Pharmacological evaluation of *Salvadora persica* on scopolamine-induced memory disorder

Prakash R. Biradar (✉ prakashrb123@gmail.com)

Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi-590010, India

Vasim Attar

Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi-590010, India

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Abstract

Background: *Salvadora persica* root has been reported for various pharmacological activities and it can also be used for memory sharpening.

Aim and Objective: The aim of the present study is to evaluate the effect of *Salvadora persica* root extract on scopolamine induced amnesia in rats

Materials and Methods: The evaluation of *Salvadora persica* (*S. persica*) on scopolamine induced memory impairment was carried out using scopolamine 3mg/kg p.o for 7 days followed by treatment with Donepezil (3mg/kg) and *S. persica* root extract (250, 500, 1000 mg/kg) for 21 days; assessed by *in vivo* and *in vitro* studies. *In vivo* models Elevated Plus Maze (EPM), Morris Water Maze (MWM) and Passive Avoidance Paradigm (PA) was used for assessment of Transfer Latency (TL), Escape Latency (EL) and Step Through Latency (STL). *In vitro* studies on rat cerebral cortex and hippocampus AChE enzyme estimation, estimation of reduced glutathione, GSH and histopathological studies.

Results: Treated animals shown increased STL whereas decreased TL and EL. *In vitro* studies of brain and parts of the brain showed decreased AChE enzyme activity in hippocampus and cerebral cortex, increased level of reduced glutathione level and reduced MDA levels. Considerable histopathological changes were seen in treated rat brain, specially in CA1 and CA3 neural intact in hippocampus region.

Conclusion: Present study describes attenuation action of *S. persica* on memory impairment induced by scopolamine may be due to increase in Acetylcholine (ACh) level in brain by inhibiting AChE enzyme and antioxidant property of *S. persica*. Histopathological changes are seen in scopolamine treated rats and treatment rats.

1. Introduction

“Memory impairment is defined as loss of ability to interfere with occupational, social activities, relationship due to the absence of gross clouding of consciousness or motor involvement” (Parle M. et al. 2011). Etiological factors associated with memory impairment such as oxidative stress, hypercholesterolemia, cerebral ischemia, energy failure, calcium overload, glutamate-mediated excitotoxicity and functional changes (Wang J. et al. 2009). AD is the most usual cause of dementia in old population (Sugimoto H. 2008). AD disease is a progressive irreversible neurological disorder occurs gradually and results in memory loss, unusual behavior, personality changes and loss of thinking ability (Parihar M. et al. 2004). AD associate with accumulation of beta-amyloid (A β) plaque and intracellular neurofibrillary tangles of hyperphosphorylated tau protein (Tiraboschi et al. 2004).

According to world Alzheimer report 2018 50 million people in worldwide suffering from dementia (World Alzheimer report 2018). Treatment of AD involves use of memantine and Acetylcholinesterase (AChE)

Inhibitors but the available treatment for AD doesn't treat the disease but reduce the symptoms, hence delays the patient's loss of autonomy. Hence new molecules are required to investigate for treatment of AD.

India is a gold mine for traditional medicine, which are having less side effects and having different phytoconstituents with mechanism of action. Beneficial effects of traditional medicines in the management of infectious and non-infectious diseases is also reported via in silico and in vitro approaches (Khanal et al 2019_{a,b}; Khanal et al 2020_{a-e}; Rodrigues 2020_{a,b}; Patil et al 2019; Ternikar et al 2020). Previous report also reported the probable action against memory defect for traditional medicine using multiple approaches reporting the acetylcholinesterase activity of folk medicine (Duyu et al 2020). Similarly, *Salvadora persica* having tradition claim on memory sharpening (Jivad N. et al. 2014). The aqueous and alcoholic extract of stem and root contain sulfate chlorides, thiocyanate, nitrates, alkaloids, resin and coloring matter, traces of tannins and saponins, fluoride, silica, sulfur, vit.C and sterols. The flavonoids rutin and quercetin were detected salvadorea has been present in the root of *s. persica* benzylisothiocynate also present. The meswak shows various pharmacological activities like antibacterial, antimycotic, analgesic, and anti-inflammatory, stimulation of salivary secretion, cytotoxic, locomotor activity, topical medication, antiulcer, anticonvulsant, sedative and antispasmodic. The therapeutic application of meswak are oral hygiene removal of smear layer, root canal, irrigant, plaque control, dental gel, gingival recession, industrial oil production etc (Ahmad H. et al. 2014) and can be used for memory sharpening (Jivad N. et al. 2014). The toxicity dose of *S. persica* is safe upto 5000mg/kg body weight (Soliman G. et al. 2017).

