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Protocatechuic acid is a potential candidate for the treatment of cognitive impairment.

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Abstract:

Cognitive dysfunction, impairing daily functioning, is a significant health concern with aging and psychiatric disorder. Protocatechuic acid (PCA), a phenolic compound in various sources, enhance antioxidant defenses by increasing glutathione enzyme trough Nrf2 activation. PCA protects against oxidative stress and provides neuroprotection. Our study aimed to investigate role of systemic PCA administration in cognitive behavior using Y-Maze, and MWM test in a scopolamine-induced memory impairment model in mice. PCA increase spontaneous alteration compared to scopolamine – treated group, indicates its potential candidate for treating cognitive impairment.

(Key words: - Cognition, cognitive impairment, protocatechuic acid, STM, learning memory)

Cognitive functions enable us to know and gather information about our surroundings. Cognition may be defined as the mental action or process of acquiring knowledge and understanding thought, experience and the senses. The basic cognitive functions are attention, memory, speech and language, decision making, processing speed, executive functioning[1,2].

Poor memory, slow recall and retention problems are common in today's stressful and competitive world. Stress and emotional problems are ever increasing in today's fast life. These problems combined with an ageing population, may lead to memory loss, amnesia, anxiety, high blood pressure, depression, Parkinson's disease (PD), Alzheimer's Disease (AD), Schizophrenia (SZ), cancer etc[3]. All these disorders have some components predisposing to cognitive decline.

Cognitive dysfunction is a major health problem in the 21st century with the increasing burden of ageing population and psychiatric disorders. It is the loss of intellectual functions such as thinking, remembering, and reasoning of sufficient severity to interfere with daily functioning. It is one of the most functionally debilitating aspect of many neuropsychiatric disorders and neurodegenerative disorders, such as SZ, depression, AD, dementia, cerebrovascular impairment, seizure disorders, head injury and the PD. Cognitive decline is an established entity in neurodegenerative disorders such as AD and PD disease and cognitive enhancers (CE) are treatment of choice[4].

Due to complex and progressive nature of neurodegenerative disorders and lack of availability of suitable medicines in conventional therapy, use of natural products seems to be more promising and acceptable[5].

PCA is a phenolic acid, one of the major benzoic acid derivatives found in apples, green, and black tea, vegetables and fruits and also naturally present in many Chinese herbal medicines such as *Salvia miltiorrhiza (Danshen), Hibiscus sabdariffa L.* and *Alpiniaoxyphylla* A high level of this compound was found in the extract from the rind of *Citrus reticulata Blanco*[6].

PCA exhibits cardioprotective effects, anti-cancer, anti-ulcer, anti-ageing, anti-inflammatory, analgesic, anti-atherosclerotic, antiplatelet[7], anti-bacterial[8], anti-metastatic, anti-diabetic activities[9], hepatoprotective activity[10], neurological and nephroprotective activity in addition to antioxidant activity associated with free radical scavenging. Further, PCA shows anti-ageing effect through its anti-oxidant potential through enhanced resistance to osmotic stress as well as increased thermo tolerance and might result in lifespan extension[7].

Earlier studies in cell lines have shown that the PCA increases the expression of glutathione peroxidase (GPx) and glutathione reeducates (GR), mainly by inducing the JNK-mediated phosphorylation of the transcription factor Nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2), a major regulator of antioxidant/detoxifying enzymes. This finding further confirms and expands the concept that PCA improves antioxidant cellular defenses. PCA protects against ischemia-reperfusion-induce oxidative injury both *In-vivo* and *In-vitro*, previous study showed that PCA act via PKCε to activates Nrf2 which promotes heme oxygenase-1 (HO-1) expression and reduce oxidative stress in ischemic/hypoxic injury, thus it provides neuroprotection[11]. PCA treatment attenuates oxidative stress by improving endogenous antioxidant system (superoxide dismutase (SOD) and catalase (CAT)) in hippocampus and cerebral cortex[11].

PCA has been reported as pharmacological agent improving cognitive deficits and attenuation of amyloid deposit in amyloid-β protein precursor/presenilins 1 (AβPP/PS1) double transgenic mice[12]. However, a detailed effect of PCA on cognitive behavior is yet not reported.

Scopolamine by blocking cholinergic neurotransmission cause amnesia in animals. The present study aims to investigate whether systemic administration of PCA improves the cognitive behavioral functions by its neuroprotective effect in normal mice along with scopolamine induced deficit model.

