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Original Article

Protective effect of hydro-alcoholic extract of *Salvia haematodes* Wall root on cognitive functions in scopolamine-induced amnesia in rats

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ABSTRACT

Diminished cholinergic transmission may be responsible for development of amnesia. Hence, the present study was undertaken to investigate the possible protective effect of hydro-alcoholic extract of *Salvia haematodes* Wall root (HESH) on cognitive functions in scopolamine-induced amnesia in adult Sprague Dawley rats. The rats were divided randomly into five groups each consisting of five rats ($n = 5$). Rats of the groups I, II, III, IV, and V received orally normal saline (10 ml/kg b. wt.), normal saline (10 ml/kg), standard drug rivastigmine (1.5 mg/kg), HESH (20 mg/kg), and HESH (40 mg/kg), respectively once a day for fourteen days. Then, they were subjected to single dose of scopolamine (1 mg/kg b. wt. *ip*) except in group I on fourteenth day 60 min after respective normal saline or drug administration. They were observed for the effects on step down latency (SDL), locomotor activity and brain AChE activity for the learning and memory. The acquisition SDL, retention SDL and locomotor activity were significantly ($p < 0.01$) decreased while AChE activity was significantly ($p < 0.01$) increased in scopolamine-treated group II as compared to normal control group I. The acquisition SDL, retention SDL and locomotor activity were significantly ($p < 0.01$) increased while, AChE activity was significantly ($p < 0.01$) decreased with all the doses of HESH and in rivastigmine-treated group as compared to scopolamine-treated group II. Hydro-alcoholic extract of *S haematodes* root possesses protective effect on cognitive functions and may prove to be a useful memory restorative agent in the management of cognitive dysfunctions as in amnesia and Alzheimer's diseases.

1. Introduction

Cognition covers human learning and memory, problem solving, conceptual processes, skilled performance, thinking and decision making.[1] The system implicated in these cognitive processes involves brain's cholinergic system.[1] Decline in these cognitive abilities results in a neurodegenerative disorder called as amnesia which may be one of the symptoms of some neurodegenerative diseases such as Alzheimer's disease.[1,2] It may happen due to brain damage either through brain injury or the use of some specific drugs specifically sedatives.[1] It may also happen due to the use of muscarinic cholinergic receptor antagonists which impair learning and memory in both the humans and rodents.[3,4] The prevalence of dissociative amnesia is approximately 1.0–2.6% of the total world's population and the incidence of global transient amnesia is 2.9–10 per 100,000 cases every year.[5,6]

The plant *Salvia haematodes* Wall (belonging to family Lamiaceae) is commonly known as red sage by folklorists, Behman Surkh in Urdu, Lal Behman in Hindi and Red Sage in English.[7] Root of the plant contains flavonoids, tannins, phenols, alkaloids, carbohydrates, sterols and essential oils such as 1,8-cineole, linalool, α- and β-pinene, carvacrol, luteolin.[7–9] A high concentration of bioflavonoid, salvinine has been found in the plants of *Salvia* species. It is known to have antioxidant, antimicrobial, antiinflammatory, cardiotonic, antidiabetic, anticonvulsant and several other pharmacological activities.[10–14] Physicians of Ayurvedic and Unani systems of medicine employ it for the treatment of several ailments. Root of the plant is used in cardiac disorders, seminal debility and as a cerebral nervine tonic by the practitioners of traditional medicine in India.[15–17] It is also used as aphrodisiac for the treatment of premature ejaculation of semen and sexual

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disorders.[17–19] It has also been recommended to use it in gout.[20] It was found clinically effective in cases of diarrhea also, supporting the anticholinergic effect of *S. haematodes* root on smooth muscles.[21] It is also an ingredient of Unani formulations such as Khamira Gaozaban Sada, Laboob Kabir, Laboob Sagheer, Majun Muravvahul and several others indicated as nervine and brain tonics.[22] There is no scientific documentation available about the protective effect of hydro-alcoholic extract of *S. haematodes* root (HESH) on cognitive functions, which is clinically relevant. Despite the severity and high prevalence of the amnesia, the allopathic system of medicine is yet to provide a suitable drug for its treatment. Hence, the present study was undertaken to investigate the memory enhancing activity of (HESH) in scopolamine-induced amnesia in rats.

