JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Evaluation of protective role of Abutilon Indicum in Aluminium chloride induced Alzheimer's disease in Rats

D N Parmar*[1], P D Sachdeva[1], M R Kukkar[2]

1. Department of Pharmacology, A. R. College of Pharmacy & G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar - 388120, Gujarat, India,
2. Department of Pharmacognosy, A. R. College of Pharmacy & G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar - 388120, Gujarat, India.

Article history:

Received 20 July 2017
Revised 13 Sept 2017
Accepted 20 Sept 2017
Available online 1 Dec 2017

Citation:
Parmar D. N., Sachdeva P. D., Kukkar M. R. Evaluation of protective role of Abutilon Indicum in Aluminium chloride induced Alzheimer's disease in Rats **J Pharm Sci Bioscientific Res**. 2017. 7(5):314-321

For Correspondence:
Devshree N Parmar

Department of Pharmacology, A. R. College of Pharmacy & G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar - 388120, Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

Alzheimer's disease is a complex neurodegenerative disease characterized by progressive decline in memory and cognitive functions, due to genetic defects, oxidative stress and increase in level of enzyme acetylcholinesterase causing reduction in level of neurotransmitter Acetylcholine. Literature survey reveals that an important constituent present in the plant Abutilon indicum is β-sitosterol which produces anti-inflammatory activity and its leaves are reported to possess good antioxidant activity. Also, spectrophotometric analysis of methanolic extract of the plant is reported to show good acetylcholinesterase inhibitory activity. Hence, methanolic extract of Abutilon indicum was evaluated for its memory retention and cognitive enhancement ability against Aluminium chloride induced Alzheimer's disease in rats. Alzheimer's disease was induced in Sprague-dawley rats by administration of Aluminium chloride (4.2mg/kg i.p.) for 28days. Effectiveness of Abutilon indicum whole plant extract [400mg/kg, 600mg/kg] was evaluated using various behavioural models and by estimating brain acetylcholineesterase enzyme level. Methanolic extract of Abutilon indicum showed significant decrease in Transfer Latency in all learning and memory models indicating its effectiveness in reversing the cognitive impairment caused by Aluminium chloride and thus improving memory. Biochemical estimation of brain homogenate revealed decrease in level of brain acetylcholinesterase enzyme in drug treated rats suggesting improvement in cholinergic function. From the results of the experiments, it can be concluded that methanolic extract of Abutilon indicum shows protective effect against Aluminium chloride induced Alzheimer's disease in rats, due to its anti-inflammatory, anti-oxidant and acetylcholinesterase enzyme inhibitory activity.

KEY WORDS: Alzheimer's disease, Abutilon indicum, Memory, Acetylcholinesterase enzyme, Aluminium Chloride.

INTRODUCTION:

Alzheimer's disease AD is a complex neurodegenerative disease characterized by progressive decline in memory, language and other cognitive functions. . Several etiologic hypotheses have been advanced for AD like, genetic defects, appearance of neurofibrillary tangles, altered amyloid precursor processing, deficiency of neurotropic factors, mitochondrial defect, trace element neurotoxicity, energy metabolism deficit and oxidative stress.[1] Alzheimer disease is the most common form of dementia, accounting for 50%-75% of the total, with a greater proportion in the higher age ranges.[2] The pathophysiology of AD is related to the

injury and death of neurons, initiating in the hippocampus brain region that is involved with memory and learning, and then atrophy affects the entire brain. Mutations in the amyloid precursor protein (APP) and/or presenilins 1 and 2 gene (PS-1, PS-2) genes lead to the production of protein, amyloid beta 42 (Aβ42) that accumulates into amyloid plaques. A microtubule-associated protein expressed in neurons called tau protein aggregates abnormally into masses inside nerve call body known as neurofibrillary tangles.[3] Both the amyloid plague and neurofibrillary tangles cause injury and death of neurons leading to memory loss and behavioural changes. The degeneration of neurological function is also due to reduction in levels of neurotransmitter acetylcholine (Ach) [3] due to depletion of the enzyme choline acetyltransferase, which catalyses the synthesis of Ach and increase in level of acetylcholinesterase enzyme (AChE) which causes rapid hydrolysis of acetylcholine.[4] Cerebral inflammation is also reported in pathogenesis of AD**.** [5] The acetylcholinesterase inhibitors (AChEI) are the only licensed agents for primary treatment of AD.[6] They inhibit or block the breakdown of acetylcholine and thus help to improve brain function.[7] Also, the use of non-steroidal anti-inflammatory (NSAIDs) drugs and high dietary intake of antioxidants have been associated with a reduced risk of AD. These different treatment strategies have been investigated to cure AD, but with relevant side effects, including nausea, vomiting, headache, insomnia, lack of appetite, stomach pain etc.[8]

Therefore it is worthwhile to choose the traditional medicines system for treatment of AD.