Scopolamine is a cholinergic antagonist which involved in the transmission of acetylcholine in CNS (El-Sherbiny et al. 2003) and can be used as pharmacological model for AD (Ebert U. et al. 1998). The present study was aimed to evaluate the action of *S. persica* on scopolamine induced memory impairment in rats. The amnesia can be induced by using scopolamine in concentration 3mg/kg body weight by intraperitoneal route for 7 days (Rahimzadegan M. et al. 2018).

2. Materials And Methods

2.1. Chemicals:

Scopolamine HBr and Donepezil HCl was provided by Vital Laboratories Pvt. Ltd. Gujarat, India and Apotex Research Pvt. Ltd. Bangalore, India as a gift sample for evaluations. DTNB and Acetylcholine iodide was purchased from Sigma-Aldrich USA.

2.2. Plant Material:

The *S. persica* root was procured from Huma Traders, Miraj (Maharashtra, India) and authenticated by Mr. Ajit Lingayat, authentication expert, KAHER Shri B.M.K. Ayurveda Mahavidyalaya. CRF code: CRF/Auth/2018/122. The roots were shade dried, pulverized into the coarse powder and subjected for extraction process.

2.3. Extraction

The dried coarse powder was extracted by maceration technique using hydro-alcohol as a solvent for seven days with occasional shaking. After 7 days, the extract was filtered & concentrated using evaporator. The obtained sticky extract was dried by using water bath; percentage yield of plant was calculated

2.4. Animals

Wistar rats of either sex were procured from CPCSEA approved invivo bioscience Bengaluru, India. They were housed in a clean and transparent polypropylene cage, divided into seven groups; each group contains six animals and was maintained under 12/12hr natural light-dark cycle at room temperature, 45-55 % relative humidity. All the animals were acclimatized one week before the experiment and allowed with free access to standard pellet and water *ad libitum*. The study protocol was reviewed and approved by the institutional Animal Ethical committee Reg.No.221/Po/Re/S/2000/CPCSEA, KLE College of Pharmacy, Belagavi. Karnataka, India.

2.5. Estimation of Acetylcholinesterase enzyme in Cerebral Cortex and Hippocampus (Ellman G; et al. 1961)

AChE enzyme was estimated by method of Ellman *et al*. The rate of moles of substrate hydrolysed per min per gram of tissue was calculated.

2.6. Lipid peroxidation estimation (Ohkawa, H., et al. 1979)

The amount of MDA present in the brain is measured by the method of Ohkawa *et al*. The MDA level was expressed as nano moles of MDA /mg of protein in brain homogenate.

2.7. Estimation of reduced glutathione (Ellman G. 1959)

Glutathione level was measured by the method of Ellman G. L. GSH level was stated in μ moles/mg of tissue.

2.8. Histopathological study

Rat brains were collected & kept in formalin. Brains stained by using haematoxylin-eosin. The hippocampal lesions studied using electronic microscope at 40 $\times$ and 10 $\times$ magnifications.

2.9. Experimental procedure

Animals were divided into seven groups containing six animals in each; Group I received normal saline. Group II received scopolamine hydrobromide (3mg/kg/day) through i.p. for 7 days. Group III received scopolamine hydrobromide (3mg/kg/day) for 7 days plus donepezil HCl (3mg/kg p.o.) for 21 days. Group IV, V & VI receives scopolamine hydrobromide (3mg/kg/day) for 7 days after that *S. persica* extract

(250, 500 and 1000mg/kg p.o. respectively) for 21 days. *S. persica* doses were selected based on acute oral toxicity study (Soliman G. et al. 2017).

