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EFFECT OF DIFFERENT EXTRACT OF ALLIUM SATIVUM (BULB) FOR THE TREATMENT OF COGNITIVE DYSFUNCTION

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ABSTRACT

The neuroprotective and memory enhancer supplement of plants are rich content of numerous mixtures which have therapeutic properties. The frequency of neurodegeneration in brain will be producing the conditions such as memory loss and cognitive dysfunction. The extract of Allium sativum exerts antioxidant and acetylcholinesterase inhibition effect. The proposed work estimated that the extract of plant may give synergistic effect on memory enhancing and treatment for cognitive disorder. The animals were assessed anti-inflammatory activity, antioxidant activity (Elevated plus Maze Model), acetylcholine Esterase inhibitory activity (Elevated plus Maze Model), open-field test, morris water maze test and step-down test on normal and aged mice. The tests showed the disabled spatial memory of the matured mice was partly justified by ethyl acetate extract of Allium sativum (bulb) (100 mg/kg; $P < 0.05$) as compared with the aged control mice. In step-down tests, the non spatial memory of the aged mice was improved by Allium sativum ethyl acetate extract (100 mg/kg; $P < 0.05$). Additionally, ethyl acetate extract of plant could inhibit acetyl cholinesterase (AChE), and also showed anti-inflammatory and anti-oxidant activity in the brain tissue of the matured mice. The results explained that ethyl acetate extract of Allium sativum (100 mg/kg; $P < 0.05$) improved memory and effective for the treatment of cognitive dysfunction of the matured mice most likely by means of its antioxidant and anti-inflammatory properties. Accordingly, the improved oxidative pressure status and cholinergic function along with signal transduction through drug particle may be dependable for the neuroprotective and memory-upgrading effects of animals. Thus the treatment on animals with Allium sativum ethyl acetate extract did not cause significant change in the cortex of brain and have no side effect. Thus the proposed work is showed that ethyl extract of Allium sativum has the ability to invert cognitive loss, which can fill in as a very strong bridge for additional investigations of the plant extract and its active compounds, as they exhibited promising results in terms of cognitive dysfunction.

KEYWORDS : Neuroprotection, Memory-enhancing effect, Allium sativum, cognitive dysfunction, Natural products.

Memory is course of action of conserves, storing and recalling experiences and data. It is closely related with learning process. Learning is mainly knowledge collection which is offered by present memories.

Memories are a primary basic part of human identity which makes them unique creature of earth. Memories also control the human behavior at every time by reminding its past actions and effects. If past events could not be remembered it would be impossible for language, relationship, or personal identity to develop. Memory loss is usually described as forgetfulness or amnesia.

An individual can record sensory stimulus, actions, information, etc preserve for dumpy or long stage of moment and while desired remind the matching at shortly owed to memory. Poor Memory is a lower preservation and slow recall. Now a day it is ordinary problems due stressful and competitive life. Factor such as period, stress, feeling are might reason recollection loss, loss of memory, concern, high blood pressure, dementia, to further aggressive virus like schizophrenia and Alzheimer's diseases.

"Cognition" is a fancy word that mental health professionals use to describe the wide range of brain-based behaviors that we rely on every day. Cognition encompasses lots of different skills, including perception memory, learning, judgment, abstract reasoning (thinking about things that aren't directly in front of us), and problem solving using language, and planning.

Cognition related to thinking skills and intellectual skills that allow observing, obtain, understand and respond to information and it includes the abilities to remember, concentration, process information, problems solving, information organization, converse and act upon information. All these abilities are inter-related and work together to function properly by any human in environment.

Cognitive skills are not similar as academic skills because academic skills include different subject's knowledge such as literature, math, history and science. Cognitive skills is mental capabilities required to learn subject matter and it helps function in daily life. Cognitive skills are the fundamental skills that helps in think, read, understand, remember, plan and organize.

METHOD AND MATERIALS:

Procurement of Chemical and solvents:

All chemicals and solvents were of analytical grade (AR Grade) and were purchased from Sigma Aldrich, Ranbaxy fine chemicals Ltd., LOBA chemicals Ltd.,S.D. fine chemicals Ltd., Spectrochem chemicals. Pre-coated TLC plates having silica gel 60 F254 thickness 0.2 mm were purchased from Merck. All the solvents used for HPLC analysis were purchased from JT Baker and Fischer scientific Ltd.

