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Research Paper

Anti-inflammatory Potential of Dapsone Loaded Chitosan Nanoparticles in Streptozotocin-Induced Experimental Dementia

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ABSTRACT: The potential of dapsone loaded characterized chitosan nanoparticles (CSNP) as a targeted delivery was investigated in memory deficits associated with dementia of Alzheimer disease type. Streptozotocin (STZ) in two doses (3 mg/kg on 1st and 3rd day) via intracerebroventricular route was used to induce dementia in swiss albino mice. The results showed that administration of STZ significantly impaired learning and memory based on Morris water-maze (MWM) test and raised myeloperoxidase (MPO) level along with neutrophils infiltration density (based on brain myeloperoxidase activity along with histological studies). Dapsone (1 mg/kg & 2 mg/kg for 11 days) loaded CSNP significantly attenuated STZ induced memory impairment as well as brain MPO activity along with increased neutrophils infiltration.

KEYWORDS: Chitosan nanoparticles; dementia; streptozotocin; dapsone; myeloperoxidase activity

Introduction

Several causes of memory impairment are known out of which more than 50% accounts for Alzheimer's disease (AD) (Maslow 2010; Brookmeyer et al., 2007; Liana and Paul 2008). The identification of compounds that have disease modifying properties along with targeted delivery system is an active area of research to alter the progression of AD (Jeffrey 2009). Cholinesterase inhibitors employed for AD therapy are only modestly effective, as earlier stages of AD might not be characterized by impaired function of the cholinergic system (DeKosky et al., 2002). Advances in understanding of the etiologies and pathogenesis of AD highlights that inflammatory changes play an important role. (Fuller et al., 2010; Antero et al., 2009; Camerib and Landreth 2010; McGeer and McGeer 2003; Magdalena et al., 2006; Michael and Kerry 2007; Leonel et al., 2008). Anti-inflammatory therapy has attracted much attention as a strategy for reducing the risk or slowing the progression of AD (Aisen 2002; McGeer and McGeer 2010; McGeer and McGeer 2007; Szekely et al., 2004; Curtis et al., 2009), as these proved effective at an early stage of AD (McGeer et al., 1996; Veld et al., 2001).

Several reports indicated association of lower prevalence of AD, in aged leprosy patients with the anti-inflammatory action of dapsone (Zhu et al., 2001; Kettle and Winterbourn 1991; McGeer et al., 1992; Chui et al., 1994; Cgui et al., 1994). Further dapsone has been demonstrated to be an irreversible inhibitor of myeloperoxidase (Kettle and Winterbourn 1991), interfere with neutrophil chemotactic migration (Harvath et al., 1986), suppression of neutrophil recruitment (Debol et al., 1997), inhibition of eosinophil peroxidase activity (Debol et al., 1992) and inhibition of 5-lipoxygenase products generation (Wozel and Lehmann 1995). However, the preventive effect of dapsone in Alzheimer's disease is still controversial probably due to poor ability to reach target site in sufficient concentration at the employed doses (Mannila et al., 2005; Goto et al., 1995; Masumi et al., 1999; Guenther et al., 2001).

Chitosan (a natural cationic polysaccharide) accumulation in neuronal cells, more preferably in pyramidal neurons of the hippocampus, as well as accumulation at extracellular structure (preferably at

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structures rich in proteins such as β-amyloid plaque) in the Alzheimer's pathology has been reported (Katja et al., 2006). Polymeric nanoparticles have shown potential in the investigation of AD as they are capable of opening tight junctions crossing the blood brain barrier (Zhuang et al., 2001), high drug loading capacities and targeting towards the mutagenic proteins of Alzheimer's (Huang et al., 1999; Ritchie et al., 1999). In this direction, chitosan nanoparticles (CSNP) are preferred due to their extended circulation time in the blood and decreased uptake by the reticuloendothelial system (Soni et al., 2005).

To our knowledge, dapsone loaded CSNP has not been exploited as therapeutic approach to reduce progression of dementia of AD type. Therefore, it is desirable to investigate the potential of dapsone loaded CSNP, in STZ induced memory deficits associated dementia of AD type.

Materials and Methods

Materials

Dapsone was a gift from Atul Ltd. (Vadodara, Gujrat, India). Chitosan (MW = 250 KDa; degree of deacetylation = 80%) was obtained as a gift sample from India Sea Foods (Cochin, India). Polysorbate 80 (Tween 80) was purchased from Spectrochem Pvt. Ltd., Mumbai, India. Acetonitrile, HPLC grade was purchased from Ranbaxy Fine Chemicals Ltd. (New Delhi, India). All other chemicals were of analytical grade and were used without further purification.

Preparation of CSNP

CSNP were prepared by the precipitation / coacervation method with slight modification (Berthold et al., 1996). Chitosan (0.15%) was dissolved in aqueous solution of acetic acid (2% v/v) containing 1% polysorbate 80. A solution of sodium sulfate (10 % w/v) was added drop wise (5 ml/min) during stirring with a blade stirrer at 1000 rpm followed by ultrasonication. After addition of sodium sulfate, stirring and sonication were continued for another one hour. The nanoparticles were separated by centrifugation for 15-20 min at 3500 rpm. The obtained sediment was then suspended in water and again centrifuged. Finally, purified nanoparticles were lyophilized (Roy et al., 1999; Van der Lubben et al., 2001; Borges et al., 2005).

Physicochemical characterization of nanoparticles

Particle size and polydispersity index

Measurements of particle size and polydispersity index were performed by photon correlation spectroscopy also known as dynamic light scattering using a Zetasizer® 3000 (Malvern instruments, UK). The size measurement was performed at 25ºC at a 90º scattering angle, and it was recorded for 180 s for each measurement. All the samples were diluted with ultra purified water before the analysis. For each sample, the mean diameter was determined in triplicate. The mean hydrodynamic diameter was generated by cumulative analysis.

