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INVESTIGATIONS ON MOLECULAR MECHANISM INVOLVED IN NEUROPROTECTIVE EFFECT OF VITAMIN D AGAINST SODIUM AZIDE INDUCED ALZHEIMER'S DISEASE IN RATS.

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ABSTRACT

The present study is undertaken to investigate the beneficial role of Vitamin D in sodium azide (SAZ) induced Alzheimer's disease (AD) in rats. This study also aimed to explore the potential role of PPAR-γ in Vitamin D mediated neuroprotection against *i.p* SAZ. administered rats. SAZ (12.5 mg/kg, *i.p*. for 5 days and 10 mg/kg, *i.p*. for next 9 days) was administered to rats, their learning and memory assessment is done by Morris Water Maze (MWM) test. After that various biochemical parameters were performed i.e acetylcholinesterase (AChE) activity, nitrite/ nitrate activity, myeloperoxidase (MPO) activity, thiobarbituric acid reactive substances (TBARS) level, glutathione (GSH) level. The study also showed that *i.p.* SAZ impaired the learning and memory and also increased the levels of brain AChE, nitrite/ nitrate, MPO and TBARS and decrease in GSH level. Pre-treatment with bisphenol-A-diglycidyl ether (BADGE), a selective PPAR-γ antagonist, significantly abolished the beneficial effect of Vitamin D in *i.p.* SAZ treated animals. This investigation lead to results, which document a potential role of PPAR-γ in Vitamin D mediated neuroprotection against *i.p*. SAZ induced Alzheimer's disease.

KEYWORDS: Morris water maze, PPAR-γ, myeloperoxidase activity, inflammation, oxidative stress, acetylcholinesterase activity, Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is slow in onset but leads to dementia, unusual behavior, personality change and ultimately, death. (Kulkarni

et al., 2010). There is destruction of neurons in the cortex and limbic structures of brain, particularly the basal forebrain, amygdala, hippocampus and cerebral cortex. These areas are responsible for learning, memory, reasoning, behavior and emotional control (Parikh *et al*., 2013). Anatomically, four major alterations in brain structure are seen: cortical atrophy, degeneration of cholinergic neurons, presence of neurofibrillary tangles (NFTs) and accumulation of neuritic plaques (Zaki *et al*., 2013). Oxidative stress and inflammation lead to degeneration of neurons in the brain (Goverdhan *et al*., 2012). The most important risk factor of AD is age. After 65 years of age the frequency of all types of dementia doubles every five years. By the time a person reaches age 85, they're at a 35% risk of dementia (Pandey *et al*., 2011).

There has been a lot of work in AD research involving various animal models. D-galactose (Kunte and Kuna, 2014), aluminium chloride (Singh *et al*., 2014), cholesterol (Hung *et al*., 2013) and high fat diet (Knight *et al*., 2014) induced models have been implicated. One of them is developed by administration of sodium azide (SAZ). It is a mitochondrial toxin which is white crystalline solid used in the manufacture of the explosive lead azide (Steven *et al*., 2007). Administration of SAZ leads to mitochondrial defects and inhibits mitochondrial key enzyme i.e cytochrome oxidase (Lalonde *et al*., 1996). This enzyme is essential for respiratory chain, its inhibition blocks mitochondrial complex-IV and deplete ATP levels which contributes to metabolic impairment and reactive oxygen species (ROS) production (Blass *et al*., 1990). This leads to neurodegeneration which mimics the AD.

The peroxisome proliferator activated receptors (PPARs) belong to the family of nuclear hormone receptors (NHR) that comprise 48 human ligand-inducible transcription factors whose activity is regulated by steroids and lipid metabolites (Gillespie *et al*., 2011). The family of PPARs is represented by the following three members: PPAR-α, PPAR-δ, and PPAR-γ (Balakumar *et al*., 2007). PPARs act principally as lipid sensors and regulate whole body metabolism, regulate energy homeostasis through its ability to stimulate the breakdown of fatty acids and cholesterol (Tyagi *et al*., 2011). They inhibit proinflammatory gene expression by mechanism of transcriptional transrepression (Mandard and Patsouris, 2013). PPAR-γ has their potential role in obesity (Badr, 2009), inflammation (DeFilippis *et al*., 2009), adipocyte differentiation (Tyagi *et al*., 2011), anticancer effect (Colin *et al*., 2010), neurodegenerative disorders (Racke *et al*., 2008), diabetes (Terauchi *et al*., 2005) and pain (Churi *et al*., 2008). PPAR-γ modulators may regulate the different aspects of AD such as