Literature survey reveals that several plants like, *Bacopa monniera, Ginkgo biloba*[9] *Myricaria elegans Royle*[10] etc possess AchE inhibiting property and hence are reported to be useful in treatment of AD.

The plant *Abutilon indicum* belongs to family Malvaceae.[11] It is reported to contain saponins, flavonoids, alkaloids, hexoses, n-alkane mixtures (C22-34), alkanol as main classes of compounds. Another important constituent reported in the plant is β-sitosterol.[12]

Traditionally the seed of the plant of *Abutilon indicum* is said to be useful as nervine tonic [13] and leaves are reported to possess good antioxidant activity .[14]

Screening of AchE inhibitory activity from natural resources using spectrophotometric method revealed that methanolic extract of whole plant of *Abutilon indicum* (MEAI) inhibits enzyme acetylcholinesterase**.**[15]

However literature survey shows that plant of *Abutilon indicum* has not been investigated for its effectiveness in treatment of AD. Hence, the present study was undertaken with the aim to evaluate potential of methanolic extract of *Abutilon indicum* in $AlCl_3$ induced AD in rats.

MATERIAL AND METHODS

Plant material

The plant of *Abutilon indicum* was collected from Petlad, District Anand, Gujarat and identified and authenticated by Dr. Sashidharan N., Head, Department of Seed Science & Technology, B. A. College of Agriculture, Anand – 388110, Gujarat. **(BACA/GPB/223/16)** . A voucher specimen of the plant **(DP/AI-12/19/ARGH-16)** has been deposited in the botany herbarium of A.R College of Pharmacy.

Preparation of plant extract

The freshly collected whole plants were chopped, shade dried and coarsely

powdered. The powder was defatted with petroleum ether ($60^{o}C$–$80^{o}C$) and

extracted with methanol using soxhlet extractor. The extract was evaporated to dryness to get a solid residue. The percentage yield of the extract was 4.2% (w/w). The residue was stored in air tight container at $2^{o}C$-$5^{o}C$ for evaluation of its pharmacological activity.

Preliminary Phytochemical Test [16]

Methanolic extract of *Abutilon indicum* was subjected to standard chemical tests for detection of various phytoconstituents present.

Drugs and reagents

Rivastigmine - Exelon Novartis Pharma – Marketed product

$AlCl_3$. AR grade - Allied Pharmaceutical Limited.

Selection and Housing of Animals

Sprauge dawley rats, of either sex, weighing 200-250gm were procured from Zydus Research Centre, Ahmedabad. The animals were housed under standard conditions, maintained on a 12hr light/dark cycle and had free access to food and water up to the time of experimentation. The protocol **(No: CPCSEA/IAEC/ARCP/2016-17/01)** of the study was approved by Institutional Animal Ethical Committee (**IAEC)** of A R College of Pharmacy and experiments were conducted as per guidelines of Committee for the Purpose of Control of Experiments on Animal (**CPCSEA)** and Ministry of Social Justice and Empowerment, Government of India.

PHARMACOLOGICAL EVALUATION

Treatment schedule: 30 rats were divided into 5 groups of 6 animals each. **Table 1** shows the grouping of animals and their treatment schedule.

Table 1: Grouping of animals and their treatment schedule

Group no	Name of group	Drug treatment and route.
I.	Vehicle control group	0.9% saline p.o
II.	Model control group	AlCl3 [4.2mg/kg] i.p
III.	Standard group	AlCl3 [4.2mg/kg] i.p +Rivastigmine p.o[0.03mg/kg]
IV.	Treatment group [low dose] (MEAI)	AlCl3 [4.2mg/kg] i.p + MEAI [low dose 400mg/kg] p.o
V.	Treatment group [high dose] (MEAI)	AlCl3 [4.2mg/kg] i.p + MEAI [high dose 600mg/kg] p.o

Induction of cognitive impairment

In rats cognitive impairment leading to Alzheimer's disease was induced by administration of $AlCl_3$ in the dose of 4.2mg/kg i.p. for 28days. Administarion of $AlCl_3$ induces neurological damage which can be confirmed by measuring cognitive impairment using behavioural models at the end of treatment period i.e 28days [10]

