

Pharmacological inhibition of inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, convalesce behavior and biochemistry of hypertension induced vascular dementia in rats

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ABSTRACT

Cognitive disorders are likely to increase over the coming years (5–10). Vascular dementia (VaD) has heterogeneous pathology and is a challenge for clinicians. Current Alzheimer's disease drugs have had limited clinical efficacy in treating VaD and none have been approved by major regulatory authorities specifically for this disease. Role of iNOS and NADPH-oxidase has been reported in various pathological conditions but there role in hypertension (Hypt) induced VaD is still unclear. This research work investigates the salutiferous effect of aminoguanidine (AG), an iNOS inhibitor and 4'-hydroxy-3'-methoxyacetophenone (HMAP), a NADPH oxidase inhibitor in Hypt induced VaD in rats. Deoxycorticosterone acetate-salt (DOCA-S) hypertension has been used for development of VaD in rats. Morris water-maze was used for testing learning and memory. Vascular system assessment was done by testing endothelial function. Mean arterial blood pressure (MABP), oxidative stress [aortic superoxide anion, serum and brain thiobarbituric acid reactive species (TBARS) and brain glutathione (GSH)], nitric oxide levels (serum nitrite/nitrate) and cholinergic activity (brain acetyl cholinesterase activity-AChE) were also measured. DOCA-S treated rats have shown increased MABP with impairment of endothelial function, learning and memory, reduction in serum nitrite/nitrate & brain GSH levels along with increase in serum & brain TBARS, and brain AChE activity. AG as well as HMAP significantly convalesce Hypt induced impairment of learning, memory, endothelial function, and alterations in various biochemical parameters. It may be concluded that AG, an iNOS inhibitor and HMAP, a NADPH-oxidase inhibitor may be considered as potential agents for the management of Hypt induced VaD.

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1. Introduction

Free radical formation and oxidative stress have been associated with the onset and progression of dementias. Brain as well as plasma in dementias display extensive oxidative stress as indexed by protein oxidation, lipid peroxidation, free radical formation, DNA oxidation, and decreased antioxidants (Pocernich and Butterfield, 2012). Agents which interfere with free radical production and oxidative stress have long been considered as one of the important approach to slow down dementia progression (Pocernich and Butterfield, 2012). Vascular dementia (VaD) is the second leading cause of dementia after Alzheimer's disease (AD) (Sharma and Singh, 2010, 2011a, 2011b), having a strong correlation with various vascular disorders (Davignus et al., 2011; Enciu et al., 2011; Gao et al., 2012; Herrmann and Obeid, 2011; Mütther et al., 2010; Sharp et al., 2011).

In the recent times, our research group has reported induction of VaD in rats with the help of experimental hypertension (Hypt), diabetes, hyperhomocysteinemia (HHcy) and hyperlipidemia (HL) (Koladiya et al., 2008, 2009; Sain et al., 2011; Sharma and Singh, 2010, 2011a, 2011b, 2012a, 2012b, 2012c). It has been reported that optimal treatment of cardiovascular risk factors such as Hypt may work as a key measure to prevent the development of VaD (Monsuez et al., 2011; Sharma and Singh, 2012a, 2012c; Sharp et al., 2011).

Nitric oxide (NO) is an important regulatory molecule for the host defense that plays a fundamental role in the cardiovascular, immune, and nervous systems. NO is synthesized by the enzyme NO synthase (NOS), which is found in three isoforms classified as neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). It has been reported that varied levels of NO has an influential role in the disruption of normal brain and vascular homeostasis and this may be involved in dementia (Aliev, et al., 2010; Austin, et al., 2010). Recently it has been suggested that three isoforms of NOS operate as central mediators of amyloid beta-peptide (A β) action, giving rise to elevated levels of NO that contributes to the maintenance, self-perpetuation and progression of the dementia of AD (Fernandez, et al., 2010).

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Inducible nitric oxide synthase (iNOS) plays an important role in neuroinflammation by generating high levels of brain nitric oxide (NO), a critical signaling and redox factor in the brain. Although NO is associated with tissue damage, it can also promote cell survival in peripheral vasculature (Cai, et al., 2008). Further many studies have implicated that up-regulation and over expression of iNOS are the most important aspects in dementia (Janda, et al., 2011; Maezawa et al., 2011; Zhang, et al., 2010). Aminoguanidine (an iNOS inhibitor) has been reported to inhibit formation of highly reactive advanced glycosylation end products (AGEs) associated with pathogenesis of secondary complications to diabetes and with cardiovascular changes in aging like arterial stiffening and cardiac hypertrophy etc. (Nilsson, 1999). It has been reported that aminoguanidine prevented hippocampal alterations in icv streptozotocin model of dementia of AD (Rodrigues et al., 2009). Aminoguanidine has also been suggested for various other pharmacological activities viz. cardio protective, anti fibrotic (Vadla and Vellaichamy, 2012); antidepressant (Montezuma et al., 2012); antianxiety (Gilhotra and Dhingra, 2010); nephroprotective (Abraham and Rabi, 2011); anti-apoptotic (Ingaramo et al., 2011); antiulcer (Sklyarov et al., 2011); anti-carcinogenic (Manikandan et al., 2009) etc. It has also been suggested that aminoguanidine prevents cerebral vasospasm after subarachnoid hemorrhage (Zheng et al., 2010a, 2010b); seizures (Rehni et al., 2009); spinal cord injury (Xu et al., 2009); hemorrhagic cystitis (Abraham et al., 2009); multiple sclerosis, demyelination (Ljubisavljevic et al., 2012); and cerebral ischemia (Harman et al., 2012) etc. Therefore, inhibitors of iNOS deserve investigation for their potential in VaD. This is the first study which investigates the possible effect of iNOS inhibitor in VaD.

NADPH oxidase is a multi-subunit enzyme complex responsible for the production of both extracellular and intracellular ROS by microglia (Block, 2008). The association of dementia with over production of reactive oxygen species (ROS) by NADPH oxidase enzymes has been noted which may result in oxidative stress that damage tissues over time (Lambeth, 2007). NADPH oxidase is an enzymatic complex that catalyzes superoxide anion O₂⁻ production from O₂ and NADPH. Further it has also been reported that NADPH oxidase is involved in the pathogenic events linked to excitotoxic/prooxidant conditions (Maldonado et al., 2010). It has been found that there is an impairment of endothelium-dependent responses in the cerebral microcirculation through ROS generated in cerebrovascular cells by the enzyme NADPH oxidase that may cause an increased susceptibility to cellular dysfunction, cellular death and dementia (Girouard et al., 2006; Iadecola et al., 2009). NADPH-oxidase inhibitors including HMAP have been reported to be useful in various pathological conditions such as atherosclerosis (Sheehan et al., 2011); hypertension (Wu et al., 2012); inflammation (Kim et al., 2012); asthma, bronchial hyperresponsiveness, ischemia-reperfusion lung injury (Lee and Yang, 2012); osteo- and rheumatoid arthritis (Kundu et al., 2012); cancer (Bonner and Arbiser, 2012); intracerebral hemorrhage (Tang et al., 2005); ischemic brain injury (Tang et al., 2012) etc. (Stefanska and Pawliczak, 2008; Yu et al., 2008). Furthermore, we have recently reported the beneficial effect of 4'-hydroxy-3'-methoxyacetophenone (HMAP), a NADPH oxidase inhibitor in diabetes induced VaD (Sharma and Singh, 2010). But the utility of NADPH-oxidase inhibitor in Hypt induced VaD has not been studied.

In the light of above the present study has been undertaken to investigate the potential of aminoguanidine, an iNOS inhibitor as well as 4'-hydroxy-3'-methoxyacetophenone (HMAP), a NADPH oxidase inhibitor in DOCA-S Hypt induced VaD in rats. Donepezil has been used as positive control in this study.

2. Material and methods

2.1. Animals

Adult albino Wistar rats (male), weighing 200–250 g were employed in the present study and were housed in animal house

with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd, Mumbai, India). The animals were exposed to 12 h light and 12 h dark cycle. The experiments were conducted between 9.00 and 18.00 h in a semi-sound-proof laboratory. The animals were acclimatized to the laboratory condition five days prior to behavioral study and were maintained in the laboratory until the completion of the study. The protocol of the study was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (reg. no. 107/1999/CPCSEA).

2.2. Drugs and reagents

All the drug solutions were freshly prepared before use. DOCA, HMAP, AG and 1,1,3,3 tetramethoxypropane were purchased from Sigma Aldrich (St. Louis, MO, USA). 5,5'-dithiobis (2-nitro benzoic acid) [DTNB], bovine serum albumin (BSA), glutathione reduced (GSH) standard and nitrobluetetrazolium (NBT) were purchased from Sisco Research Laboratories Pvt Ltd., Mumbai, India. Thiobarbituric acid was purchased from Loba Chemie, Mumbai. HMAP, AG and donepezil were dissolved in saline. HMAP was administered orally and AG and donepezil were administered intraperitoneally. DOCA was mixed with arachis oil and administered subcutaneously. Doses of all the drug agents were selected on the bases of our previous research work, pilot studies and previously published reports (Sharma and Singh, 2010, 2012a, 2012c).

