Amelioration of altered antioxidant status and membrane linked functions by vanadium and *Trigonella* in alloxan diabetic rat brains

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Trigonella foenum graecum seed powder (TSP) and sodium orthovanadate (SOV) have been reported to have antidiabetic effects. However, SOV exerts hypoglycemic effects at relatively high doses with several toxic effects. We used low doses of vanadate in combination with TSP and evaluated their antidiabetic effects on antioxidant enzymes and membrane-linked functions in diabetic rat brains. In rats, diabetes was induced by alloxan monohydrate (15 mg/100 g body wt.) and they were treated with 2 IU insulin, 0·6 mg/ml SOV, 5% TSP and a combination of 0·2 mg/ml SOV with 5% TSP for 21 days. Blood glucose levels, activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Na^+/K^+ ATPase, membrane lipid peroxidation and fluidity were determined in different fractions of whole brain after 21 days of treatment. Diabetic rats showed high blood glucose ($P < 0·001$), decreased activities of SOD, catalase and Na^+/K^+ ATPase ($P < 0·01$, $P < 0·001$ and $P < 0·01$), increased levels of GPx and MDA ($P < 0·01$ and $P < 0·001$) and decreased membrane fluidity ($P < 0·01$). Treatment with different antidiabetic compounds restored the above-altered parameters. Combined dose of *Trigonella* and vanadate was found to be the most effective treatment in normalizing these alterations. Lower doses of vanadate could be used in combination with TSP to effectively counter diabetic alterations without any toxic effects.

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1. Introduction

Diabetes is characterized by hyperglycemia and metabolic abnormalities due to decreased insulin levels, 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 (Giugliana *et al* 1996). Cu/Zn superoxide dismutase (SOD) (EC 1·15·1·1), catalase (EC 1·11·1·6) and glutathione peroxidase (GPx) (EC 1·11·1·9), are the biological antioxidant enzymes which directly scavenge free radicals and prevent their conversion to toxic products (Freeman and Crapo 1982). High oxidative stress can lead to microvascular cerebral diseases, e.g. stroke, cerebral haemorrhage, and brain infarction (Kannel and McGee 1979; Paton and Passa 1983). The reason for high risk of microvascular cerebral diseases, despite the fact that brain consumes 20% of the oxygen in the body, is that it has a low content of antioxidants and high content of unsaturated fatty acids and catecholamines that are easily oxidized, making the brain more vulnerable to oxidative damage than any

Keywords. Alloxan diabetes; antioxidants; Na^+/K^+ ATPase; sodium orthovanadate (SOV); *Trigonella foenum graecum* seed powder (TSP)

Abbreviations used: GPx, Glutathione peroxidase; MDA, malondialdehyde; ROS, reactive oxygen species; SOV, sodium orthovandate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance.

other organs in the body (Hong *et al* 2004). Diabetes represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipid peroxidation (LPO), or by indirect evidence of reduced antioxidant reserve, like SOD and catalase enzymes, in animal models (Palanivel *et al* 1998).

Na^+/K^+ ATPase (EC 3·6·3·9) a membrane-linked enzyme that 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. This pump is essential for the regulation of cell volume, uptake of nutrients, regulation of neurotransmitters release and contractibility and excitability properties of nerve tissue (Hernandez *et al* 1992; Vizi and Oberfrank 1992). Alterations in Na^+/K^+ ATPase enzyme have been documented in diabetic brain (Leong and Leung 1991). Previous study has shown that neuroblastoma cells elicit decreased Na^+/K^+ ATPase activity when exposed to high glucose for two weeks (Yorek *et al* 1991).

Membrane fluidity, which represents motional freedom of lipid molecules in the membrane bilayer, is often used as an index of membrane physical properties and controls a number of membrane functions including permeability, active transport, and ligand affinity. Since the cell membrane requires good fluidity to maintain homeostasis and metabolism in the body, fluidity is an effective index of diabetes complications. It has also been reported that free radicals generated during diabetes deteriorate membrane structure and decrease membrane fluidity. Changes in membrane fluidity have been implicated in disease process and diabetic complications (Hong *et al* 2004).

