IJPSR (2015), Vol. 6, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 February, 2015; received in revised form, 02 July, 2015; accepted, 11 November, 2015; published 01 November, 2015

EFFECT OF AGMATINE ON 6-HYDROXYDOPAMINE INDUCED MEMORY IMPAIRMENT IN PARKINSON'S DISEASE IN RODENTS

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Keywords:

6-OHDA, Parkinson Disease, Memory enhancer, Agmatine

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ABSTRACT: The core finding of the present preclinical study demonstrated that, administration of increasing doses of Agmatine is remarkably neuroprotective in male wistar rats against 6-Hydroxydopamine induced neurotoxicity. Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease with multiple motor and non-motor features that contribute to the impairment of health-related quality of life (QOL). It is associated with the loss of brown pigment neuromelanin with preferential loss of the dopaminergic neurons of the substantia nigra pars compacta results in oxidative stress and mitochondrial dysfunction. It is characterized by reduced movement, rigidity, and tremor. Agmatine (AGM) (1-Amino-4-guanidinobutane, 4 (Aminobutyl) Guanidine,1- guanidinobutane) pharmacological and electrophysiological evidence indicate that Agmatine act at N-methyl-d-aspartate (NMDAr) and nitric acid synthase (NOS) to exert anti-excitatory effects throughout the central nervous system. Agmatine act as neuroprotective agent & memory enhancer. Relevent doses of Agmatine treatment protects behavioural changes, significantly attenuated oxidative damage & improved mitochondrial complexs enzyme activities in different regions (striatum, cortex and hippocampus) of rat brain against 6-OHDA induced neurotoxicity. I.C.V. administration of 6-Hydroxydopamine is known to produce hypo activity that resembles juvenile onset and advanced Parkinson's disease in rats. The results show that Agmatine treatment is effective in various behavioral models, it could be used as an effective therapeutic agent in the management of Parkinson's disease and related conditions and thus it shows the effect of Agmatine on 6-Hydroxydopamine induced memory impairment in Parkinson's disease in Rodents.

INTRODUCTION: Parkinson's disease (PD) is a human neurodegenerative disorder which is mainly characterized by a massive and progressive degeneration of the dopaminergic neurons in the substantia nigra (SN).

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DOI:
10.13040/IJPSR.0975-8232.6(11).4743-52

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(11).4743-52

The most widely used animal models of PD involve intracranial infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) directly into the ascending dopaminergic forebrain bundle, thereby inducing severe dopaminergic neuronal degeneration associated with profound deficits in feeding, drinking, sensor motor and learning functions [9, 11, 15, 32]. 6-OHDA is a redox active neurotoxin [4], commonly used to produce a Parkinsonian pattern of neuronal loss in rodents [34]. Studies have demonstrated that the neurotoxic effects of 6-OHDA involves generation of hydrogen peroxide and hydroxyl radicals [31], reduction in glutathione (GSH) and superoxide

dismutase (SOD) activity [25] and an increase in malondialdehyde levels in the striatum [16].

To specifically damage the nigrostriatal dopaminergic pathway, 6-OHDA is injected stereotaxically into the substantia nigra, the medial forebrain bundle (that comprises the nigrostriatal tract), or the striatum [12, 13]. When injected into the striatum, 6-OHDA produces a more protracted retrograde degeneration of the nigrostriatal system which can last from 1–3 wk after lesion [26, 30,] and the dying neurons exhibit a varied morphology including some features reminiscent of apoptosis [20].

Agmatine is an endogenous polycationic amine synthesized from L-arginine by arginine decarboxylase. It is present in plasma and widely distributed in mammalian tissues [27]. Agmatine binds and activates both a2-adrenegic receptors and I1- and I2- imidazoline receptors it also blocks the ligandgated N-methyl-D-aspartate (NMDA) receptor channel in neuronal tissue [28]. Through activation of imidazoline receptors, agmatine inhibits catecholamine release from chromaffin cells and stimulates insulin release from pancreatic b-cells [6].

In earlier studies, agmatine has been shown to be a competitive inhibitor of both neuronal nitric oxide synthase (nNOS) and inducible NOS (iNOS) [2] endogenous agmatine plays a physiological role in learning and memory in a variety of cognitive tasks proposing agmatine levels in rats brain are elevated within the learning process [17, 18, 29]. These data revealed an age-related involvement of arginase and NO/NOS pathways in learning and memory leading to investigation of endogenous agmatine's possible effect on learning and memory [19].