EVALUATION PARAMETERS [17]

Radial arm maze (RAM)

Animals were divided into 5 groups with 6 animals in each group. The RAM was used to evaluate the working memory in animals. Animal was placed on the centre hub with all guillotine doors lowered. Then all the doors were simultaneously opened to allow the rat to choose arms freely. When the animal entered one of the arms, the doors to the remaining seven arms were closed. The open door was closed after the animal returned to the central hub. Then all the doors were raised again simultaneously. The trial was considered complete when the rat visited all eight arms or spent 10 min in the maze. Entry into an arm which the rat had not previously visited was recorded as a correct response and re-entry was counted as an error. A trial in which an animal made no error, or only one error at the eighth choice, was defined as a successful trial.

Elevated plus maze (EPM)

The plus maze was used to evaluate the working memory in animals. Animal was placed individually in the centre of the maze with head facing towards open arm.

Transfer latency (Time taken by animal to move from open arm to close arm with all its four legs inside) was observed for 5min.

Rodent Memory Evaluator (RME)

The passive shock avoidance is a behavioural test used to examine the long term memory of animals using Rodent Memory Evaluator (RME). This apparatus consists of a box having an electric grid floor, which has a wooden platform in the center. Each rat was placed individually on the electric grid and allowed to explore the maze for 1 minute. The stimulus (20 V) with AC current of 5mA was applied to the grid and latency to reach the shock free zone (SFZ) was recorded three consecutive times as a basal reading. Significant reduction in latency to reach SFZ after drug administration indicated improvement of memory.

At the end of 28days dosing period, on completion of all the behavioural models, rats were sacrificed by overdose of Thiopental sodium. Brains from all groups were removed and preserved in 10% formaldehyde for estimation of brain AchE level.

Estimation of Brain Acetylcholinesterase enzyme

Acetylcholinesterase enzyme activity was measured in rat brain according to the Ellman method. Treated animals were sacrificed by cervical decapitation under anesthesia and the whole brain was removed from the skull and kept in cold normal saline. Rat brain was homogenized in 0.1 m Potassium dihydrogen phosphate (KH_2PO_4) buffer, at pH 8 and kept frozen in an ice chest. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant was used for estimation of brain AchE activity. The cloudy supernatant liquid (0.4 ml) was diluted with (5,5'-dithiobis-(2-nitrobenzoic acid) DTNB solution (100 µl). The optical density of the yellow color compound formed during the reaction was measured at 412 nm. Protein estimation was done using Lowry's method. Brain acetyl cholinesterase activity was determined using the following formula.

$$R = \frac{\Delta \text{ OD} \times \text{volume of assay}}{E \times \text{mg of protein}}$$

Where

***R* –** rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein.
Δ OD - is the change in absorbance per minute and
E - extinction co-efficient, which is 13,600 $M^{-1}cm^{-1}$.

RESULTS

Preliminary phytochemical screening:

The methanolic extract of *Abutilon indicum* (MEAI) was subjected to standard chemical tests as per the reported methods and was found to contain alkaloids, phenolic compounds, flavanoids, saponins, glycosides, carbohydrates, steroids.

Effect of MEAI on Transfer Latency (TL) using Elevated Plus Maze (EPM) Apparatus

The effect of MEAI on learning and memory was investigated using TL as a parameter for retention of memory in rats. **Table: 2.** shows basal reading of TL and the change in TL shown by animals of different groups at the end of treatment period (28 days). $AlCl_3$ (4.2mg/kg) significantly (p<0.001) increased the transfer latency in model control group indicating impairment of memory.

Table 2: Effect of MEAI on TL using EPM

Treatment group	Transfer Latency (sec)	
	Basal Reading	At the end of 28days
Vehicle Control	10.20±0.47	18.33±1.87
Model control $AlCl_3$ (4.2mg/kg)i.p	40.32±0.79	102.88±0.89*
MEAI Low dose (400mg/kg) p.o.	32.45±1.18	72.13±12.65**
MEAI High dose (600mg/kg)p.o	38.44±1.96	83.21±13.98**
Rivastigmine (0.03mg/kg)p.o.	42.67±3.45	39.34±14.76#

Data are expressed as a Mean ± SEM, (n=6),*p<0.05 when compared to normal control group, **p<0.001 when compared to model control group, #p<0.0001 when compared to model control group, by One way ANOVA followed by Dunnett's test.

