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Behavioral and biochemical investigations to explore pharmacological potential of PPAR-gamma agonists in vascular dementia of diabetic rats

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ABSTRACT

Vascular dementia (VaD) is the second most common dementing illness. We have recently reported that diabetes induces VaD in rats. The present study has been designed to investigate the potential of peroxisome-proliferator-activated receptors-gamma (PPAR-γ) agonists in diabetes induced VaD of Wistar Albino rats. The rats were administered, single dose of streptozotocin (STZ) for the induction of diabetes. Morris water-maze (MWM) test was employed for testing learning and memory. Serum glucose, bodyweight, vascular endothelial function, serum nitrite/nitrate levels, aortic and brain oxidative stress levels (*viz.* aortic superoxide anion levels, brain thiobarbituric acid reactive species and brain glutathione levels) and brain acetylcholinesterase activity were also tested. STZ treated animals performed poorly on MWM hence reflecting impairment of learning and memory behavior with a significant reduction in body weight, impairment of vascular endothelial function, and decrease in serum nitrite/nitrate levels, increase in serum glucose, aortic and brain oxidative stress levels and brain acetylcholinesterase activity. Treatment of PPAR-γ agonists, pioglitazone as well as rosiglitazone significantly reversed, diabetes induced impairment of learning and memory behavior, endothelial function, and changes in various biochemical parameters. It is concluded that PPAR-γ modulators pioglitazone and rosiglitazone may be considered as potential pharmacological agents for the management of diabetes induced VaD.

1. Introduction

Diabetes and dementia have become a major public health concern worldwide due to being common diseases in the elderly population. Vascular dementia (VaD) a dementia of vascular origin is considered to be the second most common cause of dementia after Alzheimer's disease (AD) (Liu et al., 2010). Diabetes has been found to be consistently associated with the risk of VaD and there is the significant association between glucose intolerance and the risks of both VaD and AD (Sekita and Kiyohara, 2010). Diabetic people had a 1.5 to 4 fold risk for AD as well as VaD. High glucose concentration, a major pathological characteristic of diabetes, may have toxic effects on neurons in the brain through osmotic insults and oxidative stress. The insulin resistance (i.e., hyperinsulinemia) in people with impaired glucose tolerance has been one of risk factors for cognitive decline (Araki, 2010). Furthermore, diabetes is associated with an increased release of inflammatory cytokines, and the excess inflammation may be neurotoxic (Umegaki, 2010). Oxidative stress and vascular endothelial are recognized as important contributing factors in the pathogenesis of AD and dementia of vascular origin (de la Torre, 2008; Viswanathan et al., 2009). Only limited therapeutic interventions are available to reduce the incidence of VaD.

Peroxisome-proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptors super family which are present in three isoforms as α, β/δ and γ (Arck et al., 2010). PPAR-γ is present on vascular cells, exert protective role in the vascular endothelial dysfunction (Beyer et al., 2008). Disruption or down regulation of these receptors have been reported to result in vascular endothelial dysfunction (Kleinhenz et al., 2009). PPAR-γ receptors are distributed broadly in central nervous system (Sarruf et al., 2009), and activation of these receptors prevents neuronal death by reduction of oxidative stress (Zhao et al., 2009) and inflammatory mechanisms (Glatz et al., 2010). PPAR-γ agonists in addition to their anti-diabetic activity have been shown to provide beneficial effect in various CNS disorders (Chaturvedi et al., 2009; Jain et al., 2009; Kiaei, 2008; Kumari et al., 2010; Schintu et al., 2009; Zhang et al., 2010a, 2010b). Furthermore, PPAR-γ agonists have the potential to modulate various signaling molecules/pathways, including mitogen-activated protein kinases, signal transducer and activator of transcription, amyloid precursor protein degradation, beta-site amyloid precursor protein cleaving enzyme 1 and Wnt signaling (Kaundal and Sharma, 2010). Moreover, it has been recently reported that, PPAR-γ is involved in improvement of memory and

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cognitive function in AD (Hanyu and Sato, 2010). Also, cell culture studies suggest that, PPAR-γ agonists exert neuroprotective effects on cultured microglia and astrocytes by virtue of their inhibitory effect on amyloid beta elaborated pro-inflammatory cytokines (Jing and Ting, 1998; Ricote et al., 1999). However, the potential of PPAR-γ agonist in vascular dementia is still unexplored. Therefore, the present study has been undertaken to investigate the beneficial effect of PPAR-γ agonists, pioglitazone and rosiglitazone in diabetes induced vascular dementia in rats. Donepezil a well known acetylcholinesterase inhibitor served as a positive control in this investigation.

2. Material and methods

2.1. Animals

Adult male Wistar Albino rats, weighing 200–250 g (Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India) were employed in the present study. Animals were provided with standard laboratory feed (Kisan Feeds Ltd., Chandigarh, India) and water *ad libitum* and were exposed to natural cycle of light and dark. The experimental protocol was approved by institutional animal ethics committee (IAEC) and care of the animals was taken as per the guidelines of the Committee for the Purpose of control and supervision of experiments on Animals (CPCSEA), ministry of Environment and Forest Government of India, (Reg. No. 107/1999/CPCSEA). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Drugs and chemicals

Donepezil was obtained as free sample from Wokhardt Ltd., Baddi, Himachal Pradesh, India. Pioglitazone and rosiglitazone were obtained as free sample from Panacea Biotech Ltd., Lalru, India. Folin-Ciocalteu's Phenol reagent was purchased from Merck limited, Mumbai, India. 5, 5, dithiobis (2-nitro benzoic acid) (DTNB), reduced glutathione (GSH), bovine serum albumin (BSA), sulfanilamide, N-naphthylethylenediamine (NED) and thiobarbituric acid were obtained from Loba Chem, Mumbai, India. STZ, 1, 1, 3, 3-tetra methoxy propane, acetylthiocholine iodide, sodium nitropruside, phenylephrine were purchased from Sigma-Aldrich, USA. STZ was dissolved in 0.1 M citrate buffer (pH 4.5). Pioglitazone and rosiglitazone were suspended in 1% w/v of carboxy methyl cellulose (CMC) whereas donepezil was dissolved in saline water. Pioglitazone, rosiglitazone and CMC were administered orally with the help of an oral tube (canulla). Streptozotocin and Donepezil was administered intraperitoneally.