The discovery of insulin was a boon for the cure of diabetes mellitus. However, there is a need for an effective therapy, as the daily necessity for several insulin injections can be painful and it is cost effective also. Thus there is a need to seek newer and alternative approaches for effective therapy in diabetes management. Many antidiabetic compounds like vanadate salts and plant extracts have been explored as an alternative to insulin therapy for over a decade. Vanadium salts such as sodium orthovanadate mimic several of the metabolic and growth promoting effects of insulin (Shechter *et al* 1990). However, the most remarkable insulinomimetic effect of vanadium salts is their ability to normalize blood glucose in type-1 diabetic animal models (Heyliger *et al* 1987). Vanadium also improves the altered glucose and lipid homeostasis through the reversal of key glycolytic, gluconeogenic and lipogenic enzymes (Heyliger *et al* 1987; Meyerovitch *et al* 1987). The biological potential of vanadium as an insulin mimetic and antidiabetic agent is, however, hampered by its toxicity (Domingo *et al* 1993). The most common toxic effects are diarrhoea, dehydration, decreased fluid and food intake and loss in body weight (Brichard *et al* 1988; Domingo *et al* 1991). To explore the pharmacological potential of vanadium compounds and to enhance their bioavailibility by reducing their toxicity, attempts are being made to use the complex-forming capability of vanadium compounds with organic compounds (Srivastava 2000). Traditional medicines like Ayurveda and Unani in India and other countries since ancient days have employed hypoglycemic plants such as *Trigonella foenum graecum* Linn. (fenugreek) to protect against diabetic pathogenesis (Khosla *et al* 1995). Various reports have demonstrated that the *Trigonella* seeds have hypoglycemic, hypocholesterolemic and hyperinsulinomic effects on type-1 and type-2 diabetes mellitus in human and experimental animals (Puri *et al* 1995; Stark and Madar 1993; Valette *et al* 1984). This medicinal herb is considered to be an excellent candidate for oral therapy as it is effective, non-toxic and without serious side effects. The chemical constituents of *Trigonella* seeds include volatile oils, alkaloids, saponins, sapogenins, flavonoids and mucilage (Duke *et al* 1992).

In the present study, attempts have been made to reduce the toxicity of vanadate without compromising its antidiabetic effects by reducing the dose and combining it with *Trigonella* and also to explore whether this treatment can restore the altered antioxidant defense system and abnormalities of membrane-linked functions in the brains of alloxan diabetic rats.

2. Materials and methods

2.1 *Animals*

Wistar female rats weighing 200–220 *g* were used for all the experiments. Animals were kept in the animal house at temperatures of 22–26°C and relative humidity of 55%. The animals were fed standard chow (Hindustan Lever Ltd., India) and given tap water *ad libitum* until treatment or time of sacrifice. All the animal procedures were approved by the Institutional Animal Ethical Committee (IAEC) of Jawaharlal Nehru University, New Delhi, India.

2.2 *Materials*

Sodium orthovanadate (SOV), alloxan monohydrate, ATP, ouabain, 1,1,3,3-tetrahydroxypropane, 1,6-diphenyl-1,3,5-hexatriene, pyrogallol were from Sigma (St. Lewis, MO, USA). All other compounds were of analytical grade.

2.3 *Methodology*

Overnight-starved rats were made diabetic by a single subcutaneous injection of alloxan monohydrate (15 mg/100 g body weight) freshly prepared in 0·154 M sodium acetate

buffer (pH 4·5) according to the method of Sochor *et al* (1985). Control animals were given only the vehicle. The alloxan induced diabetic rats were injected with 2 IU of protamine-zinc insulin for the next 7 days to decrease the mortality and to stabilize the diabetic animals. The severity of diabetes was checked in alloxan diabetic rats by using urine glucose strips (Diastix, Bayer Diagnostic, India). Animals were then grouped into control (C), diabetic (D), diabetic treated with insulin (D + I), diabetic treated with *Trigonella* (D + T), diabetic treated with vanadate (D + V), and diabetic treated with both *Trigonella* and vanadate (D + T + V).