Table 3: Effect of MEAI on Latency to reach shock free zone using RME

Treatment group	Latency to reach SFZ	
	Basal Reading	At the end of 28days
Vehicle Control	9.32±1.32	11.33±0.12
Model control $AlCl_3$ (4.2mg/kg i.p	78.65±5.45	168±0.34*
MEAI Low dose (400mg/kg) p.o.	30±3.54	42±1.67**
MEAI High dose (600mg/kg) p.o.	23.56±0.24	30.66±1.87**
Rivastigmine (0.03mg/kg) i.p.	6.65±7.65	7.66±2.62#

Data are expressed as a Mean ± SEM, (n=6), *p<0.05 when compared to normal control group, **p<0.001 when compared to model control group, #p<0.0001 when compared to model control group by One way ANOVA followed by Dunnett's test.

Administration of MEAI (400 & 600 mg/kg p.o) successfully reversed the memory deficit induced by $AlCl_3$, by reducing the TL and the difference was found to be statistically significant (p<0.001) when compared with model control group. Standard drug Rivastigmine (0.03mg/kg p.o.) also significantly (p<0.0001) improved memory, as is evidenced by decrease in TL when compared with model control group.

Effect of MEAI on Latency to reach shock free zone (SFZ) using Rodent Memory Evaluator (RME)

RME is designed to record the latency to reach SFZ as a parameter for retention of short term memory.

Administration of $AlCl_3$ (4.2 mg/kg) increased the latency to reach SFZ indicating cognitive impairment.

Administration of MEAI (400 & 600 mg/kg p.o) reversed the cognitive impairment induced by $AlCl_3$, by reducing the latency to reach SFZ and the difference was found to be statistically significant ($p<0.001$) when compared with model control group. Similar significant difference ($p<0.0001$) in latency to reach SFZ was observed on administration of the standard drug Rivastigmine (0.03 mg/kg p.o.) **(Table: 3)**

Effect of MEAI on working memory using Radial arm maze (RAM)

RAM is designed to record working memory of rats which is essential for cognitive tasks such as comprehension and learning. It is more impaired in dementia and AD.

Entry of rat into an arm which he had not previously visited (correct arm) and time spent in correct arm is recorded as response for a period of 10min.

As is evident from **Table 4**, rats in model control group spent significantly ($p<0.005$) more time in correct arm, as compared to normal control group, indicating cognitive impairment induced by administration of $AlCl_3$(4.2 mg/kg) in model control group.

Table 4: Effect of AIE on working memory using Radial arm maze

Treatment group	Average time spent in correct arm (sec)	
	Basal Reading	At the end of 28days
Vehicle Control	40.16±1.24	34.23±4.54
Model control $AlCl_3$ (4.2mg/kg) i.p	125.64±3.54	150.68±7.64*
MEAI Low dose (400mg/kg) p.o.	90.53±3.76	84.92±3.76**
MEAI High dose (600mg/kg)p.o.	68.45±2.88	49.13±1.43**
Rivastigmine (0.03mg/kg) i.p.	60.89±8.54	41.13±7.23#

Data are expressed as a Mean ± SEM, (n=6),*p<0.05 when compared to normal control group, **p<0.001 when compared to model control group, #p<0.0001 when compared to model control group, by One way ANOVA followed by Dunnett's test.

MEAI (400 & 600 mg/kg p.o) and standard drug Rivastigmine (0.03 mg/kg p.o) significantly improved working memory by reducing average time spent by rats in correct arm as compared to model control group.($p<0.001$, $p<0.0001$ respectively)

Effect of MEAI on level of Acetylcholinesterase enzyme

Table 5 shows the result of treatment with MEAI on cholinergic markers represented by brain AchE activity in AD-induced rats. $AlCl_3$ administration induced cognitive impairment by significantly ($p<0.05$) elevating brain AchE enzyme level when compared with normal control group. Treatment with MEAI significantly ($p<0.05$) reduced AchE enzyme level in brain, indicating the counteracting action of this drug on the cholinergic system.

Table 5: Effect of AIE on Acetylcholinesterase enzyme levels

Treatment group	Acetylcholinestrase Enzyme (mol/min/mg protein)
Vehicle Control	0.481
Model control $AlCl_3$ (4.2mg/kg) i.p	1.898*
MEAI Low dose (400mg/kg) p.o.	0.783*
MEAI High dose (600mg/kg)p.o.	0.635*
Rivastigmine (0.03mg/kg) i.p.	0.353**

Data are expressed as a Mean (n=6),*p<0.05 when compared to normal control group, **p<0.01 when compared to model control group, by One way ANOVA.