Hypertension and subsequent VaD in rats was induced by administering DOCA and salt solution for 90 days (85 days + 5 days during Morris water maze exposure). Our pilot study suggests the induction of a stable hypertension in 50 days (2 months approximately). To study the effects of the therapeutics on hypertension induced VaD, all the drugs were administered for last 40 days viz. day 51 to day 90 (35 days + 5 days during MWM exposure) in DOCA treated animals. Thus, the vehicle (0.9% saline) and drug per se (AG, HMAP and donepezil) control groups were treated with the respective agent alone, for total 40 days (35 days + 5 days during MWM exposure).

2.3. Deoxycorticosterone acetate-salt (DOCA-S) hypertension induced vascular dementia

Rats, were administered DOCA (20 mg/kg, s.c., twice weekly) for 90 days and the drinking water was replaced with solution of 1% sodium chloride (NaCl) and 0.2% potassium chloride (KCl), to produce hypertension (Bockman et al., 1992; Sharma and Singh, 2012a, 2012c). The animals were used on the 86th day for the behavioral and other assessment. Mean arterial blood pressure (MABP) was measured by BIOPAC MP100, using AcqKnowledge 3.8.2. analysis system.

2.4. AG, HMAP and donepezil administration

Rats, were administered AG (75 mg kg⁻¹; 150 mg kg⁻¹ i.p., daily)/HMAP (30 mg kg⁻¹; 60 mg kg⁻¹ p.o., daily)/donepezil (0.5 mg kg⁻¹ i.p., daily) by dissolving it in saline (0.9% w/v) for 40 days in AG per se (dose 1 and dose 2)/HMAP per se (dose 1 and dose 2)/donepezil groups. Furthermore, AG (75 mg kg⁻¹; 150 mg kg⁻¹ i.p., daily)/HMAP (30 mg kg⁻¹; 60 mg kg⁻¹ p.o., daily)/donepezil (0.5 mg kg⁻¹ i.p., daily) was administered for last 40 days viz. day 51 to day 90 (35 days + 5 days during Morris water maze exposure) in DOCA treated animals.

Doses of the drugs were selected on the bases of on our pilot studies and previously published research reports (Sharma and Singh, 2010, 2012a, 2012c).

2.5. Experimental protocol

In total fourteen groups have been employed in the present study and each group consisted of eight male Wistar rats.

2.5.1. 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.

2.5.2. Group II – vehicle control group (0.9% w/v saline)

Animals were administered saline (10 ml kg^{-1} i.p., daily) for 35 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 36th to 39th day) and retrieval trials (on 40th day) on Morris water maze.

2.5.3. Group III – vehicle control group (Arachis oil)

Animals were administered with arachis oil (maximum 2.5 ml kg^{-1} s.c., twice weekly), for 85 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 86th to 89th day) and retrieval trials (on 90th day) on Morris water maze.

2.5.4. Group IV – DOCA treatment group

Animals were administered DOCA (20 mg kg^{-1} s.c., twice weekly), for 85 days followed by exposure to Morris water maze. The treatment was continued during acquisition (from 86th to 89th day) and retrieval trials (on 90th day) on Morris water maze. Drinking water of these animals was replaced with the solution of 1% NaCl and 0.2% KCl.

2.5.5. Group V and VI – 4'-hydroxy-3'-methoxyacetophenone (HMAP) dose 1/dose 2 per se

Animals were administered HMAP ($30/60 \text{ mg kg}^{-1}$ p.o., daily), for 35 days, rest of the procedure was same as described in group II.

2.5.6. Group VII and VIII – DOCA & HMAP dose 1/dose 2

HMAP ($30/60 \text{ mg kg}^{-1}$ p.o., daily) was administered to the DOCA treated rats, starting from 51st day of DOCA treatment followed by exposure to Morris water maze on 86th day of DOCA administration. The treatment was continued during acquisition (from 86th to 89th day) and retrieval trials (on 90th day) on Morris water maze. Drinking water of these animals was replaced with the solution of 1% NaCl and 0.2% KCl.

2.5.7. Group IX and X – aminoguanidine (AG) dose 1/dose 2 per se

Animals were administered AG ($75/150 \text{ mg kg}^{-1}$ i.p., daily), for 35 days, rest of the procedure was same as described in group II.

2.5.8. Group XI and XII – DOCA & AG dose 1/dose 2

AG ($75/150 \text{ mg kg}^{-1}$ i.p., daily) was administered to the DOCA treated rats; rest of the procedure was same as described in group VII and VIII.

2.5.9. Group XIII – donepezil per se

Animals were administered donepezil (0.5 mg kg^{-1} i.p., daily), for 35 days, rest of the procedure was same as described in group II.

2.5.10. Group XIV – DOCA & Donepezil

Donepezil (0.5 mg kg^{-1} i.p., daily) was administered to the DOCA treated rats; rest of the procedure was same as described in group VII and VIII.

2.6. Assessment of learning and memory by Morris water maze

Morris water maze (Morris, 1984; Parle and Singh, 2007; Sharma and Singh, 2010, 2011a, 2011b) 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 animals dislike swimming, their tendency was to escape from the water being accomplished by finding an escape platform. MWM consisted of a 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 the 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 the surface of the water. The position of the platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials on each day with a 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 the 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 the platform and allowed to remain there for 20 s . Escape latency time (ELT) to locate the hidden platform in the water maze was noted as index of acquisition or learning. Animal was subjected to acquisition trials for four consecutive days. Daily starting positions were randomized and not repeated on each day and quadrant 4 (Q4) was maintained as target quadrant in all acquisition trials. On fifth day, the 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. Each rat was subjected to four such trials and each trial was started from different quadrant. Mean time spent in all four quadrants i.e. Q1, Q2, Q3 and Q4 was recorded and the time spent in the target quadrant i.e. Q4 in search of the 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 during the light cycle i.e. between 09.00 and 18.00 h .

2.7. Biochemical parameters

2.7.1. 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 which was then used for biochemical estimation.

After 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 per the procedure defined below in Section 2.8), as well as for the estimation of superoxide anion, whereas brains were subjected to various biochemical estimations (brain TBARS, GSH, AChE and proteins). The removed brains were homogenized in phosphate buffer ($\text{pH } 7.4$, $10\% \text{ w/v}$) using Teflon homogenizer and centrifuged at 3000 rpm for 15 min to obtain the clear supernatant. This clear supernatant containing TBARS, GSH, AChE and proteins, was removed carefully from the centrifugation tube and it was then used for different biochemical estimations.

2.7.2. Estimation of serum nitrite concentration

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

2.7.3. 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 and colleagues (Sharma and Singh, 2010; Wang et al., 1998).

2.7.4. Estimation of brain acetyl cholinesterase (AChE) activity

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

2.7.5. Estimation of thiobarbituric acid reactive substances (TBARS)

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

2.7.6. 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 and colleagues (Beutler et al., 1963; Sharma and Singh, 2010).

2.7.7. 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 and colleagues (Lowry et al., 1951; Sharma and Singh, 2010).

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

Thoracic aorta was removed (as per the procedure defined above in Section 2.7.1), cut into a ring of 4 to 5 mm width, and mounted in organ bath containing Krebs–Henseleit bubbled with carbonated oxygen (95% O₂:5% CO₂), 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 et al., 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, 2011a, 2011b). 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 (Koladiya et al., 2008, 2009; Sharma and Singh, 2010, 2011a, 2011b).

2.9. Statistical analysis

Statistical analyses were done using GraphPad Prism v5.01. All results were expressed as mean ± S.E.M. Data for isolated aortic ring preparation were statistically analyzed using repeated measures of analysis of variance (ANOVA) followed by Newman Keul's test. All other results were analyzed using two way ANOVA followed by Bonferroni's post test. 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 hypertension 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 (Fig. 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 0.9% w/v saline (10 ml kg⁻¹ i.p., 26 days), arachis oil (maximum 2.5 ml kg⁻¹ s.c., twice weekly for 90 days) did not show any significant effect on ELT and TSTQ. Administration of AG (75 mg kg⁻¹ i.p./150 mg kg⁻¹ i.p., daily), HMAP (30 mg kg⁻¹ p.o./60 mg kg⁻¹ p.o., 40 days) and donepezil (0.5 mg kg⁻¹ i.p., 40 days) did not show any significant per se effect on ELT and TSTQ (Figs. 1 and 2). Furthermore, DOCA (20 mg kg⁻¹ s.c., twice weekly for 90 days) treated rats showed a significant increase in day 4 ELT (89th day of DOCA treatment), when compared to day 4 ELT of control animals (Fig. 1) indicating impairment of acquisition. Moreover, DOCA administration also produced a significant decrease in day 5 TSTQ (90th day of DOCA treatment), when compared to day 5 TSTQ of control animals (Fig. 2), indicating impairment of memory as well.