Protamine zinc insulin (2 IU) suspension was administered intraperitoneally to diabetic animals (D + I) group, each day for 21 days. The diabetic groups treated with *Trigonella* (D + T) were given 5% finely powdered *Trigonella* seeds (AGMARK brand, purchased from local market) in powdered rat feed (i.e. 5 g of dry *Trigonella* seeds in 95 g of powdered rat feed) for 21 days. SOV was given at a dose of 0·6 mg/ml in drinking water (freshly prepared) consecutively for 21 days to the diabetic animals (D + V), vanadate was dissolved in drinking water with 0·5% sodium chloride to reduce its toxicity (Heyliger *et al* 1987). The diabetic rats treated with *Trigonella* and vanadate (D + T + V) were given 0·2 mg/ml of vanadate dissolved in tap water containing 0·5% sodium chloride and 5% finely powdered *Trigonella* seeds in powdered rat feed for 21 days.

After 21 days of treatment animals from control and different experimental groups were starved overnight and subsequently sacrificed by cervical dislocation. Whole brains were dissected out, washed in normal saline, weighed, minced quickly and homogenized in 9 volumes of cold isotonic sucrose buffer using a Potter Elvehjem type of a tissue homogenizer fitted with a Teflon plunger. The homogenizing buffer contained the following in the final concentration: 0·25 M sucrose and 0·02 M triethanolamine, pH 7·4, containing 0·12 mM dithiothreitol. The entire procedure was carried out at 0–4°C. The homogenate was centrifuged at 1000 *g* for 10 min to remove the cell debris. The pellet was discarded and the supernatant was further centrifuged at 12,000 *g* for 20 min at 4°C in a refrigerated super-speed centrifuge (Sorvall RC-5B) to isolate the mitochondrial pellet. The supernatant thus obtained was centrifuged at 1,05,000 *g* for 65 min at 4°C in a Beckman ultracentrifuge (L 8–70 M) to yield the cytosolic supernatant and microsomal pellet. The final pellets were then suspended in the same buffer and used as microsomal membrane fractions.

2.4 *Estimation of enzyme activities*

2.4a *Antioxidant enzymes*: The activity of SOD was measured by the method of Marklund and Marklund (1974) with some modifications, using an assay based on the ability of SOD to inhibit the autoxidation of pyrogallol by 50%. One unit of enzyme is defined as the amount of enzyme that causes half maximal inhibition of pyrogallol autooxidation/mg protein/min. The assay of catalase was performed by following the method of Aebi (1974). One unit of enzyme activity is defined as the amount of enzyme required to decomposed 1 µmol of H_2O_2/mg protein/min. The activity of glutathione peroxidase (GPx) was measured using a coupled enzyme assay as described by Lawrence and Burk (1976). One unit of enzyme is defined as 1µmol of NADPH oxidized/mg protein/min.

2.4b *Na^+/K^+ ATPase*: Na^+/K^+ ATPase activity was measured according to the method of Mayanil *et al* (1982). The enzyme activity was calculated as the difference of the activity between total ATPase and Mg^{2+} ATPase. The Pi in the protein free supernatant was determined according to the method of Fiske and Subbarow (1925). The specific activity of the enzyme is expressed as µmol Pi released/mg protein/min.

2.4c *Lipid peroxidation*: Lipid peroxidation was assessed by measuring the formed malondialdehyde (MDA) (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (Genet et al 2002). Results are expressed as nmol MDA formed/mg protein.

2.4d *Membrane fluidity*: For measuring the fluidity, brain microsomal membranes were labelled with 1,6-diphenyl-1,3,5-hexatriene, a fluorescent probe by incubating equal volume of a membrane suspension containing 100 $\mu g \cdot ml^{-1}$ of protein in phosphate buffer and 2 µM 1,6-diphenyl-1,3,5-hexatriene suspension in the same buffer. Excitation and emission wavelengths were, respectively, 365 and 428 nm. Polarization (*P*) and anisotropy (*r*) measurements were carried out on a model SLM4800 polarization spectroflourometer as described by Chautan *et al* (1990).

2.4e *Blood glucose*: Blood glucose was estimated by Glucose Enzokit from Ranbaxy Laboratories India, using glucose oxidase method.

2.4f *Protein*: Protein contents of cytosolic and microsomal fractions were determined by Bradford (1976) method using BSA as standard.

2.4g *Statistical analysis*: The data were analysed using one-way ANOVA followed by Turkey-Kramer multiple comparison test to determine the statistical significance. The significance was calculated by comparing the controls with diabetic and treatment groups.