Collection and authentication of plant material:

The selected plant material Allium sativum Linn. Bulbs were purchased from local market of Bhopal, (M. P.) India. The specimens were identified and authenticated by the Department of Botany; Saifia College of Science & Education, Bhopal and their herbarium was deposited.

Physicochemical Evaluation: (Borhade et. al., 2017; WHO, 1998; IP, 1996)

Physiochemical qualities, for example, ashs qualities and extractive qualities were researched for each of the three

chose plant medicates as the official strategies and rules gained on WHO guidelines; quality control techniques for restorative plant materials.

Ash values (Total ash, Acid insoluble ash and Water soluble ash), Extractive values, Loss on drying and pH were determined for Allium sativum Linn. (Bulb).

Successive Solvent Extraction Of Crude Drugs

Accurately weigh 200gm of Allium sativum (bulb) was coarsely powdered and defatted with petroleum ether using Soxhlet apparatus. After complete defatting of plant material, marc was removed from the Soxhlet apparatus and dried completely. After complete drying, the marc was filled in Soxhlet apparatus and extracted with chloroform for 72 hour. After complete extraction with chloroform, extract was concentrated under reduced pressure, to obtained chloroform extract. Then, after complete extraction with chloroform, marc was removed and dried and again extracted with methanol using Soxhlet apparatus. After complete extraction with methanol, methanol extract was removed and concentrated under reduced pressure and dried in rotator evaporator to obtained methanolic extract.

The percentage yields (% Yield) of all the above obtained extracts were calculated.(Table no.1)

Prelimnary Phytochemical Analysis Of Extracts:

Qualitative test as phytochemical examination of any plant species is a vital procedure as it gives idea about the presence of different phytoconstituents and gives further possibilities of the specific plant species in its future research examinations. The concentrates acquired by progressive dissolvable extraction were exposed to different qualitative chemical tests to recognize the presence of compound constituents

Animals used for in- vivo studies:

The in-vivo animal studies were carried out on Swiss albino mice (20-25g) and wistar rat (110-150g). Mice were utilized for learn all in-vivo capacity. The animals were domicile to animal quarters previous to testing at temperature of 25±2°C and 50±5% with relative humidity in polypropylene cages through a 12 hours light/dark cycle and allowable free of charge entrance to food and water. The experiments were achieved by subsequent rules and system of CPCSEA (Committee for the purpose of Control and Supervision on Experimental Animals) approved by the IAEC (Institutional Animal Ethical Committee), RKDF University, Bhopal.

Determination of Acute Toxicity Study as per OECD Guideline (423):

Acute oral toxicity of chloroform extract of Allium Saltivum was carry out using female, mice (18-25g).All the animals were fasted for 3 hours with water ad libitium prior to the experiment. The extracts were administered in dose of 300,500 and 1000mg/kg p.o. to group of mice (n=3) and percentage mortality was noted after 24 h and daily thereafter for total 14 days. The procedure is evaluated for all the plants extract. No lethal effect or mortality was observed in animals throughout the test period following single oral administration at all selected dose levels of all extract.

Determination of in-vivo antioxidant activity (Superoxide scavenging):

Ethyl acetate extract of Allium sativum (67.24%) showed significant DPPH activity as compared to standard drug, vitamin c (86.26%). While chloroform extract of Allium sativum (65.10%) and methanolic extract of Allium sativum (74.14%) showed significant DPPH activity as compared to vitamin c (86.26%) in dose dependent manner.(Table No.2 Fig.1.)

Anti-inflammatory activity:

Anti-inflammatory activity of different extracts of Allium sativum Linn. (Bulb) was evaluated using carrageenan-induced paw oedema method in albino rats, the ethyl acetate extract of Anethum graveolens and A.sativum at the dose of 100mg/kg p.o. showed 38% and 31% inhibition in increase in paw volume, though of a short duration and intensity as compared to that of standard drug (aceclofenac sodium 10 mg/kg i.p.).