Daily administration of AG (75 mg kg⁻¹ i.p./150 mg kg⁻¹ i.p.), HMAP (30 mg kg⁻¹ p.o./60 mg kg⁻¹ p.o.) and donepezil (0.5 mg kg⁻¹ i.p.), significantly prevented DOCA-S induced rise in day 4 ELT, indicating reversal of DOCA-S induced impairment of acquisition (Fig. 1). Further treatment of these drugs also attenuated DOCA-S induced decrease in day 5 TSTQ in a significant manner, indicating reversal of DOCA-S induced impairment of memory (Fig. 2).

3.2. Effect on endothelium dependent and independent relaxation

Thoracic aorta strips of rats have been used as the representative of blood vessels. 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. DOCA-S administration significantly attenuated acetylcholine induced endothelium dependent relaxation (Fig. 3), however it did not affect SNP induced endothelium independent relaxation (data not shown).

Treatment of AG (dose 1 and dose 2), HMAP (dose 1 and dose 2) and donepezil, significantly abolished the effect of DOCA-S on endothelial dependent relaxation. However, AG, HMAP and donepezil did not show any per se effect on endothelium dependent relaxation.

3.3. Effect on mean arterial blood pressure (MABP)

Administration of DOCA-S produced a significant increase in MABP when compared to control rats. Treatment with AG (dose 1 and dose 2) as well as HMAP (dose 1 and dose 2), prevented DOCA-S induced increase in MABP in a significant manner (Table 1). But there was no significant effect of donepezil on DOCA-S induced increase in MABP (Table 1). Further, AG, HMAP and donepezil did not show any significant per se effect on MABP (Table 1).

3.4. Effect on serum nitrite, brain acetyl cholinesterase (AChE) activity and oxidative stress levels

Administration of DOCA-salt produced a significant decrease in serum nitrite & brain levels of reduced form of glutathione (GSH) with significant increase in AChE activity, aortic superoxide anion level, brain & serum thiobarbituric acid reactive species (TBARS), when compared to control rats. Treatment with AG (dose 1 and dose 2), HMAP (dose 1 and dose 2) and donepezil, prevented DOCA-salt induced impairment of these biochemical parameters, in a significant manner (Table 2). Further, AG (dose 1 and dose 2),

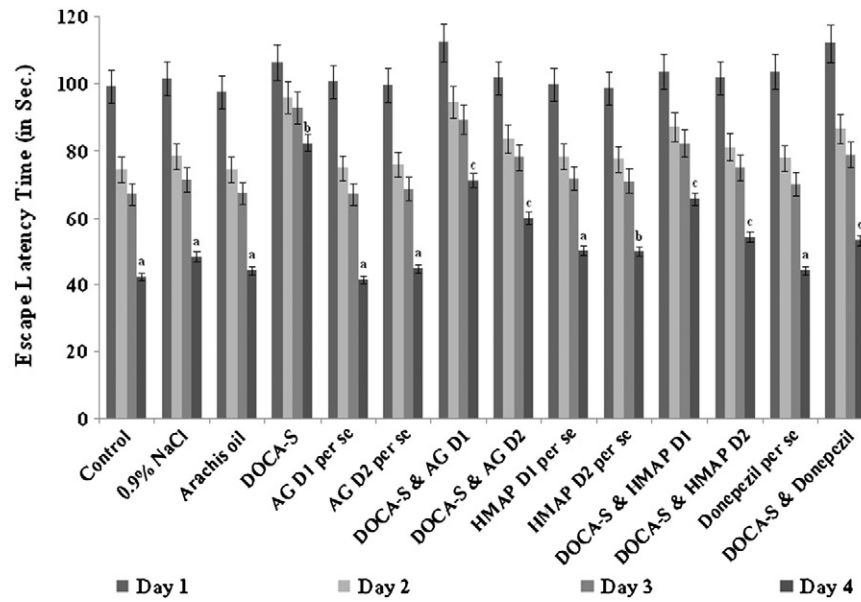


Fig. 1. Effect on day 1 to day 4 escape latency time (ELT) of animals, using Morris water maze. $n=8$, results are mean \pm standard error of means; two way ANOVA followed by Bonferroni post test. $F(5, 84) = 31.71$ $^a p < 0.001$ versus day 1 ELT in respective group; $^b p < 0.001$ versus day 4 ELT of control group; $^c p < 0.001$ versus day 4 ELT of DOCA-S treated group. NaCl – sodium chloride; DOCA-S – deoxycorticosterone acetate-salt; AG – aminoguanidine; HMAP – 4'-hydroxy-3'-methoxyacetophenone; D 1 – dose 1; D 2 – dose 2.

HMAP (dose 1 and dose 2) and donepezil, did not show any significant per se effect on any of the biochemical parameters (Table 2).

4. Discussion

Impairment of learning and memory being the primary feature of dementia, to assess learning and memory of rats, Morris water maze

(MWM) test was employed in the present study. MWM is one of the most widely used and well-accepted models to test learning and memory of rodents (Morris, 1984; Sain et al., 2011; Sharma and Singh, 2011a; Sharma et al., 2008a, 2008b). Metabolic disorders have been identified as one of the main cause of dementia; we have previously reported the development of vascular dementia (VaD) in animals by induction of metabolic disorders such as diabetes, Hypt and

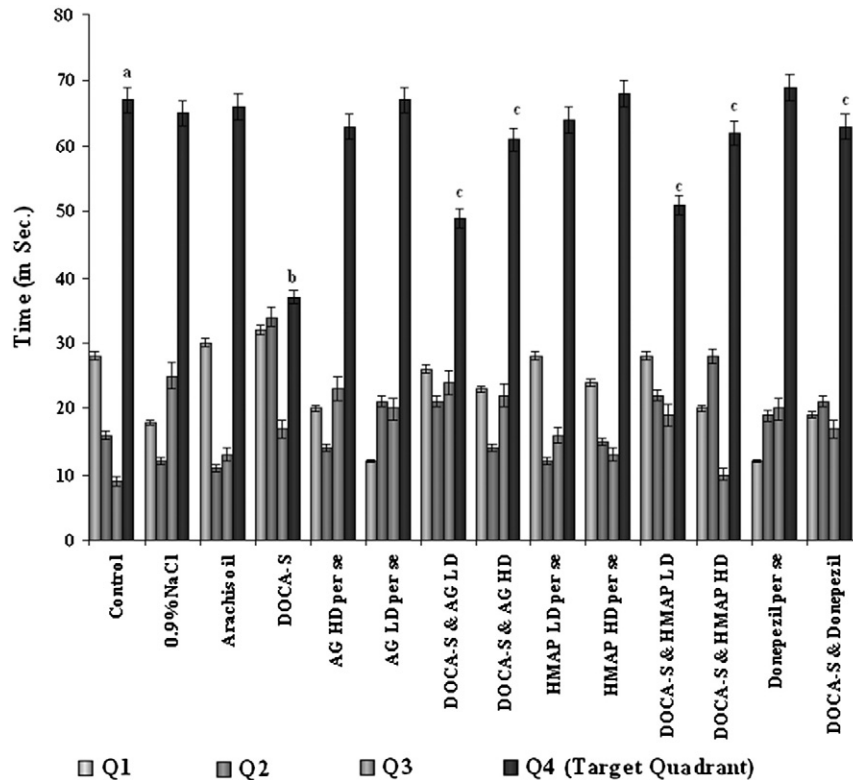


Fig. 2. Effect on time spent in target quadrant (TSTQ) of animals using Morris water-maze. $n=8$, results are mean \pm standard error of means; two way ANOVA followed by Bonferroni post test. $F(5, 84) = 14.02$; $^a p < 0.001$ versus mean time spent in other quadrants in control; $^b p < 0.001$ versus mean time spent in target quadrant in control group; $^c p < 0.001$ versus mean time spent in target quadrant in DOCA-S treated group. NaCl – sodium chloride; DOCA-S – deoxycorticosterone acetate-salt; AG – Aminoguanidine; HMAP – 4'-hydroxy-3'-methoxyacetophenone; D 1 – dose 1; D 2 – dose 2.