3. Results

3.1 *General parameters*

Animals with blood glucose levels more than 350 mg/dl were taken for the experimental analysis. These animals had glycosuria, polydipsia, polyphagia and a reduced rate of growth. Physiological parameters like body weight, tissue weight, protein and blood glucose levels as observed in control and all the experimental groups are summarized in table 1. Alloxan-induced diabetic rats showed marked hyperglycemia with almost four-fold higher blood glucose concentration when compared to control values. The combined dose of vanadate and *Trigonella* was more effective in lowering blood glucose levels in diabetic rats as compared to the separate treatment with vanadate and *Trigonella* (table 1). After 21 days of diabetes induction, body weight was significantly reduced in the diabetic group. Vanadate treatment alone could not effectively improve the weight loss, but *Trigonella* alone and in combination with vanadate was able to restore back the body weight of the diabetic animals to control levels. The brain weight, when calculated per 100 g body weight, of the control and experimental groups did not show statistical difference. The protein contents in different fractions of brain of control and experimental groups did not show any significant difference. The results of all the enzymes activity are expressed as per milligram protein.

An earlier study from our laboratory has shown the effects of 0·2 mg/ml and 0·6 mg/ml of vanadate treatment on body weight of control rats after three weeks. There was no significant change in the body weight of 0·2 mg/ml vanadate treated rats where as 0·6 mg/ml vanadate-treated rats showed a significant decrease in body weight (table 2). Effects of two doses of vanadate on blood glucose of diabetic rats were also observed, 0·2 mg/ml vanadate treatment failed to correct hyperglycemia where as treatment with 0·6 mg/ml vanadate significantly decreased hyperglycemia (Mohamad *et al* 2004).

Table 1. General parameters of control (C), diabetic (D), and diabetic rats treated with insulin (D + I), *Trigonella* (D + T), vanadate (D + V) and combined dose of *Trigonella* and vanadate (D + T + V).

Parameters	C	D	D + I	D + T	D + V	D + T + V
Body wt. (g)	218 ± 13	143 ± 16[a]	208 ± 11	210 ± 14	170 ± 12[c]	215 ± 14
Brain wt. (g)	1·68 ± 0·30	1·60 ± 0·25	1·69 ± 0·28	1·65 ± 0·29	1·60 ± 0·20	1·67 ± 0·35
Brain wt./100 g body wt.	0·77 ± 0·13	1·13 ± 0·15	0·81 ± 0·09	0·78 ± 0·10	0·97 ± 0·12	0·78 ± 0·07
Blood glucose (mg/dl)	86 ± 6·8	440 ± 16·2[a]	108 ± 8·8	137 ± 9·7[b]	118 ± 8·2	110 ± 7·2
Protein (mg/g brain)						
Cytosolic	54·5 ± 3·17	57·3 ± 4·65	55·3 ± 5·43	56·5 ± 3·95	56·0 ± 4·71	55·5 ± 2·77
Microsomes	9·8 ± 1·32	9·4 ± 1·35	9·7 ± 1·28	9·9 ± 1·36	9·8 ± 1·24	10·2 ± 1·34

Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. Fisher's *P* values are [a]$P < 0·001$, [b]$P < 0·01$ and [c]$P < 0·05$.

Table 2a. Comparison of body weights of control and different doses of vanadate treated rats after 21 days of treatment.

	Control	Control + vanadate (0·2 mg/ml)	Control + vanadate (0·6 mg/ml)
Body weight (g)	218 ± 13	213 ± 12·1	170 ± 7·5[b]

Table 2b. Comparison of plasma glucose levels of diabetic and different doses of vanadate treated diabetic rats after 21 days of treatment.

	Diabetic	Diabetic + vanadate (0·2 mg/ml)	Diabetic + vanadate (0·6 mg/ml)
Plasma glucose (mg/dl)	440 ± 15·5	380 ± 25·7	118 ± 8·2[a]

Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. The Fisher's *P* values are [a]$P < 0·001$ and [b]$P < 0·05$.

