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# Melatonin alleviates memory deficits and neuronal degeneration induced by intracerebroventricular administration of streptozotocin in rats ☆

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ABSTRACT

In the present study the effect of melatonin on intracerebroventricularly administered streptozotocin (STZ)-induced neurodegeneration was investigated in rats. STZ (3 mg/kg), administered twice with an interval of 48 h between the two doses, showed impairment in spatial memory tested by water maze test after 14 days of 1st dose. Administration of melatonin (2.5, 5.0 and 10 mg/kg, i.p.) was started 1 h prior to 1st dose of STZ and continued up to 14 days. Glutathione and malondialdehyde were used as biochemical markers of oxidative stress in different brain regions. Histopathological changes were examined by using hematoxylin and eosin stain. STZ administration caused significant decrease in glutathione and increase in malondialdehyde as compared to control and artificial Cerebrospinal Fluid treated rats indicating oxidative stress. Brain sections of STZ-treated rats showed increased vacuoles in the periventricular cortical area, damaged periventricular cells and damaged cells in the hippocampal CA4 region as compared to control and artificial Cerebrospinal Fluid treated groups. Melatonin treatment significantly attenuated the effect of STZ-induced oxidative stress and histopathological changes. The results indicate that melatonin is effective in providing protection against memory deficit, oxidative stress and neuronal damage induced by STZ.


## 1. Introduction

Melatonin, the primary secretory product of the pineal gland, is a molecule with a great functional versatility. Melatonin has anti-oxidant (free radical scavenging property) which has been linked to its oncostatic, immunomodulatory, and anti-aging properties (Reiter et al., 1997). It has been reported that melatonin levels in Cerebrospinal Fluid (CSF) was significantly decreased in aged individuals with early neuropathological changes in the temporal cortex, and suggested that the decrease in CSF melatonin levels may be an early event in the development of Alzheimer's disease possibly occurring even before the clinical symptoms (Zhou et al., 2003, Feng et al., 2004). Anti-oxidant property of melatonin may play an important role in aging and Alzheimer's disease (Wu and Swabb, 2005). Melatonin is a product that plays not only a major role in the regulation of the circadian rhythms but may also exert neuroprotective effects in Alzheimer's disease (Sanchez-Hidalgo et al., 2008).

Electrophysiological studies have reported that melatonin may regulate the electrical activity of hippocampal neurons (Wang et al., 2005, Musshoff et al., 2002) and alter synaptic transmission between hippocampal neurons (El-Sherif et al., 2003). Melatonin may influence learning and memory process through the synaptic connections within the hippocampus undergoing activity dependent changes in synaptic strength including enhancements in the strength of excitatory synaptic transmission that regulate long term potentiation (Bob and Fedor-Freybergh, 2008).

Experimental studies in rodent models also demonstrated effectiveness of melatonin on memory improvement (Gonzalez-Burgos et al., 2007). In the present study, we used the intracerebroventricular (ICV) administered streptozotocin (STZ), a glucosamine derivative of nitrosourea, induced model of dementia in rats which is based on the generation of free radicals in the brain (Sharma and Gupta, 2001a,b). STZ in subdiabetogenic dose in rats causes prolonged impairment of brain glucose and energy metabolism, decreased choline acetyltransferase levels in the hippocampus, and generation of free radicals in the brain accompanied by impairment in learning and memory (Lannert and Hoyer, 1998). The previous studies also demonstrated the effectiveness of melatonin in preventing the cognitive deficits as well as the oxidative stress caused by STZ in rats (Sharma and Gupta, 2001a,b). However, potential of melatonin against histopathological changes induced by ICV injection of STZ was not evaluated. Therefore, we thought that it would be worthwhile to correlate the effect of melatonin on behavioral and biochemical markers of oxidative stress, i.e. glutathione (GSH) and malondialdehyde with histopathological changes in the brain of STZ-treated rats.

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## 2. Material and methods

### 2.1. Animals

The experiments were carried out with adult male Sprague Dawley rats weighing 250–280 g (14–16 weeks). The animals were kept in a polyacrylic cage (22.5×37.5 cm) with 5 rats per cage and maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65%) with a 12-h light and dark cycle. Food, in the form of dry pellets, and water were available ad libitum but food was not allowed during experiments (approximate 1–2 h). The animals were procured from the Laboratory Animal Services Division of the Central Drug Research Institute, Lucknow. Experiments were performed according to internationally followed ethical standards and approved by the research ethics committee of the Central Drug Research Institute and Committee for the Purpose of Control and Supervision of Experiments on Animals.

### 2.2. Drug administration

#### 2.2.1. ICV injection of STZ

The rats were anesthetized with chloral hydrate (300 mg/kg, i.p.). STZ was procured by Sigma chemicals (St Louis, Mo, USA). STZ was injected (3 mg/kg) by ICV route with the help of Digital Lab Standard Stereotaxic Instrument (Stoelting). Briefly the head of anesthetized animal was fixed in the stereotaxic frame and bilateral injection of STZ in lateral ventricle of brain was given by using the coordinates 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, and 3.6 mm ventral from the surface of the brain. Each rat was given 20 µl on each site. The volume of the injection was kept constant, as the variation in the amount of drug in each rat was minimum as the rats were of a close weight range. The same dose of STZ was repeated after 48 h of first dose. STZ was dissolved in an artificial CSF composed of 147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$ and 2.2 mM dextrose (Sharma and Gupta, 2001a,b). This solution was made freshly just before the ICV administration.

