>1.50±0.04</td>
</tr>
<tr>
<td>LSD</td>
<td>(5.6,7)</td>
<td>(5.6,7)</td>
<td>(5.6,7)</td>
</tr>
</tbody>
</table>

Values are mean±SD of 5 independent experiments; XO is expressed in μmol mg⁻¹ protein; NO, LPO and GSH levels are expressed in μmol g⁻¹ tissue; Vitamin C is expressed in μg g⁻¹ tissue

Table 2: Levels of ATP hydrolyzing enzymes in rat brains of various studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal healthy groups</th>
<th>Diabetic groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>ATPase</td>
<td>1.90±0.042</td>
<td>1.82±0.07</td>
<td>2.01±0.104</td>
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<td>(5.6,7)</td>
<td>(5.6,7)</td>
<td>(5.6,7)</td>
</tr>
<tr>
<td>5'-nucleotidase</td>
<td>0.23±0.031</td>
<td>0.31±0.03</td>
<td>0.35±0.023</td>
</tr>
<tr>
<td>LSD</td>
<td>(5.6)</td>
<td>(5.6)</td>
<td>(5.6)</td>
</tr>
<tr>
<td>NTDPase (ADP)</td>
<td>0.45±0.021</td>
<td>0.45±0.025</td>
<td>0.43±0.017</td>
</tr>
<tr>
<td>LSD</td>
<td>(5.6,7)</td>
<td>(5.6,7)</td>
<td>(5.6,7)</td>
</tr>
<tr>
<td>NTDPase (ATP)</td>
<td>0.88±0.035</td>
<td>0.9±0.026</td>
<td>0.9±0.031</td>
</tr>
<tr>
<td>LSD</td>
<td>(5.6,7)</td>
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Values are mean±SD of 5 independent experiments; Enzymes are expressed in μmol mg⁻¹ protein

Table 3: Statistical analysis using the t-test for the parameters measured among the different studied groups

<table>
<thead>
<tr>
<th>Groups analyzed</th>
<th>XO</th>
<th>NO</th>
<th>LPO</th>
<th>GSH</th>
<th>Vitamin C</th>
<th>ATPase</th>
<th>5'-nucleotidase</th>
<th>NTDPase (ADP)</th>
<th>NTDPase (ATP)</th>
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<td>1 vs 2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>1 vs 4</td>
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<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
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<tr>
<td>1 vs 5</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>1 vs 6</td>
<td>0.05</td>
<td>0.01</td>
<td>NS</td>
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<tr>
<td>1 vs 7</td>
<td>NS</td>
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<tr>
<td>1 vs 8</td>
<td>NS</td>
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<td>NS</td>
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<td>NS</td>
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<td>NS</td>
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<tr>
<td>5 vs 6</td>
<td>0.0001</td>
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<tr>
<td>5 vs 7</td>
<td>NS</td>
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<td>6 vs 7</td>
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<td>6 vs 8</td>
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<td>7 vs 8</td>
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NS: Not significant

**DISCUSSION**

Tissue damage resulting from diabetic complications associated with oxidative stress is often seen in brain (Ratner, 2001). The present study was assessed to evaluate the ability of sildenafil citrate (Viagra) in comparison to the non-enzymatic antioxidant, vitamin E and their combination in improving brain disorders related to oxidative stress in alloxan-induced diabetic rats.

In consistent with previous published studies, the data generated in the current investigation revealed that diabetes induces oxidative stress in brain of diabetic rats which is indicated by an increase in XO activity as well as in NO and MAD (index of lipid peroxidation) levels with concomitant decrease in the levels of non-enzymatic antioxidants, GSH and vitamin C (Genet et al., 2002; Alciguzel et al., 2003; Anwar and Meki, 2003; El-Missiry et al., 2004; Preet et al., 2005; Siddiqui et al., 2005).