Morphology

The morphological examination of nanoparticles was performed by transmission electron microscopy (TEM) (Tecnai 20 G2 S TWIN at IIT, Roorkee) set at 200 kV. Nanoparticles suspension was placed on copper electron microscopy grids (Formvar filmed), allowed to sit for a few minutes, air-dried and then viewed.

Electron diffraction pattern

The arrangement of components and orientation of molecules within the nanoparticles can determine its behavior and stability and for this purpose selected area electron diffraction (Tecnai 20 G2 S TWIN at IIT, Roorkee; set at 200 kV) was obtained.

Surface charge determination

Nanoparticles were characterized with zeta potential using a Zetasizer® 3000 (Malvern instruments, UK). The zeta potential measurements were performed by using an aqueous dip cell in an automatic mode. Samples were diluted in ultra purified water and placed in the capillary measurement cell, with the cell position adjusted.

Dapsone loading

Suspension of nanoparticles was placed in an ultrasound bath for 30 min prior to loading in order to disaggregate the particles and for better loading efficiency. Drug loading was carried out after the formation of nanoparticles by soaking the preformed particles with solution of dapsone in acetonitrile-water mixture for a period of 48 hrs using orbital shaker incubator (Kumbar et al., 2002; Agnihotri et al., 2004). Different batches employing different ratios (Batch A, 1:1; Batch B, 1:3; Batch C, 1:7) of dapsone and polymeric nanoparticles were employed for dapsone loading in order to determine the dapsone release characteristics.

Evaluation of association efficiency

The dapsone loaded nanoparticles were centrifuged at a speed of 3500-4000 rpm for 20 min and the supernatant was assayed for non-bound drug concentration by spectrofluorimeter (Li et al., 2002). Loading efficiency or association efficiency was then calculated as follows:

Loading efficiency *% = {Total amount of drug-Non-bound drug/Total amount of drug}* x *100*

In-vitro release studies

Dapsone release from the chitosan nanoparticles was performed in phosphate-buffered saline (pH 7.0) (Mannila et al., 2005; Chougule et al., 2008). The release pattern was assessed using spectrofluorimeter for a period of 12 hrs. 40 mg of the lyophilized nanoparticles were dissolved in freshly prepared 100 ml release media previously equilibrated at 37^{o} C and placed in a shaker bath adjusted to 100 rpm. At preselected time intervals, suitable amount of sample was withdrawn and centrifuged at 4000 rpm for 15 min. The supernatant was then analyzed for amount of drug released from the nanoparticles.

In-vivo studies

Animals

Male swiss albino mice (20-30 gm; procured from PAU, Ludhiana, India) were employed in the present study and housed in animal house with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd, Mumbai, India). The mice were exposed to 12 hrs light and 12 hrs dark cycles. The experiments were conducted between 09.00 to 18.00 hrs in a semi sound-proof laboratory. The animals were acclimatized to the laboratory condition five days prior to behavioral study. The protocol of the study was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was taken as per Committee for the purpose of Control and Supervision of Experiments on Animals.

Drugs and reagents

All the drug solutions and suspensions were freshly prepared before use. Dapsone was suspended in 1% w/v of sodium carboxy methyl cellulose (CMC). Nanoparticles were suspended in distilled water and streptozotocin (STZ) was dissolved in artificial cerebro spinal fluid (CSF).

Intracerebroventricular (ICV) administration of streptozotocin (STZ)

Male Swiss albino mice weighing 25–30 gm were anesthetized with anesthetic ether (Haley and McCormick 1957). A polyethylene tube was placed around (except 3 mm of tip region) of hypodermic needle of 0.4 mm external diameter and it was attached to a 10 µl Hamilititon microlitre syringe (Top Syringe, Mumbai, India) which was inserted perpendicularly through the skull (not more than 3 mm) into the brain of mouse. The injection site was 1 mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into right or left ventricle randomly. The STZ (3 mg/kg) was given ICV injection bilaterally in two divided doses, on the first and the third day. The concentration was adjusted so as to deliver 10 µl at a site. Control group mice were given ICV injection of artificial cerebrospinal fluid (CSF) (Sakurada et al., 1999).

Morris water-maze test

Morris water-maze (MWM) test was employed to assess learning and memory of the animals. MWM is a swimming based model where the animal learns to escape on to a hidden platform (Morris 1984; Parle and Singh 2004; Sharma et al., 2008; Sharma et al., 2008). It consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1° C). The water was made opaque with white colored non-toxic dye. The tank was divided into four equal quadrants with help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm²), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with inter-trial gap of 5 min. The mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 seconds to locate submerged platform. Then, it was allowed to stay on the platform for 20 s. If it failed to find the platform within 120 s, it was guided gently onto platform and allowed to remain there for 20 s. Day 4 escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning. Animal was subjected to training trials for four consecutive days, the starting poison was changed with each exposure as mentioned below and target quadrant (Q 4) remained constant throughout the training period.

Day1	Q1	Q2	Q3	Q4
Day2	Q2	Q3	Q4	Q1
Day3	Q3	Q4	Q1	Q2
Day4	Q4	Q1	Q2	Q3

On fifth day, platform was removed and each mouse was allowed to explore the pool for 120 s. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrant searching for the hidden platform was noted as index of retrieval.

The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study. All the trials were completed between 09.00 to 17.00 hrs.

Collection of samples

Animals were sacrificed by cervical dislocation, brains were removed then they were homogenized in phosphate buffer (pH 7.4). The homogenate was then centrifuged at 3000 rpm for 15 min. The supernatant of homogenate was than used for biochemical estimation.

Myeloperoxidase (MPO) activity

Myeloperoxidase assay was carried out as a marker of inflammation, according to method described by (Jagtap et al., 2004).