The plant extract also showed a delayed anti-inflammatory response, this might be due to the delayed absorption of the plant extract. Carrageenan-induced paw oedema was applied as a prototype of exudative phase of acute inflammation during inflammation evaluation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of Carrageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin and the delayed phase is sustained by the leucotrienes and prostaglandins. Flavonoids and tannins are reported to inhibit PG synthesis. Most of the non steroidal anti-inflammatory drugs (NSAIDs) have well balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity.

Acetyl cholinesterase Inhibitory activity of different extracts of Allium sativum Linn. (Bulb) was evaluated using Ellman's method in albino rats, the (Table No.3 Fig No 2)

The result of scopolamine group showed significant ($p<0.01$) increase in AChE activity as compared to control group. Piracetam (100mg/kg i.p.) significantly decrease ($p<0.01$) AchE activity in scopolamine injected mice brain. Treatment with different extract of A.Sativum showed good significant result as compared to standard drug. Result showed that ethyl acetate extract of A.sativum 100mg/kg p.o.showed (0.0318 ± 1.43) significantly decreases the AChE activity as compared to standard drug piracetam 100 mg/kg p.o. (0.0247 ± 1.02).

Open-Field Test:

Mouse Locomotive Activities in the Open Field test were evaluated by using different extracts of Allium sativum Linn. (Bulb) albino mice, the results are (Table No.4 Fig.3)

Significant effect of Allium sativum Linn. Bulb, (100mg/kg p.o.) On mouse locomotive activities was observed in the open-field test ($p<0.05$ and $p<0.01$). Furthermore galantamine (3mg/kg p.o.) reduced the total distance significantly compared with the aged control group ($P< 0.05$). The overall result concluded that ethyl acetate extract of Allium sativum Linn. Bulb at the dose of 100 mg/kg p.o., showed significant result (3008±12.7) as compared to standard drug (galantamine 3 mg/kg p.o.) (2840±14.5),

SUMMARY & CONCLUSION

Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. Cognition, broadly defined, includes perception, learning, memory, and decision making, in other words, all ways in which animals take information about the world through the senses, process, retain, and decide to act on it can be called as cognition. Besides age and gender, stressful and sedentary lifestyle, dietary excess, emotional disturbances, education level, social activities and burdens are among the factors that may lead to amnesia, anxiety, high blood pressure and dementia.

Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative states such as Alzheimer's disease. The Alzheimer disease is a most ordinary

neurodegenerative disorder without an effective treatment. Dementia is the paramount feature of Alzheimer's disease. Nootropic agents are known to facilitate learning and memory, and prevent impairment of cognitive functions induced by diseases and brain insults.

Development of cognition enhancers is still a difficult task because of complexity of the brain functions, poor predictability of animal tests and lengthy and expensive clinical trials as well as the lack of a common, generally accepted, mechanism of action. Thus various nootropic agents like piracetam have not been accepted globally. After the early serendipitous discovery of first generation cognition enhancers, current research is based on a variety of working hypotheses, derived from the progress of knowledge in the neurobiopathology of cognitive processes. All these problems in the development of the new leads for the pharmacotherapy and desperate need of the treatment prompted many researchers to search the option from the traditional system of medicines for this relentless progressive and devastating illness to transform it into a manageable chronic disease.

The ethyl acetate extract of Allium sativum (67.24%) showed significant DPPH activity as compared to vitamin c (86.26%) at 100μg/ml. While chloroform extract of Allium sativum (65.10%) and methanol extract of Allium sativum (74.14%) showed DPPH activity as compared to vitamin c (86.26%).

Treatment with chloroform extract of A.sativum (0.0342 ± 1.31), ethyl acetate extract of A.sativum (0.0320 ± 1.04) and methanol extract of A.sativum (0.0343 ± 1.23) compared with standard drugs (0.0227 ± 1.23) and found that treatment with ethyl acetate of A.sativum (100mg/kg p.o.) significantly decrease the AchE activity as compared to standard drug Piracetam (100mg/kg p.o.).

The effect of different extracts Allium sativum Linn. (Bulb) on mice locomotor activities was evaluated by step down method. In this study, locomotive activity was determined on day 15, day 16 and day 28.

