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Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative–nitrosative stress in rats

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ABSTRACT

Intracerebroventricular (ICV) streptozotocin (STZ) has been shown to cause cognitive impairment, which is associated with increased oxidative stress in the brain of rats. In the present study, we investigated the effect of both the isoforms of vitamin E, α-tocopherol and tocotrienol against ICV STZ-induced cognitive impairment and oxidative–nitrosative stress in rats. Adult male Wistar rats were injected with ICV STZ (3 mg/kg) bilaterally. The learning and memory behavior was assessed using Morris water maze and elevated plus maze. The rats were sacrificed on day 21 and parameters of oxidative stress, nitrite levels and acetylcholinesterase activity were measured in brain homogenate. α-Tocopherol as well as tocotrienol treated groups showed significantly less cognitive impairment in both the behavioral paradigms but the effect was more potent with tocotrienol. Both isoforms of vitamin E effectively attenuated the reduction in glutathione and catalase and reduced the malonaldehyde, nitrite as well as cholinesterase activity in the brains of ICV STZ rats in a dose dependent manner. The study demonstrates the effectiveness of vitamin E isoforms, of which tocotrienol being more potent in preventing the cognitive deficits caused by ICV STZ in rats and suggests its potential in the treatment of neurodegenerative diseases such as Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive and irreversible neuropsychiatric disorder characterized by neuronal degeneration and cognitive deteriorations. The most prominent neurochemical change in Alzheimer's brain is a reduced concentration of acetylcholine in the hippocampus and neocortex, caused by degeneration of cholinergic neurons (Kristensen, 1990; Perry et al., 1999). It accounts for 50% of dementia cases (Areosa and Sherriff, 2003).

Oxidative stress, an imbalance between free radicals and antioxidant system, plays a critical role in the pathogenesis of AD (Gary et al., 2005; Butterfield, 2004). Oxidative stress can affect all classes of macromolecules (sugar, lipids, proteins, and DNA), leading inevitably to neuronal dysfunction (Polidori and Mecocci, 2002). Brain tissue contains a large amount of polyunsaturated fatty acids which are particularly vulnerable to free radical attacks (Gutteridge, 1995). Several other studies provide evidence to link neuronal damage with excessive generation of free radicals, which may be due to factors such as oxidative stress (Olanow, 1993), inflammation (Stuchbury and Munch, 2005) or abnormal proteins (Mandelkow et al., 2007). Antioxidant enzymes like glutathione peroxidase (GPx) and glutathione reductase (GR) have a prominent role in the management of oxidative stress within the cell; they convert superoxide radicals and peroxides to innocuous forms, often with the concomitant oxidation of reduced glutathione (GSH), to its oxidized form (GSSG) and recycles GSSG to GSH to maintain the antioxidant potential (Ishrat et al., 2006).

Intracerebroventricular injection of streptozotocin in subdiabetogenic dose in rats causes reduced energy metabolism/oxidative stress leading to cognitive dysfunction by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl-CoA. This ultimately results into cholinergic deficiency supported by reduced cholineacetyltransferase (ChAT) activity in hippocampus and an increased cholinesterase (ChE) activity in the brain of ICV-STZ rats (Blokland and Jolles, 1993; Sharma and Gupta, 2001a,b; Sonkusare et al., 2005).

Since oxidative damage is implicated in the etiology of neurological complications, treatment with antioxidants has been used as a therapeutic approach in various types of neurodegenerative diseases (Ahmad et al., 2005; Ansari et al., 2004). It has been observed that the use of antioxidants as well as dietary improvements with regard to the consumption of fruits and vegetables high in antioxidant activity and neuroprotective agents may decrease the risk of memory deficits of the Alzheimer's disease type (Weinstock and Shoham, 2004). Recently Coenzyme Q10 (CoQ10), a vitamin-like lipophilic antioxidant compound, has been reported to improve cognitive dysfunction and biochemical alterations in hippocampus and cerebral cortex of ICV-STZ treated rats (Ishrat et al., 2006).