XO is an enzyme catalyzes the oxidation of hypoxanthine to xanthine and the later to uric acid. It was reported that XO is an endogenous source of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) that can produce oxidative stress which inflicts tissue injury (Winterbourn and Sutton, 1986; Harrison, 2002). It catalyzes the reduction of nitrite to NO which exerts various influences on the pathogenesis of tissues (Mohamed et al., 2001). NO has a double-edged knife in pathophysiology, since both the abundance and paucity of NO causes diseases (Kim et al., 2002). The action of
NO depends on the relative degree of its expression by nitric oxide synthase isomers, endothelial, neuronal and inducible (eNOS, nNOS and iNOS, respectively). The eNOS expresses the eNO that causes vasodilatation (Napoli and Ignarro, 2001), inhibition of eNOS leads to vasoconstriction and atherosclerosis. NO produced by NOS is resistant to brain damage caused by vascular stroke (Nelson et al., 1995), while NO plays an important role in vascular smooth muscle dilation (Wei et al., 1995) and its over production has linked to a variety of clinical inflammatory diseases (Kim et al., 2002). The direct toxicity of NO is enhanced by reacting with superoxide radical to give powerful secondary toxic oxidizing species, such as peroxynitrite (ONOO⁻) which is capable of oxidizing cellular structure and causes lipid peroxidation (Beckman and Koppenol, 1996; Weinstein et al., 2000, Sayed Ahmed et al., 2001), a process leading to membrane damage. In this context, transformation of eNO by superoxide radical to peroxynitrite diminishes the capacity of endothelial cells to generate bioactive useful NO, which is important in maintenance of normal blood pressure, thereby decreases in NO biocactivity, causing endothelial sclerosis and subsequently hypertension (Stokes et al., 2002). Reduced eNO is implicated in diabetic macrovascular and microvascular diseases. Some authors hypothesized that increased XO activity reduces NO availability and causes the reduced blood vessel relaxation, increased vascular inflammation and endothelial proliferation observed in the later stages of diabetes (Fadillioglu et al., 2003; Alicioguzel et al., 2003). Thus from our result it can be stated that increased of XO activity which plays a crucial role in reducing the level of bioactive NO may be used as important marker for vascular disorder.

The decreased levels of the antioxidant defense system, vitamin C and GSH in brain of diabetic rats versus control ones, may be a response to the over production of ROS and RNS which may exceed the protective ability of these endogenous antioxidants. GHS participates in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates (Deneke and Fanburg, 1989). Vitamin C, an effective water soluble antioxidant, is the first to become depleted on the exposure to oxidative stress (Sies and Stahl, 1995). Normal vitamin C level is a therapeutic benefit as of its ability to reduce the oxidative stress by reacting with superoxide and hydroxide radicals as well as alkyl, peroxyl and alkoxyl radicals, thereby it can neutralize these radicals and stop the initiation and propagation of reaction chain (Buettner, 1993; Sharma and Buettner, 1993). Thus the decrease in both vitamin C and GSH presented in the current study might reflect their direct reaction with the reactive species generated in response to hyperglycemia (Yoshida et al., 1995; Buettner, 1993).