The neuroprotective potential of agmatine has been detected in a Parkinson's disease model of 1-methyl - 4 - phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) (1 mg per nostril). Agmatine has shown to attenuate dopaminergic cell loss in substantia nigra pars compacta and repeated treatment (30 mg/kg i.p.) improved short-term memory displayed by MPTP in aged mice. Observed behavioural benefits of agmatine were accompanied with the prevention of MPTP-induced decrease in hippocampus glutamate uptake. That means, one possible mechanism by which agmatine exerting its neuroprotective effects against MPTP neurotoxicity may be due to the modulation of glutamate reuptake into neurons, the main mechanism responsible for decreasing extracellular glutamate levels, thus attenuating glutamate neurotoxicity [21].

MATERIAL AND METHOD:

Subjects:

Adult male Wistar rats born and reared in the Animal House of the Agnihotri College of Pharmacy, Wardha. (India) was used in the present study. Young healthy male rats (250–300 g) were group housed (Six per cage) and maintained at 23±2 ◦C under 12:12 hrs light (08:00–20:00 h)/dark cycle with free access to rodent chow and tap water. The animal studies were approved by the Institutional Animal Ethics Committee, (Proposal no.1 and approved at dated 27/12/2012) constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naive to drug treatments and experimentation at the beginning of all studies. All tests were conducted between 08:00 and13:00 h.

Drugs and solutions:

Agmatine sulphate (Sigma-Aldrich Labs, Bangalore, India). Agmatine dissolved in double saline water. Drug solutions were prepared fresh and their doses are expressed in terms of free bases. Other chemicals used in the present investigation were of analytical grade.

Treatment schedule:

The following drugs were used in the present study. 6-OHDA (Sigma Chemical, India) was dissolved in 2 µl 0.2% ascorbic acid saline through 30 gauge stainless steel needle (pH 7.4) and administered into SNPC to animals. Agmatine sulphate (Sigma-Aldrich Labs, Bangalore, India) was diluted with double saline and administered intraperitoneally. Separate groups of rats (n=6) received i.c.v. 6-OHDA (6µg/kg), i.p. Agmatine (2.5, 5 and 10mg/kg), for 14 day.

Behavioral tests:

Morris water maze test:

Cognitive function of rats was assessed in Morris water maze test as described earlier [24, 33]. The test

apparatus included a circular water tank (180 cm in diameter and 60 cm high) made up of dark gray plastic. A escape platform (12.5 cm in diameter and 38 cm high) invisible to the rats (2 cm below the water level), was set inside the tank and filled with water (24±1 °C) made opaque by the addition of full cream milk at a height of 40 cm. The tank was located in the centre of a room that contained some prominent visual extra-maze cues; these were visible from the pool and could be used by the rats for spatial orientation.

The position of the cues remained unchanged throughout the study. The rats were received four consecutive daily training trials after 30 days (post diabetes 30 days) for next 5 days, with each trial having a cut off time of 90 s and were then placed in an empty cage for a 30s inter-trial interval. For each trial, each rat was put into the water at one of four starting positions. Each of the four trials every day was started from a different location on the edge of the pool and the order in which the start positions were used varied day to day. The rat had to swim until it climbed onto the platform submerged underneath (2 cm) the water. After climbing onto the platform, the animal remained there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 90 s, it was guided to reach the platform and allowed to remain there for the same time. The time to reach the platform (escape latency in seconds) was measured. Latency to reach the hidden platform was used as the measure of acquisition. After completion of the last trial, rats were gently dried with a towel, kept warm for an hour and returned to their home cage. .

Passive Avoidance Test:

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 cm X 27 cm X 27 cm) having three walls of wood and one wall of plexiglass, featuring a grid floor (made up of 3 mm stainless steel rods set 8-mm apart), with a wooden platform (10 cm X 7 cm X 1.7 cm) in the center of the grid floor. The box was illuminated with a 15-W bulb during the experimental period. Electric shock (20 V, AC) was delivered to the grid floor. Training (i.e. eighth day of drug treatment) was carried out in two similar sessions. Each rat was gently placed on the wooden platform set in the center of the grid floor. When the rat stepped-down placing all its paws on the grid floor, shocks were delivered for 15 seconds and the step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to the grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2-15 seconds during the first test were used for the second session and the retention test. The second session was carried out 90 minutes after the first test. During the second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from the shock-free zone, if they did not step down for a period of 60 seconds and were subjected to the retention test. Retention (memory) was tested after 24 hours (i.e. ninth day, 24 hours after last dose) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 seconds. Significant increase in SDL value indicated improvement in memory [14.]