2.3. Streptozotocin (STZ) induced diabetes and associated vascular dementia

The rats were injected with the single dose of freshly prepared streptozotocin (50 mg/kg i.p.) in 0.1 M citrate buffer (pH 4.5) to induce experimental diabetes mellitus and associated dementia (Brosky and Logothetopoulos, 1969; Rakieten et al., 1963). Serum glucose levels of the animals were measured every week. The animals were used on 52nd day for the behavioral and other assessment (Sharma and Singh, 2010, 2011).

2.4. Assessment of learning and memory by Morris water maze

Morris water maze (Morris, 1984; Sharma et al., 2008a, 2008b; Sharma and Singh, 2010, 2011) is one of the most commonly used animal models to test memory. The MWM procedure was based on a principle where the animal was placed in a large pool of water, as animal dislike swimming, their tendency was to escape from the water being accomplished by finding an escape platform. MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 °C). The water was made opaque with white colored 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 cm^2), painted 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 trials on each day with gap of 5 min. The rat was gently placed in the water of the pool between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 s 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. Escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning. Animal was subjected to acquisition trials for four consecutive days. On fifth day, platform was removed and each rat was allowed to explore in 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 is noted as index of retrieval.

2.4.1. Acquisition trial

Each rat was subjected to four trials on each day. A rest period of 5 min was allowed in between each trial. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four acquisition trials was changed as described below and Q4 was maintained as target quadrant in all acquisition trials. Mean escape latency time (ELT) calculated for each day during acquisition trials was used as an index of acquisition.

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

2.4.2. Retrieval trial

On fifth day the platform was removed. Rat was placed in water maze and allowed to explore the maze for 120 s. Each rat was subjected to four such trials and each trial was started from different quadrant. Mean time spent in all three quadrants i.e. Q1, Q2 and Q3 were recorded and the time spent in the target quadrant i.e. Q4 in search of missing platform provided an 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 and 18.00 h.

2.5. Assessment of vascular endothelial function using isolated rat aortic ring preparation

Rats were decapitated and the thoracic aorta was removed, cut into a ring of 4 to 5 mm width, and mounted in organ bath containing Krebs–Henseleit bubbled with carbonated oxygen (95% O_2:5% CO_2), and maintained at 37.8 °C. The preparation was allowed to equilibrate for 90 min under 1.5 g tension. The isometric contractions were recorded (Pieper, 1997) with a force-displacement transducer (Ft-2147) connected to Physiograph (INCO, Ambala, India). The preparation was primed with 80 mmol L^{-1} KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of acetylcholine (ACh; 10^{-8} to 10^{-4} mol L^{-1}) or sodium nitroprusside (SNP; 10^{-8} to 10^{-4} mol L^{-1}) were recorded in phenylephrine (3×10^{-6} mol L^{-1}) precontracted preparations (Koladiya et al., 2008, 2009; Sharma and Singh, 2010, 2011). The intimal layer of aortic ring was rubbed gently with a moistened filter paper for 30 s to obtain endothelium-free preparations. Loss of ACh

(1×10^{-6} mol L^{-1}) induced relaxation confirmed the absence of vascular endothelium (Sharma and Singh, 2010, 2011).

2.6. Biochemical parameters

2.6.1. Collection of sample

Blood samples for biochemical estimation were collected by retro-orbital bleeding. The blood was kept at room temperature for 30 min and then centrifuged at 4000 rpm for 15 min to separate serum.

After last retro-orbital bleeding, animals were sacrificed by cervical dislocation; thoracic aorta and brain tissue were carefully removed. Thoracic aorta was used for endothelium dependent and independent relaxation as well as for the estimation of superoxide anion, whereas brain tissue was subjected to various biochemical estimations. The removed brains were homogenized in phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer and centrifuged at 3000 rpm for 15 min to obtain the clear supernatant.

Serum and clear supernatant were then used for different biochemical estimations.

2.6.2. Estimation of serum glucose levels

The glucose levels were estimated spectrophotometerically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 505 nm by glucose oxidase peroxidase (GOD-POD) method using a commercially available kit (Reckon diagnostics Pvt. Ltd. Vadodra, India).

2.6.3. Estimation of serum nitrite concentration

Serum nitrite concentration was serum nitrite was measured spectophotometrically (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 545 nm, using method of Sastry et al. (Sastry et al., 2002; Sharma and Singh, 2010).

2.6.4. Estimation of aortic production of super oxide anion

The superoxide anion was determined spectrophotometrically (DU 640B Spectrophotometer, Beckman Coulter, Inc.) at 540 nm using method of Wang et al. (Sharma and Singh, 2010; Wang et al., 1998).

2.6.5. Estimation of brain acetyl cholinesterase (AChE) activity

The whole brain AChE activity was measured spectrophotometerically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 420 nm by the method of Ellman et al. (Ellman et al., 1961; Sharma and Singh, 2010; Voss and Sachsse, 1970).

2.6.6. Estimation of thiobarbituric acid reactive substances (TBARS)

The brain TBARS was measured spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 532 nm using method of Ohkawa et al. (Ohokawa et al., 1979; Sharma and Singh, 2010).

2.6.7. Estimation of reduced glutathione (GSH)

The reduced glutathione (GSH) content in brain was estimated spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 412 nm using method of Beutler et al. (Beutler et al., 1963; Sharma and Singh, 2010).

2.6.8. Estimation of brain total protein

The brain total protein was determined spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 750 nm using method of Lowry's et al. (Lowry's et al., 1951; Sharma and Singh, 2010).

2.6.9. Experimental protocol

Fifteen groups were employed in the present study and each group comprising of 8 male Wistar albino rats (for the schematic representation, see Fig. 1).

2.6.9.1. Group I — control group. Animals were exposed to Morris water maze for acquisition trial from Day 1 to Day 4 and retrieval trial on Day 5.