Histopathological studies

Brains were removed and fixed in 10% buffered formalin solution pH 7.2. Coronal portions were cut, embedded in paraffin and sections of 4 µm were made. This was followed by staining with hematoxylin and eosin to assess the extent of inflammatory infiltrates. The extent of neutrophilic infiltration was quantitated histologically according to the method described by (Wong et al., 2005) using Olympus optical microscope (DX-31) (Olympus Corporation, Tokyo, Japan) by analyzing images obtained from 15 consecutive fields per section of the brain sample under 400 × magnification. The numbers of neutrophils obtained from the consecutive fields were added together, and the density of the neutrophils expressed as the mean value per mm^2.

Experimental protocol

Twelve groups of mice, each group comprising of seven mice were employed in the present study.

Group I (Control group)

Mice were administered normal saline (10 ml/kg s. c.) 30 min before acquisition trials conducted from day 1 to day 4 and 30 min before retrieval trail conducted on day 5.

Group II (CMC treated control group)

Mice were administered 1% w/v CMC, (10 ml/kg s. c.) daily for 7 days and then subjected to MWM test. The vehicle was also administered 45 min before acquisition trial conducted from day 1 to day 4 and before retrieval trial conducted on day 5.

Group III (ICV CSF treated control group)

Mice were injected artificial CSF (25 mg/ml, 10 µl, ICV) in two dosage schedules that is on first and on third day followed by exposure to MWM test after 14 days.

Group IV (ICV STZ treated group)

Mice were injected STZ (3 mg/kg, 10 µl, ICV) in two dosage schedules that is on first and on third day followed by exposure to MWM test after 14 days.

Group V (Dapsone per se group)

Mice were administered dapsone (1 mg/kg s. c.) daily for 7 days and then subjected to MWM test, the administration of dapsone was also continued during acquisition trail. On day 5, the animals were administered vehicle only, before retrieval trial.

Group VI (Dapsone per se group)

Mice were administered dapsone (2 mg/kg s. c.) daily for 7 days and rest of the procedure was same as described for group V.

Group VII (Dapsone nanoparticles per se group)

Mice were administered dapsone nanoparticles (1 mg/kg s.c.) daily for 7 days and rest of the procedure was same as described for group V.

Group VIII (Dapsone nanoparticles per se group)

Mice were administered dapsone nanoparticles (2 mg/kg s. c.) daily for 7 days and rest of the procedure was same as described for group V.

Group IX (Dapsone + ICV STZ treated group)

ICV STZ mice were administered dapsone (1 mg/kg s.c.) starting after seven days from the second dose of STZ, daily for 7 days and again next for four consecutive days (day 1 to day 4, 30 min before) during acquisition trials. On day 5, the mice were administered vehicle only, before retrieval trial.

Group X (Dapsone + ICV STZ treated group)

ICV STZ mice were administered dapsone (2 mg/kg s.c.) starting after seven days from the second dose of STZ, daily for 7 days and rest of the procedure was same as described for group IX.

Group XI (Dapsone nanoparticles + ICV STZ treated group)

ICV STZ mice were administered dapsone nanoparticles (1 mg/kg s.c.) starting after seven days from the second dose of STZ, daily for 7 days and rest of the procedure was same as described for group IX.

Group XII (Dapsone nanoparticles + ICV STZ treated group)

ICV STZ mice were administered dapsone nanoparticles (2 mg/kg s.c.) starting after seven days from the second dose of STZ, daily for 7 days and rest of the procedure was same as described for group IX.

Statistical analysis

All results were expressed as mean ± standard error mean (SEM). Data were analyzed using one-way ANOVA followed by *post hoc* Tukey's multiple range test using Sigma Stat Statistical Software, version 2.0. $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Physicochemical characterization of nanoparticles

Particle size distribution, morphology and Surface charge determination

The precipitation of chitosan with sodium sulfate using ultrasounds for homogenization led to the formation of nanoparticles with the mean hydrodynamic diameter of 250 nm (Fig. 1). The zeta potential of the nanoparticles was found + 21.4 mV, which indicates stability of particle suspensions in water and in several buffer systems (Fig. 2). TEM photograph revealed the spherical particles of size 200 nm (Fig. 3). The results shown an acceptable range proving them to be physically stable and have homogeneous *in-vivo* distribution (R. Bodmeier et al., 1996).

Evaluation of association efficiency and in-vitro release studies

Different batches of drug: chitosan nanoparticles were prepared so as to obtain the best batch in terms of sustained release. Association efficiency of batch A was found to be *≈ 33%, of batch B was 48% and 72% for batch C* (Fig. 4). A drug/chitosan nanoparticles ratio of 1:3 (Batch B) and especially of 1:7 (Batch C) exhibits a significant decrease in drug release as compared to batch A (1:1) (Fig. 5).

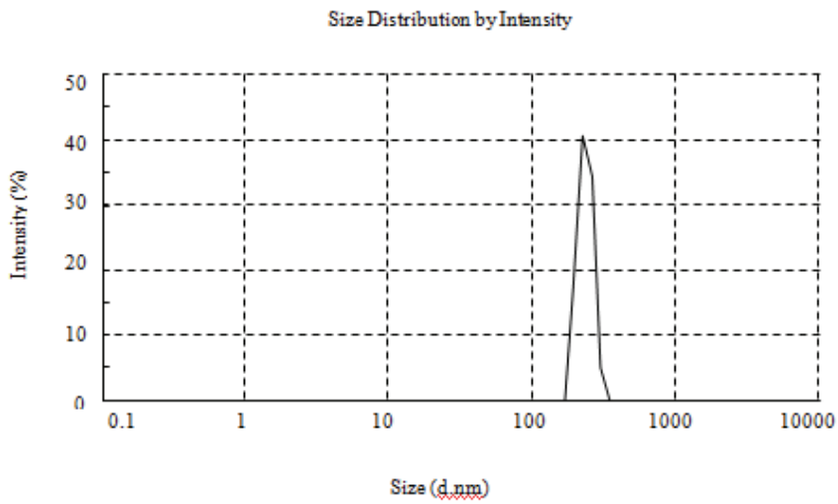

Fig. 1 Mean diameter and polydispersity index of dapsone loaded chitosan nanoparticles.