amyloid-β synthesis, inflammation, energy utilisation, lipid metabolism and insulin sensitivity (Heneka *et al*., 2011). Vitamin D plays an important role in maintaining calcium hemostasis in body. The active form of vitamin D is a seco-steroid with multiple neurotrophic and neuroprotective functions in the central nervous system. Evidence from animal studies suggests that vitamin D deficiency may impair brain physiological function and cause anatomical and behavioral adverse effects (Schlogl and Holick, 2014). On the other hand, vitamin D has been found to be protective against biological processes associated with AD and cognition, including amyloid-β deposition, inflammation, calcium homeostasis (Taqhizaden *et al*., 2013). Disruption of Vitamin D pathways mimic amyloid pathology (Gezen *et al*., 2014). Patients with AD have high prevalence of Vitamin D deficiency and it is associated with low mood and impaired cognitive performance (Nguyen *et al*., 2011). Vitamin D receptors and PPAR-γ are both ligand-activated nuclear receptors. Vitamin D receptors are present in hippocampus (Taqhizaden *et al*., 2013).

Recently, a few *in vitro* studies suggested cross talk between these two receptors with involvement of PPAR-γ in Vitamin D mediated biological responses (Woeckel *et al*., 2013). There has been a lot of research at various levels involving PPAR-γ receptors in AD and also there are some reports of Vitamin D studies in relation to AD. But, direct involvement of Vitamin D in AD and its action through PPAR-γ receptors has not been studied. So, present study is designed to investigate the activation of PPARγ receptors as potential molecular mechanism in Vitamin D-mediated protection against SAZ induced AD.

MATERIALS AND METHODS

Experimental Animals

Wistar rats of either sex (200-250 g) were used in the present study (procured from National Institute of Pharmaceutical Education and Research, Mohali) for behavioral paradigm of Alzheimer's disease and maintained in departmental animal house facility of Chandigarh College of Pharmacy, Landran, Punjab in different cages. Animals were maintained at standard laboratory chow diet and water ad libitum. The rats were exposed to 12 hr light and dark cycle. The animals were acclimatized to laboratory conditions prior to experimental study. All experiments were performed in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC). Adequate measures were taken to minimise pain or discomfort with animal experimental procedures. The care of animals were carried out as per the guidelines of Committee for the purpose of control and Supervision of Experiments on

Animals (CPCSEA), Ministry of environment and forest, Government of India (Reg. No. 1201/9/08 CPCSEA). The experimental protocol was approved by IAEC vide approval no. IAEC/ Dec, 13/ 003.

Drugs and Reagents

All reagents used in this study were of analytical grade and were freshly prepared. SAZ was purchased from Loba Chemicals (Mumbai, India). It was dissolved in 0.9% normal saline solution and was given by *i.p*. route. 1mg of Vitamin D (Fermenta biotech Ltd. Mandi, Himachal Pradesh) was completely dissolved in two drops of DMSO (Dimethyl sulfoxide) and further it was diluted with normal saline upto 100 ml. 30 mg of BADGE (Bisphenol-A-diglycidyl ether, Sigma-Aldrich) was dissolved in DMSO and was further diluted with water. The estimation kit for serum glucose was obtained from Reckon Diagnostics Pvt. Ltd., Vadodara, India.

Morris water maze test

Morris water maze (MWM) test was employed to access learning and memory of rats (Morris, 1984). MWM is a swimming based model where the animals learn to escape on to a hidden platform. It consists of large circular pool (150 cm in diameter and 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1ºC). The water was made opaque by using white non toxic dye or milk. The tank was divided in four quadrants with the help of two threads. The water pool was placed in illuminated light room. A submerged platform (10 cm^2) painted white was fixed at right angle to each other on the rim of the pool placed inside the target quadrant of this pool 1cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials with the intertrial gap of 5 min. The rat was gently placed in the water between quadrants facing the wall of the pool with the drop location changing for each trial and allowed 120 s to locate the platform. Then, it was allowed to stay on platform for 20 s. When animals failed to locate the platform in 120 s, the animals were guided gently to reach onto the platform and allowed to remain there for 20 s. The escape latency time (ELT) to locate the hidden platform in the water maze on day 4 was noted as index of acquisition and learning.