Elevated Plus Maze:

The EPM was made of dark gray PVC consisting of two opposite open arms (50 cm × 12 cm) and two opposite closed arms surrounded by 50 cm high walls of the same dimensions. The middle section that allows the animal to transit from arm to arm consisted of a square with dimensions of 12 × 12 cm. The maze was elevated 50 cm above ground and the open arms were equipped with 0.5 × 0.5 cm ledges to ensure that no animals would fall off the maze. The apparatus could be moved between rooms and it was made sure that placement and lighting conditions were identical for each trial. The trials were video recorded and computer analyzed with the ethological software viewer (Biobserve GmbH, Bonn, Germany) to measure time spent in and visits to the arms of the EPM. All other ethological analyses were performed by an experienced observer. The PC equipment was located outside of the experimental rooms. All experiments were carried out during the middle of the light phase between 1100 and 1600 h.

Biochemical estimation :

Post mitochondrial supernatant préparation :

After behavioral tests, rats were sacrificed by decapitation and brain structures were removed and separated into cerebral cortex, striatum and hippocampus for the biochemical studies. Cerebral cortex, striatum and hippocampus were rinsed with ice cold saline and homogenized in chilled 50mM phosphate buffer (pH 7.4). The homogenates were centrifuged at 4600 rpm for 10 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 15,000 rpm for 30 min at 4 °C to get the post mitochondrial supernatant, which was used to estimate biochemical parameter activity.

Estimation of nitrite:

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediame dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al. Equal volumes of supernatant and Greiss reagent were mixed, and incubated for 10 min at room temperature. The absorbance of each sample was determined at 540 nm at Perkin Elmer lambda 20 spectrophotometer. The concentration of nitrite in the supernatants was determined from a sodium nitrite standard curve and expressed as µmol/mg of Protein.

Estimation of Superoxide dismutase:

Homogenates of the prefrontal cortex were centrifuged at 25,000 × g for 15 min at 40C and supernatant dialyzed in 50mM PBS (pH 7.8) containing 1mM EDTA. SOD activity was determined based on inhibition of superoxide-dependent reactions. The reaction mixture contained 70mM potassium phosphate buffer (pH 7.8), 30 µM cytochrome c, 150µM xanthine, and tissue extract in phosphate buffer diluted 10 times with PBS in a final volume of 3 ml. The reaction was initiated by adding 10 µl of 50 units' xanthine oxidase, and the change in absorbance at 550 nm recorded. The results are expressed as unit/mg protein.

Estimation of Glutathione peroxidise:

GPX activity was estimated according to the procedure described by Mohandas et al.(1984).The reaction mixture consisted of phosphate buffer (0.05M, PH 7.0), EDTA (1m M), sodium azide (1mM), glutathione reductase (1EU/ml), glutathione (1 m M), NADPH (0.2m M), hydrogen peroxide (0.25m M), and 0.1 ml of PMS in a final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nanomoles of NADPH oxidized per minute per milligram of protein using a molar extinction coefficient of $6.22\times10^{-3}M^{-1}cm^{-1}$.

Cholinesterase activity:

Cholinergic dysfunction was assessed by measuring choline esterase (ChE) levels in cerebral cortex and hippocampus according to the method described previously by Ellman et al. [7] with slight modifications. The assay mixture contained 0.05 ml of supernatant, 3ml of 0.01M sodium phosphate buffer (pH 8.0), 0.10 ml of 0.75mM acetylthiocholine iodide (AcSCh) and 0.10 ml Ellman reagent (5_5 dithiobis [2-nitrobenzoic acid] 10mM, NaHCO3 15 mM). The change in absorbance was measured at 412nm for 5min. Results were calculated using molar extinction coefficient of chromophore (1.36×104M−1 cm−1). All samples were run in duplicate or triplicate and the enzyme activity were expressed in _mol AcSCh/min/g of protein.

Statistical analysis:

Results were expressed as mean ± S.E.M. The data were analyzed by two-way or one-way analysis of variance (ANOVA) followed by Bonferroni and Tukey's multiple comparison test respectively. Statistical significance was considered at $P<0.05$ in all the cases.

RESULTS AND DISCUSSION:

Effect of daily treatment of agmatine on 6-Hydroxydopamine-induced alterations in various behavioral parameters.