2.6.9.2. Group II — vehicle control group (0.9% saline). Animals were administered saline (10 ml kg^{-1} i.p., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

2.6.9.3. Group III — vehicle control group (1% CMC). Animals were administered CMC (10 ml kg^{-1} p.o., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

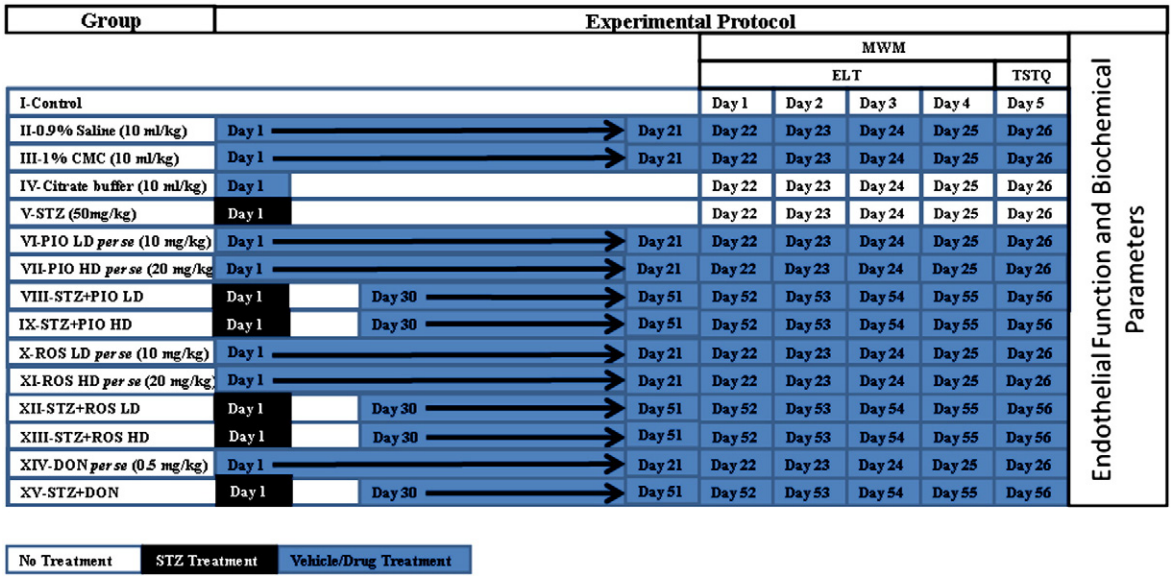

Fig. 1. Schematic representation of experimental protocol. MWM — Morris water maze; ELT — Escape latency time (Acquisition trials); TSTQ — Time spent in the target quadrant (Retrieval trial); CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

2.6.9.4. Group IV — vehicle control group (citrate buffer 0.1 M, pH 4.5). Animals were administered with single dose citrate buffer (10 ml kg^{-1} i.p.), these animals were exposed to Morris water maze on 52nd day of citrate buffer administration.

2.6.9.5. Group V — STZ treatment group. Animals were administered single dose Streptozotocin (50 mg kg^{-1} i.p.) and animals were exposed to Morris water maze on 52nd day of STZ administration.

2.6.9.6. Group VI — pioglitazone low dose per se. Animals were administered pioglitazone (10 mg kg^{-1} p.o., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

2.6.9.7. Group VII — pioglitazone high dose per se. Animals were administered pioglitazone (20 mg kg^{-1} p.o., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

2.6.9.8. Group VIII — STZ and pioglitazone low dose. Pioglitazone (10 mg kg^{-1} p.o., daily) was administered to the STZ (50 mg kg^{-1} i.p.) treated rats, starting from 30th day of STZ treatment followed by exposure to Morris water maze on 52nd day of STZ administration. The treatment was continued during acquisition (from 52nd to 55th day) and retrieval trials (on 56th day) on Morris water maze.

2.6.9.9. Group IX — STZ and pioglitazone high dose. Pioglitazone (20 mg kg^{-1} p.o., daily) was administered to the STZ (50 mg kg^{-1} i.p.) treated rats, starting from 30th day of STZ treatment followed by exposure to Morris water maze on 52nd day of STZ administration. The treatment was continued during acquisition (from 52nd to 55th day) and retrieval trials (on 56th day) on Morris water maze.

2.6.9.10. Group X — rosiglitazone low dose per se. Animals were administered rosiglitazone (10 mg kg^{-1} p.o., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

2.6.9.11. Group XI — rosiglitazone high dose per se. Animals were administered rosiglitazone (20 mg kg^{-1} p.o., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

2.6.9.12. Group XII — STZ and rosiglitazone low dose. Rosiglitazone (10 mg kg^{-1} p.o., daily) was administered to the STZ (50 mg kg^{-1} i.p.) treated rats, starting from 30th day of STZ treatment followed by exposure to Morris water maze on 52nd day of STZ administration. The treatment was continued during acquisition (from 52nd to 55th day) and retrieval trials (on 56th day) on Morris water maze.

2.6.9.13. Group XIII — STZ and rosiglitazone high dose. Rosiglitazone (20 mg kg^{-1} p.o., daily) was administered to the STZ (50 mg kg^{-1} i.p.) treated rats, starting from 30th day of STZ treatment followed by exposure to Morris water maze on 52nd day of STZ administration. The treatment was continued during acquisition (from 52nd to 55th day) and retrieval trials (on 56th day) on Morris water maze.

2.6.9.14. Group XIV — donepezil per se. Animals were administered Donepezil (0.5 mg kg^{-1} i.p., daily) for 21 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 22nd to 25th day) and retrieval trials (on 26th day) on Morris water maze.

2.6.9.15. Group XV — STZ and donepezil. Donepezil (0.5 mg kg^{-1} i.p., daily) was administered to the STZ (50 mg kg^{-1} i.p.) treated rats, starting from 30th day of STZ treatment followed by exposure to Morris water maze on 52nd day of STZ administration. The treatment was continued during acquisition (from 52nd to 55th day) and retrieval trials (on 56th day) on Morris water maze.

2.7. Statistical analysis

All the results of this study were statistically analyzed by software sigma state 3.5. The results were expressed as mean ± standard deviation of mean. The data for isolated aortic ring preparation were statistically analyzed using repeated measure ANOVA followed by Newman-Keul's test. Rest of the data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range test. The $p<0.05$ was considered to be statistically significant.