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Material and method:

The aim of study was to study the role of systemic administration of protocatechuic acid in cognitive behavior using Y-maze apparatus and MWM test behavioral models in scopolamine-induced memory impairment model

Swiss Albino mice (25-30g) were housed 3-5/cage before beginning of experimental procedures under a controlled room temperature (25±1°C), relative humidity (50-70%) and a 12:12-h light/dark cycle (lights on 07:00 h). Food (Trimurti Feeds, Nagpur) and tap water were provided *ad libitum*. Protocols used in the present study were carried out under strict compliance with Institutional Animal Ethics Committee (IAEC), IAC/UDPS2014/3-29.8.2014. Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur, Maharashtra, India.

Scopolamine (1 mg/ml) was dissolved in a 0.9% saline solution (Jeon et al., 2016). PCA (50 mg/ml) was dissolved in 10% dimethyl sulphoxide (DMSO) and 90% polyethylene glycol (PEG). All drugs were purchased from Sigma (Sigma-Aldrich, St. Louis, MO. USA).

Behavioral paradigms:

Y-Maze:

The Y-maze is three-arm maze with equal angles between all arms, which were 35 cm in length and 5 cm in width, with walls 10 cm high (Diagram 2). The maze floor and walls were constructed from dark grey polyvinyl plastic. Mice were initially placed within one arm and the sequence and number of arm entries were recorded, 10 min period for each mouse. Arms were cleaned between tests to remove odours and residues by diluted 10 % ethanol. Alternation behavior was determined from successive entries into three different arms (e.g., ABC, CAB, or BCA). An arm entry by the mice was defined as placing all four paws within a boundary of the arm.

% Alternation = [(Number of alternations) / (Total arm entries − 2)] × 100

The number of arm entries was used as an indicator of locomotor activity [13]

Morris water maze (MWM) test:

MWM task (Diagram 3). Animals received a block of four trials during four daily sessions. During the first 4 days, the platform, situated in the centre of the southwest quadrant, was submerged 1.5 cm below the surface of water and therefore invisible, for testing spatial learning. The platform position remained stable over 4 days and acquisition of this task was assessed. The task requires mice to swim to the hidden platform guided by distal cues. After mounting the platform, the mice were allowed to remain there for 20 sec until the start of the next trial. The animals were given a maximum of 90 sec to find the platform; if they failed to find the platform in this time, they would be placed by the experimenter on the platform and allowed to stay there for 20 sec. On day 5, a probe test was conducted 24h later. This probe trial consisted of a 60 sec free swim period without a platform and the time percentage spent in the target quadrant was recorded [14].

Experimental Design:

1. Dose dependent effect of PCA on scopolamine–induced alternations in working memory in Y-Maze:

Mice were trained for 10 min before 1h of experimental protocol with one closed arm in the Y-Maze apparatus and then treated with either, saline or scopolamine (2 mg/kg) via *i.p.* and placed back in the home cage. After 30 min mice were administered PCA (15, 30, 45 mg/kg) via *i.p.* route and placed back in the home cage. After 30 min of PCA administration these animals were assessed for alternation behaviour in Y-maze for 8 min in which all the arms were kept opened.

2. Dose dependent effect of PCA on scopolamine-induced memory deficits in MWM:

Mice were treated with either, saline or scopolamine (2 mg/kg *i.p*.) and placed back in the home cage. After 30 min mice were administered with PCA (15, 30 and 45 mg/kg *i.p.*) and placed back in the home cage. After 30 min of PCA administration these animals were assessed for in MWM and escape latency was measured for 1 min. Habituation was given for 4 days with hidden platform, 4 sessions/day and cutoff time was 60 sec. If mouse did not reach within 1 min on hidden platform then it was placed manually for 30 sec, and testing was done on 5th day without drug administration, and time spent in target and opposite quadrant was measured for 1 min.

The results were analyzed by a one-way or two-way ANOVA followed by Bonferroni's multiple comparison tests. A value of $P<0.05$ was considered to be statistically significant in all the cases.

1. Effect of PCA on scopolamine-induced alternations in working memory using Y-Maze:

Effect of PCA on scopolamine-induced memory deficit was investigated by using the Y-Maze task. A significant group effect was observed in spontaneous alternation behaviours (One way ANOVA $F_{4,19} = 60.31$, $P< 0.0001$, n=4, Figure1). Bonferroni multiple comparisons test revealed that spontaneous alternation (%) in scopolamine-treated group (2 mg/kg *i.p.)* was significantly lowered than that of vehicle treated control group ($p< 0.001$). We found that PCA at 15, 30 and 45 mg/kg *i.p*. ($p< 0.001$, $p< 0.001$, and $p<0.001$ respectively) significantly reversed scopolamine induced deficits in working memory.