In the consolidation trials, the aged mice (71.31±0.04) had longer time on the electric grid than the normal control mice (17.21±0.03) (< 0.01). However, no significant differences were observed in all groups on the time in safety zone. In the retrial tests, the aged control mice (150.11±1.23) still displayed significant differences for the time spent in the safety zone (220.23±1.01) as compared with the normal control mice.

Treatment with chloroform extract of A.sativum (61.22±0.06), Ethyl acetate extract of A.sativum (41.12±0.08) and methanolic extract of A.sativum ((52.31±0.01) compared with standard drugs, Galantamine 3mg/kg p.o, and found that treatment with ethyl acetate extract of A.Sativum(100mg/kg p.o.). significantly (p<0.05 &p<0.01) shorten the time spent on electric grid as compared to standard drug gelantamine 3mg/kg p.o.(28.23±0.03).

While, treatment with chloroform extract of A.sativum 100mg/kg p.o (155.23±2.2), Ethyl acetate extract of A.sativum 100mg/kg p.o. (203.21±1.3), and methanolic extract of A.sativum 100mg/kg p.o (191.14±1.7) as compared with standard drugs, galantamine 3mg/kg p.o. and found that significantly (p<0.05 &p<0.01) increased the time spent on safety zone as compared to standard drug galantamine 3mg/kg p.o. (217.13±1.3).

Overall work done in present study concluded that, ethyl acetate extract of Allium sativum showed maximum memory enhancing activity and showed good result for the treatment of cognitive dysfunction. in different pharmacological screening models, as compared to other extracts.

Table 1: Solvent extractive values (%w/w) of Allium sativum Linn. (Bulb)

S. No.	Name of extract	Extractive value
		A. Sativum
1	Chloroform Extract	3.28 % w/w
2	Ethyl acetate Extract	4.58 % w/w
3	Methanol Extract	9.08 % w/w

Table 2: In-vitro antioxidant activity of different extract of Allium sativum Linn. (Bulb).

Plant extract	Concentration (μg/ml)	DPPH free radical Inhibition
Chloroform extract (ChAS)	5	28.12±0.12
	10	38.06±1.38
	50	55.12±1.19
	100	65.10±1.14
Ethyl Acetate extract (EaAS)	5	30.23±0.13
	10	43.16±1.04
	50	60.82±1.09
	100	67.24±1.14
Methanol extract (MtAS)	5	37.21±0.11
	10	41.26±0.14
	50	48.52±0.09
	100	74.14±0.04*
Vitamin C (standard drug)	5	53.21±0.11
	10	62.21±1.11
	50	71.21±1.11
	100	86.21±1.07*

Data value are expressed as mean ± SEM *P<0.05 and **P<0.01 compared with Standard group.

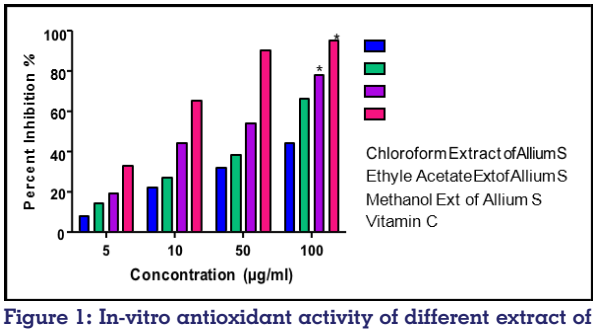

Figure 1: In-vitro antioxidant activity of different extract of Allium sativum Linn. (bulb).

Table 3: In-vivo anti-inflammatory activity of different extract of Allium sativum Linn. (Bulb).