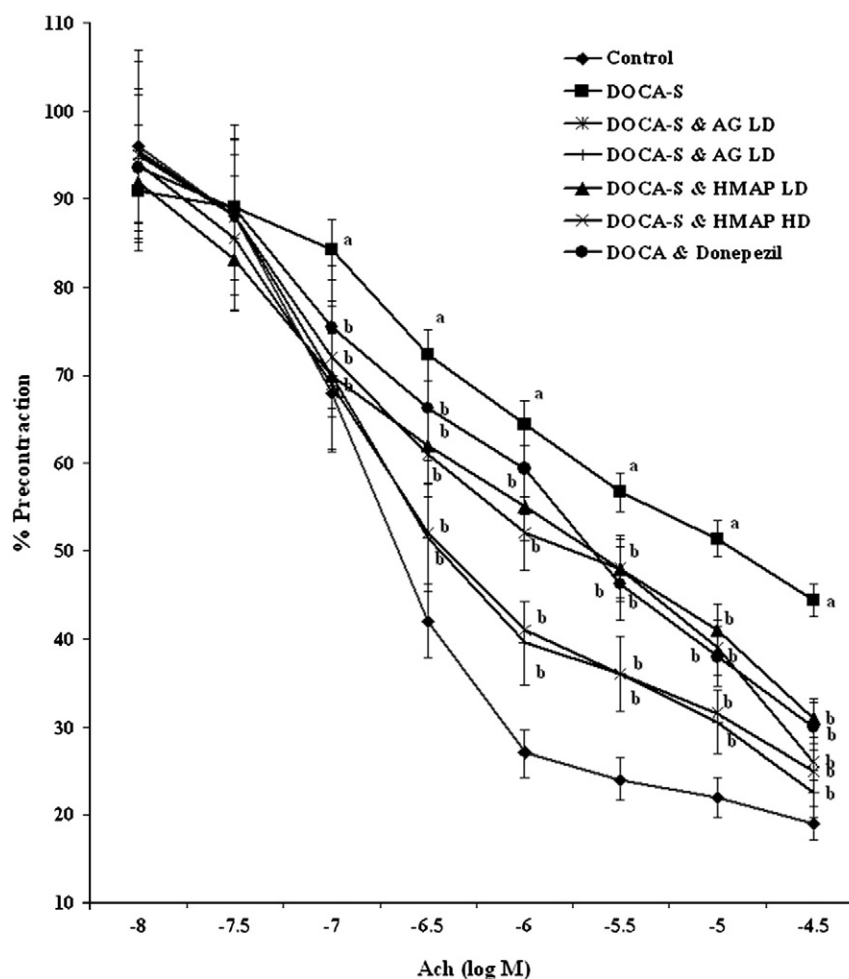


Fig. 3. Effect on acetylcholine induced endothelium dependent relaxation using aortic ring preparation. $n=8$, responses are expressed as percentage of precontraction induced by 3×10^{-6} M phenylephrine. Results are mean \pm standard error of means; repeated measure ANOVA followed by Newman Keul's test. ^a $p<0.05$ versus control; ^b $p<0.05$ versus DOCA-S treated group. Ach – acetylcholine; NaCl – sodium chloride; DOCA-S – deoxycorticosterone acetate-salt; AG – Aminoguanidine; HMAP – 4'-hydroxy-3'-methoxyacetophenone; D 1 – dose 1; D 2 – dose 2.

Table 1
Effect of various agents, on mean arterial blood pressure (MABP) of animals.

Name of the group	Mean arterial blood pressure (mm Hg)	
	Basal	Final
Control	88.1 \pm 2.3	92.2 \pm 2.9
Vehicle control (0.9% NaCl)	90.3 \pm 3.2	91.1 \pm 2.7
Vehicle control (Arachis oil)	87.7 \pm 3.3	89.1 \pm 3.1
DOCA-S	90.7 \pm 2.4	165.6 \pm 4.2 *
AG D 1 per se	89.8 \pm 2.4	92.1 \pm 3.2
AG D 2 per se	90.1 \pm 2.1	95.6 \pm 3.1
DOCA-S & AG D 1	88.3 \pm 2.4	142.7 \pm 2.8 *, **, ***
DOCA-S & AG D 2	89.4 \pm 3.7	130.6 \pm 3.3 *, **, ***
HMAP D 1 per se	90.3 \pm 2.8	94.1 \pm 2.2
HMAP D 2 per se	92.1 \pm 3.4	93.6 \pm 4.1
DOCA-S & HMAP D 1	90.5 \pm 3.2	135.8 \pm 3.7 *, **, ***
DOCA-S & HMAP D 2	89.4 \pm 3.7	121.5 \pm 4.4 *, **, ***
Donepezil per se	88.4 \pm 3.3	92.2 \pm 2.9
DOCA-S & Donepezil	91.5 \pm 3.4	161.5 \pm 3.3 *

$n=8$, results are mean \pm standard error of means; two way ANOVA followed by Bonferroni post test.

$F(5, 84) = 14.73$.

NaCl – sodium chloride; DOCA-S – deoxycorticosterone acetate-salt; AG – Aminoguanidine; HMAP – 4'-hydroxy-3'-methoxyacetophenone; D 1 – dose 1; D 2 – dose 2.

* $p<0.0001$ versus control group.

** $p<0.0001$ versus DOCA-S treated group.

*** $p<0.001$ versus dose 1 of respective drug treated group.

hyperhomocysteinemia (HHcy) (Sharma and Singh, 2011a, 2012a, 2012b, 2012c). In the present investigation deoxycorticosterone acetate-salt (DOCA-S) administration resulted in hypertension (HypT), vascular endothelial dysfunction, memory deficit along with alterations in various biochemical parameters of rats. DOCA-S is widely used for the assessment of HypT and its secondary complications including vascular endothelial dysfunction (Bockman et al., 1992; Sharma and Singh, 2012a, 2012c).

DOCA-S treated rats, performed poorly on MWM test, indicating impairment in their learning abilities and memory capacities. Furthermore, a significant rise in brain AChE activity, brain and serum TBARS, aortic superoxide anion along with a fall in brain GSH and serum nitrite/nitrate levels was also noted (Fig. 4).

Chronic administration of DOCA-S in our study has produced significant degree of vascular endothelial dysfunction reflected by impairment of acetylcholine induced endothelial dependent relaxation of aortic strips and reduction in serum nitrite/nitrate levels. DOCA in earlier studies has been demonstrated to induce endothelial dysfunction, so our findings are in line with previous findings (Sahan-Firat et al., 2010; Szasz and Watts, 2010). Further, DOCA has also been documented to enhance the production of free radicals with subsequent increase in oxidative stress (Borde et al., 2011; Szasz and Watts, 2010). DOCA 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 DOCA induced endothelial

Table 2

Effect of various agents on oxidative stress (superoxide anion, TBARS, GSH) & brain acetyl cholinesterase (AChE) activity and serum nitrite/nitrate levels of animals.

Name of the group	Serum nitrite/nitrate ($\mu\text{M/L}$)	Serum TBARS ($\mu\text{M/L}$)	Aortic superoxide anion (reduced NBT – pM/min/mg)	Brain AChE activity (μM of ACh hydrolyzed/min/mg protein)	Brain TBARS (nM/mg protein)	Brain GSH ($\mu\text{M/mg}$ of protien)
Control	12.8 \pm 2.2	3.8 \pm 0.6	3.7 \pm 0.4	3.1 \pm 0.5	4.2 \pm 0.7	18.2 \pm 2.1
Vehicle control (0.9% NaCl)	11.2 \pm 3.1	3.7 \pm 0.4	3.6 \pm 0.5	3.3 \pm 0.7	4.1 \pm 0.6	17.3 \pm 2.4
Vehicle control (arachis oil)	11.6 \pm 1.8	3.6 \pm 0.3	3.3 \pm 0.7	2.9 \pm 0.9	4.5 \pm 0.5	17.5 \pm 2.6
DOCA-S	6.5 \pm 1.2 ^a	7.3 \pm 1.1 ^a	6.9 \pm 0.7 ^a	11.6 \pm 1.7 ^a	11.2 \pm 1.2 ^a	7.2 \pm 3.1 ^a
AG D 1 per se	11.5 \pm 1.5	3.4 \pm 0.7	3.5 \pm 0.5	3.6 \pm 0.6	4.4 \pm 0.6	18.1 \pm 1.5
AG D 2 per se	12.1 \pm 1.1	3.9 \pm 0.4	3.2 \pm 0.6	3.3 \pm 0.8	4.1 \pm 0.5	17.2 \pm 1.4
DOCA-S & AG D 1	8.3 \pm 0.6 ^{a, b}	5.9 \pm 0.6 ^{a, b}	6.1 \pm 0.4 ^{a, b}	9.1 \pm 0.6 ^{a, b}	8.6 \pm 1.1 ^{a, b}	11.4 \pm 1.5 ^{a, b}
DOCA-S & AG D 2	11.1 \pm 0.4 ^{a, b}	4.2 \pm 0.7 ^{a, b}	5.2 \pm 0.5 ^{a, b}	6.7 \pm 1.8 ^{a, b}	5.7 \pm 0.7 ^{a, b}	13.7 \pm 1.2 ^{a, b}
HMAP D 1 per se	12.2 \pm 2.4	3.4 \pm 0.5	3.2 \pm 0.6	3.4 \pm 0.5	4.7 \pm 0.4	17.8 \pm 1.3
HMAP D 2 per se	11.4 \pm 3.1	3.7 \pm 0.6	3.4 \pm 0.9	3.2 \pm 0.7	4.4 \pm 0.8	18.1 \pm 1.7
DOCA-S & HMAP D 1	8.1 \pm 0.9 ^{a, b}	6.1 \pm 0.4 ^{a, b}	5.8 \pm 0.6 ^{a, b}	8.4 \pm 1.2 ^{a, b}	8.3 \pm 1.4 ^{a, b}	12.6 \pm 2.7 ^{a, b}
DOCA-S & HMAP D 2	9.2 \pm 1.1 ^{a, b}	4.7 \pm 0.5 ^{a, b}	4.4 \pm 0.8 ^{a, b}	5.4 \pm 1.5 ^{a, b}	6.4 \pm 1.2 ^{a, b}	15.1 \pm 0.9 ^{a, b}
Donepezil per se	12.1 \pm 3.2	3.7 \pm 0.4	3.9 \pm 0.7	3.4 \pm 0.6	4.2 \pm 0.7	17.5 \pm 3.3
DOCA-S & donepezil	9.9 \pm 0.5 ^{a, b}	6.1 \pm 0.7 ^{a, b}	6.0 \pm 0.3 ^{a, b}	4.5 \pm 0.9 ^{a, b}	7.6 \pm 0.8 ^{a, b}	13.7 \pm 3.2 ^{a, b}