#### 2.2.2. Intraperitoneal (i.p.) administration of melatonin

Melatonin, purchased by Sigma Chemicals (St Louis, Mo, USA), was first dissolved in absolute ethanol and then diluted with 0.9% saline; the final ethanol concentration was <0.5% dissolved in normal saline (Feng et al., 2004). It was administered i.p. in doses of 2.5, 5 and 10 mg/kg daily up to 14 days. The treatment of melatonin was started 1 h prior to 1st dose of STZ.

### 2.3. Grouping

The groups ($n=10$) involved in the study were control, CSF treated group, STZ-treated group and melatonin treated STZ group. From the 14th day of ICV administration of STZ and CSF, these groups were subjected to trials on Morris water maze. On the 21st day groups were sacrificed and brains of 5 rats were used for biochemical estimations of oxidative stress parameters i.e. GSH and malondialdehyde levels in different brain regions and the brain tissue of the rest of the 5 rats were processed for histopathological studies.

### 2.4. Learning and memory studies

Learning and memory was tested after 14 days of 1st dose of STZ by the Morris water maze test in control, CSF, STZ and melatonin treated STZ groups of rats.

#### 2.4.1. Morris water maze test

The Morris water maze (Videomax, Columbus Inc. USA) consisted of a large circular black pool of 120 cm diameter, 50 cm height, filled to a depth of 30 cm with water at 26±2 °C. Four equally spaced points around the edge of the pool were designed as North, East, South and West. A black colored round platform of 8 cm diameter was placed 1 cm below the surface of water in a constant position in the middle of the North East quadrant in the pool; the starting point was in the South West quadrant in all the trials. The water was colored with non-toxic black dye to hide the location of the submerged platform. The rats could climb on the platform to escape from the necessity of swimming. Trials were given for 3 consecutive days in order to train rats in the Morris water maze. The rats were given a maximum time of 120 s (cut-off time) to find the hidden platform and were allowed to stay on it for 30 s (Qi et al., 2009). The experimenter put the rats that failed to locate the platform onto it. The animals were given a daily session of 5 trials per day. Latency time to reach the platform was recorded in each trial. Latency time of the last trial of each session is shown in the results. A significant decrease in latency time from that of the 1st session was considered as successful learning (Saxena et al., 2007).

### 2.5. Biochemical studies

Biochemical parameters of oxidative stress, malondialdehyde and GSH level were measured in the brain regions on the 21st day after STZ and artificial CSF administration. Melatonin treated STZ group was also sacrificed on the 21st day of STZ administration.

#### 2.5.1. Brain tissue preparation

The rats were decapitated under ether anesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline and dissected into the frontal cortex, striatum, hypothalamus, thalamus, hippocampus and cerebral cortex according to Glowinski and Iversen (1966). In brief, the dissection of the brain was performed from the dorsal surface as follows: first the rhombencephalon (cerebellum, medulla and pons) was separated by a transverse section from the rest of the brain. A transverse section was made at the level of the optic chiasma on the ventral surface. This section separated the cerebrum into two parts — caudal portion and rostral portion. The caudal portion was divided into five parts. First the hypothalamus was dissected by taking the anterior commissure as horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit constituting thalamus. Striatum was dissected with the external walls of the lateral ventricles as internal limits and the corpus callosum as external limit. Hippocampus was then dissected from the remaining caudal portion by opening the flaps. The remainder caudal portion was the cerebral cortex. The frontal part of the striatum was dissected separately from the rostral portion and the rest of the rostral portion constituted the frontal cortex. A 10% (w/v) homogenate of brain samples (0.03 M sodium phosphate buffer, pH 7.4) was prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm. The homogenized tissue preparation was used to measure GSH and malondialdehyde.

#### 2.5.2. Measurement of GSH

GSH was determined by its reaction with 5, 5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically at 412 nm within 15 min (Ellman, 1959). The brain homogenate was mixed with an equal amount of 10% trichloroacetic acid and centrifuged (Remi cold centrifuge) at 2000 *g* for 10 min at 4 °C. The supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5, 5′-dithiobis (2-nitrobenzoic acid) and 0.4 ml of double-distilled water were added and the mixture was shaken vigorously on vortex.

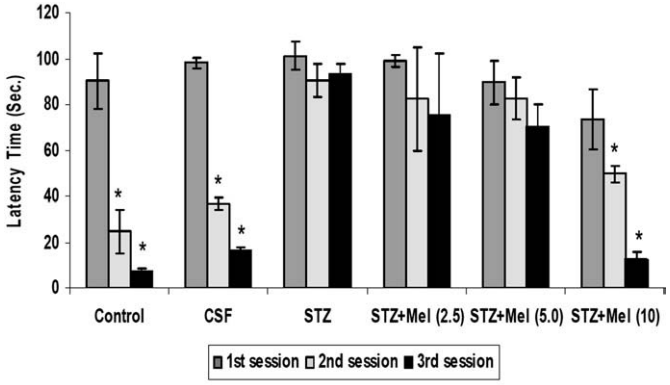


**Fig. 1.** Effect of different doses (2.5, 5 and 10 mg/kg, i.p.) of melatonin (Mel) on STZ-induced deficit in memory in Morris water maze test. *Significant difference ($P<0.001$, two-tailed) from the 1st session (Student's '*t*' test paired).