Acquisition (Training) Trial

Each rat was subjected to four trials on consecutive days, during which the starting position was changed with each exposure as shown below while the target quadrant (Q4) was remained constant in all the acquisition trials.

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

Retrieval Trial

On day 5, the platform was removed and each rat was allowed to explore the pool for 120 s. Mean time spent in all four quadrants was noted. The mean time spent in the target quadrant searching for hidden platform was noted as an index of retrieval. The experimenter always stood at the same position. Care was taken to maintain the location of the water with respect to other objects in the laboratory. All the trials were completed between 0900 to 1700 hr.

Collection of samples

Animals were sacrificed by cervical dislocation, brains were removed and then homogenized in phosphate buffer (pH=7.4). The homogenates were then centrifuged at 3000 rpm for 15 min. The supernatant of homogenates were collected and used for biochemical measures.

BIOCHEMICAL PARAMETERS

Estimation of total protein content

The total protein content was determined by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard.

Estimation of Acetylcholinesterase (AChE) levels

AChE is a marker of loss of cholinergic neurons in the forebrain. The whole brain AChE activity was measured by the method of Ellman *et al*. (1961). The enzymatic activity in the supernatant was expressed as nmol per mg protein.

Estimation of reduced glutathione (GSH) level

The whole brain GSH level was estimated by the method of Beutler *et al*. (1963). The concentration of glutathione in the supernatant expressed as µmol per mg protein.

Estimation of Thiobarbituric acid reactive species (TBARS) level

The whole brain TBARS level as an index of lipid peroxidation (Niehius and Samuelson, 1968). The amount of TBARS level will be expressed in nanomoles per mg of protein.

Estimation of brain nitrite/ nitrate concentration level

The accumulation of nitrite in the supernatant, was measured as an indicator of the production of nitric oxide, determined by the method of Green *et al*. (1982). The concentration of nitrite/ nitrate in the supernatant expressed as µg per mg protein.

Estimation of Myeloperoxidase (MPO) level

The myeloperoxidase (MPO) activity which is measured as an index of neutrophil accumulation was measured using method of Krawisz *et al*., (1984). The concentration of MPO in the supernatant expressed as U per mg protein.

Estimation of serum glucose level

At the end of the experimental protocol, the blood samples were collected and serum was separated. The serum samples were frozen until analyzing the biochemical parameters. The glucose concentration was estimated by glucose oxidase peroxidase GOD-POD method (Trinder *et al*., 1969) using commercially available kit (Reckon diagnostics Pvt. Ltd., Vadodara, India).

EXPERIMENTAL PROTOCOL

Six groups of rats were employed in the study, Each group was comprised of minimum six rats. Procedure of drug treatment was carried out for 14 days.

GROUP I (Normal control)

Rats were administered with saline (10ml/kg *i.p*.) 30 min before acquisition trial conducted from day 1 to day 4 and 30 min before retrieval trial on day 5 using Morris water maze (MWM) test.

GROUP II (Vit D *per se*)

Rats were administered with Vitamin D (10µg/kg *i.p*., for 14 days) and then subjected to MWM test. The drug was also given during acquisition trials from day 1 to day 4. During retrieval trial i.e on day 5, 0.9% normal saline was administered 30 min before trial.

GROUP III (SAZ control)

Rats were administered with SAZ (12.5mg/kg/day, *i.p.* for 5 days and 10mg/kg, *i.p.* for next 9 days) and then followed by exposure to MWM test. The SAZ was given during acquisition trials from day 1 to day 4. On day 5 rats were also administered with 0.9% normal saline 30 min before retrieval trial.

GROUP IV [SAZ + Vitamin D (LD) low dose]

SAZ treated rats were given Vitamin D at a dose of 5µg/kg, *i.p*. for 14 days and then subjected to MWM test. The Vitamin D was co-administered with SAZ during acquisition trials i.e day 1 to day 4. On day 5 i.e. retrieval trial 0.9% normal saline was administered 30 min before retrieval trial.

GROUP V [SAZ + Vitamin D (HD) high dose]

SAZ treated rats were given Vitamin D at a dose of 10µg/kg, *i.p*. for 14 days and then subjected to MWM test. The Vitamin D was co-administered with SAZ during acquisition trials i.e day 1 to day 4. On day 5 i.e. retrieval trial 0.9% normal saline was administered 30 min before retrieval trial.