2. Materials and methods

2.1. Reagents and instrumentations

The entire chemicals used were of analytical grade. 0.9% normal saline (Albert David Ltd, Ghaziabad, India), ethanol (Changshu Yangyuan chemical, China), formaldehyde (Fisher scientific Ltd, Mumbai, India), ethyl acetate (Himedia chemicals), methanol (Fisher scientific Ltd, Mumbai, India), diethyl ether (SD fine-chemical Ltd Mumbai, India), acetic acid (SD fine-chemical Ltd Mumbai, India), formic acid (Rankem, New Delhi), Bovine serum albumin fraction-V (Himedia chemicals), 99% anhydrous potassium dihydrogen phosphate (Chemikabiochemika reagent), 5,5-dithiobis (2-nitro benzoic acid) (DTNB, Himedia chemicals), disodium hydrogen phosphate (SD fine chem. Ltd), Folincoicalteau phenol reagent (Fisher scientific), sodium nitrite (Sigma-Aldrich), rivastigmine (Dr. Reddy's), UV- Spectrophotometer (PharmaSpec UV-1700 Shimatzu), micropipette (10–100 μl & 100–1000 μl) (Superfit), centrifuge (Shimatzu AUX220), digital balance (Unibloc, PAT 1987), refrigerator (Intello cool LG).

2.2. Procurement and authentication of the plant materials

The plant material was procured from Hamdard Dawakhana, Amina Bad, Lucknow of Uttar Pradesh (India) and authenticated by the botanists, authentication office, Faculty of Pharmacy, Integral University Lucknow, India. A voucher specimen of *S. haematodes* Wall root (IU/PHAR/HRB/15/25) was deposited there for further reference.

2.3. Preparation of plant extract and evaluation of extractive value

The procured dried root was powdered to a coarse drug powder with the help of a mechanical grinder and was extracted with 50% hydro-alcoholic solvent by cold maceration for 72 h with concomitant agitation. The obtained extract was filtered and concentrated to dryness under reduced pressure and temperature using rotary evaporator (Buchi Rotavapor-R; Labco, India). The extractive value was calculated and the dried extract (HESH) was stored in a refrigerator below 5 °C for further studies.[23]

2.4. Experimental animal

Adult rats, *Rattus norvegicus* starin Sprague Dawley, (140 ± 20 g) were procured from Central Drug Research Institute (CDRI), Lucknow (India). They were kept in departmental animal house, Integral University, Lucknow (India). The animals were housed separately in polypropylene cages for acclimatization at a temperature and relative humidity of 23 ± 2 °C and 50–60%, respectively with a 12 h light/dark cycle for one week before and during the commencement of the experiment. Animals were kept on standard pellet diet (Dayal animal feed Unnao, India) and provided drinking water *ad libitum* throughout the study period. All the experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) and ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC), Faculty of Pharmacy, Integral University Lucknow (Approval No. IU/Pharm/M.Pharm/IAEC/15/11).

2.5. Acute toxicity study

The procedure was followed as per the Organization for Economic Cooperation and Development (OECD) 423 guidelines. The extract at the doses of 5, 50, 300 and 2,000 mg/kg b. wt. *po* were administered to different groups of rats and observed for 14 days for signs of neurological, behavioral toxicity and mortality.[23]

2.6. Experimental protocol

The protective effect of hydro-alcoholic extract of *S. haematodes* root (HESH) on cognitive function was evaluated using five groups of adult Sprague Dawley rats each consisting of five rats ($n = 5$).[4,24] Group I served as normal control and received normal saline (10 ml/kg b. wt. *po*) once a day for 14 days. Group II served as stress control and received normal saline (10 ml/kg b. wt. *po*) once a day for 14 days. Group III served as standard drug-treated group and received standard drug rivastigmine (1.5 mg/kg b. wt. *po*) once a day for 14 days.[25] Groups IV and V served as test drug-treated groups and received HESH (20 and 40 mg/kg b. wt. *po*, respectively) once a day for 14 days. Then, animals of all the groups except group I were subjected to single dose of scopolamine (1 mg/kg b. wt. *ip*) on 14th day 60 min after the respective normal saline or drug administration.[3] Then, 45 min after the scopolamine administration, all the behavioral activities were evaluated using the passive avoidance model. This was termed as acquisition trail (AT) which corresponds to learning. Further, the retention trail (RT) was carried out after 24 h of scopolamine administration. In the RT, the above mentioned parameter was reassessed as an index of memory. Additionally, locomotor activity was assessed using an actophotometer. Then, the animals were euthanized by cervical decapitation and the brains were isolated for evaluation of the brain acetylcholine esterase (AChE) activity.