Administartion of standard drug Rivastigmine (0.03 mg/kg) also significantly ($p<0.01$) reduced AchE enzyme level in brain.

DISCUSSION

The most well known type of neurodegenerative dementia in elderly is AD.[18] The pathological hall marks of AD are senile plagues of protein b-amyloid and neurofibrillary tangles.[19] Also Ach reduction directly correlates with deterioration of cognition [20], which gave rise to the "cholinergic hypothesis" of AD[21]. Cerebral inflammation is also reported in pathogenesis

of AD. [22] Evidence is also available which states that oxidative stress causes deposition of b-amyloid plagues which increase production of pro-inflammatory cytokines leading to a local inflammatory response causing neurodegeneration and cell death [1] . Beta-amyloid plagues also induce DNA damage and protein aggregation by increasing oxidative stress.[23]

Chronic exposure to $AlCl_3$ causes oxidative deterioration of cellular lipids, proteins and DNA leading to tissue damage [24, 25]. Results of the present study reveal that $AlCl_3$ caused significant deterioration in memory in model control group. Similar effects produced by $AlCl_3$ (4.2mg/kg) are reported by other researchers. [26] Also $AlCl_3$ significantly increased the level of AchE in brain homogenate and our results are in agreement with results of other researcher. [27]

Treatment of $AlCl_3$ induced AD rats with Rivastigmine showed improvement in memory [27] and a significant decline in brain AchE activity. Other researchers have reported similar results with use of Rivastigmine.[28]. Rivastigmine is known to correct neurological dysfunction and improve memory in animals by its anti-inflammatory, immunomodulatory activity [29] and AchE inhibiting activity.

Treatment of $AlCl_3$ induced AD rats with MEAI exhibited significant improvement in Alzheimer's status of rats as evidence by decrease in TL on EPM and RAM, and time taken to reach SFZ as compared to model control rats. It also reduced brain AchE levels more than the model control group

These findings correlate with previous research studies using *Centella asiatica, Glycyrrhiza glabra and Zingiber officinale* which showed a link between memory improving effect and AchE inhibitory activity[9] Hence it is possible that memory improving activity of MEAI is mediated by its AchE inhibitory activity.

Wide range of phytoconstituents are reported to be responsible for anti-inflammatory and anti-oxidant activity which include steroids, phenolic coumpounds, alkaloids, tannins and saponnins .[30] Flavanoids exert antioxidant[31] activity and steroids posses anti-inflammatory activity.[32] Phytochemical screening of MEAI revealed the presence of streroids. phenolic compounds, alkaloids, carbohydrates, flavonoids and tannins. Thus the plant *Abutilon indicum* has both antioxidant and anti inflammatory phyto-constituents which may be responsible for its neuroprotective role evidenced by improvement in memory.

CONCLUSION

MEAI significantly improved memory by reversing the cognitive impairment caused by $AlCl_3$.

Biochemical estimation of brain homogenate revealed decrease in level of brain AchE suggesting improvement in cholinergic function.

Thus, it can be concluded that AIE, by its anti-inflammatory, anti-oxidant and AchE inhibitory activity exerts protective effect against $AlCl_3$ induced AD, which is a neurodegenerative disease associated with increase in AchE enzyme activity.

REFERENCES

1) De la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neuron 2004; 3:184–190.

2) Alzheimer society of Canada, Alzheimer's disease: The progression of Alzheimer's disease. Introduction 2005; 05: 332-31.

3) Jarvick L, Greenson H. About a peculiar disease of cerebral cortex. Alzheimer's disease associated disorder 1987; 1: 7-8.

4) Guather S. Alzheimer's disease: Current and future therapeutic perspective progression. Neuropsychopharma /bio/ psychiatry 2001: 25: 73-89.

5) Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia 2012:7; 647-53.

6) Briks JS, Melzer D, Beppo H. Donezepil for mild and moderate Alzheimer disease in the Cochrane library 2003; 3: 65

7) Tariot PN, Cummings JL, Katz IR, Mintzer J, Perdomo CA, Schwam EM et al. A randomized double-blind, placebo-controlled study of the efficacy and safety of donepezil in patients with Alzheimer's disease in the nursing home setting. J Am Geriatr Soc 2001; 49: 1590-99.