3.2 *Antioxidant enzymes*

The diabetic state is associated with a generalized increase in tissue oxidative stress, which might be reflected in the changes in the tissue antioxidant system. Results of the changes in antioxidant enzymes are presented in table 3. As can be seen, SOD and catalase activities showed a significant decrease ($P < 0{\cdot}01$, $P < 0{\cdot}001$), however, GPx showed an increased activity ($P < 0{\cdot}01$) after 21 days of diabetes.

Treatment of the diabetic animals with insulin, *Trigonella*, vanadate, and the combination of *Trigonella* and vanadate reversed the changes of these enzymes to control levels. Diabetic rats treated with the combination therapy showed the maximum restoration in SOD, catalase, and GPx activities.

3.3 *Na^+/K^+ ATPase activity*

The activity of Na^+/K^+ ATPase enzyme in control and different experimental groups are presented in table 3. The effect of diabetes on Na^+/K^+ ATPase enzyme in brain microsomal membrane fractions at 21 days of diabetes induction showed a significant decrease ($P < 0{\cdot}01$), when compared to control rats. Treatment with *Trigonella* and vanadate alone and in combination reversed the altered activity of Na^+/K^+ ATPase enzyme of diabetic rats.

3.4 *Lipid peroxidation and membrane fluidity*

The level of lipid peroxidation and membrane fluidity were determined in control, diabetic and diabetic treated rats with different antidiabetic compounds and results are

Table 3. Changes in enzyme activities in control (C), diabetic (D), diabetic rats treated with insulin (D + I), *Trigonella* (D + T), vanadate (D + V) and *Trigonella* with vanadate (D + T + V) in different fractions of rat brains.

Fractions/Enzymes	C	D	D + I	D + T	D + V	D + T +V
Cytosol						
SOD	8·63 ± 0·5	6·5 ± 0·47^b	8·26 ± 0·48	8·0 ± 0·50	8·1 ± 0·45	8·43 ± 0·49
CAT	4·99 ± 0·27	3·0 ± 0·29^a	4·92 ± 0·23	4·07 ± 0·24	4·76 ± 0·27	4·84 ± 0·25
GPx	0·02 ± 0·002	0·043 ± 0·007^b	0·02 ± 0·002	0·026 ± 0·003	0·022 ± 0·003	0·02 ± 0·002
Microsomal						
Na^+/K^+-ATPas	2·0 ± 0·10	1·46 ± 0·08^b	2·01 ± 0·12	1·9 ± 0·08	1·95 ± 0·09	2·10 ± 0·10

Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. Fisher's *P* values are $^aP < 0{\cdot}001$ and $^bP < 0{\cdot}01$. Enzyme activities are given as U/mg protein.

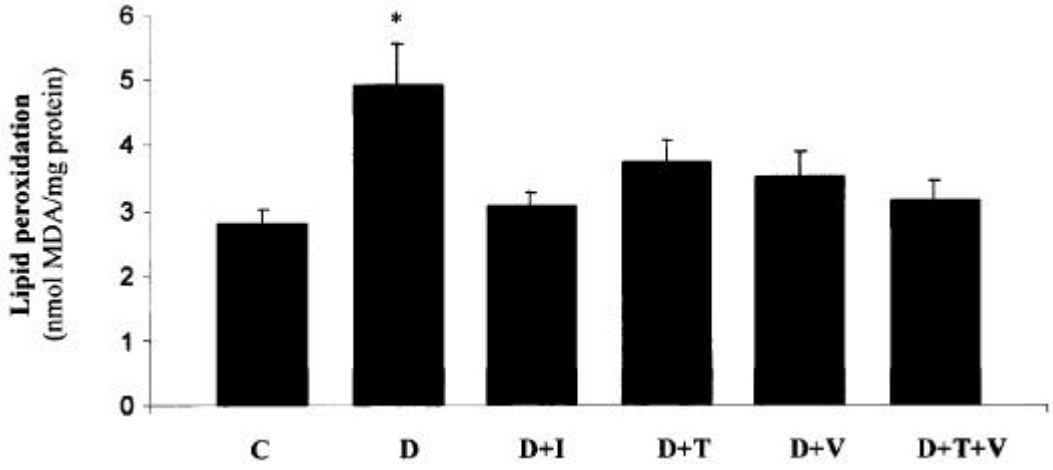

Figure 1. Changes in MDA levels in control (C), diabetic (D), diabetic rats treated with insulin (D + I), *Trigonella* (D + T), vanadate (D + V) and *Trigonella* with vanadate (D + T + V) in microsomal fractions of rat brains. Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. Fisher's *P* values are $^*P < 0{\cdot}001$.