Effect of agmatine on Morris water maize:

In Morris water maze test, the mean escape latency of trained rats gradually decreased during training session in 6-OHDA treated animals. However, escape latency was significantly increased [$F(5, 30) = 232.1$, $P<0.0001$] on the 15th day in Morris

water maze as compared to the vehicle treated group ($P < 0.05$). Agmatine (2.5,5 and 10 mg/kg, ip) [$F (5, 30) = 126.9$, $P<0.0001$] treatment showed a significant improvement in memory performance on the 15th day as compared to the 6-OHDA treated group ($P < 0.05$) However, lower dose of Agmatine (2.5mg/ kg, ip) did not show any significant effect on memory performance as compared to 6-OHDA treated rats. (**Fig.1 & 2**).

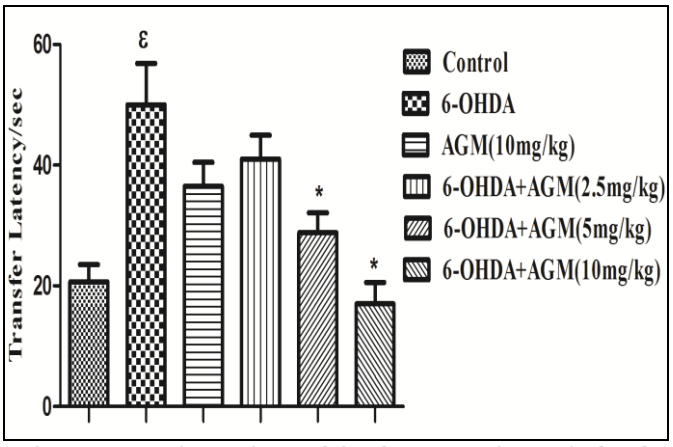

FIG.1: EFFECT OF AGMATINE ON SPATIAL NAVIGATION TASK IN 6-OHDA TREATED RATS

Influence of agmatine treatment on transfer latency in Morris water maze. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

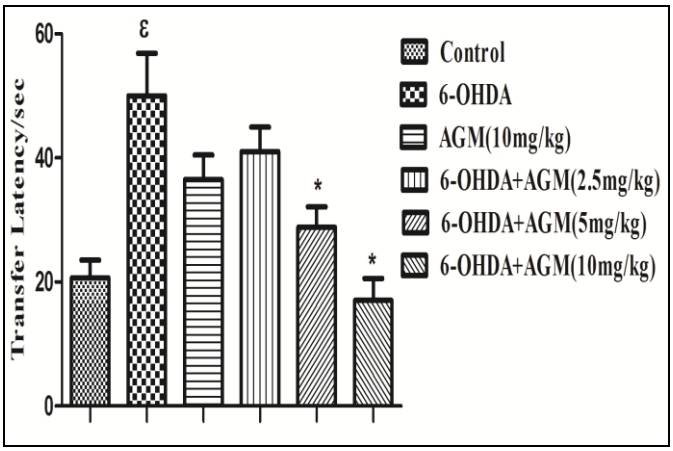

FIG.2: INFLUENCE OF AGMATINE TREATMENT ON TIME SPENT IN TARGET QUADRANT IN MORRIS WATER MAZE.

Influence of agmatine treatment on time spent in target quadrant in Morris water maze. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

Effect of agmatine on passive avoidance test:

Fig.3 & 4 shows the investigation ratios for the two retention intervals (30 min and 24 h, respectively), for the passive avoidance test. One-way ANOVA revealed a significant effect of Parkinson induction and agmatine treatment at the short-term 30 min retention interval [$F (5, 30) = 112.6$, $P<0.0001$] as well as at 24h retention trial [$F (5, 30) = 139.9$, $P<0.0001$]. Post hoc test revealed that 6-OHDA induced Parkinson's disease rats explored less to the passive avoidance compared to non Parkinson's rats during both retention trials ($P<0.05$). Further, agmatine treated Parkinson's disease rats explored more to passive avoidance as compared to vehicle treated group at both retention trials ($P<0.05$).

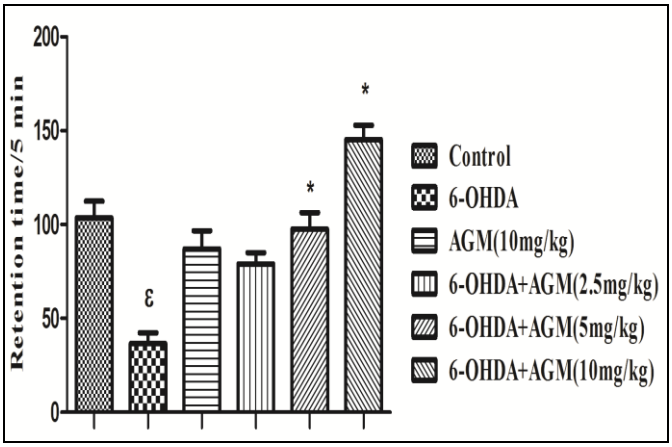

FIG.3: INFLUENCE OF TREATMENT OF AGMATINE ON THE RETENTION TIME FOR THE TWO RETENTION INTERVALS (30 MIN), FOR THE PASSIVE AVOIDANCE TEST