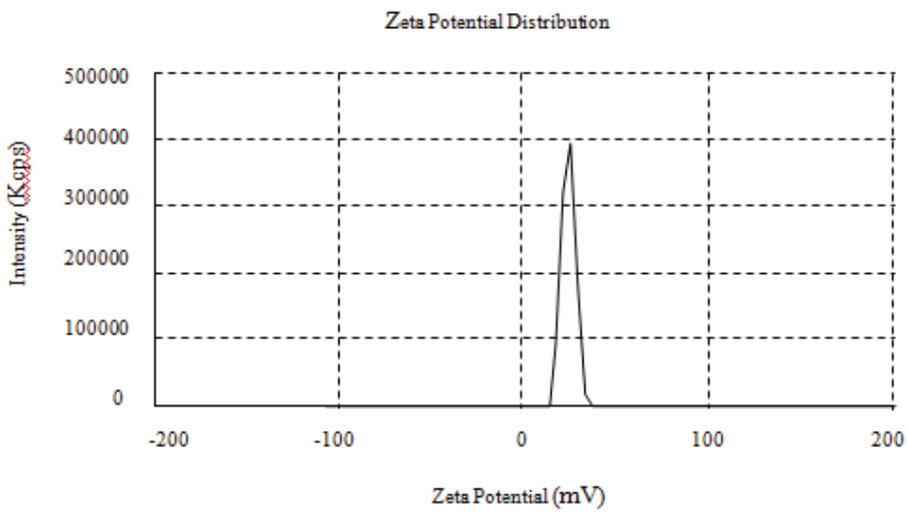

Fig. 2 Zeta potential value of dapsone loaded chitosan nanoparticles.

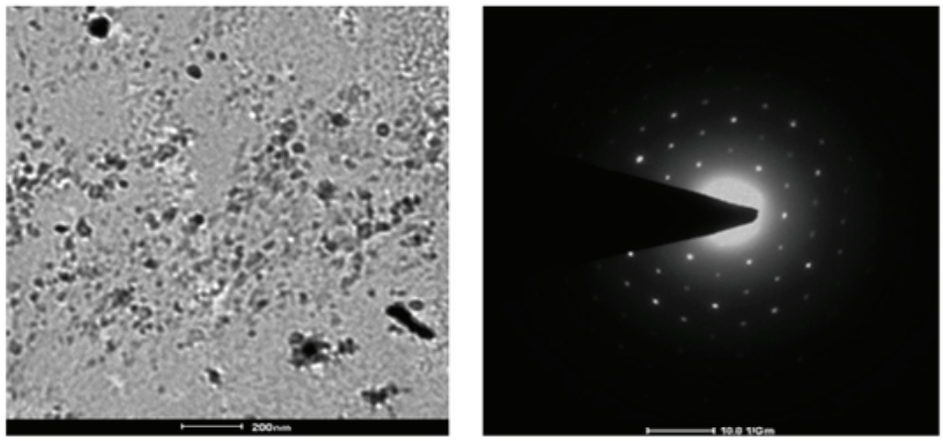

Fig. 3 (a) TEM photograph shows particle size distribution size 200 nm. (b) The selected area electron diffraction shows well defined crystalline pattern & highly oriented attachment structure.

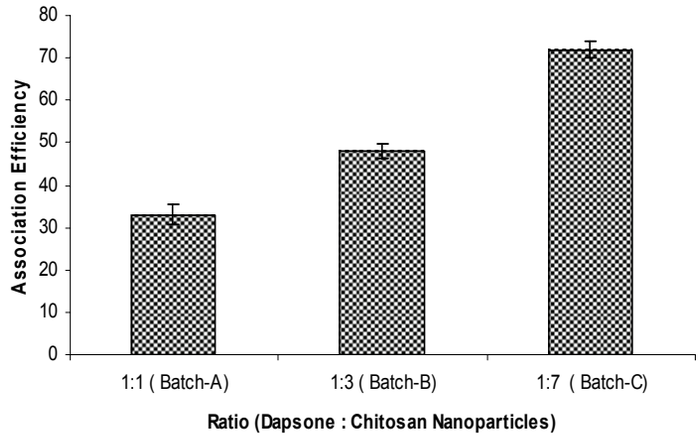

Fig. 4 Composition and association efficacy of dapsone-chitosan nanoparticles. Values represent Mean ± S.D. (n=3)

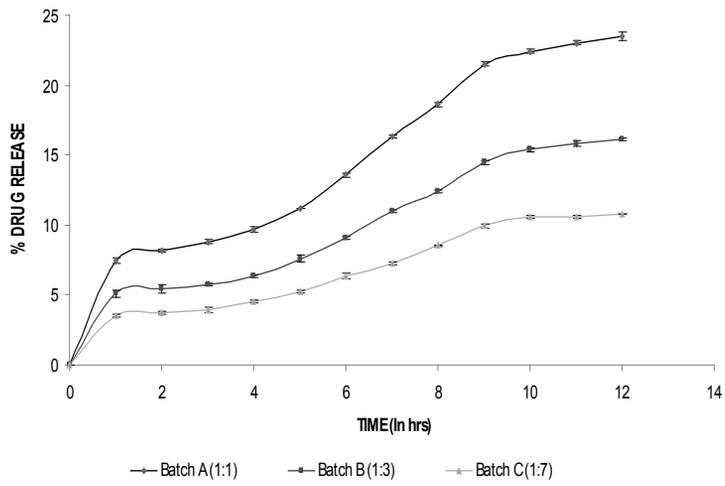

Fig. 5 *In-vitro* release profile of dapsone from chitosan nanoparticles. Values represent mean ± S.D. (n=3)