3. Results

3.1. Effect on escape latency time (ELT) and time spent in target quadrant (TSTQ), using Morris water maze (MWM)

Before subjecting the animals to MWM test, their motor coordination scores were measured by employing Rota rod test. However, no significant difference was noted between scores of diabetic and control animals (data not shown). Control rats showed a downward trend in their ELT. There was a significant fall in day 4 ELT, when compared to day 1 ELT of these rats (Table 1), reflecting normal learning ability. Further on day 5 a significant rise in TSTQ was observed, when compared to time spent in other quadrants (Fig. 2), reflecting normal retrieval as well. Administration of various vehicles did not show any significant effect on ELT and TSTQ as compared to control rats. Administration of pioglitazone (10 mg kg^{-1} p.o./20 mg kg^{-1} p.o., 26 days)/rosiglitazone (10 mg kg^{-1} p.o./20 mg kg^{-1} p.o., 26 days)/donepezil (0.5 mg kg^{-1} i.p., 26 days) did not show any

Table 1
Reversal of STZ diabetes induced increase in Day 4 Escape Latency Time (ELT) of animals by pioglitazone and rosiglitazone.

S. No.	Group	Treatment	Escape Latency Time (ELT)	
			Day 1 (in sec.)	Day 4 (in sec.)
1	I	Control	99.2 ± 2.3	42.2 ± 2.6[a]
2	II	Vehicle control (distilled water)	101.5 ± 3.2	48.2 ± 4.3[a]
3	III	Vehicle control (citrate buffer)	103.4 ± 2.7	49.6 ± 3.6[a]
4	IV	Vehicle control (CMC)	98.2 ± 4.1	47.3 ± 3.2[a]
5	V	STZ	100.4 ± 3.2	91.6 ± 7.5[b]
6	VI	Pioglitazone low dose *per se*	105.2 ± 4.2	49.2 ± 3.8[a]
7	VII	Pioglitazone high dose *per se*	103.2 ± 2.4	45.6 ± 3.1[a]
8	VIII	STZ and pioglitazone low dose	101.3 ± 3.5	69.6 ± 3.4[a,c]
9	IX	STZ and pioglitazone high dose	102.3 ± 3.7	60.5 ± 3.6[a,c]
10	X	Rosiglitazone low dose *per se*	105.8 ± 3.6	44.2 ± 2.9[a]
11	XI	Rosiglitazone high dose *per se*	102.1 ± 3.8	42.8 ± 3.4[a]
12	XII	STZ and rosiglitazone low dose	102.4 ± 5.1	70.2 ± 3.8[a,c]
13	XIII	STZ and rosiglitazone high dose	103.3 ± 4.6	65.3 ± 3.3[a,c]
14	XIV	Donepezil *per se*	103.5 ± 2.7	44.2 ± 3.6[a]
15	XV	STZ and donepezil	110.2 ± 4.3	51.2 ± 3.2[a,c]

Each group comprised of 8 rats. As noted on Morris water maze, STZ diabetic rats have shown a significant increase in Day 4 ELT, which was significantly reduced by pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil. All data of ELT are represented as mean ± standard error of means (S.E.M) and were statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p<0.05$ was considered to be statistically significant.
CMC — Carboxymethylcellulose; STZ — Streptozotocin.
[a] $p<0.05$ versus day 1 escape latency time in respective groups.
[b] $p<0.05$ versus day 4 escape latency time in control group.
[c] $p<0.05$ versus day 4 escape latency time in STZ treated group.

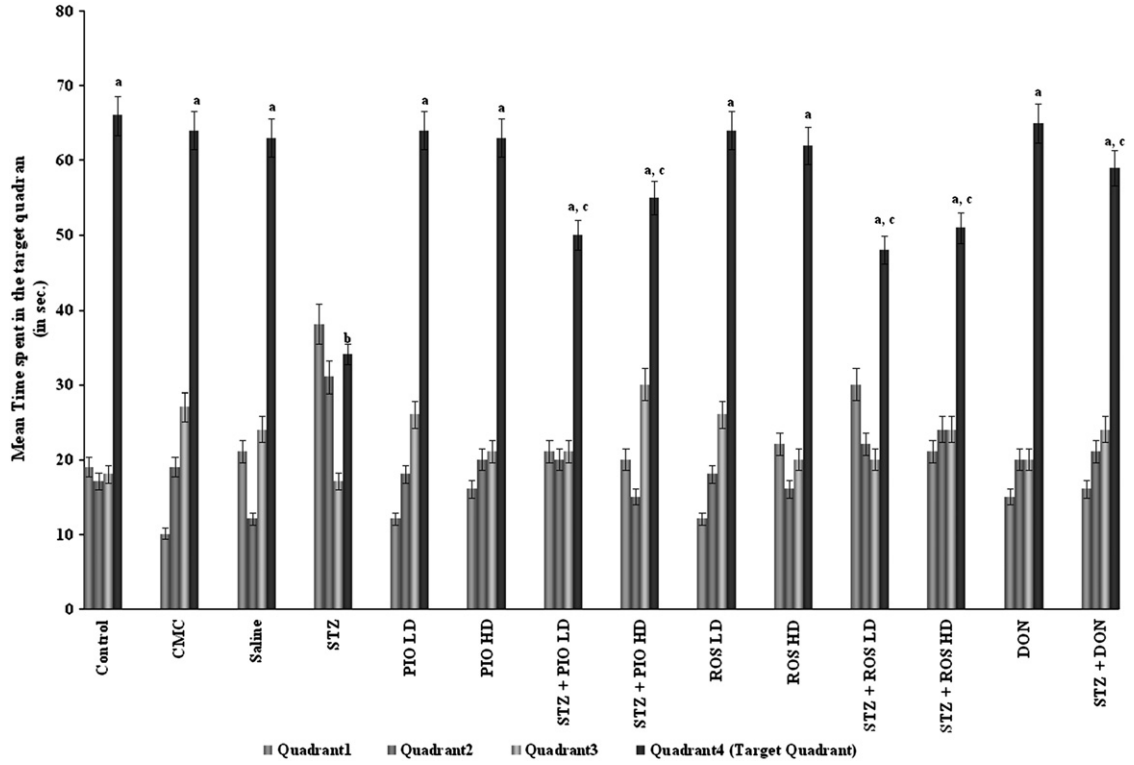

Fig. 2. Reversal of STZ diabetes induced reduction in mean time spent in target quadrant (TSTQ) of animals by pioglitazone and rosiglitazone. Each group comprised of eight rats. As noted on Morris water maze, STZ diabetic rats have shown a significant reduction in Day 5 TSTQ, which was significantly increased pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil. All data of TSTQ are represented as mean ± standard error of means (S.E.M) and were statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p<0.05$ was considered to be statistically significant. $^{a}p<0.05$ versus mean time spent in other quadrants; $^{b}p<0.05$ versus mean time spent in target quadrant in control group; $^{c}p<0.05$ versus mean time spent in target quadrant in STZ treated group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

significant *per se* effect on ELT and TSTQ as compared to control rats (Table 1and Fig. 2). However streptozotocin (STZ) (50 mg kg^{-1} i.p., single dose) treated rats showed a significant increase in day 4 ELT (55th day of STZ treatment), when compared to day 4 ELT of control animals (Table 1) indicating impairment of acquisition. Further STZ administration also produced a significant decrease in day 5 TSTQ (56th day of STZ treatment), when compared day 5 TSTQ of control animals (Fig. 2), indicating impairment of memory as well.