2.7. Evaluation of effect of hydro-alcoholic extract of Salvia haematodes root on behavioral activity by passive shock avoidance paradigm in rats

Passive shock avoidance to examine the long term memory based on negative reinforcement was evaluated.[4] The apparatus [a box ($27 \times 27 \times 27$ cm^3) having three wall of wood, one wall of Plexiglas and grid floor made up of 3 mm stainless steel rod set 8 mm apart with wooden platform ($10 \times 7 \times 1.7$ cm^3) in the centre] used in the test was illuminated with a 15 W bulb. Each rat during training was placed on the wooden platform. Electric shock (50 Hz, 1.5 mA) for 1 s was delivered to the grid floor when the rat stepped down and placed its paw on the grid floor. The step down latency (SDL, time taken by the rat to step down and place all four paws on grid floor) was recorded and the rats showing it in the range of 2–15 s were taken for the acquisition and retention tasks. 90 min after the training session, the acquisition task was carried out and the animals were removed from the shock free zone if they did not step down for the period of 60 s. After 24 h, retention task was tested in a similar manner except with an upper cut of time of 180 s.

2.8. Evaluation of effect of hydro-alcoholic extract of Salvia haematodes root on locomotor activity in rats

The locomotor or horizontal activity was evaluated using an actophotometer.[4,26] Each rat of all the groups before respective treatments was placed individually in the actophotometer for 5 min and basal activity was obtained. Subsequently, each rat of all the groups was given their respective treatment according to experimental protocol. Then, 60 min after the scopolamine administration, the rats were again placed in the actophotometer for recording the activity score.

2.9. Evaluation of effect of hydro-alcoholic extract of Salvia haematodes root on brain acetylcholine esterase activity in rats

Each rat of all the groups after 2 h of their respective treatment was sacrificed by instant decapitation. The whole brain was quickly removed and kept in an ice bath. A known weight of the brain tissue was homogenized in 0.32 M aqueous sucrose solution to get a 10% homogenate that was centrifuged at 3,000 rpm for 15 min followed by centrifugation at 10,000 rpm for 10 min at a constant temperature of 4 °C. Following centrifugation, 1 ml of the supernatant was mixed with 9 ml of sucrose solution to get a 1% post-mitochondrial supernatant (PMS). AChE activity was measured in this 1% PMS based on the principle that the rate of formation of yellow colored thiocholine from acetylthiocholine iodide in presence of dithiobis(2-nitrobenzoic acid) (DTNB) increases with the increase in tissue cholinesterase.[26–28] The 2.7 ml of phosphate buffer (0.1 M, pH 8.0), 0.1 ml of 10 mM DTNB solution prepared in disodium phosphate buffer (0.1 M, pH 8.0), 0.1 ml of 1% PMS were taken in a test tube and mixed. Then, the reaction mixture was taken in a cuvette and pre-incubated at 37 °C for 5 min. The reaction was initiated by the addition of 0.1 ml of the substrate 30 mM acetylthiocholine iodide, prepared in phosphate buffer, in the reaction mixture and the absorbance was recorded at the wavelength of 412 nm against reagent blank for 3 min after every 1 min interval. Reagent blank was prepared by mixing 2.8 ml of phosphate buffer, 0.1 ml of DTNB solution and 0.1 ml of acetylthiocholine iodide in another test tube. AChE activity was calculated using the formulae, $[R = (\delta_{OD} \times V)/(E \times \text{mg of protein})]$; Where, R = rate of enzyme activity in 'n' mole of acetylthiocholine iodide hydrolyzed per min per mg of protein, δ_{OD} = change in absorbance per minute, E = extinction co-efficient, 13,600/M/cm, V = Volume of assay, 3 ml.