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Vitamin E is one of the essential, fat-soluble nutrient that functions as an antioxidant in the human body. Burton and Ingold (1989) presented the first comprehensive review article discussing that α-tocopherol has near optimal activity as a chainbreaking antioxidant and that both the phenolic head and phytyl tails contributed to the biological properties of the vitamin E molecule. α-Tocopherol gained recognition as the most important lipophilic radical-chain-breaking antioxidant in tissues *in vivo*. Deficiency of α-tocopherol in membranes made them highly permeable and therefore vulnerable to degradation (Sen et al., 2000). Tocotrienol, another isoform of vitamin E possesses numerous functions that are not shared by α-tocopherol. At nanomolar concentrations, α-tocotrienol uniquely prevents inducible neurodegeneration by regulating specific mediators of cell death (Khanna et al., 2003; Sen et al., 2000). Micromolar amounts of tocotrienol suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis (Pearce et al., 1994; Pearce et al., 1992). Tocotrienols are thought to have more potent antioxidant properties than α-tocopherol (Serbinova et al., 1991; Serbinova and Packer, 1994).

Thus, the present study was designed to investigate the influence of both the isoforms of vitamin E i.e. α-tocopherol and tocotrienol, on learning and memory impairments induced by intracerebroventricular injection of STZ and on biochemical markers of oxidative stress and acetylcholinesterase activity, in the brains of rats with streptozotocin-induced dementia.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (320–350 g) bred in Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a 12:12 h light: dark cycle and had free access to food (Ashirwad Industries, Mohali, India) and water. Animals were acclimatized to laboratory conditions before the tests. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

2.2. Intracerebroventricular administration of streptozotocin

ICV injection of STZ was performed as described previously (Sonkusare et al., 2005). Rats were anesthetized with thiopentone (Neon Laboratories, India, 45 mg/kg, i.p.). The head was placed in position in the stereotaxic apparatus and a midline sagittal incision was made in the scalp. Following coordinates were used for ICV injection: 0.8 mm posterior to bregma, 1.5 mm lateral to saggital suture and 3.6 mm ventral from the surface of the brain. STZ (Sigma, USA) was dissolved in citrate buffer (pH 4.4) just prior to injection. The STZ group was injected bilaterally with ICV STZ (3 mg/kg) in two divided doses, on days 1 and 3. The concentration of STZ in citrate buffer was adjusted so as to deliver 10 µl of the solution. Rats in control group were given ICV injection of the same volume of citrate buffer on the first and third day as in STZ injected rat. The cut skin was sutured after second injection followed by daily application of antiseptic powder (Neosporin®). One sham group (without surgery) was also included in the study to nullify the effect of surgery, if any. Post operatively, the rats were fed with milk by oral gavage and normal pellet diet for 4 days, followed by normal pellet diet alone.

2.3. Drugs and treatment schedule

α-Tocopherol (Sigma, USA) and tocotrienol (mixture of α-, β-, γ-tocotrienol, Golden Hope Biogenic, Malaysian Palm Oil Board, Malaysia) was freshly prepared in double distilled water after triturating with 5% Tween 80 and administered by oral route from day 1 (day of 1st STZ ICV injection) to day 21. "Triturating" means to mix something (drug) having poor solubility with the help of mortar and pestle so as to form a clear solution and thus to increase its absorption upon oral administration. Streptozotocin (St. Louis, MO, USA) was prepared in citrate buffer (pH 4.4, 0.1 M). The animals were randomly divided into six experimental groups with 5–8 animals in each viz. Sham group, ICV citrate buffer injected (control), vehicle treated ICV STZ rat, α-tocopherol (100 mg/kg) and α-tocotrienol (50 and 100 mg/kg) treated ICV STZ rats. The doses employed in the present study were decided on the previous studies conducted in our laboratory (Kuhad et al., 2009).

2.4. Behavioral tests

2.4.1. Morris water maze test

Animals were tested in a spatial version of Morris water maze test (Morris et al., 1982; Tuzcu and Baydas, 2006). The apparatus consisted of a circular water tank (180 cm in diameter and 60 cm high). A platform (12.5 cm in diameter and 38 cm high) invisible to the rats, was set 2 cm below the water level inside the tank with water maintained at 28.5 ± 2 °C at a height of 40 cm. The tank was located in a large room where there were several brightly colored cues external to the maze; these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. The water maze task was carried out for five consecutive days from day 15th to day 19th. The rats received four consecutive daily training trials in the following 5 days, with each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. For each trail, each rat was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, rats were placed into the tank at the same starting point, with their heads facing the wall. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 90 s, it was guided with the help of a rod and allowed to remain on the platform for 20 s. The time to reach the platform (escape latency in seconds) was measured.