Group VII (Preventive group) *S. persica* extract (1000mg/kg p.o.) for first 21 days, followed by Scopolamine (3mg/kg i.p.) along with *S. persica* extract (1000mg/kg p.o.) for next 7 days. All the animals were subjected for exteroceptive behavioural models of memory using Elevated plus Maze, Passive avoidance paradigm, Morris Water Maze on 0th, 7th, 14th and 21st day. At the end of the experiment, animals were sacrificed by overdose of anaesthesia, brains were isolated and cerebral cortex, hippocampus Acetylcholinesterase enzyme activity, Lipid Peroxidation, Glutathion level in brain were measured and histopathological study was performed.

2.10. Behavioral experiments

2.10.1. Elevated plus Maze (EPM):

During the assessment of memory via EPM, rats were individually placed at one end of the open arm facing away from the central platform and the time taken for the rat to move from open to closed arm (Initial Transfer latency, ITL) was recorded using stopwatch. The animals were allowed to explore the maze for 90s. If the animal did not enter the closed arms within 90s, it was guided on the back into one of the closed arm and transfer latency was given as 90s. Later the rat was allowed to explore the maze for 30s to become familiar with the maze and then returned to its cage. A drop in transfer latency time during treatment sessions was taken as an index of memory improvement.

2.10.2. Morris Water Maze (MWM):

It consists of a large circular water tank having size of 110cm diameter and 60cm height. It is made of black opaque polyvinyl chloride or hard board coated with fiberglass or resin with a white surface and filled with water ($26 \pm 2^{\circ}\text{C}$) to a depth of 30cm. the floor of the circular tank is marked off into 4 equal quadrants and were designed as North, East, West and South. In all trials, 2cm below the surface of the water, a black round platform of diameter 10cm was placed in a constant position. The cut off time given to individual rat is 60s. The time taken for the rat to locate the escape platform is noted. If the rat is unable to locate the platform within cut off time, it was gently guided to the platform and was allowed to stay on it for 10s. Escape Latency Time (Time taken for the rat to locate hidden platform in the water maze) was taken as an index of learning.

2.10.3. Passive Avoidance Test (PA):

Each test consists of two distinct trials such as acquisition trial and retention trial. For acquisition trial, individual rat is placed in the light compartment, as soon as the rat entered into the dark compartment, an electrical shock of 0.4mA was applied for 3s, the latency time once the rat had entered the dark compartment was recorded as Escape Latency (EL). After 24h of acquisition trial, retention trial was conducted, in which no shock was applied when the rat entered into the dark compartment and retention

latency is recorded by time taken the rat to re-enter into the dark chamber. Animals which didn't enter dark compartment even after 180s were removed from the apparatus.

2.11. Statistical Analysis:

Outcomes were represented as Mean $\pm$ SEM. The difference among mean was determined using one way ANOVA followed by Tukey's Multiple comparison Test, using GraphPad Prism software version 5.0. Variations between sets readings was deemed significant (probability level) at the degree of freedom at 0.05.

3. Results

3.1. Effect of *S. Persica* on transfer latency (TL) using EPM

The scopolamine brought mental disturbance assessed by behavioral models. The rats were exposed to transfer latency on EPM. The transfer latency was significant increased ($p < 0.001$) from acquisition period to 0th day after the treatment of scopolamine (26.83 ± 0.600). The PC, SP250 and SP1000 showed significantly decreased TL on 7th, 14th and 21st ($p < 0.001$) on comparing with NC. SP500 showed significant decreased activity on 14th and 21st day were as preventive group showed significant decreased TL from 0th to 21st day ($p < 0.001$). The TL on 7th, 14th, and 21st of all groups showed dose dependent decrease in TL. The effect of *S. persica* TL is shown in Figure 1.

3.2. Effect of *S. persica* on ELT using MWM

Scopolamine treated group i.e. negative control (NC) showed significant increased ELT ($p < 0.001$) on 0th day on compared with acquisition period (21.25 ± 1.499). SP250, SP500 and SP1000 on 7th 14th and 21st day shows significant decreased ELT when correlated with NC ($p < 0.001$). The Donepezil treated group or positive control (PC) showed significantly decreased ELT on 14th and 21st day ($p < 0.001$) but not notable on 7th day on compared with NC. The preventive group showed significant activity from day 0th to 21st day which is compared with NC ($P < 0.001$). The ELT on 7th 14th and 21st day decrease exponentially due to all treatment groups showed dose dependent decrease in ELT. The effect of *S. persica* ELT is shown in Figure 2.