GROUP VI (BADGE + Vitamin D + SAZ)

SAZ treated rats were given BADGE at a dose of 30 mg/kg, *i.p.* and vitamin D was co-administered with SAZ for 14 days then subjected to MWM test. On day 5 i.e. retrieval trial 0.9% normal saline was administered 30 min before retrieval trial.

Statistical analysis

The observations were statistically analyzed with the help of Sigmastat software version 12.5. All of the results were expressed as mean±standard error of the mean (SEM). Results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple range test and $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of Vitamin D on brain reduced GSH level

GSH is an intracelluler reductant and plays major role in catalysis, metabolism and transport. SAZ treated rats showed a significant decrease in brain reduced GSH level in comparison with control group animals. Administration of Vitamin D for 14 days to SAZ treated rats showed a significant decline in brain reduced GSH level in comparison to SAZ treated

control group animals. The BADGE pretreatment abolished the protective effect of vitamin D in rats. However, administration of vitamin D *per se* did not show any significant changes in brain reduced GSH level when compared with control group animals.

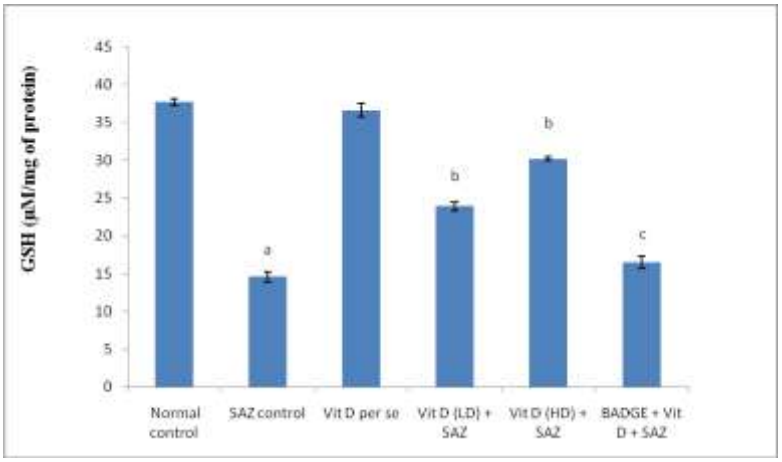

Figure 1: Effect of Vitamin D on reduced GSH level.

Values were mean ± S.E.M.; a = *P*<0.05 versus control group; b = *P*<0.05 versus SAZ treated group, c = *P*<0.05 versus SAZ + vitamin D (HD).

Effect Vitamin D on brain AChE level

SAZ treated rats showed a significant rise in brain AChE activity in comparison to control animals. Administration of Vitamin D for 14 days to SAZ treated rats showed a significant decline in brain AChE activity in comparison to SAZ treated control group animals. The BADGE pretreatment abolished the protective effect of vitamin D in rats. However, administration of vitamin D *per se* did not show any significant changes in brain AChE activity when compared to control animals.

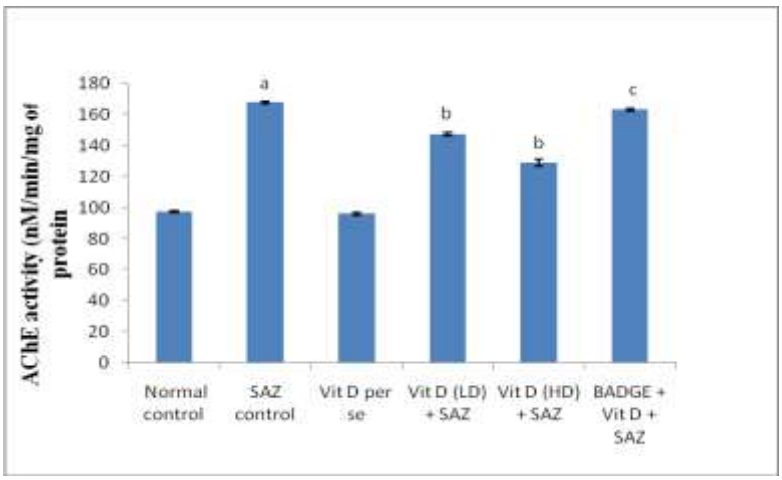

Figure 2: Effect of Vitamin D on brain AChE level.