8) Poryamout L, Dams J. Evaluation of prescriber drugs used to treat Alzheimer's disease, comparing effectiveness, safety and price. Consumer Reports Best Buy Drugs 2012; 6: 789

9) Das, A, Shanker G, Nath C, Pal R , Singh S, Singh HK. A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba* anticholinesterase and cognitive enhancing activities. Pharmacol. Biochem. Behav. 2002; 73: 893–900.

10) Ahmad W, Ahmad B, Ahmad M, Iqbal Z, Nisar M, Ahmad M. In vitro inhibition of acetylcholinesterase, butyrylcholinesterase and lipoxygenase by crude extract of *Myricaria elegans Royle*. J Biol Sci 2003; 3: 1046–49.

11) Dash GK, Samanta A, Kanungo SK, Shau SK, Suresh P, Ganpathy S. Hepatoprotective activity of leaves of *Abutilon indicum*. Indian Journal of Natural Products 2000; 16: 25-27.

12) Kuo PC, Yang ML, Pei-Lin Wu, Shih HN, Thang TD, Dung NX, Wu TS. Chemical constituents from *Abutilon indicum*. Journal of Asian Natural Products Research 2008; 10: 689-93.

13) Gatuam GK, Vidyasagar G, Dwivedi SC. Study of medicinal plants from Indian origin, A Text Book of Indian medicinal plants. Germany, Lambert Academic Publication 2012; 46-48.

14) Harborne JB. In Phytochemical Methods. Chapman and Hall Ltd, London. 1st ed. 1973; 4:34.

15) Ingkaninan K , Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J. Ethnopharmacol. 2003; 89: 261–64.

16) Khandelwal KR. Practical Pharmacognosy - Preliminary phytochemical screening. 10th ed. Pune : Nirali Prakashan 2006; pp 149-56.

17) H Gerhard Vogel(Eds). Drug discovery and evaluation- Pharmacological assays. 3rd ed. New York : Springer; 2011: 338-530.

18) Arvanitakis Z. Update on dementia. Neurologist 2010; 10: 16-22.

19) Zhu X, Perry G, Moreira PI, Aliev G, Cash AD et al. Mitochondrial abnormalities and oxidative imbalance in Alzheimer disease. J Alzheimers Dis 2006; 9: 147-53.

20) Auld DS, Kornecook TJ, Bastianetto S. Alzheimer's disease and basal forebrain cholinergic system: relations to beta-amyloid peptides. Prog Neirobiol 2002; 68: 209-45.

21) Terry AV Jr, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer's disease related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 2003; 06: 821-27

22) Salminen A. Ojala J, Kauppinen A, Karniranta K. Inflammation in Alzheimer's disease: amyloid b-oligomers trigger innate immunity defence. Prog Neurolbiol.2009; 87: 181-82.

23) Mattons MP, Maguns T. Aging and neuronal vulnerability. Net Rev Neurosci. 2006; 7: 278-94.

24) Sargazi M, Shenkin A, Roberts NB. Kidney proximal tubular cells: Effects on markers of oxidative damage. J Trace Elem Med Biol 2006; 19: 267-73.

25) Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia 2012: 647-53.

26) Madhy KA, Nadia AM, Gouda. Protective effect of ginger on Alzheimer disease induced in rats. Journal of Neuroinfectious disease 2014; 5: 3-10.

27) Kumar A, Dogra A. Protective effect of curcumin against aluminium toxicity possible behavioural and chemical alternation in rats. Behav Brain Res 2009; 205: 384-90.

28) Ahmed A, Gliane M. Amelioration of neuroinflamatory tangles characterising Alzheimer's disease by natural products. Int J of Pharm and Pharm Sci 2013; 5: 87-93.

29) Nizri E, Franesh N, Lavi E. Suppression of neuroinflammation and immunomodulation by the Acetylcholineastrase inhibitor Rivastigmine. J of neuroimmunol. 2008; 203: 12-22.

30) Arya V, Arya ML. A review on anti-inflammatory plants barks. Int J Pharm Tech Res 2011; 3: 899-908.

31) Prochazkova D, Bousova I. Antioxidant and Prooxidant properties of Flavanoids. Fitoterapia 2011; 82 : 513-23.

32) Tripathi P, Chaiham NS. Antiinflammatory activity of *Abutilon indicum* extract. Natural product research 2012; 6: 231-34.