3.3. Effect of *S. persica* on STL using PA

All scopolamine treated groups showed significantly decreased in STL on 0th day as compared with acquisition period ($p < 0.01$). The PC, SP250, SP500, SP1000 and preventive groups showed significant increased STL on 21st day, compared against NC ($p < 0.001$). The effect of *S. persica* STL is shown in Figure 3.

3.4. Effect of *S. Persica* on AChE level in rat hippocampus

The scopolamine treated group or NC animals showed significantly raised AChE ($P < 0.001$) enzyme action when contrast with control group (12.00 ± 1.471). PC group treated with donepezil 3mg/kg compared with NC showed significant decreased AChE enzyme activity ($P < 0.001$) (5.613 ± 0.414), SP250 showed significant decreased ($P < 0.01$) AChE enzyme activity on compared with NC (6.718 ± 0.9868), were as SP500, SP1000 and preventive group showed significantly decreased AChE enzyme activity in contrast with NC and when compared with SP250 & PC showed significant decreased AChE enzyme activity ($p < 0.01, 0.05$) respectively. Action of *S. persica* on AChE level in rat hippocampus is shown in figure 4.

3.5 Effect of *Persica* on AChE level in rat cerebral cortex

NC group treated with scopolamine showed significant increased AChE enzyme activity compared along with cerebral cortex from control group animals (11.75 ± 0.573). Whereas PC ($P < 0.001$), SP250 ($P < 0.01$), SP500 ($P < 0.01$), SP1000 ($P < 0.001$) and Preventive ($P < 0.01$) showed significant decreased cerebral cortex AChE enzyme level. Effect of *s. Persica* on ache level in rat cerebral cortex is shown in figure 5.

3.6. Effeect of *S. Persica* extract on reduced GSH in rat brain

The NC animal treated with scopolamine showed significant reduce in reduced GSH level ($P < 0.001$) as contrasted with control group (0.56 ± 0.083). PC group treated with donepezil 3mg/kg showed increased in reduced GSH level significantly ($P < 0.001$) compared along with NC (1.35 ± 0.065), were as animals treated with *S. Persica* extract with 500, 1000mg/kg denoted significant rise in the reduced GSH concentration when compared with NC ($P < 0.001$) but not by SP250. The preventive group showed significant increase in reduced GSH level ($P < 0.01$) when contrasted with NC. Effeect of *s. Persica* on reduced GSH in rat brain is shown in figure 6.

3.7. Action of *S. persica* extract on brain MDA level

NC group in respect of control group showed significantly increased in MDA level (44.87 ± 4.533), were as PC, SP250, SP500, SP1000 and PREV groups resulted in significant reduced MDA level in brain when compared with NC ($p < 0.001$) (Figure 7).

3.8 Histopathological study of rat brain

The NC group when compared with control group histogram indicated that severe cerebral congestion, cerebral odema, moderate meningeal congestion, neuronal eosinophilia and mild neuronal micro vacuolization, neuronal nuclear pyknosis, neuronal karyorrhexis, neutrophilic infiltration, RBC extravasation, macrophage influx, vascular proliferation, and reactive gliosis. PC and treatment groups did not show any severe change in anatomy; were as mild changes observed in cerebral congestion, meningeal congestion, neuronal eosinophilia and neuronal nuclear pyknosis compared with NC. However, the 10X magnification of hippocampus shows integrity of the neurons in the region of CA1 and CA3 of all the treated groups are maintained as compared with NC and PC. These neurons are involved in memory and cognitive functions. Histopathology of rat brain is shown in figure 8.