Administration of pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, to STZ rats, significantly prevented STZ induced rise in day 4 ELT, indicating reversal of STZ induced impairment of acquisition (Table 1). Further treatment of these drugs also attenuated STZ induced decrease in day 5 TSTQ in a significant manner, indicating reversal of STZ induced impairment of memory as well (Fig. 2).

3.2. Effect on endothelium dependent and independent relaxation

Acetylcholine (ACh) and sodium nitroprusside (SNP) in a dose dependent manner produced endothelium dependent and independent relaxation in phenylephrine (3×10^{-6} M) precontracted isolated rat aortic ring preparation. STZ administration significantly attenuated acetylcholine induced endothelium dependent relaxation (Fig. 3), however it did not affect SNP induced endothelium independent relaxation (Fig. 4). Treatment of pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, significantly attenuated the effect of STZ on endothelial dependent relaxation. Further pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, did not show any *per se* effect on endothelium dependent relaxation.

3.3. Effect on serum glucose level and body weight

Administration of STZ produced a significant increase in serum glucose (Fig. 5) and a significant decrease in body weight (Fig. 6) as compared to control rats. Treatment with pioglitazone (low and high dose)/rosiglitazone (low and high dose) to STZ treated rats significantly reduced the serum glucose levels with a significant increase in body weight. Donepezil did not show any significant change in STZ induced increase in serum glucose level and decrease in body weight (Figs. 5 and 6). Furthermore, pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, did not show any significant *per se* effect on serum glucose level and body weight of the animals (Figs. 5 and 6).

3.4. Effect on serum nitrite level

Administration of STZ produced a significant decrease in serum nitrite, when compared to control rats. Treatment with pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, prevented STZ induced decrease in serum nitrite level in a significant manner (Fig. 7). Moreover, pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, did not show any significant *per se* effect on serum nitrite level (Fig. 7).

3.5. Effect on brain acetyl cholinesterase (AChE) activity

Administration of STZ produced a significant, increase in brain AChE activity, when compared to control rats. Treatment with pioglitazone (low and high dose)/rosiglitazone (low and high dose)/

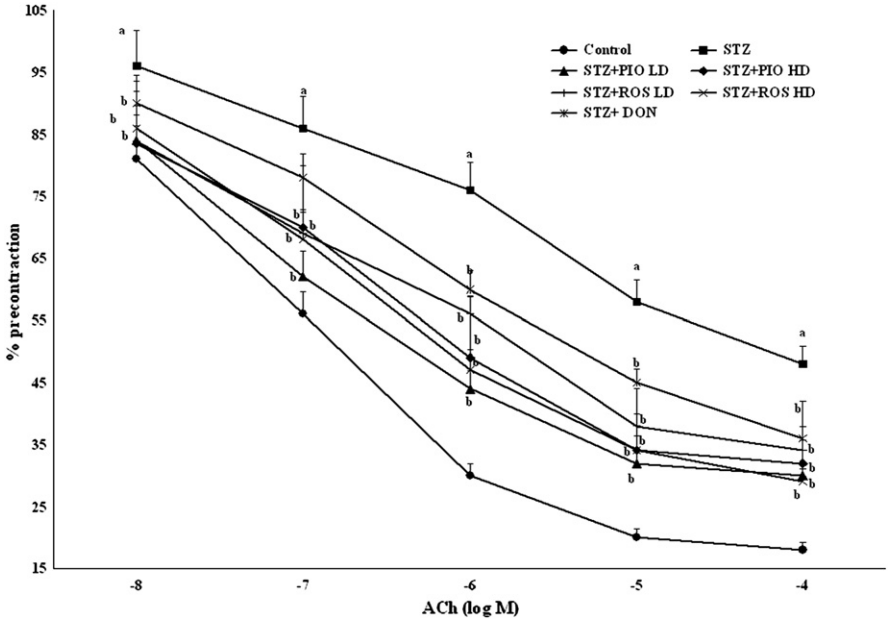

Fig. 3. Attenuation of STZ induced impairment of Acetylcholine induced endothelium dependent relaxation by pioglitazone and rosiglitazone. Each group comprised of eight rats and all the responses are expressed as percentage of precontraction induced by 3×10^{-6} M phenylephrine. As noted on aortic ring preparation using student physiograph, STZ diabetic rats have shown a significant reduction in acetylcholine induced endothelium dependent relaxation, which was significantly reduced by pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil. All data were represented as mean ± standard error of means (S.E.M) and were statistically analyzed using repeated measure analysis of variance (ANOVA) followed by Newman Keul's test. $p<0.05$ was considered to be statistically significant. [a]$p<0.05$ versus control; [b]$p<0.05$ versus STZ treated group. STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

donepezil, significantly prevented STZ induced rise in brain AChE activity. Furthermore, pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, did not show any significant *per se* effect on brain AChE activity (Fig. 8).

3.6. Effect on oxidative stress levels

Administration of STZ produced a significant increase, in aortic superoxide anion level (Fig. 9)/brain thiobarbituric acid reactive species (TBARS) (Fig. 10) and a significant decrease, in the brain levels of reduced form of glutathione (GSH) (Fig. 11), when compared to control rats; hence reflecting induction of oxidative stress. Treatment with pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, significantly prevented STZ induced oxidative stress. Further, pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil, did not show any significant *per se* effect on oxidative stress level (Figs. 9, 10 and 11).