2.10. Statistical analysis

The data were expressed as mean ± SEM (Standard Error of Mean) and the results were analyzed by one way ANOVA followed by Dunnett's test. The p values less than 0.05 were considered as statistically significant.

3. Results

The hydro-alcoholic extract of *S. haematodes* root (HESH) during acute toxicity study was found to be devoid of any mortality to any animals. The extractive value of hydro-alcoholic extract of *S. haematodes* root was found to be 7.62% w/w. The acquisition step down latency, retention step down latency and locomotor activity of scopolamine-treated group significantly decreased ($p < 0.01$) as compared to normal control group. While, these were significantly ($p < 0.01$) increased with all the doses of hydro-alcoholic extract of *S. haematodes* root and in rivastigmine-treated group as compared to scopolamine-treated group. The acetylcholine esterase (AChE) activity was significantly ($p < 0.01$) increased in scopolamine-treated group as compared to normal control group. While, it was significantly ($p < 0.01$) decreased with all doses of hydro-alcoholic extract of *S. haematodes* root and in rivastigmine-treated group as compared to scopolamine-treated group (Table 1, Fig. 1).

4. Discussion

The extractive value in the hydro-alcoholic solvent of *S. haematodes* root indicated the nature and quantity of the constituents in the extract. The hydro-alcohol soluble extractive value was found to be 7.62% w/w indicating the presence of polar constituents like steroids, phenols, alkaloids, flavonoids, glycosides in it.[29]

The extract at 5, 50, 300 and 2,000 mg/kg b. wt. *po* doses during acute toxicity study was found devoid of mortality of any animals, which reveal the safety of the HESH in doses up to 2,000 mg/kg b. wt *po*. Hence, an optimal dose of 20 and 40 mg/kg b. wt. *po* of *S. haematodes* root extract was selected here for the experimental study.

Scopolamine, a muscarinic cholinergic receptor antagonist, impairs learning and short-term memory in both the humans and rodents.[3,4] Thus, 1 mg/kg b. wt. of scopolamine was used to induce cognitive dysfunctions and amnesia in rats. It is clear from the present study that acquisition SDL, retention SDL and locomotor activity were significantly decreased ($p < 0.01$) while the AChE activity was significantly ($p < 0.01$) increased in scopolamine-treated group as compared to normal control group which led to cognitive dysfunctions and amnesia in rats as seen in Alzeimer's disease. Increased AChE activity led to reduced level of acetylcholine in the brain causing Alzheimer's disease.

Brain acetylcholine (ACh) deficiency is principal pathogenic factor in Alzeimer's disease (AD). AD is non-curable, because, once neuron is degenerated it does not regenerate again by any means. Hence, symptoms of AD is tried to remove by protecting ACh through employing (pre-treatment) of the drugs. Scopolamine is anticholinergic drug that reduces rapidly ACh response to its own receptors and memory impairment in rats which can even lead to loss of learned behavior during training of rats. Hence, post-treatment was not used in the current study while pretreatment was performed to evaluate the neuroprotective effect of HESH and not the curative effect (post-treatment).

Rivastigmine is one of the drugs belonging to pseudo-irreversible or slow reversible AChE inhibitor that increases the concentration of acetylcholine in the brain by blocking the enzyme AChE that destroys acetylcholine.[25] This increase is believed to be responsible for the improvement in memory. Thus, 1.5 mg/kg b. wt. of rivastigmine was used for the improvement in memory. It is clearly evident from the present study that the acquisition SDL, retention SDL and locomotor activity were significantly ($p < 0.01$) increased while AChE activity was significantly ($p < 0.01$) decreased in rivastigmine-treated group as compared to scopolamine-treated group.

The acquisition SDL, retention SDL and locomotor activity were significantly ($p < 0.01$) increased while AChE activity was significantly ($p < 0.01$) decreased with all the doses of HESH as compared to scopolamine-treated group. The long term administration of HESH showed pronounced effect in reversal of the scopolamine-induced amnesia. This suggested that the animal has retention of memory of the shock once entered in the shock free zone.