4. Discussion

Scopolamine is the muscarinic receptor antagonist causes inhibition of cholinergic signaling (Vogel, H. 1998, Ebert, U. et al. 1998). The mechanism of scopolamine is still not known but the various studies shows that it act on various neurotransmitters either by stimulatory or blocking action on glutamatergic (Gutierrez, J. et al. 2019, Mahmoodi, G. et al. 2010), adrenergic (Azami N. et al. 2010), dopaminergic (Alto S. et al. 2005, Dash P. et al. 2007), serotonergic (Dash P. et al. 2007, Barnes N. et al. 2007, Da silva costa-Aze V. et al. 2012) and histaminergic (Zong C. et al. 2000) system could all together scopolamine induced memory impairment suggesting that these neurotransmitters may be involved in induction of memory impairment (Rahemzadegan M. 2018). It also induces oxidative stress (Jang Y et al. 2013), apoptosis (Jahanshahi M. et al. 2013) and inflammatory responses (Ahmad A. et al. 2014). The present study explains effect of *S. persica* on scopolamine induced amnesia in rats was studied using MWM, EPM, & Passive Avoidance (PA) and biochemical estimations. However, the NC group treated with scopolamine significantly increase ELT and TL in MWM and EPM respectively, decreased STL in PA. This recommended that scopolamine interfere with acquisition, retention and consolidation of learning task. Treatment with donepezil 3mg/kg, SP 250, 500, 1000mg/kg and preventive group (SP1000mg/kg and scopolamine 3mg/kg) produced significant decreased ELT, TL and increased STL. This suggested that donepezil and *S. persica* root extract prevented rats from impaired learning and memory induced by scopolamine with dose dependent action and showed treatment and preventive learning and memory activity is sharpened.

The degradation of ACh by AChE causes decrease in neurotransmission that leads to decreased binding of ACh with M1 receptors which further leads to decrease in generation of secondary messenger system that causes the decreased neurotransmission and convert tau protein to hyperphosphorylated tau protein (Francis P. et al. 1999). ACh degraded by AChE to maintain a constant concentration in brain, were as excess action of AChE consequences in decrease in ACh causes memory impairments. The learning and memory can be improved by increasing the level ACh in brain (Zhang Z. et al. 2004). AChE inhibitors rises level of ACh in the brain which is required for nerve cells communication. Examples: Donepezil, and Rivastigmine. Donepezil is powerful, discriminating, noncompetitive & reversible antagonist of AChE used especially in the treatment of AD. Donepezil improve cognition of patient with mild to moderate AD and its effectiveness can be maintained up to 50 weeks (Jelic V. et al. 2010). In present study estimation of AChE enzyme activity in rat hippocampus and cerebral cortex were performed. However Donepezile and *S. persica* root extract showed significant decreased AChE enzyme activity compared with NC.

Oxidative stress performs key role in amnesia & one of the main cause for memory loss in AD (Parihar M. et al. 2004). Free radicals are responsible for oxidative stress and it is also known as reactive oxygen species (ROS) generated by oxygen and nitrogen depend on unpaired electron. The oxidative stress alters cells and its mechanism causing alteration of cellular properties like "fluidity, ion transport, enzyme activity & protein cross linking". Excess oxidation leads to cell death normal body handles radicals by antioxidant and antioxidant enzymes (Floyd R. et al. 2002). In this study in vitro antioxidant activity such as reduced glutathione level and lipid peroxidation in rat brain was performed. In NC group treated with

scopolamine did not showed increase in reduced glutathione, were as significant increased in reduced glutathione was seen in the PC and preventive group but not in Sp250, 500, 1000mg/kg, were as significantly increased in MDA in dose dependent manner was observed in *S. persica* root extract treated rats. Hence,the phytochemical constituents of *S. persica* like Flavanoids, alkaloids, traces of tannins and saponins, vit.C and sterols, showing Antioxidants and Antiinflammatory property to overcome oxidative stress and neuronal inflammation which damage the neurons and degeneration of neurons, Beta amyloid and Tau protein formation in hippocampus region which are involved in memory pathway.