Table 4. Polarization and anisotropy measurements in brain membrane fraction of control (C), diabetic (D), and diabetic rats treated with insulin (D + I), *Trigonella* (D + T), vanadate (D + V) and combined dose of *Trigonella* and vanadate (D + T + V).

Groups	Polarization (*P*)	Anisotropy (*r*)
C	0·237 ± 0·004	0·170 ± 0·003
D	0·274 ± 0·006[a]	0·197 ± 0·005[a]
D + I	0·240 ± 0·002	0·173 ± 0·002
D + T	0·254 ± 0·0028[b]	0·182 ± 0·002[b]
D + V	0.251 ± 0·005	0·180 ± 0·004
D + T + V	0·245 ± 0·003	0·176 ± 0·003

Each value is a mean of ± SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. Fisher's *P* values are [a]$P < 0{\cdot}01$ and [b]$P < 0{\cdot}05$.

summarized in figure 1 and table 4 respectively. In the present study, lipid peroxidation was studied and found to increase in rat brains after 21 days of diabetes induction and the results showed the significant ($P < 0{\cdot}001$) increase in brain MDA levels. The polarization (*P*) and anisotropy (*r*) values were found to be higher in 21 days diabetic group than in control group, indicating significant ($P < 0{\cdot}01$) decreases in fluidity of the membrane in the diabetic group.

Treatment of the diabetic animals with *Trigonella*, vanadate alone and with *Trigonella* reversed the above-altered parameters to normal values. The combined administration of *Trigonella* and lower dose of vanadate was found to be the most effective in controlling the diabetis-induced changes in lipid peroxidation and membrane fluidity.

4. Discussion

The present study explores the effect of experimental diabetes on different parameters like SOD, catalase, GPx, Na^+/K^+ ATPase, lipid peroxidation and fluidity of membrane lipid in rat brains, and whether the co-administration of *Trigonella* and vanadate can normalize the alterations occurred during diabetes.

Oxidative stress is the imbalance between production and removal of reactive oxygen species (ROS). Increased oxidative stress, which contributes substantially to the pathogenesis of diabetic complications, is the consequences of either enhanced ROS production or attenuated ROS scavenging capacity. Several reports have shown the alterations in the antioxidant enzymes during diabetic condition (Genet *et al* 2002; Preet *et al* 2005). The antioxidative defense system like SOD and catalase showed lower activities in brain during diabetes and the results agree well with the earlier published data (El-Missiry *et al* 2004). The decreased activities of SOD and Catalase may be a response to increased production of H_2O_2 and O_2^- by the auto oxidation of excess glucose and non-enzymatic glycation of proteins (Argano *et al* 1997). Hodgson and Fridovich (1975) and Pigleot *et al* (1990) have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The decreased activity of SOD and catalase could also be due to their decreased protein expression levels in the diabetic condition as reported recently in liver (Sindhu *et al* 2004). However, activity of GPx is enhanced in diabetic rat brains. This result is consistent with the studies of Ulusu *et al* (2003), the increased GPx activity represent a compensatory mechanism to degrade H_2O_2, which is produced in excess during the metabolism of catecholamines. Treatment of the diabetic animals with antidiabetic compounds like insulin, vanadate, *Trigonella* and combined therapy of *Trigonella* with lower dose of vanadate restored the altered activities of SOD, catalase and GPx.

Na^+/K^+ ATPase plays an important role in the functional activity of nervous cells. The present study has shown that diabetes decreased Na^+/K^+ ATPase activity in brain microsomal membrane. This is in agreement with the earlier published data (Mayanil *et al* 1982). Hyperglycemia has been shown to generate free radicals from auto-oxidation of glucose, formation of advance glycated end products (AGEs) and increased polyol pathway, with concomitant increase in cellular lipid peroxidation and damage of membrane in diabetes. One of the consequences of lipid peroxidation degenerative processes can result in enzyme activity changes. In the present study the formation of TBARS, a product of lipid peroxidation reaction, was significantly increased in diabetic brain tissues, as reported earlier (El-Missiry *et al* 2004). This increased lipid peroxides formation during diabetes disturbs the anatomical integrity of the membrane, leading to the inhibition of several membrane bound enzymes. Previously it has been reported that the inhibition of mouse cerebral Na^+/K^+ ATPase activity by ultraviolet C (UV-C) generated OH^- and a proxyl (ROO^-) radical is mediated via lipid peroxidation induced disruption of membrane integrity (Jamme *et al* 1995). The reduction in the activity of Na^+/K^+ ATPase observed in diabetic tissue may be due to the membrane peroxidative damage induced by increased lipid peroxidative status.