2.4.1.1. Memory consolidation test. A probe trial was performed (Tuzcu and Baydas, 2006) wherein the extent of memory consolidation was assessed. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. In the probe trial, the rat was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The total time spent in target quadrant in a time period of 90 s was recorded.

2.4.2. Elevated plus maze test

Memory acquisition and retention was tested using elevated plus maze test on days 19 and 20. The apparatus consisted of two crossed arms, one closed and the other, open. Each rat was placed on the open arm, facing outwards. The time taken by the rat to enter the closed arm in the first trial (acquisition trial) on 19th day was noted and was called as initial transfer latency. Cut-off time was fixed as 90 s and in case a rat could not find the closed arm within this period, it was gently pushed in to one of the closed arms and allowed to explore the maze for 30 s. Second trial (retention trial) was performed 24 h after the acquisition trial and retention transfer latency was noted (Sharma and Gupta, 2002; Kumar and Gupta, 2002). The retention trial latency was expressed as percentage of initial trial latency.

2.4.3. Closed field activity

Closed field activity was measured to rule out the interference of change in locomotor activity in the parameters of learning and memory. Spontaneous locomotor activity was measured on day 20 using digital photoactometer and values expressed as counts per 5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room (Sharma and Gupta, 2001a,b).

2.5. Acetylcholinesterase activity

Cholinergic dysfunction was assessed by acetylcholinesterase activity. The quantitative measurement of acetylcholinesterase levels in the whole brain homogenate were estimated according to the method of Ellman et al. (1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide and 0.10 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman reagent). The change in absorbance was measured at 412 nm for 5 min. Results were calculated using molar extinction coeffecient of chromophore (1.36×10^4 M^{-1} cm^{-1}) and expressed as percentage of control.

2.6. Assessment of oxidative stress

2.6.1. Post mitochondrial supernatant preparation

Brain samples were rinsed with ice cold saline (0.9% sodium chloride) and homogenized in chilled phosphate buffer (pH 7.4). The homogenates were centrifuged at 800 ×*g* for 5 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10,500 ×*g* for 20 min at 4 °C to get the post mitochondrial supernatant, which was used to assay lipid perioxidation, reduced glutathione, nitrite, catalase and superoxide dismutase activity.

2.6.2. Estimation of lipid peroxidation

The malondialdehyde content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances by the method of Wills (1965). Briefly, 0.5 ml of post-mitochondrial supernatant and 0.5 ml of Tris–HCl were incubated at 37 °C for 2 h. After incubation 1 ml of 10% trichloro acetic acid was added and centrifuged at 1000 ×*g* for 10 min. To 1 ml of supernatant, 1 ml of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling, 1 ml double distilled water was added and absorbance was measured at 532 nm. Thiobarbituric acid-reactive substances were quantified using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol of malondialdehyde per mg protein. Tissue protein was estimated using the Biuret method and the brain malondialdehyde content expressed as percentage of control.

2.6.3. Estimation of reduced glutathione

Reduced glutathione was assayed by the method of Jollow et al. (1974). Briefly, 1.0 ml of post-mitochondrial supernatant (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4 °C for at least 1 h and then subjected to centrifugation at 1200 ×*g* for 15 min at 4 °C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm.

2.6.4. Estimation of superoxide dismutase

Cytosolic superoxide dismutase activity was assayed by the method of Kono (1978). The assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of above mixture was taken and to it 0.05 ml of post mitochondrial supernatant and 0.05 ml of hydroxylamine hydrochloride (adjusted to pH 6.0 with NaOH) were added. The auto-oxidation of hydroxylamine was observed by measuring the change in optical density at 560 nm for 2 min at 30/60 s intervals.

2.6.5. Estimation of catalase

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml post mitochondrial supernatant (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of K min^{-1} and expressed as percentage of control.