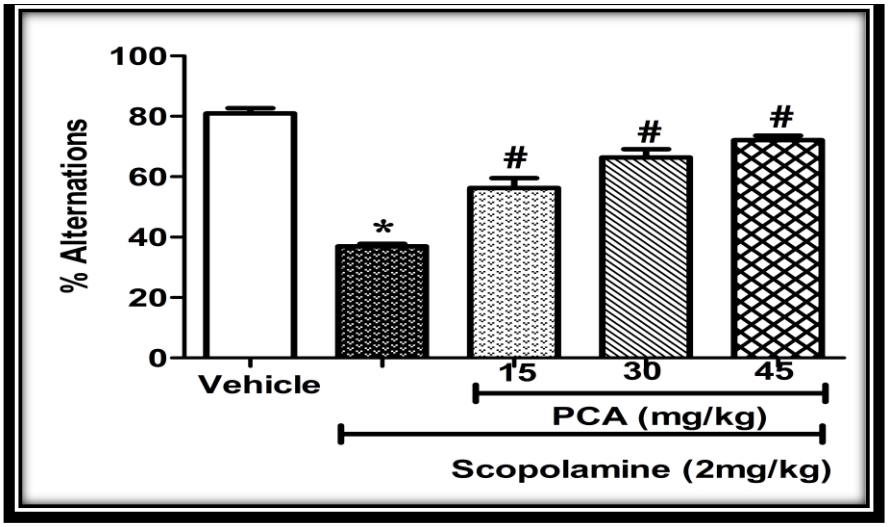

Figure 1: Effect of PCA on scopolamine-induced memory impairment in mice using Y-Maze. Spontaneous alternations behaviour during 8 min session was recorded. Data represent mean± SEM (n =4). *p <0.001 vs vehicle treated group, #p <0.001vs scopolamine treated group.

2. Dose dependent effect of PCA on scopolamine-induced memory deficits in MWM:

MWM test represent the model of memory especially spatial memory. We observed that, scopolamine (2mg/kg, *ip*) dose significantly decreased the escape latency in acquisition trial as compared to vehicle treated control group. Application of two-way ANOVA showed significant effects of treatment ($F_{4,80}$ = 7.812, p<0.0001), days ($F_{3,80}$ = 28.77, p<0.0001) and their interaction ($F_{12,80}$ =1.267, p<0.05) on escape latency. Further, post-hoc Bonferroni multiple comparison test revealed that scopolamine 2 mg/kg treatment significantly increased the escape latency on day 2 and 3 (p<0.05, p<0.05 respectively), and day 4 (p<0.01) whereas PCA at dose 30 mg/kg *i.p.* treatment significantly decreased the escape latency on day 2 (p< 0.05) and day 3 and 4 (p<0.01, p<0.01 respectively), and PCA at dose 45 mg/kg *i.p.* treatment significantly decreased the escape latency on day 3 and 4 (p<0.001, p<0.001 respectively) during acquisition trial compared to scopolamine-treated group. However, lower dose of PCA (15 mg/kg) had no effect on all four acquisition trial, thus considered as ineffective (Figure 2A, Table 2A).

Data obtained in probe test (24-hr later acquisition trial) reflected the effect of PCA treatment on retrieval of the memory. Animals with PCA at doses (15, 30, 45 mg/kg *i.p.*) increased the time spent in target quadrant and decreased the time spent in opposite quadrant (Fig 12B, Table 1b). Application of one way ANOVA showed significant effect of PCA on time spent in target quadrant ($F_{9,49}$ = 5.685, p< 0.0001).The post-hoc Bonferroni test revealed that PCA 15 (p<0.05), 30, 45 mg/kg *i.p.* (p<0.001, p<0.001 respectively) significantly increased the time spent in target as compared to and in opposite quadrant as compared to scopolamine treated group. Whereas PCA had no effect on time spent in opposite quadrant compare to (Figure 2B, Table 2B).