Supplementation of diabetic rats with either Viagra or vitamin E or their synergistic combination, was effective in modulating the alteration of brain XO activity, NO and MDA levels as well as the decrease in vitamin C and GSH contents. The best results were obtained with synergistic combination of both drugs. In agree with some authors (Daven et al., 2004; Hong et al., 2004), these results confirm that the used drugs have excellent effects against oxidative damage and reactive species induced by diabetes even for brain which is sensitive to oxidative stress due to high oxygen consumption, high unsaturated fatty acid content and poor antioxidants (Halliwell, 1996). Two mechanisms can be postulated to effects of the used drugs. The first one (proved from our data) is that both drugs may exert their beneficial actions through the direct inhibition of XO as a source of ROS and RNS. The second mechanism is based on the ability of either one to control hyperglycemia which promotes free radicals production or directly inactivate free radical species. These are supported by previous studies have been shown that, although Viagra is poor hypoglycemic control (Ojemole et al., 2006), it has long term protective effects on brain oxidative stress (Daven et al., 2004), it inhibits the formation of ROS by direct inhibition of oxidases activities (Muzaffar et al., 2005; Shukla et al., 2005). The antioxidant action of Viagra may have a crucial role in modifying the vascular complication in brain associated with diabetic state as it has transient vasodilatory properties in vivo (Ojemole et al., 2006; Silva et al., 2006). This may be explained on the basis that, the inhibitory effect of Viagra on lipid peroxidation and improving the antioxidant levels (vitamin C and GSH) presented in the current study may be accompanied by an increase in bioactive NO. The main of its action is to activate the soluble guanylyl cyclase which results in increased level of cGMP (Pfeifer et al., 1998). On the other hand, it was reported that Viagra is a potent and selective inhibitor of cGMP-specific phosphodiesterase, the metabolizing enzyme of cGMP (Beavo, 1995; Nakamizo et al., 2003), leading to cGMP availability. So it stimulates the NO-cGMP pathway (through stimulation of bioactive NO and inhibition of cGMP hydrolysis) which promotes the physiological relaxation of vascular smooth muscles. Vitamin E, the major lipid phase antioxidant vitamin, is glycemic control (Kheir-Eldin et al., 2001), can prevent lipid peroxidation and maintains GSH and vitamin C levels in the damaged tissue by inhibiting free radicals formation (Duval and Poelman, 1994; Kulkarni and Byczkowski, 1994; Hong et al., 2004). Thus, it can normalize brain endothelial function (Siman and Eriksson, 1997) and modify ischemic and postischemic brain damage (Abe et al., 1998).
One of the consequences of free radical species and lipid peroxidation degenerative processes, is the change of brain cell membranes (Stefanello et al., 2005). The free radicals oxidize polyunsaturated fatty acid, a main component of the cell membrane, thereby destroying the spatial arrangement of the membrane and removing the biological activities, as the result its functions deteriorate and fluidity decrease, impairing the functions of the bound enzymes like Na+/K+ ATPase which plays an important role in the functional activity of nerve cells (Kuella et al., 1997; Yousef et al., 2002; Hong et al., 2004; deAssis et al., 2006).

In consistent with some investigators, the current data has shown that diabetes induces a decrease in Na+/K+ ATPase activity in brain of diabetic rats compared with normal animals, a fact that may be an important factor in the pathogenesis of the central nervous system in the diabetic state (Franzon et al., 2005; Siddiqui et al., 2005). Na+/K+ ATPase is a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It catalyzes the hydrolysis of ATP and couples it to the transport of Na+ and K+ across the cell membrane, thereby generating the transmembranous Na+/K+ gradient (Freceinska and Silver, 1994). This pump is essential for the regulation of cell volume, uptake of nutrients, regulation of neurotransmitter release and excitability properties of nerve tissue (Vizi and Oberfrank, 1992). It has been proposed that alteration in this enzyme activity may represent an important neurotoxic mechanism for neurons (Lees, 1993). In this context, there are some data showing that inhibition of such enzyme provokes increased Na+ uptake and cytosolic free Ca+2 concentrations, releasing acetylcholine, decreasing membrane potential of synaptosomes from cerebral cortex (Satoh and Nakazato, 1992). Decreased Na+/K+ ATPase activity leads to neuron-selective lesion in the brain (Lees et al., 1990) and occur in brain of patients of Alzheimer's disease (Hattori et al., 1998), suggesting that inhibition of this enzyme may be used as useful indicator of brain neurodegenerative pathophysiology related to memory and cognitive disorders of diabetic state.

Administration of either Viagra or vitamin E or their combination prevents the inhibition of Na+/K+ ATPase activity of diabetic rat brains and consequently would attenuate the resultant neurotoxicity. This result suggest that the inhibition of both drugs on reactive species and lipid peroxidation may restore membrane fluidity and hence the functional ability of associated enzymes. In support with previous authors revealed that treatment with Viagra has been shown to have beneficial effects in promoting brain functional recovery, it exerts its action through inhibition of NADPH oxidase activity and free radicals formation (Shukla et al., 2005) and stimulation of NO-cGMP pathway which has been implicated in neuroprotection and neurogenesis (Zhang et al., 2002; Davenport, 2004). Also, previous reports proved the importance of antioxidants, such as vitamin E, treatment in preventing neuronal damage and membrane fluidity induced by oxidative stress (Low et al., 1997; Abe et al., 1998; Kheir-Eldin et al., 2001; Hong et al., 2004). It has the ability to improve Na+ and K+ ions in the Central Nervous System (CNS) necessary to maintain the ionic gradient for neuronal excitability (Freceinska and Silver, 1994).