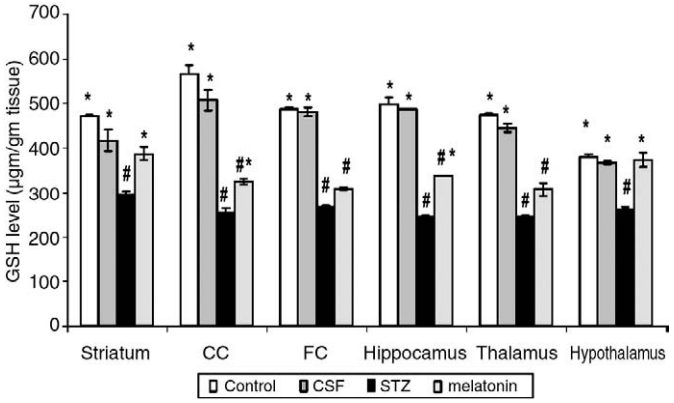


**Fig. 2.** GSH level in brain areas of control, artificial CSF, STZ and melatonin treated STZ group. *Significant difference ($P<0.001$) in GSH from STZ. (CC — cerebral cortex, FC — frontal cortex). #Significant difference ($P<0.001$) in GSH from control; one-way ANOVA followed by Tukey test.

### 2.5.3. Measurement of malondialdehyde

Malondialdehyde, which is a measure of lipid peroxidation, was measured spectrophotometrically by the method of Colado et al. (1997), using 1, 1, 3, 3-tetraethoxypropane as standard. Malondialdehyde is expressed as nmol/g tissue. To 500 µl of tissue homogenate in phosphate buffer (pH 7.4), 300 µl of 30% trichloroacetic acid, 150 µl of 5 N hydrochloric acid and 300 µl of 2% w/v 2-thiobarbituric acid were added and then the mixture was heated for 15 min at 90 °C. The mixture was centrifuged at 12,000*g* for 10 min. Pink colored supernatant was obtained, which was measured spectrophotometrically at 532 nm immediately.

### 2.5.4. Blood glucose estimation

Blood was collected by tail prick and glucose was measured by accu-check sensor comfort glucostrips (Roche Diagnostic, India) in control, CSF, STZ and melatonin treated STZ, 14 days after first dose of STZ.

### 2.6. Histopathological studies

A histopathological study in brain tissue was conducted according to Li et al. (1998). Rats were deeply anesthetized under ether anesthesia. The brain was fixed by transcardial perfusion, first with 50 ml of phosphate-buffered saline (0.02 M, pH 7.4), then with 220 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline, pH 7.4 for pre-fixation of the tissue. Then the brain tissue was dissected out carefully and was kept in 4% paraformaldehyde overnight for post-fixation. After post-fixation the tissue was dehydrated and embedded in paraffin for 4 h in infiltration unit. Block was prepared in block preparation unit (Shandon Histocenter-2) and coronal sections (10 µm) were cut with the help of a microtome (Leica RM 2255, Lab India) and picked up on poly-L-lysine coated slides. Sections from the rostral to the caudal portion of the brain were stained with hematoxylin and eosin (HE) (Li et al., 1998). Stained sections of Fig. 4 were captured (light microscopy) at ×100 magnification while the rest of the stained sections were captured at ×200 magnification by Leica Application suite V3.1.0 software. Three identical microscopic fields of each section were selected for count of dead cells in per $mm^2$ area by Leica QWin V3 software (Charriaut-Marlangue et al., 1996). Dead cells were identified morphologically by blebbing of plasma membrane, diffused pallor of eosinophilic background, alterations in size and shape of cells, vacuolation, chromatin condensation and condensed nucleus.

### 2.7. Statistical analysis

The results are expressed as mean ± S.E.M. Statistical analysis of data of Morris water maze test, biochemical values and histological data was performed by one-way ANOVA followed by Tukey test and level of significance was $P<0.05$ in all statistical evaluations.

## 3. Results

### 3.1. Morris water maze test

#### 3.1.1. Effect of different doses of melatonin on STZ-induced memory deficits in Morris water maze test

In control and CSF groups latency times in the 2nd and 3rd retention sessions were significantly lower than in the 1st session [control group: $F$ (2, 12) = 24.59, CSF group: $F$ (2, 12) = 413.49, $P<0.001$]. There was no significant change in latency to reach the platform in STZ-treated rats in retention sessions (2nd and 3rd) in comparison to the 1st session [$F$ (2, 12) = 0.8313, $P>0.05$]. Daily treatment with melatonin was started from the day of first ICV injection of STZ in three different doses i.e. 2.5 mg/kg, 5.0 mg/kg and 10 mg/kg. There was no significant decrease in latency time to reach the platform in 2.5 mg/kg [$F$ (2, 12) = 4.19, $P>0.05$] and 5 mg/kg [$F$ (2, 12) = 1.604, $P>0.05$] melatonin treated STZ group. A significant decrease in latency time from that in the 1st session was observed from the 2nd session onward following the administration of melatonin at 10 mg/kg dose [$F$ (2, 12) = 14.62, $P<0.0001$] (Fig. 1).