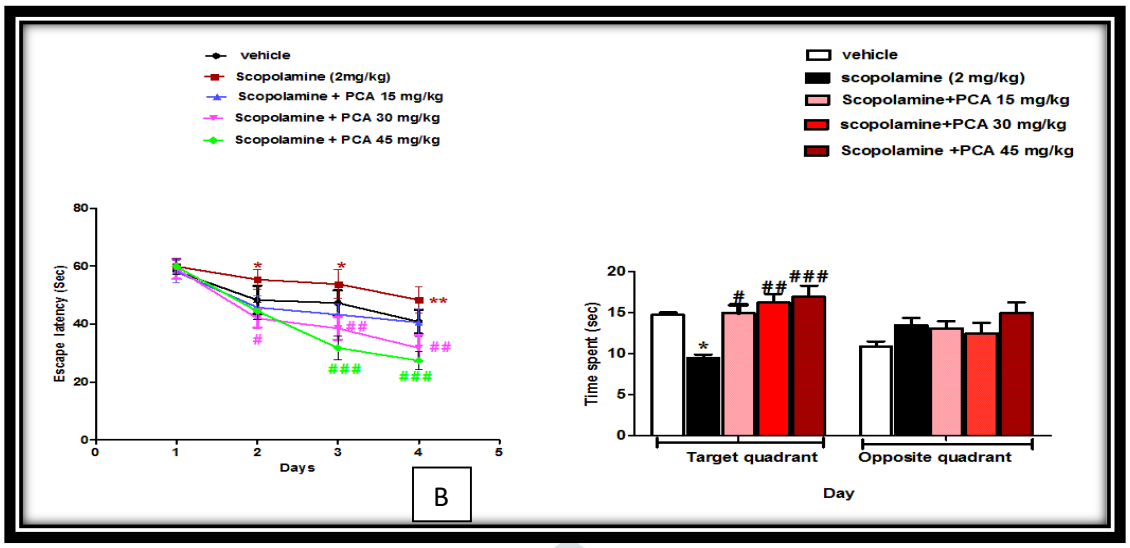

Figure 2: The effect of PCA on A) escape latencies during the training trial sessions and B) on swimming time during probe trial session in MWM in scopolamine-induced memory dysfunction in mice. Data represent mean± SEM (n =4).*p<0.05, *p<0.001. vs vehicle treated control group, #p <0.05, ## p<0.01, ### p <0.001vs scopolamine treated group respectively.

Sr. No.	Treatment *(ip)*	Mean escape latency (sec ± SEM)			
		Day 1	Day 2	Day 3	Day 4
1	Saline+ vehicle (control)	58.24 ±0.13	48.31 ±4.86	47.31 ± 4.39	40.81 ± 4.15
2	Scopolamine (2 mg/kg)	59.87 ± 0.73	55.38 ± 3.39*	53.85 ± 4.95*	48.38 ± 4.66**
3	PCA (15 mg/kg)	58.12 ± 3.73	45.75 ± 3.97	43.31 ± 3.58	40.56 ± 4.81
	PCA (30 mg/kg	59.06 ± 3.44	42.00 ± 3.20#	38.56 ± 3.98##	31.81 ± 4.51###
5	PCA (45 mg/kg)	59.87 ± 2.62	44.50 ± 2.97	31.81 ± 4.10###	27.43 ± 3.06###

Table 2A: Dose dependent effect of PCA on acquisition performance in MWM *p<0.05, *p<0.001. vs vehicle treated control group, #p<0.05, ##p<0.01, ###p<0.001vs scopolamine treated group respectively.

Sr. No.	Treatment *(ip)*	Time spent (sec ± SEM)	
		24-hr (Post-acquisition)	
		Target quadrant	Opposite quadrant
1	Saline + vehicle (control)	14.75± 0.3	10.87 ± 0.67
2	Scopolamine (2 mg/kg)	9.50± 0.40*	13.50 ± 0.91
3	PCA (15 mg/kg)	15± 0.9#	13.13 ± 1.2
4	PCA (30 mg/kg)	16.00± 1.08##	12.50 ± 1.2
5	PCA (45 mg/kg)	17.00± 1.33##	15.00 ± 1.29

Table 2B: Dose dependent effect of PCA in probe performance in MWM *p<0.05 vs vehicle treated control group, #p <0.05, ## p <0.001vs scopolamine treated group respectively.

Loss of learning and memory is a common thread in various cognitive disorders like delirium, amnesia, and dementia. Patient may show psychiatric symptoms secondary to cognitive problems like depression, anxiety. Decreased cholinergic firing, oxidative stress, hypercholesterolmia cause memory impairment[12]. Scopolamine, an anti-cholinergic agent, reduces the effective action of a given concentration of acetylcholine at a synapse without changing the acetylcholine concentration, and produces memory deficits can be used for model for amnesia [15].