ROS also affect membrane-linked enzyme activity through modification of membrane fluidity because the activity of most membrane bound enzymes is regulated by the physiochemical state of their membrane lipid environment. Na^+/K^+ ATPase has also been reported to be sensitive to changes in membrane fluidity (Sutherland *et al* 1988). This enzyme shows the increased activity in response to enhanced membrane fluidity. In the present

study, polarization and anisotropy measurement of membranes from diabetic brains indicate decreased fluidity in the lipid structure, which agrees well with earlier published report (Hong *et al* 2004). The decrease in membrane fluidity of diabetic brain could be due the peroxidation of membrane phospholipids through free radicals, which is generated by persistent hyperglycemia. Treatment of the diabetic animals with *Trigonella*, vanadate and combined therapy of *Trigonella* with 0·2 mg/ml of vanadate restored the decreased activity of Na^+/K^+ ATP, increased lipid peroxides and altered membrane fluidity after 21 days of treatments.

The mechanism by which *Trigonella* exerts its effects, is still not clear. *Trigonella* may exhibit its therapeutic effects through modulation of insulin secretion. 4-Hydroxyisoleucine, an amino acid extracted and purified from *Trigonella* seed displays an insulinotropic property *in vitro* and stimulate insulin secretion (Broca *et al* 1999). Furastanol, saponins in *Trigonella* seeds increase food consumption and induce hypocholesterolemia in streptozotocin diabetic rats (Petit *et al* 1995). Diasgenin (saponin) and trigonelline (alkaloid) are found to inhibit intestinal glucose uptake in vitro (Al-Habori *et al* 2001). *Trigonella* like other antidiabetic plant extract such as *Momordica charantia* are known to rejuvenate the *b* cells in the islets of Langerhans, thus increasing the capacity of insulin secretion in type 1 diabetes (Ahmed *et al* 1998). *Trigonella* also has antioxidant properties (Genet *et al* 2002). A reduction in the production of free radicals and lipid peroxides formation by restoring the antioxidant enzymes can beneficially prevent the decreased activity of Na^+/K^+ ATPase enzyme. The beneficial effects observed in this study might also be attributed to the insulin mimetic effects of vanadate. Vanadate stimulates phosphorylation of the insulin receptor either directly by activation of the tyrosine kinase present in the beta subunit of the insulin receptor, or through its inhibitory effect on phosphotyrosyl phosphatases (Swarup *et al* 1982). Fantus *et al* (1996), have reported that vanadate exerts its insulin mimetic effects by promoting insulin receptor endocytosis and inhibiting intracellular ligand receptor degradation. Treatment of diabetic rats with vanadate (0·6 mg/ml) was more effective in normalizing the above altered parameters as compared to 5% *Trigonella* when given separately, but resulted in a significant weight loss of the diabetic treated animals. Lower doses of vanadate (0·2 mg/ml) alone did not result in weight loss when given to control rats but when administered to diabetic rats it was not very effective in reversing hyperglycemia. Therefore, the dose of vanadate was reduced without compromising with its antidiabetic potential by combining it with *Trigonella* in order to reduce its toxicity. Earlier it has been shown by Shinde *et al* (2001) that treatment with an organic complex of vanadium such as bis (maltolato) oxo-vanadium (IV) (BMOV) was effective in improving glucose homeostasis. It can be suggested that there may be some *in vivo* complex formation by vanadate with organic compounds of *Trigonella.* This combined treatment of lower dose of vanadate and *Trigonella* was found to be the most effective treatment in stabilizing the physiological parameters, antioxidative defense system and membrane-linked functions; therefore the combined therapy can be considered a better alternative to be explored further as a means of diabetic control.

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