Influence of treatment of agmatine on the Retention time for the two retention intervals (30 min), for the passive avoidance test. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM

(5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

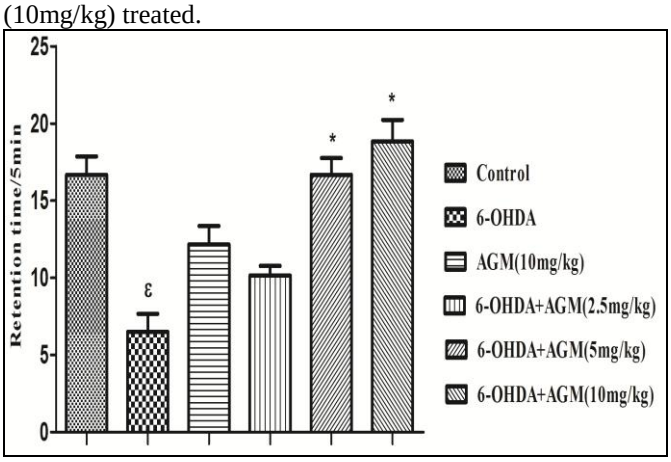

FIG.4: INFLUENCE OF TREATMENT OF AGMATINE ON THE RETENTION TIME FOR THE TWO RETENTION INTERVALS (24 H), FOR THE PASSIVE AVOIDANCE TEST

Influence of treatment of agmatine on the Retention time for the two retention intervals (24 h), for the passive avoidance test. Each value represents mean ± S.E.M. of 6 observations.

$^{\varepsilon}$P<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

Effect of Agmatine on elevated plus maze test:

Fig.5 & 6 shows the time spend in open arm for the two retention intervals (30 min and 24 h, respectively), for the elevated plus maze test. One-way ANOVA revealed a significant effect of Parkinson induction and agmatine treatment at the short-term 30 min retention interval [F (5, 30) =112.6, P<0.0001] as well as at 24h retention trial [F (5, 30) =139.9, P<0.0001]. Post hoc test revealed that 6-OHDA - induced Parkinson's disease rats explored less to the elevated plus maze compared to non Parkinson's rats during both retention trials (P<0.05). Further, agmatine treated Parkinson's disease rats explored more to novel objects as compared to vehicle treated group at both retention trials (P<0.05).

Effect of daily treatment of agmatine on 6-Hydroxydopamine induced alterations in various biochemical parameters.

Effect of agmatine on nitrite concentration:

I.C.V. administration of 6-OHDA significantly increased nitrite concentration in striatum as compared to vehicle treated group. However, Agmatine (5 and 10 mg/kg) treatment significantly attenuated nitrite concentration as compared to 6-OHDA treated group (P < 0.05) (**Fig. 7**). However lower dose of Agmatine (2.5 mg/kg) did not produce any significant effect on these oxidative stress parameters in 6-OHDA treated group.

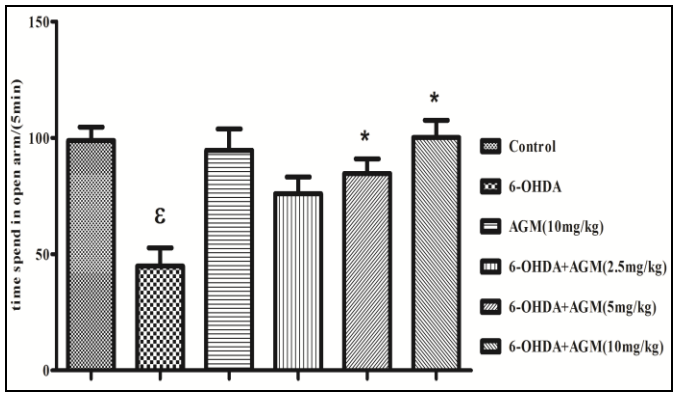

FIG.5: INFLUENCE OF TREATMENT OF AGMATINE ON THE TIME SPEND IN OPEN ARM FOR THE TWO RETENTION INTERVALS (30 MIN), FOR THE ELEVATED PLUS MAZE TEST

Influence of treatment of agmatine on the Time spend in open arm for the two retention intervals (30 min), for the elevated plus maze test. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