***In-vivo* studies**

In present studies, Intracerebroventricular (ICV) administration of Streptozotocin (STZ) significantly impaired learning and memory in mice. The ICVSTZ model has been described as an appropriate animal model for AD (Lannert and Hoyer 1998). Cerebral glucose and energy metabolism is associated with oxidative stress (Mayer et al., 1990; Feillet-Coudray et al., 1990). After ICV administration, the highest concentration of STZ reaches the fornix and periventricular white matter at the level of third ventricle, which shows the greatest damage and ICV STZ induced amnesia is independent of its hyperglycemic effect (Sharma and Gupta 2001; Shoham et al., 2003; Mayer et al., 1990). Although the mechanism of action of STZ on memory impairment is not yet known, it probably involves the induction of oxidative stress to which myelin is particularly vulnerable (Feillet-Coudray et al., 1990; Reagan et al., 2000; Smith 2003). Damage to myelin by oxidative stress is seen in disorders such as AD with cognitive impairment (Braak et al., 2000). Oxidative stress has also been documented to amplify inflammatory process (Usachev et al., 1993). ICV STZ in rats causes desensitization of insulin receptors and biochemical changes similar to that of AD or ageing brain (Hoyer et al., 1994; Hoyer 2000; Hoyer 2000. In addition, reduced energy metabolism and synthesis of acetyl CoA ultimately results in cholinergic deficiency and thereby memory deficit in ICV STZ treated rats.

Effect of vehicles on acquisition and retrieval of memory

Administration of normal saline (10 ml/kg s.c.) 30 min before acquisition trials conducted on day 1 to day 4, significantly decreased escape latency time (ELT) as compared with its value noted on day 1 indicating normal acquisition of memory (Table 1). Mice, administered normal saline (10 ml/kg s.c.) 30 min before retrieval trial conducted on day 5 have significantly more time in the target quadrant (Q 4) in search of missing platform as compared to other quadrants (Q1, Q2 Q3) indicating retrieval of memory (Fig. 6). Mice treated with 1% w/v CMC as well as with artificial CSF (25 mg/ml, 10µl, ICV) did not show any significant effect on day 4 ELT (Table 1) and day 5 mean time in target quadrant of control animals (Fig. 6). These observations are in agreement with the results of our earlier studies and reports from other laboratory (Sharma et al., 2008; Sharma et al., 2008; Parle and Singh 2007; Packard et al., 1996).

Table 1 Effect of dapsone nanoparticles on STZ (ICV)-induced increase in day 4 escape latency time (ELT).

Group	Treatment	Dose	Day 1 ELT (in sec)	Day 4 ELT (in sec)
I	Control (Saline)	10 ml/Kg s.c.	95.5 ± 4.1	40.14 ± 1.5[a]
II	CMC control 1% w/v	10 ml/Kg s.c.	97.8 ± 3.5	39.71 ± 1.7
III	CSF control	25 ml/kg, 10µl, ICV	97.2 ± 3.6	41.85 ± 2.0
IV	Dapsone per se	1 mg/kg s.c.	96.7 ± 3.8	39.0 ± 2.6
V	Dapsone per se	2 mg/kg s.c.	97.4 ± 4.2	38.85 ± 1.6
VI	Dapsone nanoparticles per se	1 mg/kg s.c.	98.0 ± 3.9	39.57 ± 1.8
VII	Dapsone nanoparticles per se	2 mg/kg s.c.	97.8 ± 3.7	40.0 ± 1.2
VIII	STZ (ICV)	3 mg/kg, 10µl, ICV	99.1 ± 2.3	77.5 ± 0.81[b]
IX	STZ (ICV) + Dapsone	3 mg/kg, 10µl, ICV + 1 mg/kg	97.0 ± 2.4	71.5 ± 0.84
X	STZ (ICV) + Dapsone	3 mg/kg, 10µl, ICV + 2 mg/kg	96.1 ± 3.3	72.2 ± 1.6
XI	STZ (ICV) + Dapsone Nanoparticles	3mg/kg, 10µl, ICV + 1 mg/kg	95.1 ± 4.3	52.2 ± 0.91[c]
XII	STZ (ICV) + Dapsone Nanoparticles	3mg/kg, 10µl, ICV + 2 mg/kg	95.7 ± 4.0	52.4 ± 1.08[c]
XI	STZ (ICV) + Dapsone Nanoparticles	3mg/kg, 10µl, ICV + 1 mg/kg	95.1 ± 4.3	52.2 ± 0.91[c]
XII	STZ (ICV) + Dapsone Nanoparticles	3mg/kg, 10µl, ICV + 2 mg/kg	95.7 ± 4.0	52.4 ± 1.08[c]