It is clear from the present study that the AChE activity was significantly ($p < 0.01$) decreased with all doses of HESH and in rivastigmine-treated group as compared to scopolamine-treated group indicating the stimulatory action of these drugs on the cholinergic system. Hence, the memory enhancing effect of HESH can be attributed to its anti-AChE activity. Hence, *S. haematodes* may be used to delay the onset and to reduce the severity of

Table 1
Effect of hydro-alcoholic extract of *Salvia haematodes* root on different cognitive functions specific variables in the rats.

Treatment groups & Cognitive functions specific variables		I Normal control: normal saline, 10 ml/kg b. wt., *po*	II Stress control: normal saline, 10 ml/kg b. wt., *po* + scopolamine, 1 mg/kg b. wt., *ip*	III Standard drug treated: rivastigmine, 1.5 mg/kg b. wt., *po* + scopolamine, 1 mg/kg b. wt., *ip*	IV Test drug treated: HESH, 20 mg/kg b. wt., *po* + scopolamine, 1 mg/kg b. wt., *ip*	V Test drug treated: HESH, 40 mg/kg b. wt., *po* + scopolamine, 1 mg/kg b. wt., *ip*
Passive shock avoidance paradigm	Acquisition step down latency (sec)	4.89 ± 0.47	2.17 ± 0.17*	3.5 ± 0.72#	3.00 ± 0.58#	3.16 ± 0.87#
	Retention step down latency (sec)	9.16 ± 0.60	4.83 ± 0.30*	7.00 ± 2.13#	6.00 ± 1.51#	6.67 ± 2.35#
Activity score on 14th day		157.75 ± 6.22	100.5 ± 4.5*	165.25 ± 4.09#	163.5 ± 3.62#	160.75 ± 2.87#
Brain acetylcholine esterase activity (μmoles/mg of protein)		129.19 ± 5.498	305.62 ± 20.99*	183.85 ± 5.336#	144.94 ± 9.778#	156.55 ± 10.27#

Values were expressed as mean ± SEM ($n = 5$). * indicates $p < 0.01$ as compared to normal control, #$p < 0.01$ as compared to toxic control.