n = 8, results are mean \pm standard error of means; two way ANOVA followed by Bonferroni post test.Serum nitrite/nitrate – F (5, 84) = 7.3; ^ap < 0.05 versus control group; ^bp < 0.05 versus DOCA-S treated group.Serum TBARS – F (5, 84) = 32; ^ap < 0.05 versus control group; ^bp < 0.05 versus DOCA-S treated group.Aortic superoxide anion – F (5, 84) = 41.1; ^ap < 0.05 versus control group; ^bp < 0.05 versus DOCA-S treated group.Brain AChE activity – F (5, 84) = 50.2; ^ap < 0.05 versus control group; ^bp < 0.05 versus DOCA-S treated group.Brain TBARS – F (5, 84) = 49.2; ^ap < 0.05 versus control group; ^bp < 0.05 versus DOCA-S treated group.Brain GSH – F (5, 84) = 20.3; ^ap < 0.05 versus control group; ^bp < 0.05 versus DOCA-S treated group.

TBARS – thiobarbituric acid reactive species; GSH – reduced form of glutathione; ACh – acetylcholine; AChE – acetylcholinesterase; NBT – nitrobluetetrazolium; NaCl – sodium chloride; DOCA-S – deoxycorticosterone acetate-salt; AG – aminoguanidine; HMAP – 4'-hydroxy-3'-methoxyacetophenone; D 1 – dose 1; D 2 – dose 2.

dysfunction. 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 (Morris, 1984; Sain et al., 2011; Sharma and Singh, 2012a, 2012c). In the present study we have observed a significant increase in brain AChE activity in hypertensive rats. It has been reported previously that oxidative stress induces increase in brain AChE activity (El-Demerdash, 2011; Sharma and Singh, 2010, 2011a, 2012a). Thus, increased oxidative stress is one of the important reasons which is responsible for the increase in brain AChE activity in the present study. Furthermore, it has also been reported that hypertension causes

cholinergic deficits and to oppose this, up-regulation of vesicular acetylcholine transporter (VACHT) has been observed (Tayebati et al., 2008). Administration of AChEIs has been reported to counteract the hypertension induced cholinergic deficits and our recent studies have documented the beneficial effect of AChEIs in hypertension induced increase in brain AChE activity (Sharma and Singh, 2012a, 2012c). Thus, at this point of time we may not be able to rule out the possibility of involvement of hypertension in enhancement of brain AChE activity as well. But this aspect needs further clarifications and thus further research is required on this aspect. Therefore, the observed DOCA induced vascular dementia may be due to increase in oxidative

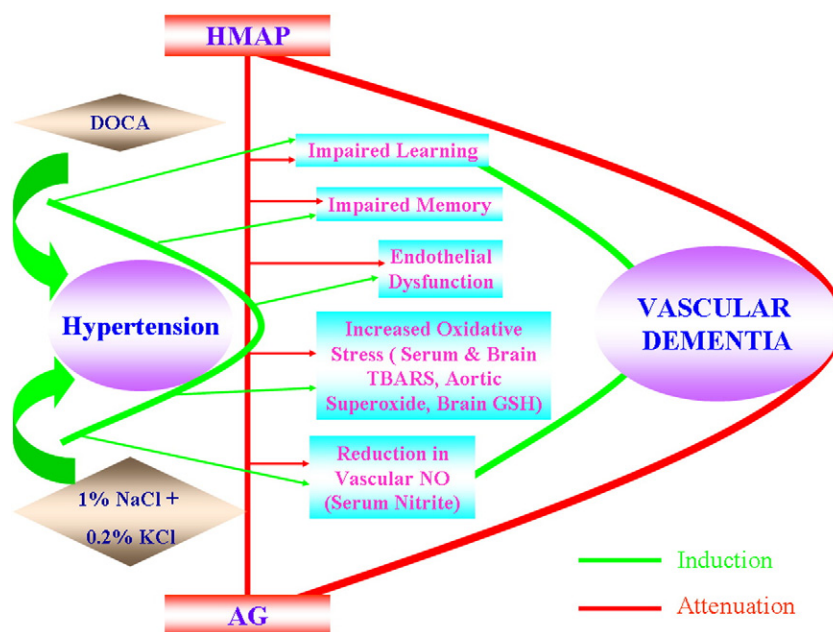


Fig. 4. Improvement of experimental hypertension induced vascular dementia by aminoguanidine and 4'-hydroxy-3'-methoxyacetophenone. HMAP – 4'-hydroxy-3'-methoxyacetophenone; DOCA – deoxycorticosterone acetate; NaCl – sodium chloride; KCl – Potassium chloride; AChE – acetylcholinesterase; TBARS – thiobarbituric acid reactive species; GSH – reduced form of glutathione; NO – nitric oxide; AG – Aminoguanidine.

stress levels both peripherally as well as centrally, impairment of endothelial function and increase in brain acetylcholinesterase activity.

Treatments of AG (an, iNOS inhibitor), HMAP (a NADPH-oxidase inhibitor) and donepezil (an acetylcholinesterase inhibitor) have attenuated DOCA-S induced endothelial dysfunction, impairment of learning & memory, serum nitrite/nitrate, oxidative stress as well as brain AChE activity. AG and HMAP in addition to above effects also significantly reduced MABP of DOCA-S treated animals.

It has been suggested that inducible nitric oxide synthase (iNOS), is upregulated in hypertension (Smith et al., 2011), which may contribute to the inflammatory response, increased reactive oxygen species, vascular remodeling, decreased aortic blood flow attenuation of endothelium dependent relaxation, endothelial cell damage or cell death, reduced NO bioavailability etc (Steed et al., 2010). Reactive oxygen species cause structural membrane damage, induce inflammation, and scavenge NO to yield peroxynitrite (ONOO^-). This activates the inducible NO synthase, which further compounds ONOO^- formation (Berg et al., 2011). Reactive oxygen species and ONOO^- cause mitochondrial dysfunction by inhibiting the mitochondrial electron transport chain and uncoupling oxidative phosphorylation, which ultimately leads to neuronal bioenergetic failure. Furthermore, in certain 'at risk' areas of the brain, free radicals may induce neuronal apoptosis (Berg et al., 2011; Steed et al., 2010).

It has been reported that hypertension is associated with endothelial dysfunction and reduced NO bioavailability (Ghiadoni et al., 2012). Further this has been suggested that this reduction in NO bioavailability is due to reactions of this molecule with free radicals generated by oxidative respiration (H_2O_2 and O_2^-), leading to the production of peroxynitrite, a powerful oxidant (Celotto et al., 2010). Aminoguanidine, an iNOS inhibitor has been reported to attenuate these abnormalities (Celotto et al., 2010; Zheng et al., 2010a, 2010b).

Recently it has also been reported that blockage of iNOS by aminoguanidine caused enhancement of cytoprotective mechanisms, reduction of iNOS activity and oxidative stress, and an increase in blood L-arginine level (Sklyarov et al., 2011). It has previously been reported that aminoguanidine attenuates memory impairment and reduction in brain nitrite, AChE activity in global cerebral ischemia, hypoxia, neurotoxicity and in various other neurodegenerative disorders (Mori et al., 2001; Stevanović et al., 2010; Tran et al., 2001; Udayabanu et al., 2008; Yamada et al., 1999). Therefore, the beneficial effect of aminoguanidine in the present study is may be attributed to its inhibitory activity on iNOS, preventive action on oxidative stress, neuroprotective and anti-acetylcholinesterase activity.

Nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) has a dedicated function of generating reactive oxygen species (ROS) (Jiang et al., 2011). NADPH-oxidases are an important source of ROS in both cerebral (Miller et al., 2006) and systemic vasculatures (Rajagopalan et al., 1996). Accumulating evidence suggests that NADPH-oxidase has an important role in signal transduction in cellular stress responses. NADPH-oxidase can be activated by a collection of chemical, physical, and biological cellular stresses (Jiang et al., 2011). NADPH-oxidase is involved in conditions such as hypertension, hyperhomocysteinemia, hypercholesterolaemia, diabetes, aging etc (Drummond et al., 2011). It is an important contributor to the oxidative stress, endothelial dysfunction and vascular inflammation that underlies arterial remodeling and atherogenesis (Drummond et al., 2011).

Higher NADPH-oxidase level may cause hippocampal neuronal damage (Park et al., 2008), cerebrovascular deregulation due to increase in oxidative stress (Iadecola et al., 2009), activation of extracellular signal-regulated kinase 1/2, phosphorylation of cytosolic phospholipase A_2 , and arachidonic acid (AA) release (Shelat et al., 2008). Studies have shown that Nox1 (NADPH oxidase 1), Nox2 (also known as gp91phox) and Nox4 are all expressed in cerebral arteries, suggesting that multiple isoforms of NADPH-oxidase may be important for reactive oxygen species production by cerebral arteries

(Block, 2008; Miller et al., 2006). Activation of microglial NADPH-oxidase causes neurotoxicity through two mechanisms one is extracellular ROS produced by microglia that are directly toxic to neurons and another is intracellular ROS that function as a signaling mechanism in microglia to amplify the production of several pro-inflammatory and neurotoxic cytokines (for example, tumor necrosis factor- α , prostaglandin E_2 , and interleukin-1 β) (Block, 2008). Activation of NADPH-oxidase is a characteristic feature of microglial activation both in vitro and in vivo, and experimental evidence suggests that ROS generated by activated microglia could directly contribute to brain injury by inducing lipid peroxidation, DNA fragmentation and protein oxidation in surrounding cells – a phenomena called “bystander lysis” (McGeer and McGeer, 1997).

Oxidative stress seems to play an important role in the brain damage (Bomboi et al., 2010). Further oxidative stress may inhibit nitric oxide production (De Vriese et al., 2000) and the available NO may rapidly react with superoxide anion (O_2^-) to form peroxynitrite (ONOO^-), simultaneously reducing the availability of NO for relaxation and increasing ONOO^- induced toxicity, ONOO^- has been reported to uncouple eNOS, thus further promoting the synthesis of O_2^- (Zou et al., 2004). Although different enzyme systems participate in ROS generation, it is generally recognized that NADPH-oxidase is a predominant source in the vasculature interfering with endothelial dysfunction (Guzik et al., 2000).

The widespread expression of NADPH-oxidase in neurons has led to the recognition that deliberate ROS production by NADPH-oxidase plays an important role in many biological events, including neuronal signaling (Kishida et al., 2005). Indeed, there is substantial evidence that NADPH-oxidase based ROS can regulate synaptic plasticity and memory formation (Kishida and Klann, 2007). NADPH-oxidase may become promising pharmacological targets for the treatment and prevention of dementia (Zekry et al., 2003). Enhanced NADPH-oxidase activity may be associated with vascular dementia and other cerebrovascular complications (Miller et al., 2006). It has been reported that HMAP is also an important antioxidant and acts as free radical scavenger (Heumüller et al., 2008). HMAP treatment has been documented to reverse the decreased eNOS expression and to attenuate the over expression of iNOS, both at protein and at mRNA levels, in rat aorta without affecting eNOS or iNOS expression (Olukman et al., 2010). Therefore, the observed beneficial effect of HMAP in DOCA-S Hypertension induced VaD may primarily be attributed to its NADPH-oxidase inhibitory action, anti-acetylcholinesterase activity, antioxidant action, modulation of eNOS and iNOS expression and improvement of endothelial function.

It is important to note here that both AG and HMAP have attenuated the DOCA-salt induced hypertension and thus there are the chances that the beneficial effect of these drugs on vascular dementia may be due to their normotensive effect. But as the previous reports suggest the beneficial effect of HMAP in diabetes induced VaD (Sharma and Singh, 2010) and AG in dementia of AD (Rodrigues et al., 2009), and also other results of this present study suggest that there is reduction of oxidative stress, acetyl cholinesterase activity, and improvement of endothelial function with a significant increase in serum nitrite/nitrate levels. Therefore, it may be suggested that, the beneficial effects of these drugs is not just because of their normotensive effect alone. Rather, it is the multifunctional response of these agents which has provided benefit in DOCA-salt induced VaD. Furthermore, we have used the two different doses of AG as well as HMAP. It has been clearly seen from the results that there is an increase in the beneficial effect of these agents on higher dose. Higher effect of AG and HMAP, on blood pressure, learning, memory and biochemical parameters has been observed on higher doses.

Acetylcholinesterase inhibitors are the main class of drugs which are frequently used for the management of memory deficits. In our previous reports we have demonstrated that donepezil in addition to its usefulness in dementia of AD (Sharma et al., 2008a, 2008b) also exert beneficial effect in different animal models of VaD (Sharma and Singh,

2010, 2011a, 2012a, 2012c). Donepezil is already in clinical use for management of dementia of various etiologies.

5. Conclusions

On the basis of results of this study and above discussions, it is concluded that experimental Hypt has induced endothelial dysfunction and subsequent VaD. Treatments of AG (an iNOS inhibitor) and HMAP (a NADPH-oxidase inhibitor) have convalesce Hypt induced VaD in rats. As this is the first report which suggests the salutary effect of inhibitors of iNOS and NADPH-oxidase in hypertension induced VaD, thus further studies are required to explore the full potential of AG and HMAP for the management of Hypt associated VaD.