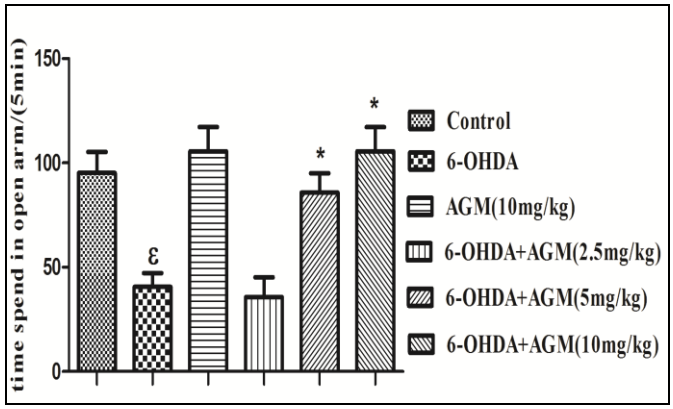

FIG.6: INFLUENCE OF TREATMENT OF AGMATINE ON THE TIME SPEND IN OPEN ARM FOR THE TWO RETENTION INTERVALS (24 H), FOR THE ELEVATED PLUS MAZE TEST.

Influence of treatment of agmatine on the time spends in open arm for the two retention intervals (24 h), for the elevated plus maze test. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

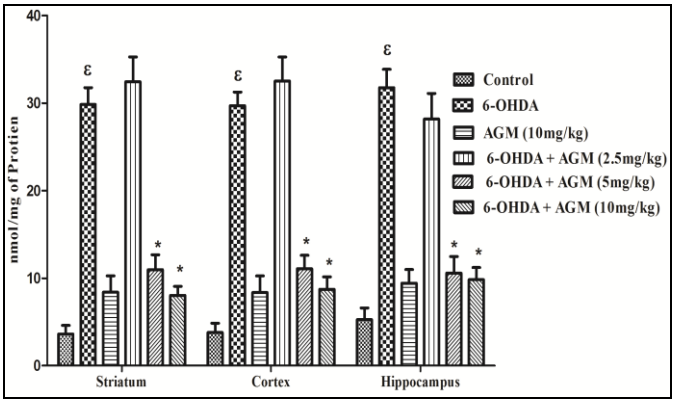

FIG.7: EFFECT OF AGMATINE ON NITRITE CONCENTRATION

Influence of treatment with agmatine on nitrite concentration in striatum, cerebral cortex and hippocampus of rat brain (mean ± S.E.M. of 6 observations) in the different groups of rats. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non-Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

Effect of agmatine on Superoxide Dismutase Estimation levels in 6-OHDA treated rats I.C.V. administration of 6-OHDA significantly decreased Superoxide Dismutase concentration in striatum as compared to vehicle treated group. However, Agmatine (5 and 10 mg/kg) treatment significantly attenuated SOD concentration as compared to 6-OHDA treated group (P < 0.05) **(Fig. 8**).

Effect of agmatine on Glutathion Peroxidase Estimation levels in 6- OHDA treated rats I.C.V. administration of 6-OHDA significantly decreased Glutathion Peroxidase concentration in striatum as compared to vehicle treated group. However, Agmatine (5 and 10 mg/kg) treatment significantly attenuated nitrite concentration as compared to 6-OHDA treated group (P < 0.05) (**Fig. 9**). However lower dose of Agmatine (2.5 mg/kg) did not produce any significant effect on these oxidative stress parameters in 6-OHDA treated group.

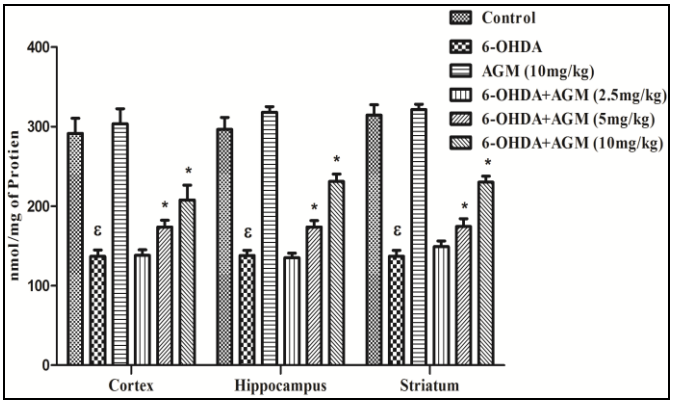

FIG. 8: EFFECT OF AGMATINE ON SUPEROXIDE DISMUTASE ESTIMATION LEVELS IN 6-OHDA TREATED RATS

Influence of treatment with agmatine on Superoxide Dismutase concentration in striatum, cerebral cortex and hippocampus of rat brain (mean ± S.E.M. of 6 observations) in the different groups of rats. Each value represents mean ± S.E.M. of 6 observations.