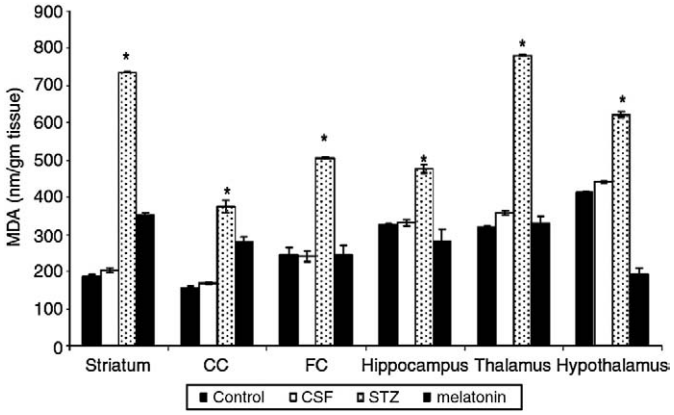


**Fig. 3.** Malondialdehyde (MDA) level in the brain areas of control, artificial CSF, STZ and melatonin treated STZ group. (CC — cerebral cortex, FC — frontal cortex). *Significant difference ($P<0.001$) in malondialdehyde (MDA) from control, CSF and melatonin treated STZ group; one-way ANOVA followed by Tukey test.

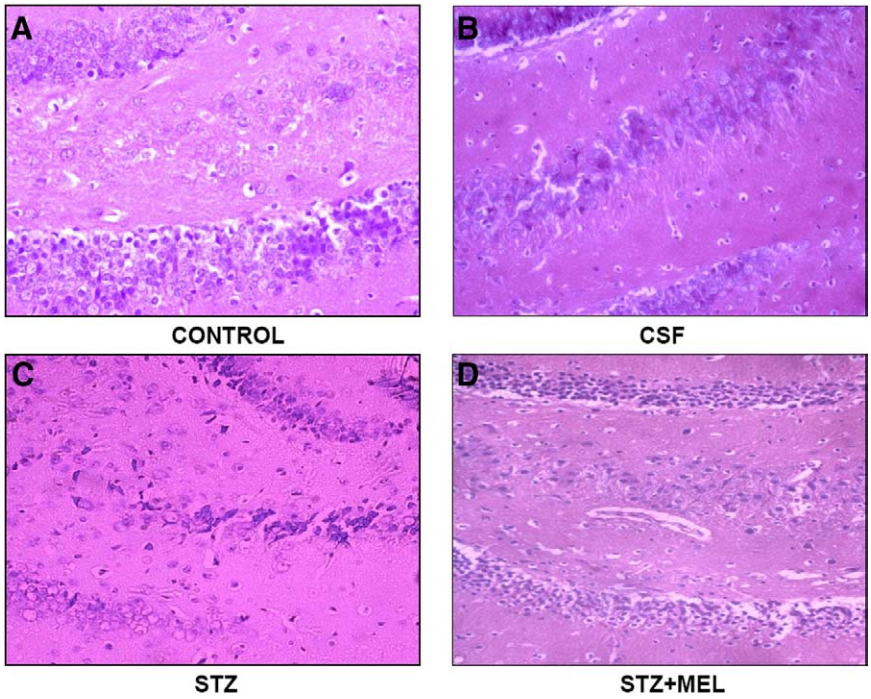


**Fig. 4.** Micrographs showing effect of melatonin on STZ-induced histopathological changes in rat hippocampal neurons (all three layers). (A) Pyramidal neurons in the CA4 subfield of the hippocampus in a control case. (B) The same hippocampal subfield in CSF. (C) There is an obvious decrease in the number and density of CA4 region neurons in STZ-treated rat. (D) The same hippocampal subfield in melatonin treated STZ case.

### *3.2. Biochemical parameters of oxidative stress in the brain*

#### *3.2.1. GSH level*

The *GSH* level (μg/g tissue) in the brain regions was measured on day 21 after the first dose of STZ. The level of GSH was significantly lower in different brain regions of STZ-treated rats from control and CSF. In melatonin treated STZ group GSH level was significantly increased from STZ-treated group in the striatum, cerebral cortex, hippocampus and hypothalamus [striatum – $F\ (3,\ 16) = 74.032$, $P < 0.001$, cerebral cortex – $F\ (3, 16) = 52.81$, $P < 0.001$, hippocampus – $F\ (3,\ 16) = 121.81$, $P < 0.001$ and hypothalamus – $F\ (3,\ 16) = 52.66$, $P < 0.001$] regions while in the frontal cortex and thalamus there