2.7. Nitrite estimation

Nitrite was estimated in the whole brain using the Greiss reagent and served as an indicator of nitric oxide production. A measure of 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to 100 µl of post mitochondrial supernatant and absorbance was measured at 546 nm (Green et al., 1982). Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as percentage of control.

2.8. Statistical analysis

Results were expressed as mean ± S.E.M. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was considered at $p<0.05$. The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

3. Results

3.1. Behavioral observations

3.1.1. Effect on performance in Morris water maze task

The change in escape latency was observed onto a hidden platform produced by training trials. Although the latencies to reach the submerged platform decreased gradually in all the groups during 5 days of training in Morris water maze test, the mean latency (days 2–5) was significantly ($p<0.05$) prolonged in ICV STZ group, as compared to sham and control group, showing a poorer learning performance due to ICV-STZ infusion. This disrupted performance of ICV STZ group was significantly ($p<0.05$) improved by the chronic treatment with tocopherol (100 mg/kg) and tocotrienol (50 and 100 mg/kg) in a dose dependent manner (Fig. 1).

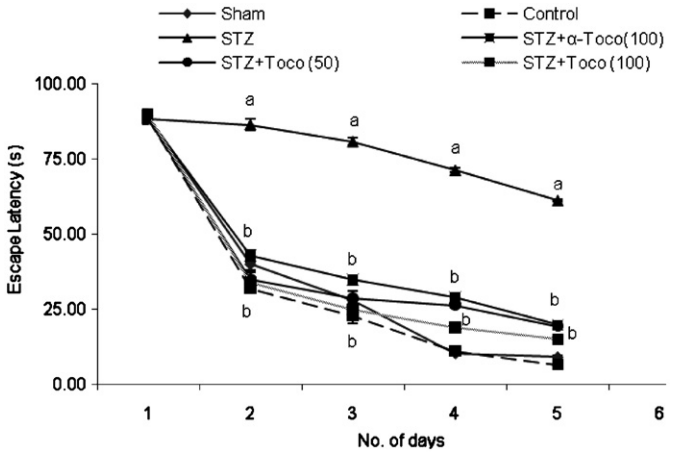

Fig. 1. Effect of α-tocopherol (α-Toco-100 mg/kg) and tocotrienol (Toco-50 and 100 mg/kg) treatment on the performance of spatial memory acquisition phase in intracerebroventricular streptozotocin treated rats. Data is expressed as mean ± S.E.M. [a]$p<0.05$, compared to control and treatment groups from second day of the training sessions; [b]$p<0.05$, compared to intracerebroventricular streptozotocin treated group (one-way ANOVA followed by Tukey's test).

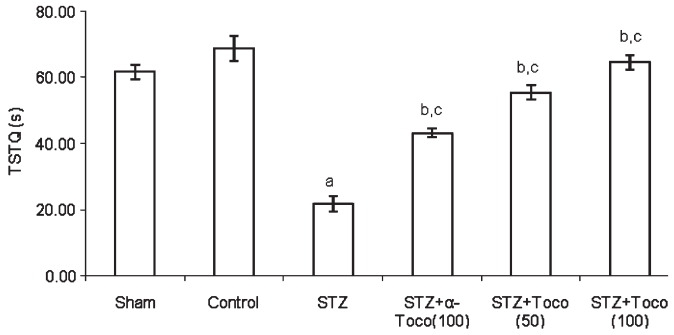

Fig. 2. Effect of α-tocopherol and tocotrienol treatment on time spent in target quadrant in which the platform had previously been located during acquisition. Both tocopherol and tocotrienol significantly inhibited streptozotocin-induced memory deficits. [a]$p<0.05$, compared to control and treatment groups; [b]$p<0.05$, compared to intracerebroventricular streptozotocin treated group; [c]$p<0.05$, compared to one another (one-way ANOVA followed by Tukey's test).

On the probe trial, with the platform removed, ICV STZ group failed to remember the precise location of the platform, spending significantly ($p<0.05$) less time (21.80 ± 2.35) in the target quadrant than sham group (61.67 ± 2.19) and control group (68.80 ± 3.72). The total time spent in the target quadrant was significantly ($p<0.05$) increased by the chronic tocopherol (100 mg/kg) and tocotrienol (50 and 100 mg/kg) treatment, 49.60 ± 2.34, 58.50 ± 2.96 and 63.00 ± 2.61 respectively as compared to ICV STZ treated rats (Fig. 2).