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References

- Abraham P, Rabi S. Protective effect of aminoguanidine against cyclophosphamide-induced oxidative stress and renal damage in rats. *Redox Rep* 2011;16(1):8–14.
- Abraham P, Rabi S, Selvakumar D. Protective effect of aminoguanidine against oxidative stress and bladder injury in cyclophosphamide-induced hemorrhagic cystitis in rat. *Cell Biochem Funct* 2009;27(1):56–62.
- Aliev G, Palacios HH, Lipsitt AE, Fischbach K, Lamb BT, Obrenovich ME, et al. Nitric oxide as an initiator of brain lesions during the development of Alzheimer disease. *Neurotox Res* 2010;16(3):293–305.
- Austin SA, Santhanam AV, Katusic ZS. Endothelial nitric oxide modulates expression and processing of amyloid precursor protein. *Circ Res* 2010;107(12):1498–502.
- Berg RM, Möller K, Bailey DM. Neuro-oxidative-nitrosative stress in sepsis. *J Cereb Blood Flow Metab* 2011;31(7):1532–44.
- Beutler E, Duron O, Kelly B. Reduced glutathione estimation. *J Lab Clin Med* 1963;61:82.
- Block ML. NADPH oxidase as a therapeutic target in Alzheimer's disease. *BMC Neurosci* 2008;9(Suppl 2):S8. <http://dx.doi.org/10.1186/1471-2202-9-S2-S8>.
- Bockman CS, Jeffries WB, Pettinger WA, Abel PW. Reduced contractile sensitivity and vasopressin receptor affinity in DOCA-salt hypertension. *Am J Physiol* 1992;262:1752–8.
- Bombai G, Castello L, Cosentino F, Giubilei F, Orzi F, Volpe M. Alzheimer's disease and endothelial dysfunction. *Neurol Sci* 2010;31(1):1–8.
- Bonner MY, Arbiser JL. Targeting NADPH oxidases for the treatment of cancer and inflammation. *Cell Mol Life Sci* 2012;69(14):2435–42.
- Borde P, Mohan M, Kasture S. Effect of myricetin on deoxycorticosterone acetate DOCA-salt-hypertensive rats. *Nat Prod Res* 2011;7:1–11.
- Cai ZY, Yan Y, Sun SQ, Zhang J, Huang LG, Yan N, et al. Minocycline attenuates cognitive impairment and restrains oxidative stress in the hippocampus of rats with chronic cerebral hypoperfusion. *Neurosci Bull* 2008;24(5):305–13.
- Celotto AC, Fukada SY, Laurindo FR, Haddad R, Eberlin MN, de Oliveira AM. Chronic hyperhomocysteinemia impairs vascular function in ovariectomized rat carotid arteries. *Amino Acids* 2010;38(5):1515–22.
- Davignul ML, Plassman BL, Pirzada A, Bell CC, Bowen PE, Burke JR, et al. Risk factors and preventive interventions for Alzheimer disease: state of the science. *Arch Neurol* 2011;68(9):1185–90.
- De Vries AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoute PM. Endothelial dysfunction in diabetes. *Br J Pharmacol* 2000;130:963–74.
- Drummond GR, Selemidis S, Griendling KK, Sobey CG. Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov* 2011;10(6):453–71.
- El-Demerdash FM. Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. *Food Chem Toxicol* 2011;49(6):1346–52.
- Ellman GL, Courtney DK, Andres V, Feathstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;7:88–95.
- Enciu AM, Constantinescu SN, Popescu LM, Muresanu DF, Popescu BO. Neurobiology of vascular dementia. *J Aging Res* 2011;2011:401604.
- Fernandez AP, Pozo-Rodríguez A, Serrano J, Martínez-Murillo R. Nitric oxide: target for therapeutic strategies in Alzheimer's disease. *Curr Pharm Des* 2010;16(25):2837–50.
- Gao L, Zeng XN, Guo HM, Wu XM, Chen HJ, Di RK, et al. Cognitive and neurochemical alterations in hyperhomocysteinemic rat. *Neurol Sci* 2012;33(1):39–43.
- Ghiadoni L, Taddei S, Virdis A. Hypertension and endothelial dysfunction: therapeutic approach. *Curr Vasc Pharmacol* 2012;10(1):42–60.
- Gilhotra N, Dhirga D. GABAergic and nitriergic modulation by curcumin for its antianxiety-like activity in mice. *Brain Res* 2010;1352:167–75.
- Girouard H, Park L, Anrather J, Zhou P, Iadecola C. Angiotensin II attenuates endothelium-dependent responses in the cerebral microcirculation through nox-2-derived radicals. *Arterioscler Thromb Vasc Biol* 2006;26(4):826–32.
- Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, et al. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. *Circ Res* 2000;86:E85–90.
- Harman F, Hasturk AE, Duz B, Gonul E, Korkmaz A. An evaluation of the effectiveness of pre-ischemic hyperbaric oxygen and post-ischemic aminoguanidine in experimental cerebral ischemia. *Neurosciences (Riyadh)* 2012;17(2):121–6.
- Herrmann W, Obeid R. Homocysteine: a biomarker in neurodegenerative diseases. *Clin Chem Lab Med* 2011;49(3):435–41.
- Heumüller S, Wind S, Barbosa-Sicard E, Schmidt HH, Busse R, Schröder K, et al. Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. *Hypertension* 2008;51(2):211–7.
- Iadecola C, Park L, Capone C. Threats to the mind: aging, amyloid, and hypertension. *Stroke* 2009;40(3):S40–4.
- Ingaramo PI, Ronco MT, Francés DE, Monti JA, Pisani GB, Ceballos MP, et al. Tumor necrosis factor alpha pathways develops liver apoptosis in type 1 diabetes mellitus. *Mol Immunol* 2011;48(12–13):1397–407.
- Janda E, Visalli V, Colica C, Aprigliano S, Musolino V, Vadalà N, et al. The protective effect of tianeptine on Gp120-induced apoptosis in astroglial cells: role of GS and NOS and NF-κB suppression. *Br J Pharmacol* 2011;164(6):1590–9.
- Jiang F, Zhang Y, Dusting GJ. NADPH oxidase-mediated redox signaling: roles in cellular stress response stress tolerance and tissue repair. *Pharmacol Rev* 2011;63(1):218–42.
- Kim SY, Moon KA, Jo HY, Jeong S, Seon SH, Jung E, et al. Anti-inflammatory effects of apocynin, an inhibitor of NADPH oxidase, in airway inflammation. *Immunol Cell Biol* 2012;90(4):441–8.
- Kishida KT, Klann E. Sources and targets of reactive oxygen species in synaptic plasticity and memory. *Antioxid Redox Signal* 2007;9(2):233–44.
- Kishida KT, Pao M, Holland SM, Klann E. NADPH oxidase is required for NMDA receptor-dependent activation of ERK in hippocampal area CA1. *J Neurochem* 2005;94(2):299–306.
- Koladiya RU, Jaggi AS, Singh N, Sharma BK. Ameliorative role of atorvastatin and pitavastatin in L-methionine induced vascular dementia in rats. *BMC Pharmacol* 2008;8:14.
- Koladiya RU, Jaggi AS, Singh N, Sharma BK. Beneficial effects of donepezil on vascular endothelial dysfunction-associated dementia induced by L-methionine in rats. *J Health Sci* 2009;552:215–25.
- Kundu S, Ghosh P, Datta S, Ghosh A, Chattopadhyay S, Chatterjee M. Oxidative stress as a potential biomarker for determining disease activity in patients with Rheumatoid Arthritis. *Free Radic Res* 2012;46(12):1482–9. <http://dx.doi.org/10.3109/10715762.2012.727991>.
- Lambeth JD. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic Biol Med* 2007;43(3):332–47.
- Lee IT, Yang CM. Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases. *Biochem Pharmacol* 2012;84(5):581–90.
- Ljubisavljevic S, Stojanovic I, Pavlovic R, Stojnev S, Stevanovic I, Sokolovic D, et al. The reduced glutathione and S-nitrosothiols levels in acute phase of experimental demyelination—pathophysiological approach and possible clinical relevancy. *Neuroscience* 2012;219:175–82.
- Lowry OH, Rosebrough NJ, Far AL, Randall RJ. Protein measurement with folin-phenol reagent. *J Biol Chem* 1951;193:265–75.
- Maezawa I, Zimin PI, Wulff H, Jin LW. Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity. *J Biol Chem* 2011;286(5):3693–706.
- Maldonado PD, Molina-Jijón E, Villeda-Hernández J, Galván-Arzate S, Santamaría A, Pedraza-Chaverrí J. NAD(P)H oxidase contributes to neurotoxicity in an excitotoxic/prooxidant model of Huntington's disease in rats: protective role of apocynin. *J Neurosci Res* 2010;88(3):620–9.
- Manikandan R, Thiagarajan R, Beulaja S, Chindhu S, Mariammal K, Sudhandiran G, et al. Anti-cataractogenic effect of curcumin and aminoguanidine against selenium-induced oxidative stress in the eye lens of Wistar rat pups: an in vitro study using isolated lens. *Chem Biol Interact* 2009;181(2):202–9.
- McGeer PL, McGeer EG. The role of the immune system in neurodegenerative disorders. *Mov Disord* 1997;12:855–8.
- Miller AA, Drummond GR, Sobey CG. Novel isoforms of NADPH-oxidase in cerebral vascular control. *Pharmacol Ther* 2006;111(3):928–48.
- Monsuez JJ, Gesquière-Dando A, Rivera S. Cardiovascular prevention of cognitive decline. *Cardiol Res Pract* 2011;2011:250970. <http://dx.doi.org/10.4061/2011/250970>.
- Montezuma K, Biojone C, Lisboa SF, Cunha FQ, Guimarães FS, Joca SR. Inhibition of iNOS induces antidepressant-like effects in mice: pharmacological and genetic evidence. *Neuropharmacology* 2012;62(1):485–91.
- Mori K, Togashi H, Ueno KI, Matsumoto M, Yoshioka M. Aminoguanidine prevented the impairment of learning behaviour and hippocampal long-term potentiation following transient cerebral ischemia. *Behav Brain Res* 2001;120:159–68.
- Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 1984;11(1):47–60.
- Müther J, Abholz HH, Wiese B, Fuchs A, Wollny A, Pentzek M. Are patients with dementia treated as well as patients without dementia for hypertension, diabetes, and hyperlipidaemia? *Br J Gen Pract* 2010;60(578):671–4.