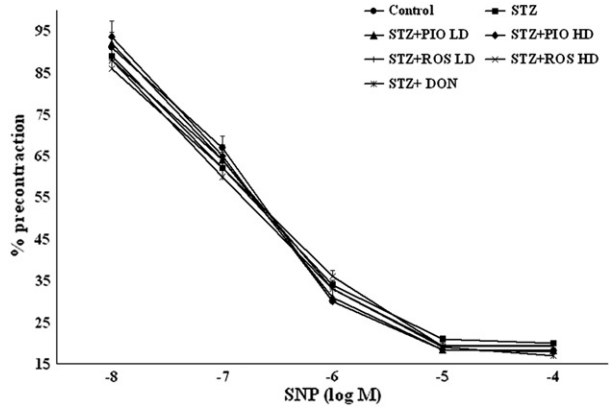

Fig. 4. Effect of various treatments on sodium nitroprusside induced endothelium independent relaxation. Each group comprised of eight rats and all the responses are expressed as percentage of precontraction induced by 3×10^{-6} M phenylephrine. As noted on aortic ring preparation using student physiograph, there was no effect of any of the treatments on endothelium independent relaxation. All data were represented as mean ± standard error of means (S.E.M) and were statistically analyzed using repeated measure analysis of variance (ANOVA) followed by Newman Keul's test. $p<0.05$ was considered to be statistically significant. STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

4. Discussion

Morris water maze employed in the present study is one of the most accepted models to evaluate learning and memory of the rodents (Morris, 1984; Parle and Singh, 2007). Control untreated animals in our study have shown marked reduction in day 4 escape

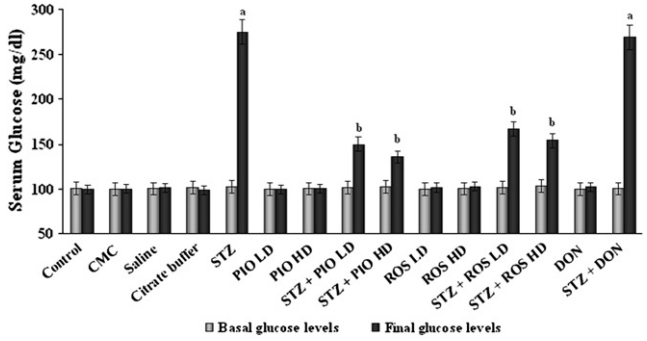

Fig. 5. Attenuation of STZ diabetes induced rise in serum glucose of animals by pioglitazone and rosiglitazone. Each group comprised of 8 rats. STZ diabetic rats have shown a significant rise in serum glucose as measured on day 1 of Morris water maze exposure, which was significantly attenuated by pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil. All data of serum glucose are represented as mean ± standard error of means (S.E.M) and were statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p<0.05$ was considered to be statistically significant. [a]$p<0.05$ versus basal values in respective groups; [b]$p<0.05$ versus values in control group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

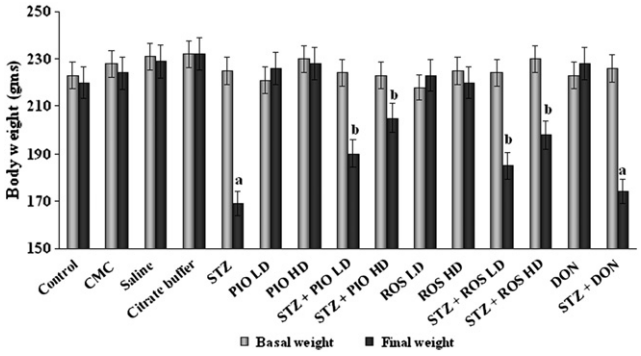

Fig. 6. Attenuation of STZ diabetes induced decrease in body weight of animals by pioglitazone and rosiglitazone. Each group comprised of 8 rats. STZ diabetic rats have shown a significant decrease in body weight as measured on day 1 of Morris water maze exposure, which was significantly attenuated by pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil. All data of body weight are represented as mean ± standard error of means (S.E.M) and were statistically analyzed using one way ANOVA followed by Tukey's multiple range test. $p<0.05$ was considered to be statistically significant. $^{a}p<0.05$ versus basal values in respective groups; $^{b}p<0.05$ versus values in control group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

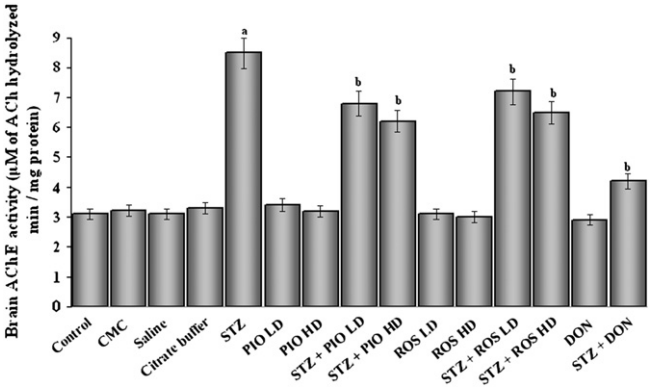

Fig. 8. Reversal of STZ induced increase in brain acetyl cholinesterase activity by pioglitazone and rosiglitazone. Each group comprised of 8 rats. Pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil significantly reverse the STZ diabetes induced increase in brain acetyl cholinesterase activity. $^{a}p<0.05$ versus control group; $^{b}p<0.05$ versus STZ treated group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

latency time (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 mean time spent in target quadrant (TSTQ) when compared to time spent in other quadrants, indicating normal retrieval (memory) as well. These results are in lines to previous studies from our own lab (Koladiya et al., 2008; Sharma and Singh, 2010, 2011) as well as from other labs (Packard et al., 1996; Camarasa et al., 2010).

Results of the present investigation state that Streptozotocin (STZ) in a single dose of 50 mg kg^{-1} i.p. has produced hyperglycemia, vascular endothelial dysfunction, memory impairment and abnormalities in various biochemical parameters of rats. Single dose STZ administration is a very well documented and an accepted model of diabetes in rats (Leung et al., 2010; Sharma and Singh, 2010, 2011; Sokolovska et al., 2010). STZ induced experimental diabetes is widely used for the assessment of diabetic conditions and its secondary complications including endothelial dysfunction (Chopra et al., 2010; Feng et al., 2010; Huynh et al., 2010; Marotta et al., 2010; Olukman et al., 2010; Rao et al., 2010; Yohannes et al., 2010; Zhang et al., 2010a, 2010b).