Values were mean ± S.E.M.; a = *P*<0.05 versus control group; b = *P*<0.05 versus SAZ group; c = *P*<0.05 versus SAZ + vitamin D (HD).

Effect of Vitamin D on brain TBARS level

Sodium azide treated rats showed a significant rise in brain TBARS level in comparison to control animals. Administration of Vitamin D for 14 days to SAZ treated rats showed a significant decline in brain TBARS level in comparison to SAZ treated control group animals. The BADGE pretreatment abolished the protective effect of vitamin D in rats. However, administration of vitamin D *per se* did not show any significant changes in brain TBARS level when compared to control group animals.

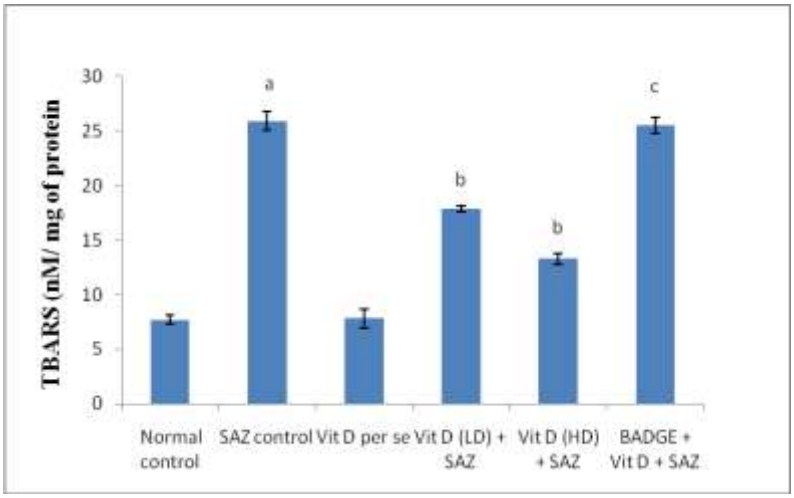

Figure 3: Effect of Vitamin D on brain TBARS level.

Values are mean ± S.E.M.; a = *P*<0.05 versus control group; b = *P*<0.05 versus SAZ treated group; c = *P*<0.05 versus SAZ + vitamin D (HD).

Effect of Vitamin D on brain Nitrite/ Nitrate level

SAZ treated rats showed a significant rise in brain nitrite/nitrate level when compared with control group animals indicating neurogenic inflammation. Administration of Vitamin D for 14 days to SAZ treated rats showed a significant decline in brain nitrite/ nitrate level in comparison to SAZ treated animals. The BADGE pretreatment abolished the protective effect of vitamin D in rats. However, administration of vitamin D *per se* did not show any significant changes in brain nitrite/ nitrate level when compared with control group animals.

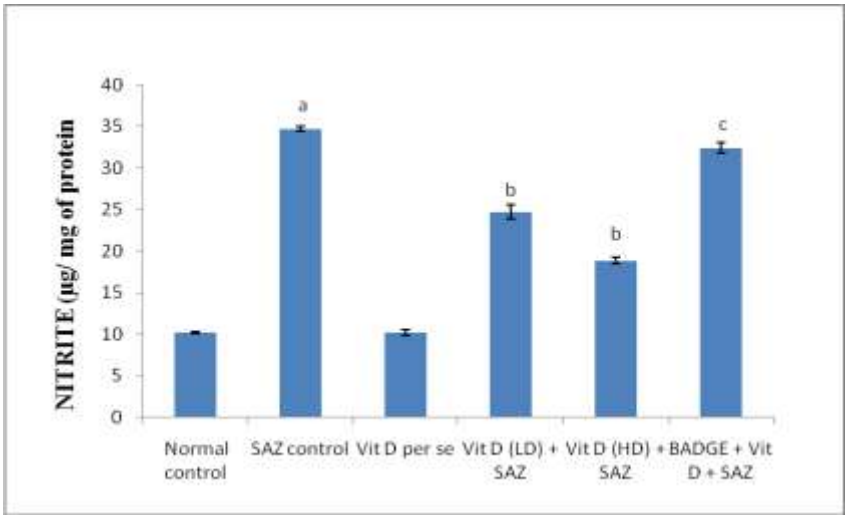

Figure 4: Effect of Vitamin D on brain nitrite/ nitrate level.

Values are mean ± S.E.M.; a = *P*<0.05 versus control group,b = *P*<0.05 versus SAZ treated group; c = *P*<0.05 versus SAZ + vitamin D (HD).