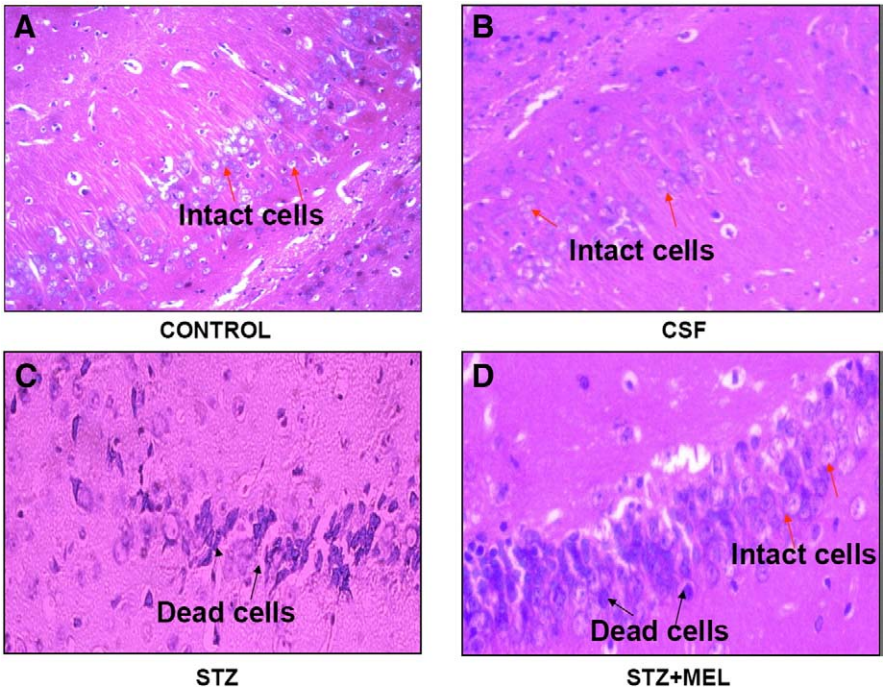


**Fig. 5.** Micrograph showing effect of STZ on neurons of the CA4 region of the hippocampus. (A) Control and (B) CSF did not reveal difference in cell numbers in the neurons of CA4 region. (C) Note: shrinkage of CA4 region in STZ-treated rat brain (black arrow in C) revealed degeneration in the hippocampus of STZ-treated rats. (D) Less degeneration is seen in melatonin treated STZ rats in the CA4 region.

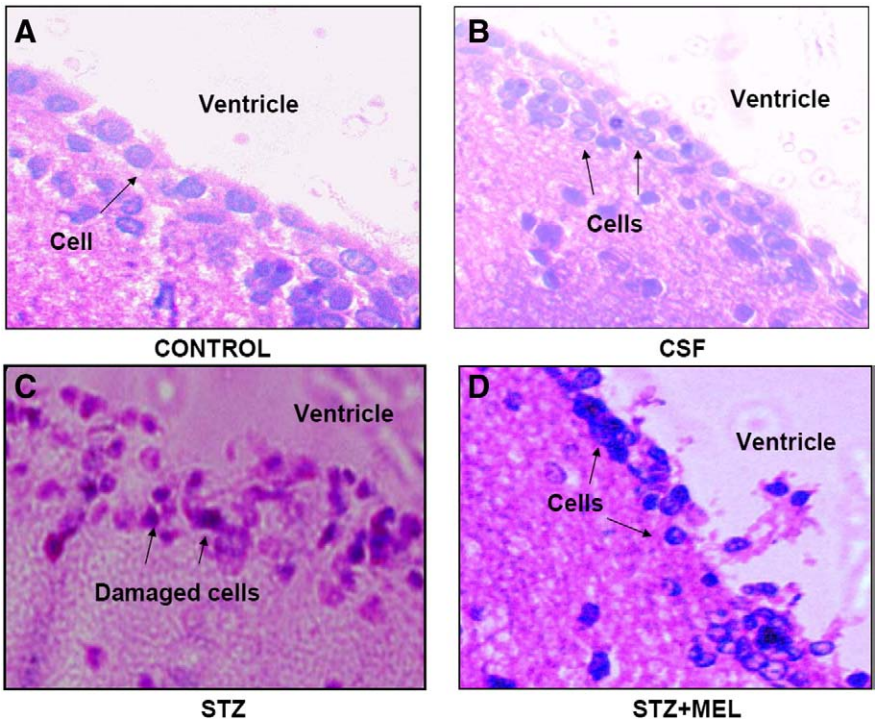


**Fig. 6.** Micrograph showing effect of STZ on periventricular neurons. (A) Control and (B) CSF did not reveal difference in morphology of periventricular cells while (C) STZ showed remarkable degeneration of periventricular neurons. (D) Melatonin treated STZ rats showed less periventricular neuronal degeneration than STZ rats.

was insignificant increase in GSH level from STZ-treated group [frontal cortex — $F$ (3, 16) = 9.49, $P > 0.001$, thalamus — $F$ (3, 16) = 8.88, $P > 0.001$] (Fig. 2).

*3.2.2. Malondialdehyde level*

The *malondialdehyde* level (nmol/g tissue) in the brain regions was measured on day 21 after the first dose of STZ. The level of *malondialdehyde* was significantly higher in different brain regions of STZ-treated rats as compared to control, CSF and melatonin treated STZ group [striatum — $F$ (3, 16) = 55.3, $P < 0.001$, cerebral cortex — $F$ (3, 16) = 72.44, $P < 0.001$, frontal cortex — $F$ (3, 16) = 58.99, $P < 0.001$, hippocampus — $F$ (3, 16) = 22.59, $P < 0.001$, thalamus — $F$ (3, 16) = 425.68, $P < 0.001$ and hypothalamus — $F$ (3, 16) = 404.88, $P < 0.001$] (Fig. 3).