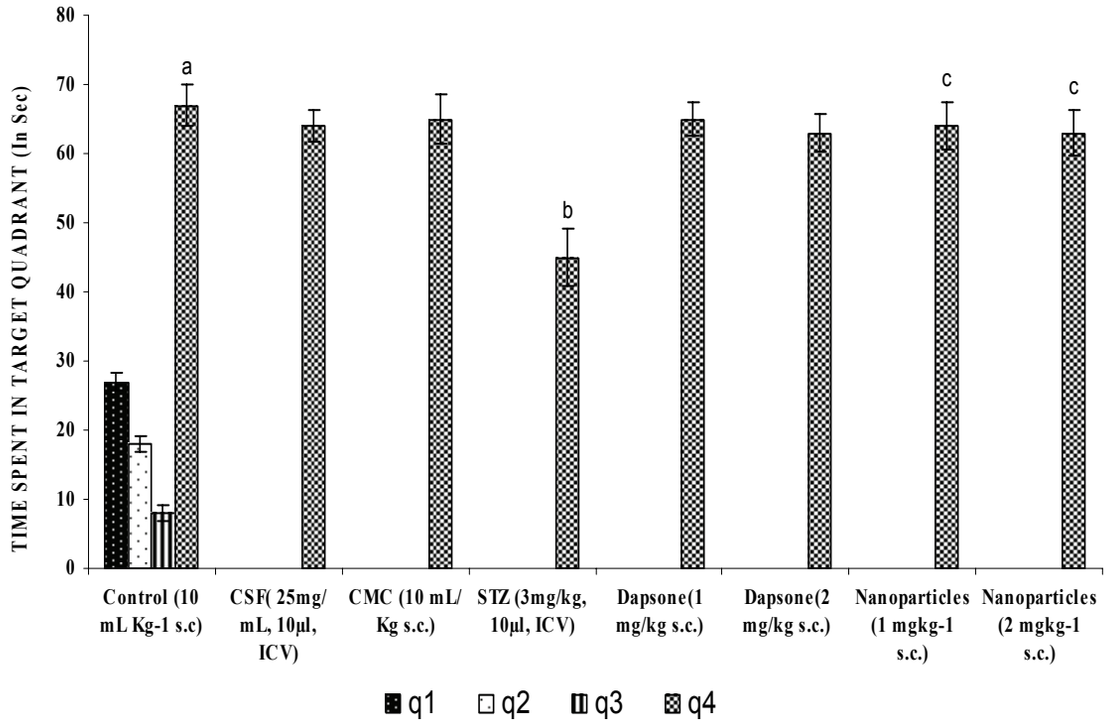

Fig. 6 Effect of Dapsone and Dapsone nanoparticles on time spent in target quadrant on day 5. Each group (*n*=7) represents Mean±S.E.M. ^{a}P< 0.05 versus time spent in other quadrants; ^{b}P < 0.05 versus time spent in target quadrant of control group; ^{b}P =0.05 versus time spent in target quadrant of control group ^{c}P > 0.05 versus time spent in target quadrant of STZ (ICV) treated group; ANOVA followed by Tukey's Multiple range test.

Each group (n = 7) represents mean ± S.E.M. ^{a}P< 0.05 versus Day 1 ELT in control;^{b}P < 0.05 versus Day 4 ELT in control;

^{c}P < 0.05 versus Day 4 ELT in STZ (ICV); ANOVA followed by Tukey's multiple range test.

Effect of STZ on acquisition and retrieval of memory

Administration of STZ (3 mg/kg, 10 µl, ICV) in two doses scheduled on first and on third day significantly prevented decrease in day 4 ELT of vehicle control group (Table 1) and markedly reduced time in target quadrant (Q4) in search of missing platform during retrieval trial, thereby reflecting impairment of learning and memory (Figures 6).

Effect of dapsone and dapsone nanoparticles on acquisition and retrieval of memory

Dapsone (1 mg/kg s.c. and 2 mg/kg s.c.) / dapsone nanoparticles (1 mg/kg s.c. and 2 mg/kg s.c..), *per se* did not produce any significant change on decrease in day 4 ELT (Table 1) and increase in time in target quadrant in search of missing platform during retrieval trial conducted on day 5 of control mice (Fig. 6).

Effect of dapsone and dapsone nanoparticles on STZ induced dementia

Treatment of Dapsone (1 mg/kg s.c. and 2 mg/kg s.c.) did not produce any significant effect on STZ induced rise in day 4 ELT (Table 1) and decrease in time in target quadrant in search of missing platform during retrieval trial conducted on day 5 of STZ treated animals (Figure 7). However dapsone nanoparticles (1 mg/kg s.c. and 2 mg/kg s.c.) significantly attenuated STZ induced rise in day 4 ELT (Table 1) and decrease in day 5 time in target quadrant (Fig. 7).

Effect of STZ, dapsone and dapsone nanoparticles on MPO levels of brain

STZ (3 mg/kg, 10 µl, ICV administered in two doses schedules that is on first and on third day) significantly, increased the brain MPO level, when compared to control group, reflecting involvement of inflammation in ICV STZ mediated memory impairment. Dapsone (1 mg/kg s.c. and 2 mg/kg s.c.) / dapsone nanoparticles (1 mg/kg s.c. and 2 mg/kg s.c..) did not produce any significant per se effect on, brain MPO level of control animals (Tables 2).