STZ in previous reports has been well reported to induce endothelial dysfunction (Olukman et al., 2010; Schafer et al., 2010; Woodman and Malakul, 2009). In line with these studies, STZ in our investigation has induced significant endothelial dysfunction as reflected by impairment of endothelial dependent relaxation and reduction in nitrite/nitrate level. STZ treatment has been documented to enhance production of oxidative free radicals eventually leading to high oxidative stress (Pari and Srinivasan, 2010; Wang et al., 2011). STZ induced rise in superoxide anion in aortic strip of present study is a reflection of oxidative stress and probably is one of the major contributing factors in STZ induced endothelial dysfunction. STZ diabetic rats in our study performed poorly on MWM indicating impairment in their learning abilities and memory capabilities. Further there was a significant rise in brain acetyl cholinesterase (AChE) activity and brain oxidative stress levels (indicated by an increase in TBARS and decrease in GSH levels). Studies have shown that STZ induced diabetes may cause impairment of learning and memory and exacerbate post stroke dementia (Zhang et al., 2010a, 2010b; Zhou et al., 2007). The STZ diabetic animals have also been reported to suffer from diabetic encephalopathy (Saravia et al., 2006; Xu et al., 2008). Moreover, a strong association of diabetes with vascular dementia in humans has been documented (Luchsinger, 2010). In our previous studies intra-cerebroventricular injection of STZ has been reported to produce memory impairment

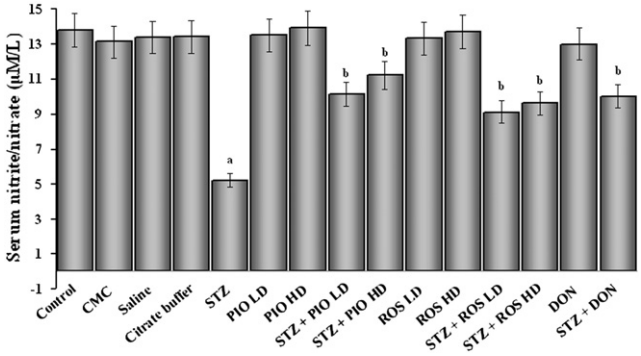

Fig. 7. Reversal of STZ induced decrease in serum nitrite/nitrate levels by pioglitazone and rosiglitazone. Each group comprised of 8 rats. Pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil significantly reverse the STZ diabetes induced reduction in serum nitrite/nitrate levels. $^{a}p<0.05$ versus control group; $^{b}p<0.05$ versus STZ treated group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

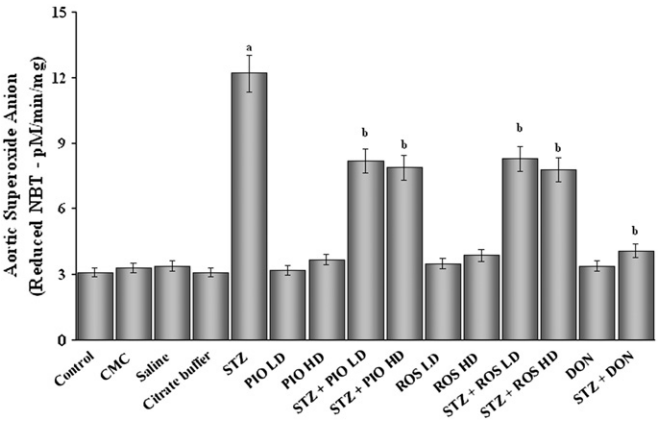

Fig. 9. Reversal of STZ induced increase in aortic superoxide anion generation by pioglitazone and rosiglitazone. Each group comprised of 8 rats. Pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil significantly reverse the STZ diabetes induced increase in aortic superoxide anion generation. $^{a}p<0.05$ versus control group; $^{b}p<0.05$ versus STZ treated group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

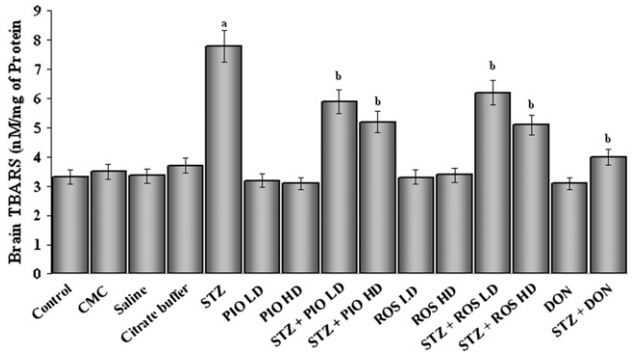

Fig. 10. Reversal of STZ induced increase in brain thiobarbituric acid reactive species (TBARS) levels by pioglitazone and rosiglitazone. Each group comprised of 8 rats. Pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil significantly reverse the STZ diabetes induced increase in brain TBARS levels. $^{a}p<0.05$ versus control group; $^{b}p<0.05$ versus STZ treated group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

of rodents (Sharma et al., 2008a, 2008b). Further, endothelial dysfunction (vascular defects) has been reported to induce varying degree of memory impairment in animals as well as humans (Bomboi et al., 2010; Isingrini et al., 2009; Kearney-Schwartz et al., 2009). Endothelial dysfunction has also reported to be related to higher oxidative stress levels (Javeshghani et al., 2009; Ozkul et al., 2010; Weseler and Bast, 2010). Moreover in our earlier studies we have demonstrated that vascular endothelial dysfunction in addition to impairment of memory and oxidative stress produces rise in brain AChE activity (Koladiya et al., 2008, 2009). Furthermore, very recently we have reported that single diabetogenic dose of STZ produces vascular dementia manifested in the terms of endothelial dysfunction; memory impairment as well as brain oxidative stress and rise in brain AChE activity. Therefore, the observed STZ diabetes induced vascular dementia is in line with our previous finding (Sharma and Singh, 2010, 2011).

In the present study, administration of pioglitazone, rosiglitazone (both PPAR-γ agonists) and donepezil (an AChE inhibitor), significantly attenuated the effect of STZ on learning and memory of rats. In addition these drugs also attenuated STZ induced endothelium dysfunction, hyperglycemia and other biochemical changes.