*3.2.3. Blood glucose*

There was no significant difference in blood sugar level expressed in mg/dl among control (92.2 ± 4.59), and on 14 days after the first

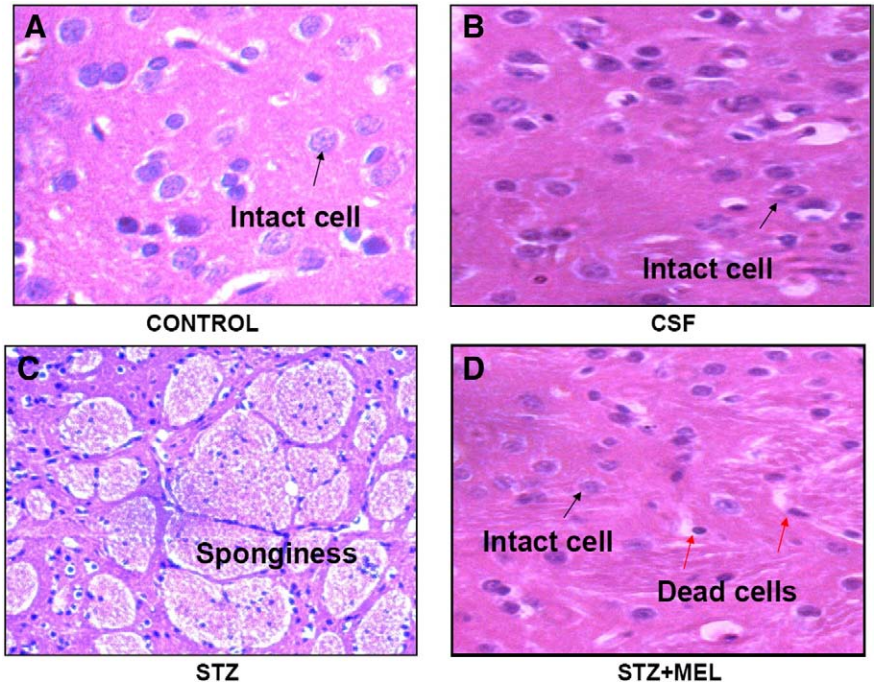


**Fig. 7.** Micrograph showing effect of STZ on periventricular cortical neurons. (A) Control and (B) CSF did not reveal difference in periventricular cortical cells while (C) STZ showed remarkable degeneration of periventricular cortical neurons. (D) Melatonin treated STZ rats showed less periventricular cortical neuronal degeneration than STZ rats.

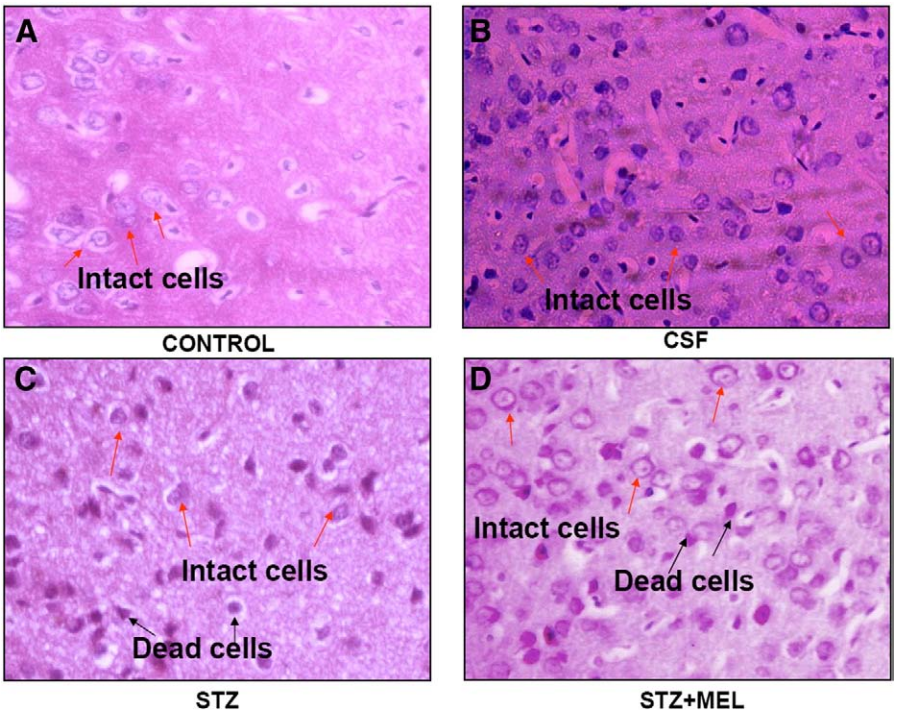


**Fig. 8.** Micrograph showing effect of STZ on deep seated cortical neurons (entorhinal cortex, subicular complex). (A) Control and (B) CSF did not reveal difference in cortical cells (red arrow showing intact cells) while (C) STZ showed degeneration in deep seated cortical neurons (black arrows showing dead cells and red arrows showing live cells). (D) Melatonin treated STZ rats showed less cortical neuronal degeneration than STZ rats. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dose of CSF (79.8 ± 5.71), STZ (82.0 ± 9.43) and STZ in melatonin pre-treated (89 ± 3.87) groups [$F$ (3, 16) = 0.86, $P$ > 0.05, Tukey test].

### 3.3. Histopathological changes

Microscopic analysis of HE stained sections in Fig. 4 showed medium magnification views of coronal sections taken from control, CSF, STZ and melatonin treated STZ rat brain sections showing all the three layers of the hippocampus. In CA1 and CA3 regions there was neuronal degeneration in STZ as compared to control, CSF and STZ-treated melatonin rats, although these changes were mild.