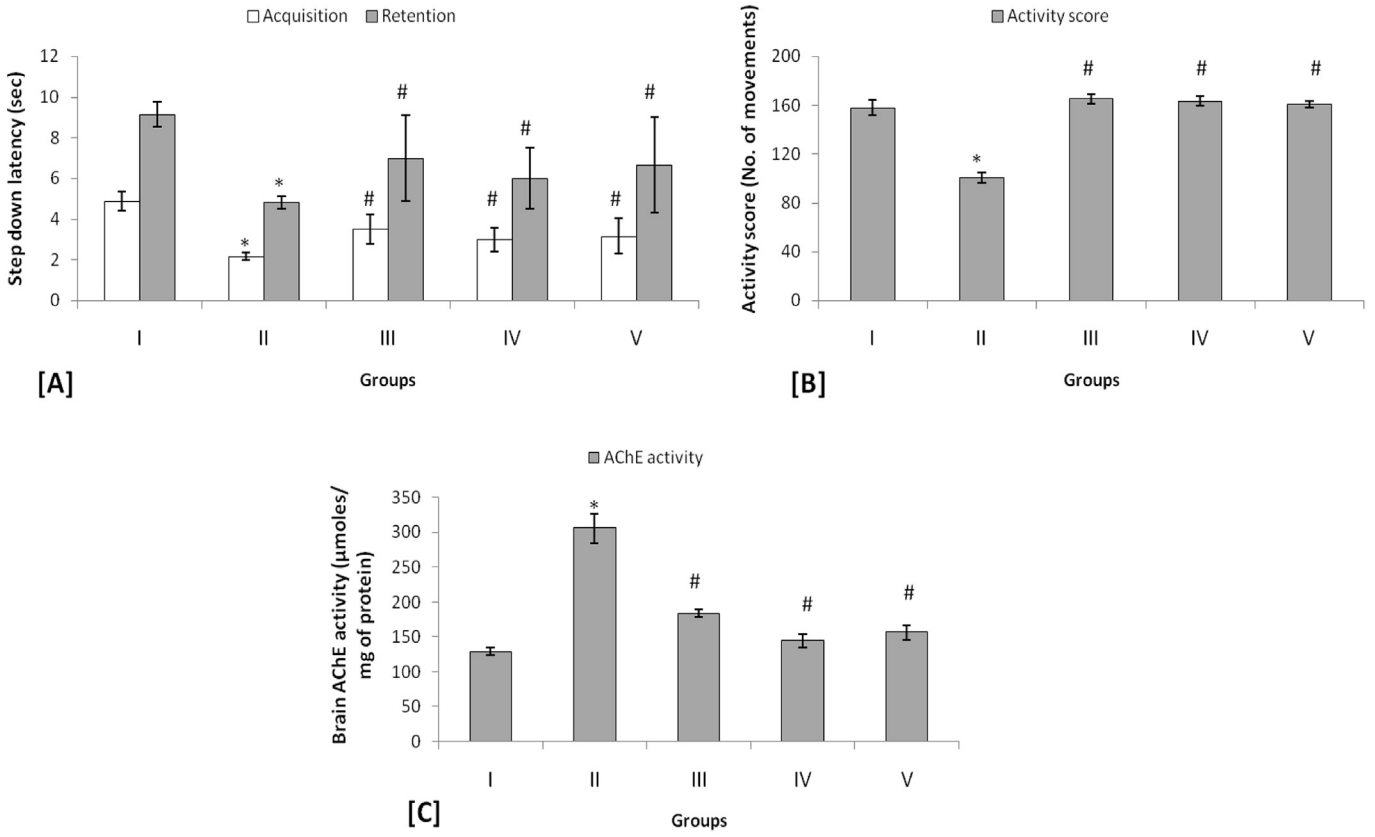

Fig. 1. Effect of hydro-alcoholic extract of *Salvia haematodes* root on different cognitive functions specific variables in the rats: [A] Effect of hydro-alcoholic extract of *Salvia haematodes* root on step down latency by passive shock avoidance paradigm in rats, **[B]** Effect of hydro-alcoholic extract of *S. haematodes* root on locomotor activity in rats, **[C]** Effect of hydro-alcoholic extract of *S. haematodes* root on brain acetylcholine esterase (AChE) activity in rats [Values were expressed as mean ± SEM ($n = 5$). * indicates $p < 0.01$ as compared to normal control, #$p < 0.01$ as compared to toxic control].

Alzeimer's disease. However, further investigation is warranted to explore the possible involvement of other neurotransmitters such as glutamate, γ-aminobutyric acid and catecholamines responsible for the memory improving property of *S. haematodes*. It may be carried out to isolate the active principle(s) from *S. haematodes* roots and to determine the mechanism of action.

5. Conclusion

Present study concluded that hydro-alcoholic extract of *S. haematodes* Wall root possesses protective effect on cognitive function against scopolamine-induced memory impairments by elevating acetylcholine level in the brain. Thus, it may be explored as a useful memory restorative agent in the management of cognitive dysfunctions as in amnesia and Alzheimer's diseases.

Conflict of interest

None to declare.