In the present study, we examined the effects of PCA on memory impairment induced by scopolamine treatment using Y-Maze task and MWM task in mice. Spontaneous alteration behavior in the Y-Maze has been considered an indicator of STM. It was observed that PCA increased spontaneous alternation as compared to scopolamine-treated group. Similarly, MWM learning task was used to assess hippocampal-dependent spatial learning ability[16]. The escape latency observed from day to day means reference or LTM (Morris, 1984). Impairment in LTM was observed in the scopolamine treated group. PCA (30, and45 mg/kg) significantly shortened the escape latencies prolonged by scopolamine treatment. At the probe trial session, PCA improved time spent in target quadrant as compared to scopolamine-treated group. If the animals spent more time in the pool quadrant where the platform had previously been placed during the training sessions, this would indicate that the animals acquired the MWM task, showing the spatial memory improvement[17].

It was observed that systemic administration of PCA could improve the spatial learning, and working memory in scopolamine-induced memory impairment. Present study signifies PCA as a potential candidate for the treatment of cognitive impairment.

1. Harada CN, Natelson Love MC, Triebel KL. Normal cognitive aging. Clinics in Geriatric Medicine. 2013;29(4):737–52. doi:10.1016/j.cger.2013.07.002
2. Glisky EL, Glisky ML. Memory rehabilitation in older adults. Cognitive Neurorehabilitation. 2008;541–62. doi:10.1017/cbo9781316529898.037
3. 1. Vasudevan M, Parle M. Pharmacological actions of Thespesia populnea relevant to alzheimer's disease. Phytomedicine. 2006;13(9–10):677–87. doi:10.1016/j.phymed.2006.01.007
4. 1. Ceskova E. Novel treatment options in depression and psychosis. Clinical Therapeutics. 2017;39(8). doi:10.1016/j.clinthera.2017.05.317
5. 1. Lamptey RN, Chaulagain B, Trivedi R, Gothwal A, Layek B, Singh J. A review of the common neurodegenerative disorders: Current therapeutic approaches and the potential role of Nanotherapeutics. International Journal of Molecular Sciences. 2022;23(3):1851. doi:10.3390/ijms23031851
6. Areosa Sastre A, Sherriff F, McShane R. Memantine for dementia. Cochrane Database of Systematic Reviews. 2005; doi:10.1002/14651858.cd003154.pub4
7. Kim J-H, Kim G-H, Hwang K-H. Monoamine oxidase and dopamine β-hydroxylase inhibitors from the fruits of gardenia jasminoides. Biomolecules and Therapeutics. 2012;20(2):214–9. doi:10.4062/biomolther.2012.20.2.214
8. 1. Stojković DS, Živković J, Soković M, Glamočlija J, Ferreira ICFR, Janković T, et al. Antibacterial activity of Veronica Montana L. extract and of protocatechuic acid incorporated in a food system. Food and Chemical Toxicology. 2013;55:209–13. doi:10.1016/j.fct.2013.01.005

9. Harini R, Pugalendi KV. Antihyperglycemic effect of protocatechuic acid on streptozotocin-diabetic rats. Journal of Basic and Clinical Physiology and Pharmacology. 2010;21(1). doi:10.1515/jbcpp.2010.21.1.79
10. Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacology. 2014;2014:1–9. doi:10.1155/2014/952943
11. Szwajgier D, Borowiec K, Pustelniak K. The neuroprotective effects of phenolic acids: Molecular mechanism of action. Nutrients. 2017;9(5):477. doi:10.3390/nu9050477
12. Prashar Y, Gill NS, Kakkar S (2014). Anti-amnesic activity of protocatechuic acid in scopolamine induced amnesia in rats. JPPS 4(4):65-76.
13. Lam LM, Nguyen MT, Nguyen HX, Dang PH, Nguyen NT, Tran HM, et al. Anti-cholinesterases and memory improving effects of Vietnamese xylia xylocarpa. Chemistry Central Journal. 2016;10(1). doi:10.1186/s13065-016-0197-5
14. 1. Frye CA, Walf A. Progesterone, administered before Kainic acid, prevents decrements in cognitive performance in the Morris Water Maze. Developmental Neurobiology. 2011;71(2):142–52. doi:10.1002/dneu.20832
15. Ebert, Kirch. Scopolamine model of dementia: Electroencephalogram findings and cognitive performance. European Journal of Clinical Investigation. 1998;28(11):944–9. doi:10.1046/j.1365-2362.1998.00393.x
16. Vorhees CV, Williams MT. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nature Protocols. 2006;1(2):848–58. doi:10.1038/nprot.2006.116
17. 1. Blokland A, Geraerts E, Been M. A detailed analysis of rats' spatial memory in a probe trial of a morris task. Behavioural Brain Research. 2004;154(1):71–5. doi:10.1016/j.bbr.2004.01.022