Treatment	Dose	Mean Paw Volume ±SEM (ml)				
		Before carrag-eenan	1h	2h	3h	4h
Saline	0.5ml p.o	0.88± 0.01	1.24± 0.06	1.31± 0.08	1.75 ±0.05	1.81± 0.04
Aceclofenac sodium	10 mg/kg i.p.	0.74± 0.02	0.81± 0.03	0.88± 0.04	0.91± 0.07*	1.02± 0.06
Chloroform extract	100 mg/kg p.o.	0.70± 0.04	1.26± 0.02	1.42± 0.05	2.08± 0.11	2.19± 0.07
Ethyl Acetate extract	100 mg/kg p.o.	0.78± 0.04	1.31± 0.01	1.12± 0.05	1.13± 0.05*	2.21± 0.04
Methanol extract	100 mg/kg p.o.	0.79± 0.01	1.34± 0.06	1.82± 0.04	1.60± 0.03	2.09± 0.06
% Inhibition In edema±SEM (%)						
Saline	0.5ml p.o	0.81± 0.02	-	-	-	-
Aceclofenac sodium	10 mg/kg i.p.	0.74± 0.03	53.70 ±1.91	61.60 ±4.61	58.76 ±4.87	58.54 ± 4.66

Chloroform extract	100 mg/kg p.o.	0.80± 0.02	15.70 ±4.95	25.25 ±4.05	16.80 ±4.11	14,76 ±4.07
Ethyl Acetate extract	100mg/kg p.o.	0.78± 0.04	12.28 ±8.84	27.62 ±6.21	31.01 ±4.05	29.54 ±2.75
Methanol extract	100 mg/kg p.o.	0.79± 0.01	13.81 ±0.08	14.80 ±2.84	24.60 ±2.83	23.75 ±2.86
Values are mean ± SEM, (n=5), *P<0.01and **P<0.05,						

Values are mean ± SEM, (n=5), *P<0.01and **P<0.05

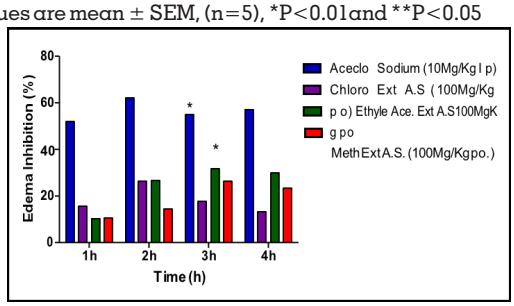

Figure 2: In-vivo anti-inflammatory activity of different extract of Allium sativum Linn. (Bulb).

Table 4: Effect of different extracts of Allium sativum Linn. (Bulb) on acetyl cholinesterase inhibitory activity in mice brain.

S. No.	Group of animals	Treatment	Dose	AChE activity of brain
1	G1	Normal Control	0.9 % Nacl, i.p	0.0379 ± 1.03
2	G2	Scopolamine	2mg/kg, i.p	0.0489 ± 1.12#
3	G3	Piracetam	100 mg/kg, p.o.	0.0227 ± 1.23*
4	G4	Chloroform extract (ChAS)	100 mg/kg p.o.	0.0342 ± 1.31
5	G5	Ethyl Acetate extract (EaAS)	100 mg/kg p.o	0.0320 ± 1.04*
6	G6	Methanol extract (MtAS)	100 mg/kg p.o.	0.0343 ± 1.23

Table 5: Effect of different extracts of Allium sativum Linn. (Bulb) on locomotive activities of mice.

S.No.	Group of animals	Treatment	Dose	Total distance (cm)
1	G1	Normal Control	Dist. Water p.o.	3712±11.7
2	G2	Aged group	Dist. Water p.o.	3523±11.2
3	G3	Galantamine	3.0 mg/kg p.o.	2412±12.2*
4	G4	Chloroform extract(ChAS)	100 mg/kg p.o.	3054±12.6
5	G5	Ethyl Acetate extract(EaAS)	100 mg/kg p.o.	3217±10.4
6	G6	Methanol extract (MtAS)	100 mg/kg p.o.	2778±11.3*
Values are mean±SEM, (n=5), *P<0.01and **P<0.05,				

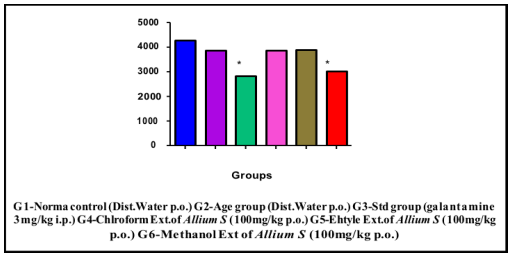

Figure 3: Effect of different extract of Allium sativum Linn. (Bulb) on locomotive activities of mice.