ƐP<0.001 vs. non- Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

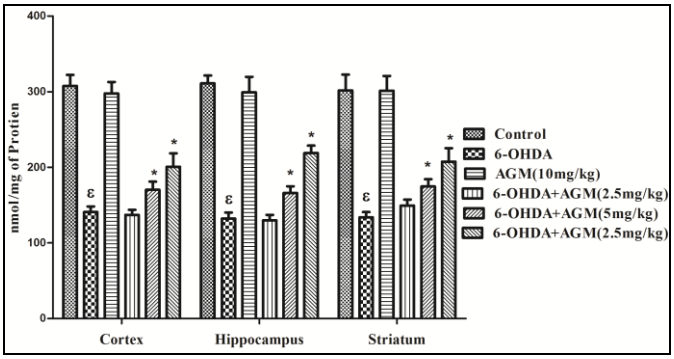

FIG. 9: EFFECT OF AGMATINE ON GLUTATHION PEROXIDASE ESTIMATION LEVELS IN 6- OHDA TREATED RATS

Influence of treatment with agmatine on Glutathion Peroxidase concentration in striatum, cerebral cortex and hippocampus of rat brain (mean ± S.E.M. of 6 observations)

in the different groups of rats. Each value represents mean ± S.E.M. of 6 observations.
ƐP<0.001 vs. non- Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated.

Effect of agmatine on acetylcholine esterase levels in 6-OHDA treated rats:

Cholinesterase (ChE) activity was expressed as AcSCh formed. The changes in ChE activity in striatum, cerebral cortex and hippocampus after administration of Agmatine are presented in **Fig. 10.** As can be observed, ChE activity was significantly increased in the striatum [F (5, 30) =381.2 P<0.0001], cortex [F (5, 30) =187.0, P<0.0001] and hippocampus [F (5, 30) =132.4, P<0.0001] of Parkinson control group compared to the non-Parkinson control group. Treatment with Agmatine (2.5, 5 and 10 mg/kg) significantly decreased the ChE activity in striatum compared to Parkinson's control rats (P<0.01). Treatment with Agmatine (2.5, 5 and 10 mg/kg) significantly decreased the ChE activity in cortex compared to Parkinson's control rats (P<0.01). Similarly, treatment with Agmatine (5 and 10 mg/kg) significantly decreased the ChE activity in hippocampus compared to Parkinson control rats (P< 0.01). Agmatine treatment in non-Parkinson rats did not influence the ChE activity as compared to non-Parkinson control rats (P>0.05). (**Fig.10**).

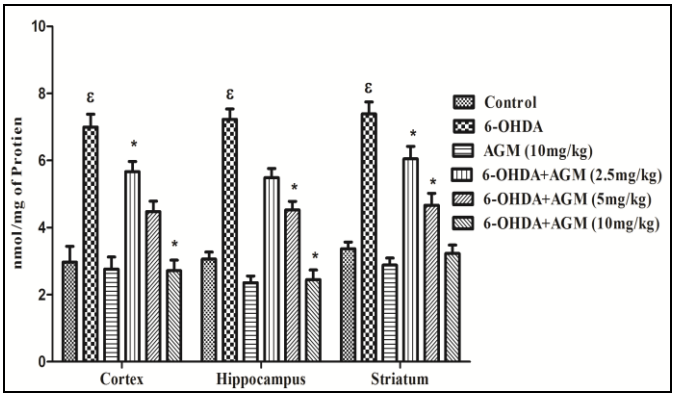

FIG.10: EFFECT OF AGMATINE ON ACETYLCHOLINE ESTERASE LEVELS IN 6-OHDA TREATED RATS

Effect of treatment with agmatine on Acetyl cholinesterase level in striatum, cerebral cortex and hippocampus of rat brain (mean ± S.E.M. of 6 observations) in the different groups of rats. Each value represents mean ± S.E.M. of 6 observations.
ƐP<0.001 vs. non- Parkinson's test group; *P<0.001 vs. Parkinson's test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); 6-OHDA: 6- Hydroxy Dopamine; AGM (10mg/kg): Agmatine (10mg/kg); 6-OHDA+ AGM (2.5mg/kg): 6-Hydroxy Dopamine Agmatine (2.5mg/kg) treated; 6-OHDA+ AGM (5mg/kg): 6-Hydroxy Dopamine Agmatine (5mg/kg) treated; 6-OHDA+ AGM (10mg/kg): 6-Hydroxy Dopamine Agmatine (10mg/kg) treated

PD is often complicated by a variety of cognitive symptoms that ranges from isolated memory and thinking problems to severe dementia. While, the motor symptoms of PD are well-known for tremor, rigidity, slowness of movement and imbalance [10].