- Nilsson BO. Biological effects of aminoguanidine: an update. *Inflamm Res* 1999;48(10): 509–15.
- Ohokawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351–8.
- Olukman M, Orhan CE, Celenk FG, Ulker S. Apocynin restores endothelial dysfunction in streptozotocin diabetic rats through regulation of nitric oxide synthase and NADPH oxidase expressions. *J Diabetes Complications* 2010;24(6):415–23.
- Park KW, Baik HH, Jin BK. Interleukin-4-induced oxidative stress via microglial NADPH oxidase contributes to the death of hippocampal neurons in vivo. *Curr Aging Sci* 2008;1(3):192–201.
- Parle M, Singh N. Reversal of memory deficits by Atorvastatin and Simvastatin in rats. *Yakugaku Zasshi* 2007;127(7):1125–37.
- Pieper GM, Langenstroer P, Siebeneich W. Diabetic-induced endothelial dysfunction in rat aorta: role of hydroxyl radicals. *Cardiovasc Res* 1997;34(1):145–56.
- Pocernich CB, Butterfield DA. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. *Biochim Biophys Acta* 2012;1822(5):625–30. <http://dx.doi.org/10.1016/j.bbadis.2011.10.003>.
- Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griending KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. *J Clin Invest* 1996;97:1916–23.
- Rehni AK, Singh TG, Kalra R, Singh N. Pharmacological inhibition of inducible nitric oxide synthase attenuates the development of seizures in mice. *Nitric Oxide* 2009;21(2):120–5.
- Rodrigues L, Biasibetti R, Swarowsky A, Leite MC, Quincozes-Santos A, Quilfeldt JA, et al. Hippocampal alterations in rats submitted to streptozotocin-induced dementia model are prevented by aminoguanidine. *J Alzheimers Dis* 2009;17(1):193–202.
- Sahan-Firat S, Jennings BL, Yaghini FA, Song CY, Estes AM, Fang XR, et al. 23' 45'-tetramethoxystilbene prevents deoxycorticosterone-salt-induced hypertension contribution of cytochrome P-450 1B1. *Am J Physiol Heart Circ Physiol* 2010;299(6):H1891–901.
- Sain H, Sharma B, Jaggi AS, Singh N. Pharmacological investigations on potential of peroxisome proliferator-activated receptor-gamma agonists in hyperhomocysteinemia-induced vascular dementia in rats. *Neuroscience* 2011;192:322–33.
- Sastry KV, Moudgal RP, Mohan J, Tyagi JS, Rao GS. Spectrophotometric determination of serum nitrite and nitrate by copper–cadmium alloy. *Anal Biochem* 2002;306(1): 79–82.
- Sharma B, Singh N. Pitavastatin and 4'-hydroxy-3'-methoxyacetophenone (HMAP) reduce cognitive dysfunction in vascular dementia during experimental diabetes. *Curr Neurovasc Res* 2010;7(3):180–91.
- Sharma B, Singh N. Attenuation of vascular dementia by sodium butyrate in streptozotocin diabetic rats. *Psychopharmacology* 2011a;215(4):677–87.
- Sharma B, Singh N. Behavioral and biochemical investigations to explore pharmacological potential of PPAR-gamma agonists in vascular dementia of diabetic rats. *Pharmacol Biochem Behav* 2011b;100(2):320–9.
- Sharma B, Singh N. Experimental hypertension induced vascular dementia: pharmacological biochemical and behavioral recuperation by angiotensin receptor blocker and acetylcholinesterase inhibitor. *Pharmacol Biochem Behav* 2012a;102(1): 101–8.
- Sharma B, Singh N. Salutary effect of NFrB inhibitor and folacin in hyperhomocysteinemia-hyperlipidemia induced vascular dementia. *Prog Neuropsychopharmacol Biol Psychiatry* 2012b;38(2):207–15. <http://dx.doi.org/10.1016/j.pnpb.2012.03.013>.
- Sharma B, Singh N. Defensive effect of natrium diethyldithiocarbamate trihydrate (NDDCT) and lisinopril in DOCA-salt hypertension-induced vascular dementia in rats. *Psychopharmacology (Berl)* 2012c;223(3):307–17. <http://dx.doi.org/10.1007/s00213-012-2718-9>.
- Sharma B, Singh N, Singh M, Jaggi AS. Exploitation of HIV protease inhibitor Indinavir as a memory restorative agent in experimental dementia. *Pharmacol Biochem Behav* 2008a;89(4):535–45.
- Sharma B, Singh N, Singh M. Modulation of celecoxib- and streptozotocin-induced experimental dementia of Alzheimer's disease by Pitavastatin and Donepezil. *J Psychopharmacol* 2008b;22(2):162–71.
- Sharp SI, Aarsland D, Day S, Sønnesyn H, Alzheimer's Society Vascular Dementia Systematic Review Group, Ballard C. Hypertension is a potential risk factor for vascular dementia: systematic review. *Int J Geriatr Psychiatry* 2011;26(7):661–9.
- Sheehan AL, Carrell S, Johnson B, Stanic B, Banfi B, Miller Jr FJ. Role for Nox1 NADPH oxidase in atherosclerosis. *Atherosclerosis* 2011;216(2):321–6.
- Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, et al. Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. *J Neurochem* 2008;106(1):45–55.
- Sklyarov AY, Panasyuk NB, Fomenko IS. Role of nitric oxide-synthase and cyclooxygenase/lipoxygenase systems in development of experimental ulcerative colitis. *J Physiol Pharmacol* 2011;62(1):65–73.
- Smith CJ, Santhanam L, Bruning RS, Stanhewicz A, Berkowitz DE, Holowatz LA. Upregulation of inducible nitric oxide synthase contributes to attenuated cutaneous vasodilation in essential hypertensive humans. *Hypertension* 2011;58(5):935–42.
- Steed MM, Tyagi N, Sen U, Schuschke DA, Joshua IG, Tyagi SC. Functional consequences of the collagen/elastin switch in vascular remodeling in hyperhomocysteinemic wild-type eNOS^{-/-} and iNOS^{-/-} mice. *Am J Physiol Lung Cell Mol Physiol* 2010;299(3):L301–11.
- Stefanska J, Pawliczak R. Apocynin: molecular aptitudes mediators. *Inflammation* 2008;2008:106507.
- Stevanović ID, Jovanović MD, Colić M, Jelenković A, Bokonić D, Ninković M. Nitric oxide synthase inhibitors protect cholinergic neurons against AICD excitotoxicity in the rat brain. *Brain Res Bull* 2010;81(6):641–6.
- Szasz T, Watts S. W Uric acid does not affect the acetylcholine-induced relaxation of aorta from normotensive and deoxycorticosterone acetate-salt hypertensive rats. *J Pharmacol Exp Ther* 2010;333(3):758–63.
- Tang J, Liu J, Zhou C, Ostanin D, Grisham MB, Neil Granger D, et al. Role of NADPH oxidase in the brain injury of intracerebral hemorrhage. *J Neurochem* 2005;94(5): 1342–50.
- Tang XN, Cairns B, Kim JY, Yenari MA. NADPH oxidase in stroke and cerebrovascular disease. *Neurol Res* 2012;34(4):338–45.
- Tayebati SK, Di Tullio MA, Amenta F. Vesicular acetylcholine transporter (VACHT) in the brain of spontaneously hypertensive rats (SHR): effect of treatment with an acetylcholinesterase inhibitor. *Clin Exp Hypertens* 2008;30(8):732–43.
- Tran MH, Yamada K, Olariu A, Mizuno M, Ren XH, Nabeshima T. Amyloid beta-peptide induces nitric oxide production in rat hippocampus: association with cholinergic dysfunction and amelioration by inducible nitric oxide synthase inhibitors. *FASEB J* 2001;15:1407–9.
- Udayabanu M, Kumaran D, Nair RU, Srinivas P, Bhagat N, Aneja R, et al. Nitric oxide associated with iNOS expression inhibits acetylcholinesterase activity and induces memory impairment during acute hypobaric hypoxia. *Brain Res* 2008;1230: 138–49.
- Vadla GP, Vellaichamy E. Anti-fibrotic cardio protective efficacy of aminoguanidine against streptozotocin induced cardiac fibrosis and high glucose induced collagen up regulation in cardiac fibroblasts. *Chem Biol Interact* 2012;197(2–3):119–28.
- Voss G, Sachsse K. Red cell and plasma cholinesterase activities in microsomes of human and animal blood determined simultaneously by a modified acetylthiocholine-DTNB procedure. *Toxicol Appl Pharmacol* 1970;16(3):764–72.
- Wang HD, Pagano PJ, Du Y. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. *Circ Res* 1998;82:810–8.
- Wu TC, Chao CY, Lin SJ, Chen JW. Low-dose dextromethorphan, a NADPH oxidase inhibitor, reduces blood pressure and enhances vascular protection in experimental hypertension. *PLoS One* 2012;7(9):e46067.
- Xu WB, Lv G, Wang YF, Lu XH, Huang T, Zhu Y, et al. Combination of dexamethasone and aminoguanidine reduces secondary damage in compression spinal cord injury. *Cell Mol Neurobiol* 2009;29(5):683–9.
- Yamada K, Komori Y, Tanaka T, Senzaki K, Nikai T, Sugihara H, et al. Brain dysfunction associated with an induction of nitric oxide synthase following an intracerebral injection of lipopolysaccharide in rats. *Neuroscience* 1999;88:281–94.
- Yu J, Weiwer M, Linhardt RJ, Dordick JS. The role of the methoxyphenol apocynin, a vascular NADPH oxidase inhibitor, as a chemopreventative agent in the potential treatment of cardiovascular diseases. *Curr Vasc Pharmacol* 2008;6(3):204–17.
- Zekry D, Epperson TK, Krause KH. A role for NOX NADPH oxidases in Alzheimer's disease and other types of dementia? *IUBMB Life* 2003;55(6):307–13.
- Zhang ZL, Zuo YM, Wang QH, Xiao HB, Kuang HX. Effects of Valeriana amurensis on the expressions of iNOS COX-2 and IkappaCB-alpha in Alzheimer's disease model rat's brain. *Zhong Yao Cai* 2010;33(4):581–3.
- Zheng B, Zheng T, Wang L, Chen X, Shi C, Zhao S. Aminoguanidine inhibition of iNOS activity ameliorates cerebral vasospasm after subarachnoid hemorrhage in rabbits via restoration of dysfunctional endothelial cells. *J Neurol Sci* 2010a;295(1–2): 97–103.
- Zheng YF, Dai DZ, Dai Y. NaHS ameliorates diabetic vascular injury by correcting depressed connexin 43 and 40 in the vasculature in streptozotocin-injected rats. *J Pharm Pharmacol* 2010b;62(5):615–21.
- Zou MH, Cohen R, Ullrich V. Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. *Endothelium* 2004;11(2):89–97.