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References

1. Bowen DM, Smith CB, White P, Dawson AN. Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain*. 1976;99:459–496.
2. Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol*. 1982;10:122–126.
3. Stevens R. Scopolamine impairs spatial maze performance in rats. *Physion Behav*. 1981;27:385–386.
4. Kulkarni KS, Kasture SB, Mengi SA. Efficacy study of *Prunus amygdalus* (almond) nuts in scopolamine-induced amnesia in rats. *Indian J Pharmacol*. 2010;42(3):168–173.
5. Anonyms. *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: DSM-5*. Washington, D.C: American Psychiatric Association; 2013. http://dx.doi.org/10.1176/appi.books.9780890425596.
6. Quinette P, Guillery-Girard B, Dayan J, et al. What does transient global amnesia really mean? Review of the literature and thorough study of 142 cases. *Brain*. 2006;129(Part 7):1640–1658.
7. Bordilau R, Spiridon L, Teaca CA, et al. Anti-inflammatory constituents from different medicinal plants. *JCIV Eng Manag*. 2009;8(4):785–792.
8. Ageel AM, Mossa JS, Tariq M, Al-Yahya MA, Al-Saeed MS. *Plants used in Saudi folk medicine*. Riyadh, Saudi Arabia: King Saud University; 1987:71.
9. Adam JN, Sivropoulov A, Kokkini S, Lanaras T, Arsenakis M. Antifungal activities of *Origarnum vulgare* subsp. Hirtum, *Mentah spicata, Lavandula angustifolia* and *Salvia fruticose* essential oils against human pathogenic fungi. *J Agric Food Chem*. 1998;46:1739–1745.
10. Akbar A, Tariq M, Nisa M. Pharmacological studies on *Salvia haematodes* Wall. *Acta Trop*. 1985;42(4):371–374.
11. Baylac S, Racine P. Inhibition of 5-lopoxygenase by essential oils and other natural fragrant extracts. *Int J Aromather*. 2003;13:138–142.
12. Abdille MH, Singh RP, Jayaprakasha GK, Jena BS. Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chem*. 2005;90(4):891–896.
13. Raja R, Jeeva S, Prakash J, Marimuthu J, Irudayaraj V. Antibacterial activity of selected ethnomedicinal plants from South India. *Asian Pac J Trop Med*. 2011;4(5):375–378.
14. Hamidpour M, Hamidpour R, Hamidpour S, Shahlari M. Chemistry, Pharmacology, and medicinal property of Sage (*Salvia*) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer. *J Tradit Complement Med*. 2014;4(2):82–88.
15. Dymock W, Warden CH, Hooper D. *Pharmacographia Indica*vol. 1. Karachi, Pakistan: Institute of Health and Tibbi Research, Hamdard National Foundation; 1972:239.
16. Nadkarni AK. *Indian Materia Medica*. 3rd ed. vol. 1. Bombay, India: Dhootapapeshwar Prakashan Ltd; 1954:2205.
17. Avicena, 1048. Al-Qanoon-fil-rib (vol. 2, Urdu translation). Nawal Kishore, Lucknow, India; p. 52.
18. Baitar I. *Jame-ul-Mufradar-al-Advia-wal-Agh:ia*. Egypt: Amara Press; 1871:121.
19. Said M. *Pharmacopoeia of Eastern Medicine*. Hamdard National Foundation, Hamdard. Karachi, Pakistan: The Times Press; 1969:69.
20. Khan MA. *Muheet-e-Azam I*. Kanpur, India: Matba Nizami; 1886:378.
21. Khan AQ. *Personal Communication*. 1980.
22. Kabir H. *Shamsher's Morakkabat (Unani Formulations)*. 1st ed. Mirza Aligarh, India: Shamsher Publisher and Distributors; 2003:93.
23. Usmani A, Mujahid M, Khushtar M, Siddiqui HH, Rahman MA. Hepatoprotective effect of *Anacyclus pyrethrum* Linn against antitubercular drug-induced hepatotoxicity in SD rats. *J Complement Integr Med*. 2016;13(3):295–300.
24. Badruddeen, Fareed S, Siddiqui HH, Haque SE, Khalid M, Akhtar J. Effects of *Onosma bracteatum* Wall. (Gaozaban) on stress model in Sprague Dawley rats. *J Clin Diagn Res*. 2012;6(suppl 7):1356–1360.
25. Bejar C, Wang R, Weinstock M. Effect of rivastigmine on scopolamine-induced memory impairment in rats. *Eur J Pharmacol*. 1999;383:231–240.
26. Badruddeen, Fareed S, Siddiqui HH, Haque SE. Psychoimmunomodulatory activity of *Salvadora persica* L. (Miswak) extract on stress model in rats. *Asian J Trad Med*. 2012;7(3):109–117.
27. Ellman GL, Courtney KD, Anders F, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 1961;7:88–95.
28. Lowry OH, Rosenbrough NJ, Forr AL, Randall RJ. Protein measurement with the Folin's phenol reagent. *J Biol Chem*. 1951;193:265–275.
29. Rahman MA, Kamal M, Hussain A. Phytochemical and analytical evaluation of Kachnal (*Bauhinia racemosa* Lam.) Bark. *Curr Trad Med*. 2015;1:136–144.