Neuronal density and number in CA4 region of hippocampus is markedly decreased in STZ-treated animal (C) as compared to the control (A) and CSF (B). Similar changes with much less intensity are seen in the melatonin treated group (D). Quantitative analysis of cell counts in CA4 region showed a significant reduction in the number of cells of the lesioned rats compared to control [$F$ (3, 56) = 232.08, $P$ < 0.001] (Fig. 5).

The periventricular region showed a decrease in number of neurons and ependymal cells lining the ventricle in the STZ-treated group (C) in comparison to control (A) and CSF (B). Melatonin treated group showed a less degree of similar changes (D) [$F$ (3, 56) = 112.25, $P$ < 0.001] (Fig. 6).

Control (A) and CSF (B) did not reveal any remarkable difference while STZ (C) showed marked degeneration of neurons. Sponginess is also seen in periventricular cortical region due to degeneration. The changes were less in melatonin treated rats (D) (Fig. 7).

In deep seated cortical region (subicular region) (×200) control (A) and CSF (B) did not reveal any remarkable difference while STZ showed degeneration of neurons (black arrows) (C). The changes were less in melatonin treated rats (D) [$F$ (3, 56) = 88.389, $P$ < 0.001] (Fig. 8). The quantitative comparison in terms of dead cell counts per mm$^2$ of different brain regions is depicted in Fig. 9.

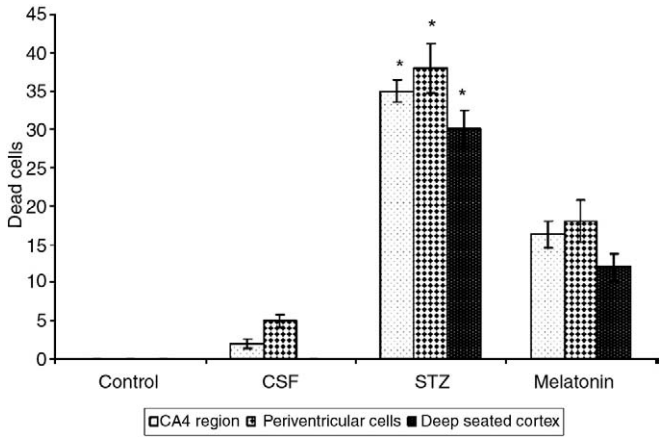


**Fig. 9.** Dead cell counts in the brain of control, artificial CSF, STZ and melatonin treated STZ group. *Significant difference ($P$ < 0.001) of dead cells from control, CSF and melatonin treated STZ group; one-way ANOVA followed by Tukey test.

## 4. Discussion

The present study was designed to provide histopathological evidence for neuroptotective activity of melatonin along with memory improvement and anti-oxidant effect against neuronal damage caused by STZ given by ICV route in brain areas. Memory deficit induced by STZ in Morris water maze test in rats is a commonly used rodent model of dementia. STZ causes neuronal damage in the brain by producing free radicals, thereby inducing the state of oxidative stress, impairment of glucose utilization and demyelination (Sharma and Gupta, 2002; Shoham et al., 2003).

STZ showed a persistent significant deficit in Morris water maze tests after 14 days of the first dose without altering blood glucose level. Administration of artificial CSF by ICV in a manner similar to

STZ did not hinder the learning that rules out the possibility of interference by procedures adopted during ICV administration on learning activity. These results of STZ memory deficits are in conformity with other workers who have demonstrated cognitive impairment after STZ in rats (Blokland and Jolles, 1993; Lannert and Hoyer, 1998). Melatonin pre-treatment showed a dose related reversal of STZ-induced memory deficits as demonstrated by a significant decrease in the latency time in reaching the platform in Morris water maze test. The most effective dose of melatonin (10 mg/kg) was used in subsequent biochemical and histopathological studies.

Several studies suggest that oxidative stress plays an important role in pathogenesis of neurodegenerative disorder like Alzheimer's disease (Zeevalk et al., 1998; Guela, 1998). GSH is an important antioxidant component of the cellular detoxification of free radicals. Malondialdehyde is a biochemical marker of lipid peroxidation. Results of oxidative stress parameters malondialdehyde and GSH levels in different brain regions are in conformity with other workers who have demonstrated that STZ caused neuronal degeneration by exerting its effect by producing free radicals (Shoham et al., 2003). Decrease in GSH and increase in malondialdehyde indicates a production of free radicals. In the present study STZ-treated rats showed significant lower level of GSH which was significantly increased following the melatonin treatment in all the areas except frontal cortex and thalamus. Melatonin brought down malondialdehyde level in STZ-treated group to that extent where there was no significant difference from control as well as CSF treated group. Thus melatonin pre-treatment countered the STZ-induced oxidative stress and promotes neuroprotection via direct and indirect mechanisms in numerous in vitro or in vivo neural injury systems (Feng et al., 2004).

The effect of melatonin was also seen on histopathological changes in brain areas of rats treated with STZ. The histopathological changes were examined by using HE stain in sequential brain sections to confirm the extent of damage induced by STZ. Brain sections of STZ-treated rats stained by HE staining showed increased vacuoles in cortical area, damaged periventricular cells, and disorganization of hippocampus as compared to control and CSF treated groups. STZ caused severe damage to the periventricular cells and cortex. In the periventricular cortex of STZ-treated rats, sponginess can be seen clearly but melatonin treatment prevents the damage to some extent. In the deeper cortical regions the extent of damage was found less than the periventricular cortical region in STZ-treated rat brain and at this level melatonin treatment showed that the morphology of cells looks like the morphology of control and CSF treated ones.