Effect of Vitamin D on brain MPO level

Sodium azide treated rats showed a significant rise in MPO level, when compared with control group animals indicating neurogenic inflammation. Administration of Vitamin D for 14 days to SAZ treated rats showed a significant decline in brain MPO level in comparison to SAZ treated animals. The BADGE pretreatment abolished the protective effect of vitamin D in rats. However, administration of vitamin D *per se* did not show any significant changes in brain MPO level when compared with control group animals.

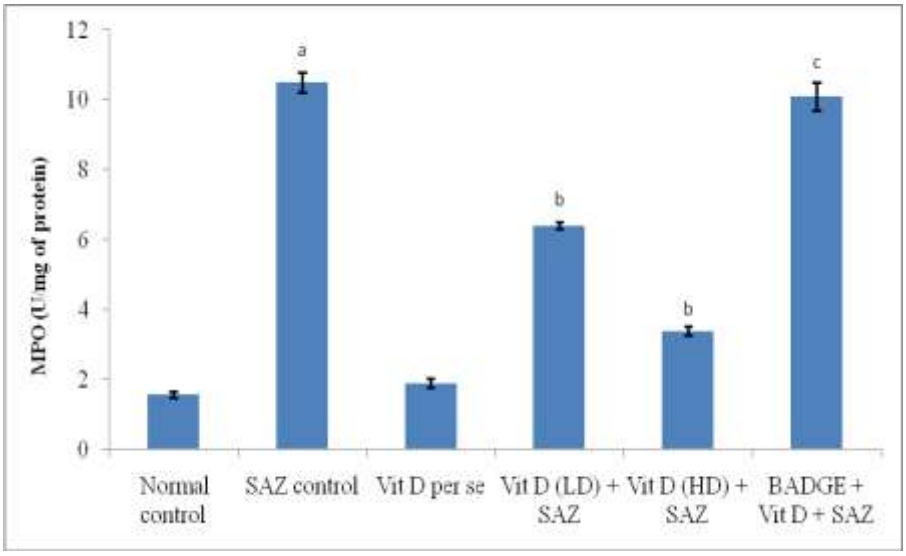

Figure 5: Effect of Vitamin D on brain MPO level.

Values are mean ± S.E.M.; a = *P*<0.05 versus control group; b = *P*<0.05 versus SAZ treated group; c = *P*<0.05 versus SAZ + vitamin D (HD).

Effect of Vitamin D on memory

Control rats showed a significant decline in their day 4 escape latency time (ELT) on subsequent exposure to Morris Water Maze (MWM), when compared to Day 1 ELT reflecting normal learning. Moreover, these animals showed a significant increase in Time spent in target quadrant (TSTQ) in comparison to time spent in other quadrants conducted on Day 5, reflecting normal memory. Vehicle control group did not show any significant effect on Day 4 ELT and Day 5 TSTQ when compared to control group animals. Rats treated with sodium azide showed a significant increase in Day 4 ELT in comparison to control group animals and decrease in Day 5 TSTQ indicating impairment of memory and learning respectively. Administration of Vitamin D for 14 days to SAZ treated rats showed a significant fall in day 4 ELT and rise in day 5 TSTQ indicating reversal of learning and memory respectively. The BADGE pretreatment abolished the protective effect of vitamin D in rats. However, administration of Vitamin D *per se* did not exhibit any significant effect on day 4 ELT and day 5 TSTQ indicating normal acquisition and retrieval.

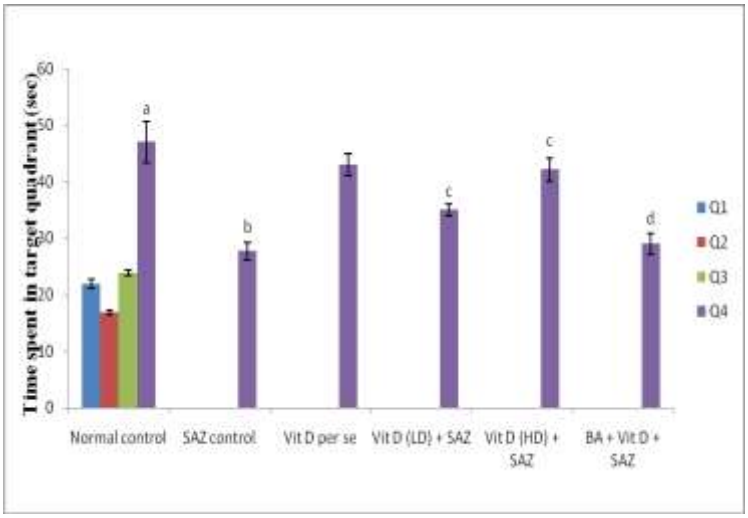

Figure 6: Effect of Vitamin D on mean time spent in target quadrant (TSTQ) using Morris water maze.