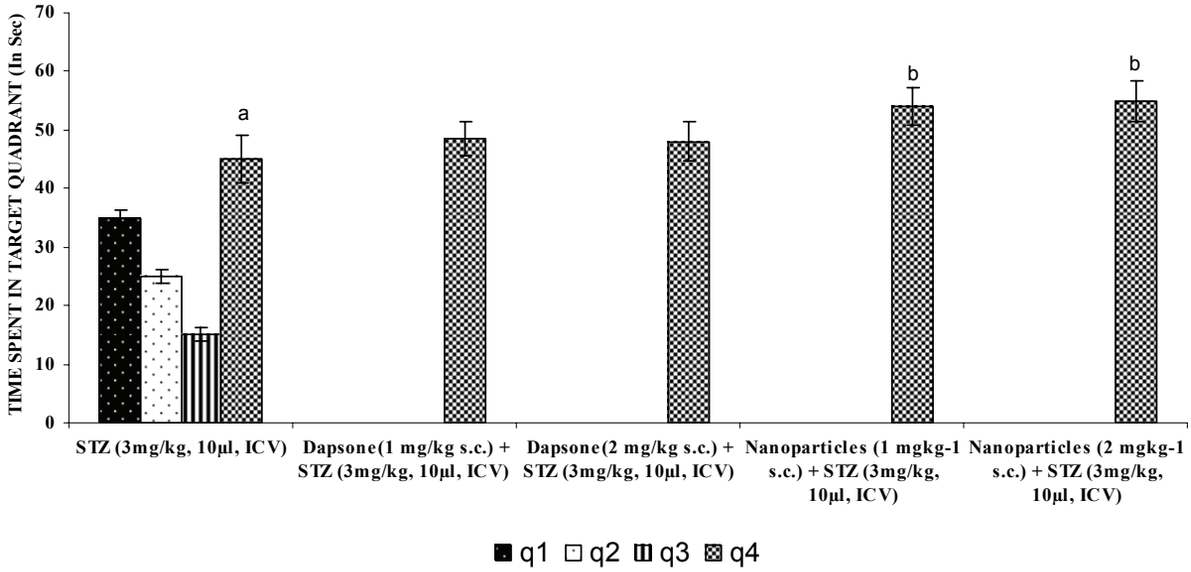

Fig. 7 Effect of dapsone and dapsone nanoparticles on STZ (ICV) induce decrease in time spent in target quadrant on day 5. Each group (*n*=7) represents Mean ± S.E.M. [a]*P*< 0.05 versus time spent in other quadrants; [b]*P* < 0.05 versus time spent in target quadrant of STZ (ICV) treated group; ANOVA followed by Tukey's Multiple range test.

Table 2 Effect of dapsone nanoparticles on STZ (ICV) induced increase in myeloperoxidase (MPO) activity

Group	Treatment	Dose	MPO activity of brain (U/g)
I	Control (Saline)	10 ml/kg s.c.	0.89 ± .02
II	CMC control 1% w/v	10 ml/kg s.c.	0.93 ± .01
III	CSF control	25 mg/ml, 10µl, ICV	0.92 ± .02
IV	Dapsone per se	1 mg/kg s.c.	0.93 ± .01
V	Dapsone per se	2 mg/kg, s.c.	0.95 ± .02
VI	Dapsone nanoparticles per se	1 mg/kg, s.c.	0.92± .02
VII	Dapsone nanoparticles per se	2 mg/kg, s.c.	0.93 ± .04
VIII	STZ (ICV)	3 mg/kg, 10µl, ICV	2.2 ± .02[a]
IX	STZ (ICV) + Dapsone	3 mg/kg, 10µl, ICV + 1 mg/kg, s.c.	2.1 ± .02
X	STZ (ICV) + Dapsone	3 mg/kg, 10µl, ICV + 2 mg/kg, s.c.	2.07 ± .02
XI	STZ (ICV) + Dapsone Nanoparticles	3 mg/kg, 10µl, ICV + 1 mg/kg, s.c	1.5 ± .03[b]
XII	STZ (ICV) + Dapsone Nanoparticles	3 mg/kg, 10µl, ICV + 2 mg/kg, s.c	1.5 ± .03[b]

Each group (n =7) represents mean ± S.E.M. [a]P < 0.05 versus brain MPO activity of control;
[b]P < 0.05 versus brain MPO activity of STZ (ICV); ANOVA followed by Tukey's multiple range test

Effect of dapsone and dapsone nanoparticles on STZ induced increased MPO levels

Treatment of dapsone (1 mg/kg, s.c. and 2 mg/kg, s.c.) did not produce any significant effect on STZ induced rise in brain MPO levels, whereas dapsone nanoparticles (1 mg/kg s.c. and 2 mg/kg, s.c.) significantly reversed the STZ induced rise in MPO levels (Tables 2).

Effect of STZ on neutrophilic infiltrate density of brain

STZ (3 mg/kg, 10 µl, ICV administered in two doses schedules that is on first and on third day) significantly,

increased the brain neutrophilic density, when compared to control group, reflecting inflammation. Dapsone (1 mg/kg, s.c. and 2 mg/kg, s.c.) / Dapsone Nanoparticles (1 mg/kg, s.c. and 2 mg/kg, s.c..) did not produce any significant per se effect on, brain neutrophilic density of control animals (Fig. 8 and 9).

Effect of dapsone and dapsone nanoparticles on STZ induced increase in brain neutrophilic density

Treatment of dapsone (1 mg/kg, s.c. and 2 mg/kg, s.c.) did not produce any significant effect on STZ induced rise in brain neutrophilic density, whereas dapsone nanoparticles (1 mg/kg, s.c. and 2 mg/kg, s.c.) significantly reversed the STZ induced rise in brain neutrophilic density (Fig. 8 and 10).

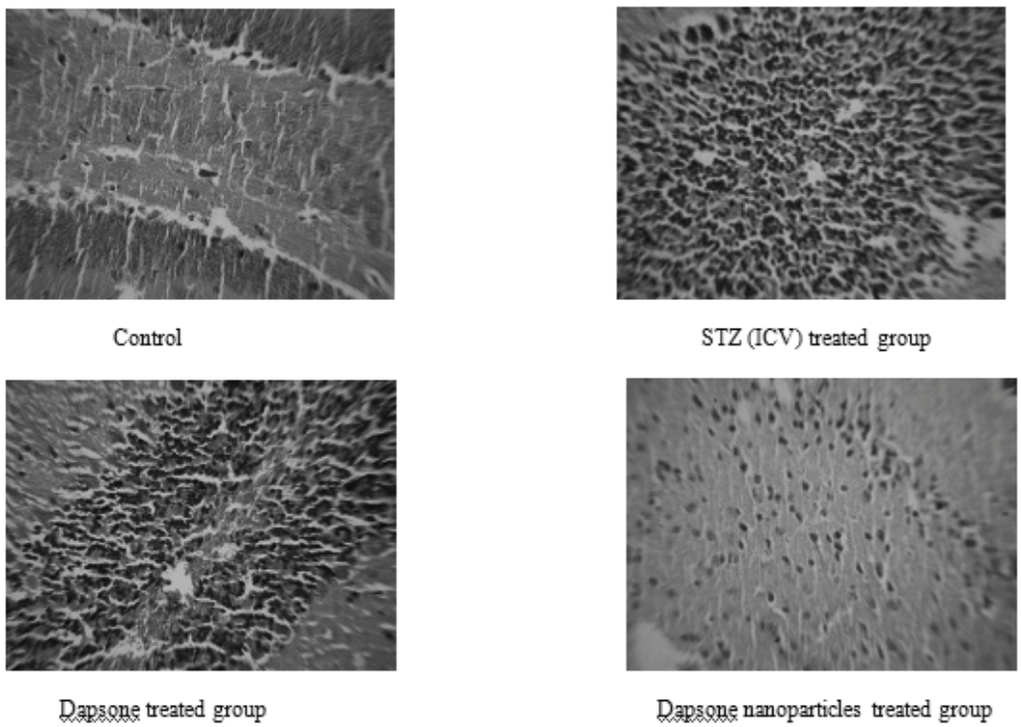

Fig. 8 Micrographs of the brain tissue after hematoxylin and eosin staining representing various study groups.