PPAR-γ agonists, pioglitazone and rosiglitazone are better known as insulin sensitizers that constitute an important class of drugs currently being used clinically in type II diabetes (Bermudez et al., 2010; Krieger-Hinck et al., 2010). These drugs act by binding to the PPAR-γ, a member of the nuclear receptors super family that has a

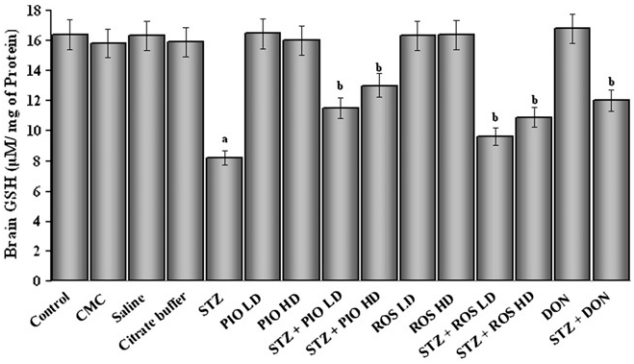

Fig. 11. Reversal of STZ induced decrease in brain glutathione (GSH) levels by pioglitazone and rosiglitazone. Each group comprised of 8 rats. Pioglitazone (low and high dose)/rosiglitazone (low and high dose)/donepezil significantly reverse the STZ diabetes induced reduction in brain GSH levels. $^{a}p<0.05$ versus control group; $^{b}p<0.05$ versus STZ treated group. CMC — Carboxymethylcellulose; STZ — Streptozotocin; PIO — Pioglitazone; ROS — Rosiglitazone; DON — Donepezil; LD — Low dose; HD — High dose.

key function in glucose regulation, lipid metabolism, vascular tone and inflammation (Arck et al., 2010; Halvorsen et al., 2010; Yu et al., 2010; Zhang et al., 2010a, 2010b). In the present PPAR-γ is expressed widely in CNS, where it has a prominent role in the regulation of neuroprotection (Fatehi-Hassanabad and Tasker, 2011; Glatz et al., 2010). PPAR-γ agonists, has been shown to exert neuroprotective effect (Fatehi-Hassanabad and Tasker, 2011; Glatz et al., 2010; Zhao et al., 2009). PPAR-γ agonists have also been found to have excellent antioxidant activity (Li et al., 2011; Nicolakakis et al., 2008). In recent reports PPAR-γ has been demonstrated to play a vital role in the vasculature (Cipolla et al., 2010). Activation of PPAR-γ receptors has been shown to inhibit endothelial dysfunction (Tsuchiya et al., 2009) and improve the cerebral blood flow in brain areas (Nicolakakis et al., 2008). Further, PPAR-γ is being considered as novel target to manage cognitive decline in Alzheimer's disease patients and animals (Heneka and Landreth, 2007; Landreth, 2007; Landreth et al., 2008). Pioglitazone has been reported to reverse memory deficits in experimental animals by virtue of their potential anti-oxidative, anti-inflammatory, neuroprotective and anti-acetylcholinesterase activity (Kaur et al., 2009; Pathan et al., 2006). Also, in our earlier study we have demonstrated that pioglitazone mediated beneficial effect in intra-cerebro-ventricular STZ induced dementia, involves nitric oxide dependent pathway (Kaur et al., 2009). In addition many other reports have documented usefulness of PPARγ agonists in memory deficits which is independent of their glucose lowering property (Heneka and Landreth, 2007; Kaur et al., 2009; Landreth, 2007; Landreth et al., 2008; Nikolakakis et al., 2008; Glatz et al., 2010; Zhao et al., 2009).

Pioglitazone and rosiglitazone in addition to above actions have also been reported to improve endothelial function via activation of endothelial PPAR γ (Duan et al., 2008; Pasceri et al., 2000). PPAR γ activation has been shown to decrease the expression of adhesion molecules that induce endothelial inflammation by adherence of monocytes to the endothelium (Jackson et al., 1999; Pasceri et al., 2000; Verrier et al., 2004). Furthermore, PPAR γ agonists have been shown to directly enhance NO production in cultured endothelial cells via PPAR γ-dependent mechanism (Polikandriotis et al., 2005). These findings suggest that PPAR γ agonists could directly improve endothelial function by decreasing local inflammation and increasing NO production.

Therefore, with support from literature and data in hand it appears quite evident that pioglitazone and rosiglitazone mediated reversal of STZ diabetes induced vascular dementia involves coordinated activity of their multiple actions *viz*; anti-diabetic, anti-oxidative, anti-AChE activity, anti-inflammatory and neuroprotective actions.

Pioglitazone and rosiglitazone not only reduced the serum glucose levels of the animals (as shown in Fig. 5), but at the same time these two agents have significantly improved the endothelial function, enhanced the levels of serum nitrite/nitrate, along with significant reduction of brain acetylcholinesterase activity, serum, aortic and brain oxidative stress. All these effects of above drugs eventually lead to improvement of learning and memory scores of the diabetic animals. At this point it can be said that probably both glucose lowering as well as glucose independent actions of above drugs are important. Perhaps this is the first report documenting potential of PPAR-γ agonists in STZ diabetes induced vascular dementia.

Donepezil (an AChE inhibitor) is a well-established drug for the management of memory dysfunction and being clinically used for memory deficits of AD patients and dementia of other etiologies. Therefore it has been used as a positive control in the present study. Although it is an anti-cholinesterase agent but number of studies (including from our lab) have shown that it has many additional actions viz; anti-oxidative, anti-inflammatory, etc., which are equally important in mediating its beneficial effect in memory loss (Sharma et al., 2008a, 2008b). Further it has also been shown to be effective in

experimental vascular dementia (Koladiya et al., 2008, 2009; Sharma and Singh, 2010, 2011). Hence we selected donepezil as standard, the best agent available for this purpose. Here, it is used for the comparison of pioglitazone and rosiglitazone and our results are showing almost similar effect of these two drugs as that of donepezil.

In lieu of the above discussion it may be concluded that PPAR-γ agonists, pioglitazone and rosiglitazone provide beneficial effects by improving learning, memory, endothelial function, brain cholinergic activity and lowering blood glucose as well as oxidative stress in STZ diabetes induced, vascular dementia. Modulation of PPAR-γ may be considered as important target for vascular dementia. Nevertheless further studies are needed to substantiate these findings.

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