STZ caused damage to the hippocampal CA4 region cells and the morphology of cells in this particular region was also changed in comparison to that of control and CSF treated ones. Melatonin treatment again prevents the damage and showed less extent of damage than STZ-treated rats in hippocampal CA4 region cells. These histopathological changes following STZ administration indicate neuronal degeneration in the hippocampal region of the brain, which is mainly involved in memory regulation. Therefore, on the basis of the present findings we may suggest that these degenerative changes in the hippocampal region caused by STZ may be an important factor for disrupted memory functions.

In conclusion, melatonin proffered a salutary effect against memory deficit, oxidative stress and histopathological changes in rat brain areas caused by STZ, which is commonly used as rodent model of dementia. Thus melatonin emerges as a potential neuroprotective drug for treatment of dementia disorders because it may also interfere with progress of ongoing neurodegeneration processes.

## Acknowledgement

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## References

Blokland A, Jolles J. Spatial learning deficit and reduced hippocampal ChAT activity in rat after an ICV injection of streptozotocin. Pharmacol Biochem Behav 1993;44:491–4.
Bob P, Fedor-Freybergh P. Melatonin, consciousness, and traumatic stress. J Pineal Res 2008;44:341–7.
Charriaut-Marlangue C, Margaill I, Represa A, Popovici T, Plotkine M, Ben-Ari Y. Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. J Cereb Blood Flow Metab 1996;16:186–94.
Colado MI, O'shea E, Granados R, Misra A, Murray TK, Green AR. A study of the neurotoxic effect of MDMA ('ecstasy') on 5-HT neurons in the brains of mothers and neonates following administration of the drug during pregnancy. Br J Pharmacol 1997;121: 827–33.
Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959;82:70–7.
El-Sherif Y, Tesoriero J, Hogan MV, Wieraszko A. Melatonin regulates neuronal plasticity in the hippocampus. J Neurosci Res 2003;72:454–60.
Feng Z, Cheng Y, Yong C, Bin-Ling Z, Zhi-Wei Q, Chuan Q, et al. Long-term effects melatonin or 17b-estradiol on improving spatial memory performance in cognitively impaired, ovariectomized adult rats. J Pineal Res 2004;37:198–206.
Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain: I. The disposition of [3H] norepinephrine, [3H] dopamine and [3H] DOPA in various regions of the brain. J Neurochem 1966;13:655–70.
Gonzalez-Burgos I, Letechipia-Vallejo G, Lopez-Loeza E, Morali G, Cervantes M. Long term study of dendritic spines from hippocampal CA1 pyramidal cells, after neuroprotective melatonin treatment following global cerebral ischemia in rats. Neurosci Lett 2007;423:162–6.
Guela C. Abnormalities of neural circuitry in Alzheimer's disease. Neurol 1998;51.
Lannert H, Hoyer S. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behav Neurosci 1998;112:1199–208.
Li Y, Powers C, Jiang N, Chopp M. Intact, injured, necrotic and apoptotic cells after focal cerebral ischemia in the rat. J Neurol Sci 1998;156:119–32.
Musshoff U, Riewenherm D, Berger E, Fauteck JD, Speckmann EJ. Melatonin receptors in rat hippocampus: molecular and functional investigations. Hippocamp 2002;12: 165–73.
Qi D, Zhu Y, Wen L, Liu Q, Qiao H. Ginsenoside Rg1 restores the impairment of learning induced by chronic morphine administration in rats. J Psychopharmacol 2008;23: 74–83.
Reiter RJ, Tang L, Garcia JJ. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 1997;60:2255–71.
Sanchez-Hidalgo M, Guerrero Montavez JM, Carrascosa-Salmoral MP, Naranjo Gutierrez MC, Lardone PJ, de la Lastra Romero CA. Decreased MT1 and MT2 melatonin receptor expression in extrapineal tissues of the rat during physiological aging. J Pineal Res 2009.
Saxena G, Singh SP, Pal R, Singh S, Pratap R, Nath C. Gugulipid, an extract of *Commiphora whighitii* with lipid-lowering properties, has protective effects against streptozotocin induced memory deficits in mice. Pharmacol Biochem Behav 2007;86: 797–805.
Sharma M, Gupta YK. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sci 2001a;68:1021–9.
Sharma M, Gupta YK. Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. Pharmacol Biochem Behav 2001b;70:325–31.
Sharma M, Gupta YK. Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. Life Sci 2002;7:2489–98.
Shoham S, Bejar C, Kovalev E, Weinstock M. Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. Exp Neurol 2003;184:1043–52.
Wang LM, Sulthana NA, Chaudhury D. Melatonin inhibits hippocampal long-term potentiation. Eur J NeuroSci 2005;22:2231–7.
Wu YH, Swabb DF. The human pineal gland and melatonin in aging and Alzheimer's disease. J Pineal Res 2005;38:145–52.
Zeevalk GD, Bernard LP, Niklas WJ. Role of oxidative stress and the glutathione system in loss of dopamine due to impairment of energy metabolism. J Neurochem 1998;70:1421–30.
Zhou JN, Liu RY, Kamphrost W, Hofman MA, Swaab DF. Early neuropathological Alzheimer's changes in aged individuals are accompanied by decreased cerebrospinal fluid melatonin levels. J Pineal Res 2003;35:125–30.