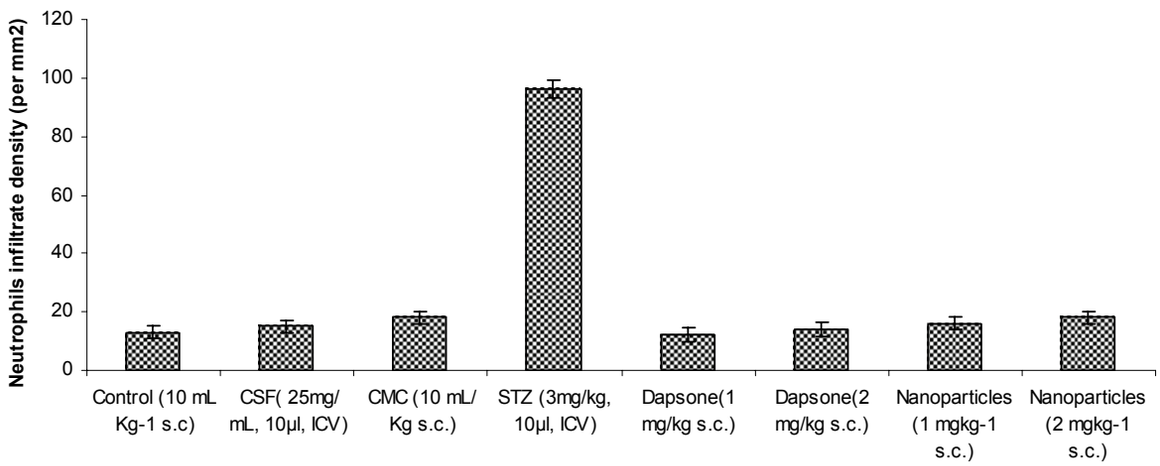

Fig. 9 Effect of dapsone and dapsone nanoparticles on neutrophils infiltrate density. Values are mean ± SEM. [a]*P*< 0.05 versus control; [b]*P* > 0.05 versus STZ (ICV) treated group; ANOVA followed by Tukey's Multiple range test.

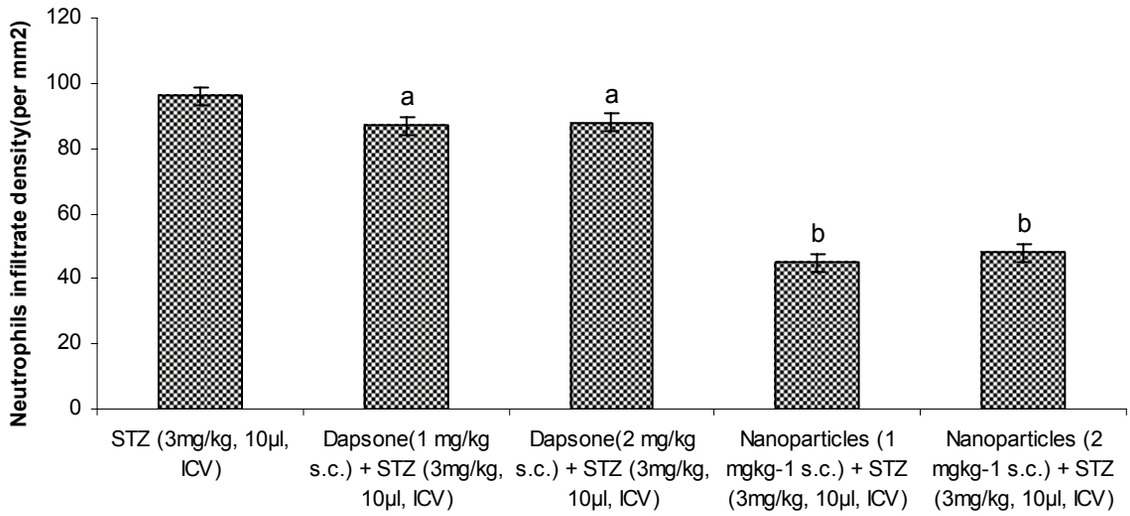

Fig. 10 Effect of dapsone and dapsone Nanoparticles on STZ (ICV) induce increase in neutrophils infiltrate density. Values are mean ± SEM $^{a}P > 0.05$ versus STZ (ICV) treated group; $^{b}P < 0.05$ versus STZ (ICV) treated group; ANOVA followed by Tukey's Multiple range test.

Conclusion

The noted reversal of STZ induced dementia by dapsone loaded chitosan nanoparticles appears to be associated with its targeted anti-inflammatory action via chitosan nanoparticles that enables it to target the potential regions in Alzheimer's disease. The probable reason why dapsone failed to modify STZ induced dementia may probably be related to its poor ability to reach target site in sufficient concentration at the employed doses i.e 1 mg/kg and 2 mg/kg. On the other hand, CSNP loaded dapsone might have attained concentration in the target sites sufficient enough even at a dose of as low as 1 mg/kg which significantly attenuated STZ induced impairment of learning and memory along with reversal of STZ mediated rise in MPO level and neutrophils infiltrate density. Anti-dementing potential of dapsone loaded chitosan nanoparticles requires further investigations.

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