3.1.2. Elevated plus maze test

Initial transfer latency (ITL) did not differ significantly in any of the groups. Retention transfer latency (RTL) of sham and control group was significantly less than that of ICV STZ injected group. Treatment with tocopherol (100 mg/kg) and tocotrienol (50 and 100 mg/kg) significantly ($p<0.05$) lowered the RTL in ICV STZ injected rats signifying improvement in learning and memory (Fig. 3A). The reduction in RTL with tocotrienol (50 mg/kg) was more pronounced than with tocopherol (100 mg/kg).

3.1.3. Closed field activity

The spontaneous locomotor activity did not differ significantly between the sham, control, vehicle treated ICV STZ group and tocopherol (100 mg/kg) and tocotrienol (50 and 100 mg/kg) treated ICV STZ groups on day 20. The mean values in the sham, control vehicle treated ICV STZ and tocopherol (100 mg/kg) and tocotrienol (50 and 100 mg/kg) treated ICV STZ group were 238.67 ± 8.29, 237.67 ± 1.76, 242.75 ± 4.33, 232.4 ± 5.13 248 ± 3.54 and 237.6 ± 7.5 respectively.

3.2. Biochemical observations

3.2.1. Effect of treatment on acetylcholinesterase activity in rat brain

Acetylcholinesterase activity was increased by 2 fold in the brains of ICV STZ treated (199.2 ± 13.9) rats as compared to sham (96.41 ± 9.00) and control group (100 ± 9.5). Tocopherol (100 mg/kg) and tocotrienol (50 and 100 mg/kg) treatment dose-dependently decreased cholinesterase activity in brain of ICV STZ injected rats (Fig. 4A, $p<0.05$). However, inhibition of cholinesterase activity was more with both the doses of tocotrienol (50 and 100 mg/kg) as compared to tocopherol (100 mg/kg).

3.2.2. Effect of treatment on ICV-STZ induced changes in lipid peroxidation

Malonaldehyde (MDA) levels were increased significantly in the brain of ICV STZ treated rats as compared to sham and control group. Chronic treatment with tocopherol and tocotrienol produced a significant ($p<0.05$) and dose-dependent reduction in MDA levels in brain of STZ-treated rats (Fig. 4B).

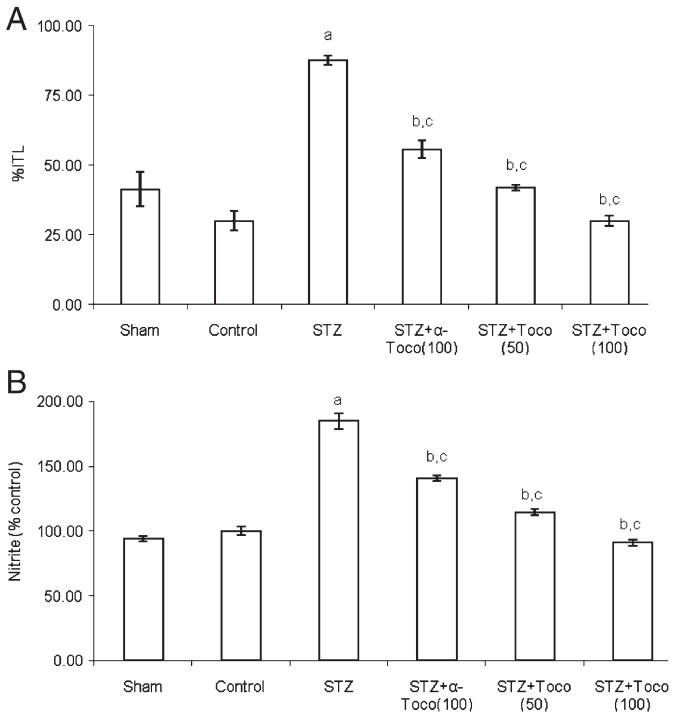

Fig. 3. Effect of α-tocopherol and tocotrienol treatment on percentage initial transfer latency (A) and nitrite (B) levels in intracerebroventricular streptozotocin treated rats. Data are expressed as mean ± S.E.M. [a]$p<0.05$, compared to control group; [b]$p<0.05$, compared to intracerebroventricular streptozotocin treated group; [c]$p<0.05$, compared to one another (one-way ANOVA followed by Tukey's test).