Ectonucleotidases such as NTPDases and 5' nucleotidase are other enzymes participate in ATP hydrolysis in the brain synaptic cleft as well as in the control of purinergic neurotransmission (Zimmerman, 1999; Schetinger et al., 2001; Blaz, 2003). NTPDases hydrolyze the extracellular ATP and ADP in the presence of Ca+2 and Mg+2, while 5' nucleotidase catalyzes the hydrolysis of AMP, playing an important role in adenosine production. The present data revealed marked increased NTPDases and 5' nucleotidase activities in brain of diabetic rats versus normal healthy groups, implying alteration of nucleotide hydrolysis and attenuation of purinergic neurotransmission (Lunekes et al., 2004). Similar results were obtained in synaptosomes from diabetic rats (Lunekes et al., 2004). Simulation of these enzymes is double-edged weapon, as their stimulation, leading to enhancement of ATP, ADP and AMP hydrolysis and consequently an increment of adenosine production. It is known that ATP is an excitatory neurotransmitter (Cunha and Ribeiro, 2000) and ADP activates platelet aggregation where as adenosine is a neuromodulator and cytoprotective agent, that it promotes vasodilatation and inhibition of platelet aggregation (Lunekes, 2003). Thus the increment of adenosine production and ADP depletion are considered as a compensatory response against some brain pathological condition associated with diabetes (e.g., thrombosis and hypertension) (Lunekes et al., 2004). However, this physiological response is not always sufficient to prevent the pathological events in brain microenvironment related to diabetes (Lunekes et al., 2003). At the same time, adenosine inhibits the release of neurotransmitters and neuronal excitability (Bruun dege and Dunwiddie, 1997). The potential increase of brain adenosine level and a lower availability of ATP as an excitatory neurotransmitter affect in particular, the hippocampus synaptosomal fraction, since this region
of brain plays a key role in memory and learning (Bruno et al., 2005). Thus, stimulation of brain ectonucleotidases may be used as another useful markers for behavior and cognitive disturbances found diabetes (Gradman et al., 1993; Greenwood et al., 2003). So, the enzymatic control of nucleotide levels is important in the process of brain haemostasis.

Supplementation of diabetic rats with either Viagra or vitamin E significantly reduces the activities of brain ectonucleotidases, however their synergistic combination more or less down regulate the enzyme activities to near normal levels. This regulatory mechanism may have a crucial effect on the physiological function of both ATP as neurotransmitter and adenosine as cytotoxicant agent, since it may alleviate the imbalance in nucleotide levels which associated with cognitive disorder, aging and memory impairment related to diabetes. Previous investigation proved that Viagra has been shown to have beneficial effects on learning and memory (Daven et al., 2004), beside it could attenuate platelet aggregation (Przyklenk and Klener, 2001). Also, it was reported that vitamin E has the ability to modify aging process and platelet hyperaggregatibility related to oxidative stress induced by diabetes (Siman and Eriksson, 1997; Hong et al., 2004). Administration of the used drugs to normal rats caused no changes in any of the measured parameters versus control untreated group, indicating their safety.

In conclusion, the current investigation revealed that Viagra, which is already in widespread use for treatment of sexual dysfunction, has the same beneficial influence of vitamin E in ameliorating the oxidative stress and associated complications induced in brain of diabetic rats, however this positive response is potentiated with the synergistic combination of both drugs and this may render Viagra to have an additional curative properties and considered as a novel therapeutic strategy to modulate the pathogenesis of brain in diabetic state.

REFERENCES