The core finding of the present study is that, administration of relevant doses of agmatine is remarkably neuroprotective in rats against 6-Hydroxydopamine-induced neurotoxicity. We have chosen the dose of agmatine (2.5, 5 and 10 mg/kg., i.p.) according to the previous studies done in our laboratory [3]. There are no previous reports on the protective effect of agmatine in 6-Hydroxydopamine induced neurotoxicity, an animal model for Parkinson's disease. In the present study, agmatine attenuated various behavioral and biochemical alterations due to 6-Hydroxydopamine and thus providing the first evidence regarding its beneficial effect in Parkinson's disease.

In the present study, the administration of 6 µg of 6- OHDA into the right unilateral ventricle led to a decrease in DA levels of approximately 50–60% in the right striatum of rats at all ages. Nigral DA levels were reduced to a slightly less extent. In contrast to more severe bilateral lesions, the pattern of changes in body weight after surgery was similar between the vehicle and lesioned animals [35].

The present study employed 6-OHDA as animal model of Parkinson disease. 6-OHDA induce nigrostrital dopaminergic lesion via the generation of hydrogen peroxide and derived hydroxyl radicals. 6-OHDA could induce catecholaminergic cell death by three main mechanisms: reactive oxygen species generated by intra or extracellular auto-oxidation, hydrogen peroxide formation induced by MAO activity or direct inhibition of the mitochondrial respiratory chain. These events lead

to strong oxidative stress amplified by cytoplasmic free calcium and leads to a decrease in cellular ATP avaibility, both resulting to cell death. [5]

Impairment of mitochondrial activity also contributes to both ROS generation and nigral cell loss. The main mitochondrial defect observed in degenerating PD concerns complex I (nicotinamide adenine dinucleotide coenzyme Q reductase) of the mitochondrial respiratory chain. Complex I is located in the inner mitochondrial membrane and forms a part of the oxidative phosphorylation system (OXPHOS) responsible for the production of cellular ATP. Decreases in the activity and immunoreactivity of the reduced form of the complex I was observed in the SNpc of PD patients [23].

Unilateral 6-OHDA-induced SNpc degeneration produces an asymmetric and quantifiable motor behavior after unilateral lesion induced by systemic administration of either DA receptor agonists, l-dopa or dopamine releasing drugs amphetamine [22].

In the present study, administration of 6-Hydroxydopamine decreased the ambulatory movements (in actophotometer) and causes a delay in retraction time of the passive avoidance test (in passive avoidance test apparatus), thus representing the motor abnormalities. Daily treatment with agmatine for 14 days dose-dependently attenuated 6-Hydroxydopamine-induced hypolocomotion and motor incoordination.

I.C.V. administration of 6-Hydroxydopamine also decreased the SOD levels in the whole brain, suggesting mitochondrial damage and pretreatment with agmatine attenuated this decrease in SOD levels. These results show that agmatine may prevent mitochondrial deterioration and maintain synaptic integrity against damage induced by 6-Hydroxydopamine.

Cholinergic neurotransmission is a central process underlying memory and cognitive function. Cholinergic basal forebrain neurons in the nucleus basalis magnocellularis innervate the cerebral cortex, amygdaloid complex and hippocampus, and are essential for learning and memory formation [8]. One of the most important mechanisms responsible for correct cholinergic function is performed by enzyme choline esterase (ChE) [1]. In the present study, treatment with agmatine partially decreased the levels of ChE in cerebral cortex and hippocampus of PD rats.

CONCLUSION: The findings demonstrated the significant role of agmatine in PD by reversal of various behavioral and biochemical alterations induced by 6-Hydroxydopamine in rats. However, further studies are required to understand the exact mechanism involved in its neuroprotective role of agmatine in this animal model of Parkinson's disease.

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How to cite this article:
Kahale VP, Shelat P, Mhaiskar A, Gaikwad NJ and Mundhada DR: Effect of Agmatine on 6-Hydroxydopamine Induced Memory Impairment in Parkinson's Disease In Rodents. Int J Pharm Sci Res 2015; 6(11): 4743-52.doi: 10.13040/IJPSR.0975-8232.6(11).4743-52.