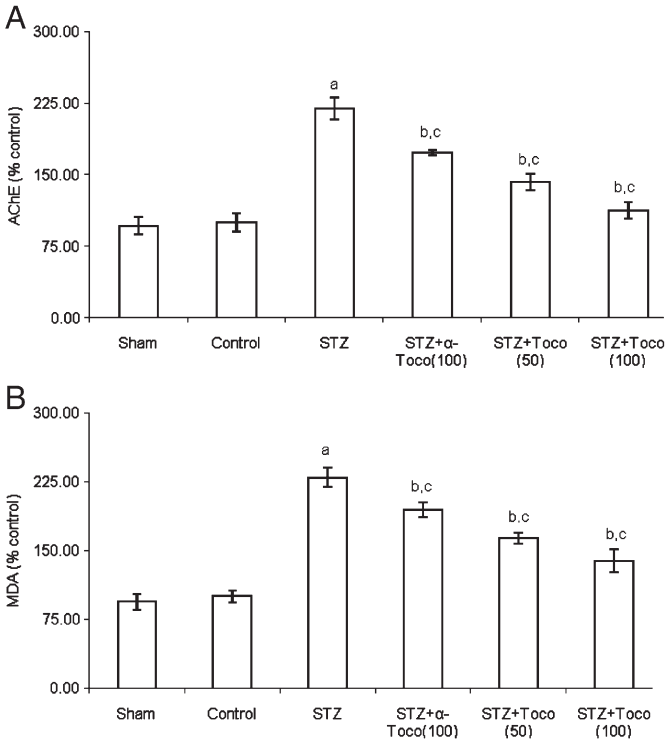

Fig. 4. Effect of α-tocopherol and tocotrienol treatment on acetylcholine esterase (A) and malonaldehyde (B) in brain of intracerebroventricular streptozotocin treated rats. Data are expressed as percentage of control. [a]$p<0.05$, compared to control group; [b]$p<0.05$, compared to intracerebroventricular streptozotocin treated group; [c]$p<0.05$, compared to one another (One-way ANOVA followed by Tukey's test).

3.2.3. Effect of treatment on antioxidant profile in the rat brain

The reduced glutathione levels and enzyme activity of superoxide dismutase and catalase significantly decreased in the brains of ICV STZ treated rats as compared to sham and control group rats. This reduction was significantly ($p<0.05$) and dose dependently improved by the treatment with tocopherol and tocotrienol in brain of STZ-treated rats (Fig. 5A, B and C).

3.2.4. Effect of treatment on ICV STZ-induced nitrosative stress

Nitrite levels were significantly elevated in brains of ICV STZ treated animals as compared to sham and control group. Tocopherol and tocotrienol treatment significantly ($p<0.05$) and dose dependently inhibited this increase in nitrite levels in brains of STZ-treated rats (Fig. 3B).