Values are mean ± S.E.M.; a = *P*<0.05 versus time spent in other target quadrant in 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 SAZ; d = *P*<0.05 versus time spent in target quadrant of SAZ + Vit D (HD).

Table: 1. Effect of Vitamin D on Day 4 ELT of SAZ treated rats using Morris Water Maze.

GROUPS	DAY 1 ELT (sec)	DAY 4 ELT (sec)
Normal Control	105.0 ± 0.99	53.7 ± 0.99[a]
SAZ Control	109.6 ± 0.99	92.6 ±0.99[b]
Vit D per se	106.4 ± 0.99	55.8 ± 0.99
SAZ + Vit D (LD)	104.8 ± 0.99	68.5 ± 0.99[c]
SAZ + Vit D (HD)	104.6 ± 0.99	56.9 ± 0.99[c]
SAZ + Vit D + BADGE	112.9 ± 0.99	92.2 ± 0.99[d]

Values are mean ± S.E.M.; a = P<0.05 versus Day 1 ELT in control group; b = P<0.05 versus Day 4 ELT in control group; c = P<0.05 versus Day 4 ELT in SAZ treated group; d = P<0.05 versus Day 4 ELT in SAZ + HD group.

Effect of Vitamin D on Serum glucose level

Rats treated with SAZ showed significant (p< 0.05) increase in serum glucose level which was measured by glucose oxidase peroxidase GOD-POD method when compared to serum glucose levels of control animals. Administration of Vitamin D for 14 days to sodium azide treated rats showed a significant decline in serum glucose level when compared to SAZ treated animals. However administration of Vitamin D per se did not exhibit any significant effect on serum glucose level.

Table: 2. Effect of Vitamin D on Serum glucose levels.

GROUPS	SERUM GLUCOSE LEVELS (mg/dl)
Normal Control	97.20 ± 0.60
SAZ Control	181.8.3 ± 0.48[a]
Vit D *per se*	95.50 ± 1.4
SAZ + Vit D (LD)	161.5 ± 3.1[b]
SAZ + Vit D (HD)	136.0 ± 3.2[b]
SAZ +VitD(HD) +BADGE	176.5 ± 1.17[c]

Values are mean ± S.E.M.; a = *P*<0.05 versus control group; b = *P*<0.05 versus SAZ treated group; c = *P*<0.05 versus SAZ + Vitamin D (HD).

DISCUSSION

The SAZ model used in present study is a well proven animal model for inducing AD and mimics the clinical situation of memory deficits and behavioral changes as seen in AD patients (Megyeri *et al*., 2008). Administration of SAZ leads to mitochondrial defects and inhibits mitochondrial key enzyme i.e cytochrome oxidase (Lalonde *et al*., 1996). This enzyme is essential for respiratory chain, its inhibition blocks mitochondrial complex-IV and

deplete ATP levels which contributes to metabolic impairment and ROS production (Blass *et al*., 1990; Davis *et al*., 1997). This leads to neurodegeneration which mimics the AD.

The SAZ administration to rats has been reported to result in neurodegeneration in the hippocampus neurons (Mehta *et al*., 2007) and the hippocampus is responsible for memory regulation. Hence, MWM was employed to determine memory deficits (Morris, 1984; Parle & Singh, 2007) associated with AD. Control untreated animals have shown marked reduction in day 4 ELT as compared to day 1 ELT during acquisition trial, suggesting normal acquisition or learning ability. Further, these animals have shown significant increase in day 5 TSTQ when compared to time spent in other quadrants, indicating normal retrieval (memory) as well.

Results of present study have shown that intraperitoneal administration of SAZ for 14 days (12.5 mg/kg for 5 days & 10 mg/kg *i.p.* for 9 days) resulted in impairment of learning & memory in rats as indicated by significant increase in day 4 ELT & decrease in day 5 TSTQ. Moreover SAZ treated animals have shown a significant increase in brain MPO activity, AChE activity, nitrite/nitrate levels & TBARS level along with depletion of brain GSH levels.