Histopathological examination of hippocampus and cerebral cortex of rat brain were performed. The changes induced by scopolamine in NC group like cerebral congestion, cerebral odema, moderate meningeal congestion, neuronal eosinophilia and mild neuronal micro vacuolisation, neuronal nuclear pyknosis, neuronal karyorrhexis, neutrophilic infiltration, RBC extravasation, macrophage influx, vascular proliferation, and reactive gliosis was observed. PC and *S. persica* root extract treatment groups reversed the damage induced by scopolamine. However, the 10X magnification of hippocampus shows integrity of the neurons in the region of CA1 and CA3 of all the treated groups are maintained as compared with NC and PC.

Conclusion

Present study describe that *S. persica* improve scopolamine induced amnesia in rats studied via behavioral, biochemical and histopathological studies. However persistent administration of *S. persica* improves scopolamine induced amnesia may be due to rising ACh concentration, antioxidant and antiinflammatory activity in hippocampus and cerebral cortex by inhibiting AChE and lipid peroxidation,tau protein and beta amyloids formation which helps in improving the memory. Histopathological findings provide influential impact of *S. persica* on scopolamine induced amnesia. Effect of *S. persica* in scopolamine induced raise in microtubule associated tau protein which needs to be further confirmation, also further approaches are made to identify the potential phytoconstituents for the inhibition of AChE and lipid peroxidation.

Declarations

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Conflict of interest

Author of this manuscript do not possess any conflict of interest in any financial and non-financial way.

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Figures

Figure 1

The effect of *S. persica* on amnesia using Elevated plus Maze: results: mean SD: (n=6): analysed by one way ANOVA followed by Tukey's Multiple comparison test whereas *** p < 0.001 compared to control, ### p < 0.001 compared to NC, @@@p < 0.001 compared to PC, %p < 0.05, %%p < 0.01, %%%p < 0.001 compared to SP250 <<< p < 0.001 compared to SP500, >>>p < 0.001 compared with SP1000 on 0th, 7th 14th and 21st day.

Figure 2

The effect of *S. persica* on amnesia using Morris Water Maze: results: mean SD: (n=6): analysed by one way ANOVA followed by Tukey's multiple comparison test whereas *** p< 0.001 compared to control; ##p< 0.01, ### p<0.001 compared to NC, @p<0.05, @@p<0.01, @@@p< 0.001 compared to PC, %p<0.05, %%p<0.01, %%%p<0.001 compared to SP250, <<< p< 0.001 compared to SP500, >>>p<0.001 compared with SP1000 on 0th, 7th 14th and 21st day

Figure 3

The effect of *S. persica* on amnesia using Passive avoidance: results: mean SD: (n=6): analysed by one way ANOVA followed by Tukey's multiple comparison test whereas ** p< 0.01, ***p< 0.001 compared to control; ###p<0.001 compared to NC, %%p<0.01 compared to SP250 on 0th, 21st day.

Figure 4

The effect of *S. persica* on hippocampus AChE: mean SEM: (n=6): analysed by one way ANOVA followed by Tukey's multiple comparison test whereas *** p< 0.001 compared to control, ##p<0.01, ###p<0.001 compared to NC, @p<0.05 compared to PC, %%p<0.01 compared to SP250.

Figure 5

The effect of *S. persica* on Cerebral cortex AChE: mean SEM: (n=6): analysed by one way ANOVA followed by Tukey's Multiple comparison test whereas ** $p < 0.01$ compared to control, ## $p < 0.01$, ### $p < 0.001$ compared to NC, % $p < 0.05$ compared to SP250, < $p < 0.05$ compared to SP500, > $p < 0.05$ compared to SP1000.

Figure 6

The effect of *S. persica* on GSH: mean SEM: (n=6): analysed by one way ANOVA followed by Tukey's Multiple comparison test whereas ***p< 0.001 compared to control, ##p<0.01, ###p<0.001 compared to nc, @@@p<0.001 compared to pc, %p<0.05 compared to SP250, <<<p<0.001 compared to SP500, >>>p<0.001 compared to SP500.

Figure 7

The effect of *S. persica* on MDA level in rat brain: mean SEM: (n=6): analysed by one way ANOVA followed by Tukey's multiple comparison test whereas *** $p < 0.001$ compared to control, ## $p < 0.01$, ### $p < 0.001$ compared to NC

Figure 8

Histopathological study of rat brain (Hippocampus and cerebral cortex 40X magnification)