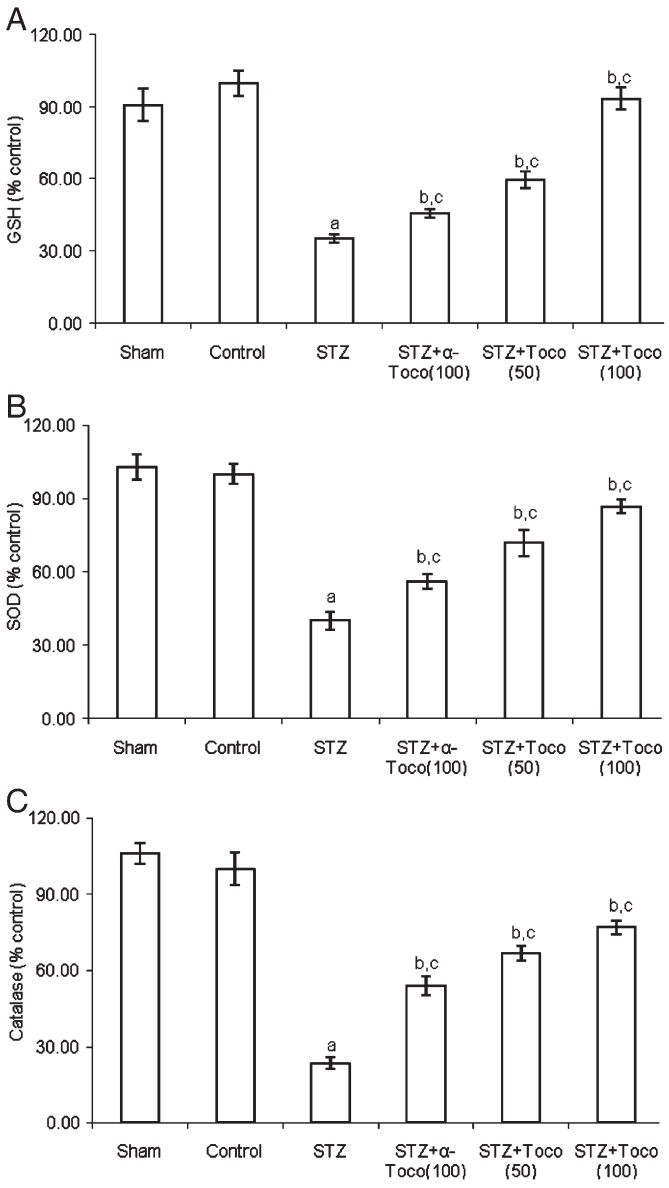

Fig. 5. Effect of α-tocopherol and tocotrienol treatment on reduced glutathione (A), superoxide dismutase (B) and catalase (C) levels in brain of intracerebroventricular streptozotocin treated rats. Data are expressed as percentage of control. [a]$p<0.05$, compared to control group; [b]$p<0.05$, compared to intracerebroventricular streptozotocin treated group; [c]$p<0.05$, compared to one another (one-way ANOVA followed by Tukey's test).

4. Discussion

Experimental research examining the antioxidant, free radical scavenging effects of tocopherol and tocotrienols has revealed that tocotrienols appear superior due to their better distribution in the fatty layers of the cell membrane (Suzuki et al., 1993). Roy et al. (2002) found that, oral tocotrienol can crosses the blood-brain barrier to reach brain tissue of rats; more so for fetal brain while pregnant mother is supplemented with tocotrienol. At nM concentrations α-tocotrienol, in contrast with α-tocopherol, protects against glutamate-induced neuronal death in mice by suppressing inducible pp60 c-src kinase activation (Sen et al., 2000) as well as by suppressing inducible 12-lipoxygenase activation (Khanna et al., 2003). α-Tocotrienol provided the most potent neuroprotection among vitamin E analogs on cultured striatal neurons of rats (Osakada et al., 2004). In our recent findings we found that tocotrienol can prevent the diabetes associated cognitive deficits by decreasing the oxidative stress and suppressing the NFkβ expression in diabetic rats (Kuhad et al., 2009).

Thus, we found it worthwhile to investigate the potential of tocopherol vs tocotrienol in preventing the streptozotocin (i.c.v) induced dementia in rats, because free radical generation is a major component of neurodegeneration along with memory impairment in this model. The ICV STZ model in rat has been described as an appropriate animal model for sporadic Alzheimer type dementia characterized by a progressive deterioration of memory, and presence of oxidative stress in the brain of rats (Sharma and Gupta, 2001a,b; Lannert and Hoyer, 1998).