In the present investigation treatment with Vitamin D attenuated the effect of SAZ on learning & memory of rats. In addition, it also improved SAZ induced reduction in GSH levels, increased AChE, TBARS, nitrite & MPO levels. AD is a progressive neurodegenerative disorder characterized by early cognitive dysfunction associated later by behavioral and social deterioration (Fadil *et al*., 2009). Patients with AD have high prevalence of Vitamin D deficiency and it is associated with low mood and impaired cognitive performance (Nguyen *et al*., 2011). Generally, vitamin D receptors are widely distributed in the cortex and hippocampus and accumulated data provide evidence for unpredicted roles for vitamin D in brain development and function (Llewellyn *et al*., 2009; Kumar *et al*., 2009; Teresita *et al*., 2012). It has antioxidant action (Harmes *et al*., 2011). Its beneficial effect can be hypothesized by the roles of Vitamin D in rheumatoid arthritis (Azzeh, 2012), chronic kidney disease (Williams *et al*., 2009), diabetes (Talaei *et al*., 2013), depression (Li *et al*., 2013), metabolic syndrome (Wang, 2013), crohn's disease (Joseph *et al*., 2009) and cancer (Matsuda *et al*., 2013). This finding illustrates the importance of use of vitamin D in the improvement of learning and memory performance.

PPAR-γ is expressed widely in CNS where it has a prominent role in regulation of neuroprotection (Fatehi *et al*., 2011; Glatz *et al*., 2010). PPAR-γ receptors have been implicated in various neuroprotective studies involving huntington disease (Jin *et al*., 2013), Parkinson's disease (Dehmer *et al*., 2004), amyotrophic lateral sclerosis (Kiaei *et al*., 2005) and cerebral ischemia (Xu *et al*., 2013). It has been reported in various studies that PPAR-γ agonists exhibit anti-inflammatory properties which are due to negative regulation of the expression of pro-inflammatory molecules such as interleukin-1b (IL-1b), IL-6 and TNF-α (Halvorsen *et al*., 2010; Zhang *et al*., 2010). These studies have proven the protective effect of PPAR-γ agonistic activity in neurodegenerative disorders. Further, it has been reported that activation of PPAR-γ upregulates Bcl-2, enhances its cell survival pathway and prevents neuronal degeneration, along with increase in mitochondrial viability (Chiang *et al*., 2011). There are studies suggesting that activation of PPAR-γ modulate the target genes of inflammation (iNOS, NF-κB and COX-2), oxidative stress and apoptosis and provides neuroprotection (Kiaei *et al*., 2008). In recent past PPAR-γ has been considered as a novel target to manage cognitive decline in AD patients (Landreth *et al*., 2008; Landreth, 2007; Singh *et al*., 2011).

Loss of glutathione had been suggested as an early signaling event in mitochondrial dysfunction and apoptotic cell death involved in neurodegeneration and ageing process. Vitamin D leads to up-regulation of the activity and expression of brain gamma glutamyl transpeptidase; a key enzyme involved in brain glutathione cycle, thus it ameliorates oxidative stress by increasing brain GSH. Moreover, it had been reported that vitamin D inhibits the expression of inducible nitric oxide synthase (iNOS) that is thought to play a role in AD pathogenesis (Soliman *et al*., 2015). In the meanwhile, vitamin D had been demonstrated to interact with reactive oxygen and nitrogen species in various models of brain oxidative challenges, suggesting its role in brain detoxification pathways (Harmes *et al*., 2011).

Therefore with support from literature & data in hand, it may be suggested that Vitamin D an antioxidant has shown ameliorative effects against SAZ induced neurodegeneration as well as loss of learning & memory in rats. However, pretreatment with BADGE, a selective PPAR-γ antagonist abolished the Vitamin D mediated protection that is signified by decrease in GSH, increase in AChE, TBARS, MPO and nitrite levels when compared to high dose treated animals.

Donepezil, an AChE inhibitor is a well-established drug for the management of dementia and being clinically used for memory deficits associated with AD and other conditions.

Hence, it is concluded that Vitamin D provides neuroprotection against SAZ induced AD in rats and this protection is mediated via activation of PPAR-γ receptors.

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