In the present study, the Morris water maze and elevated plus maze test were used for the assessment of learning and memory. A decreased escape latency in Morris water maze task in repeated trials demonstrates intact learning and memory function. We measured the escape latency manually as due to some technical problems during the time period of behavioral test we were unable to use video recording software. To avoid this we did two things, first we have measured the time spent in target quadrant by the rats in the probe trial and secondly, we have measured the memory performance in elevated plus maze test also, a widely used behavioural animal model for assessment of memory. Streptozotocin (i.c.v)-treated rats did not show a significant decline in the escape latency as compared to sham group and the group administered (i.c.v) artificial CSF, whereas tocopherol and tocotrienol treatment of streptozotocin (i.c.v)-treated rats decreased the time to reach the hidden platform. In probe trial also, the time spent in target quadrant is significantly decreased in streptozotocin treated rats as compared to sham and control group, which was significantly reversed dose dependently on treatment with both the isoforms of vitamin E. The results from elevated plus maze further substantiate the findings of Morris water maze test as both the treatments reduced the increased percent initial transfer latencies after 24 h in ICV STZ treated rats. However, in both the above memory assessment paradigms, tocotrienol showed more potent activity as compared to tocopherol. The locomotor activity of sham, control, vehicle treated ICV STZ group and tocopherol and tocotrienol treated ICV STZ rats showed no significant difference. As we didn't get any difference in the locomotor activity of any group in closed field apparatus, so we conclude that it is less likely based on the locomotor data that swim speed was an issue. Moreover, we have also performed the probe trial which is less affected by swim speed.

The results from the biochemical estimation indicate the significant increase in MDA levels and marked decrease in the activity of reduced glutathione, superoxide dismutase and catalase in the brains of ICV STZ treated rats. Treatment with tocopherol and tocotrienol returned the levels of lipid peroxides, reduced glutathione, superoxide dismutase and catalase towards their control values. Again, the effect was more pronounced with tocotrienol treatment comparable with tocopherol treated ICV STZ group. Besides the enhanced level of reactive oxygen species, NO levels were also increased in the brains

of ICV STZ treated rats. Peroxynitrite, a harmful oxidant, formed by reaction between superoxide and NO, reacts with a variety of molecules, including protein and non-protein-thiols, unsaturated fatty acids and DNA, thus affecting energy conservation mechanisms and oxidative post-translation modification of protein, and ultimately causing neuronal cell death (Murray et al., 2003). Our results showed an increase in nitrite levels in the brain of ICV STZ treated rats and chronic treatment with tocopherol and tocotrienol reduced these elevated levels dose-dependently.

Acetylcholine, a neurotransmitter associated with learning and memory, is degraded by the enzyme acetylcholinesterase, terminating the physiological action of the neurotransmitter. In addition to their role in cholinergic transmission, cholinesterases may also play a role during morphogenesis and neurodegenerative diseases (Reyes et al., 1997; Layer et al., 1987). In our results, we got significant increase in acetylcholinesterase activity in streptozotocin (i.c.v)-treated rats as compared to sham and CSF (i.c.v)-treated rats, which is in accordance with the findings from Sonkusare et al. (2005) and this increase in acetylcholinesterase activity may lead to diminished cholinergic transmission due to a decrease in acetylcholine level. Both tocopherol and tocotrienol-treated streptozotocin (i.c.v) groups showed a significant decrease in elevated acetylcholinesterase activity as compared to streptozotocin (i.c.v)-treated group. The inhibition of acetylcholinesterase activity was more prominent with tocotrienol treatment as compared with tocopherol.

All above findings pointing to more potent effects (behavioral and biochemical) of tocotrienol are in agreement with the previous findings from other research groups (Sen et al., 2000; Khanna et al., 2003; Suzuki et al., 1993; Serbinova et al., 1991; Serbinova and Packer, 1994). Tocotrienol was found to be far more potent than tocopherol and the answer to this question lies in its chemical structure. Tocopherol is a molecule with single bonds present throughout the structure while tocotrienol is having a total five double bonds in its structure which makes it highly unsaturated and due to this unsaturation by double bonds, tocotrienol can efficiently penetrate into the saturated fatty layers of tissues such as brain and liver (Suzuki et al., 1993).

This suggests that antioxidant property of both the isoforms of vitamin E may be responsible for protecting against the oxidative stress, possibly by increasing the endogenous defensive capacity of the brain to combat oxidative stress induced by ICV STZ. In addition to potent antioxidant activity the suppression of nitrosative stress and acetylcholinesterase activity also contributes significantly in preventing the cognitive impairment in ICV STZ model in rats. The findings suggest the therapeutic potential of vitamin E isoforms especially tocotrienol in age related neurodegenerative disorders where oxidative stress and cognitive impairment are involved.

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