



## Lornoxicam Alone and with Selegiline Improves the Neuroprotective Effect and Cognition in Scopolamine Induced Neurodegeneration and Cognitive Impairment in Rats

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### Abstract

Alzheimer is a progressive neurodegenerative disorder in which Oxidative stress plays a major role. The present study was designed to investigate Neuroprotective effect of Lornoxicam, Selegiline and co-administration of both drugs in Scopolamine induced cognitive impairment and neurodegeneration. Scopolamine (1.4mg/kg) was administered intraperitoneally in male Wistar rats. Rectangular maze performance test was used to assess the memory performance test. Various biochemical parameters such as Catalase, 1, 1-diphenyl-2-picrylhydrazine (DPPH), Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and acetylcholine esterase (AChE) were also assessed. Intraperitoneal Scopolamine results marked memory impairment and oxidative damage. Sub-acute treatment with Lornoxicam (1.3mg/kg, p.o) and Selegiline (0.49mg/kg, p.o) and co-administration of these two drugs for 8 days significantly attenuated scopolamine induced oxidative damage and neurodegeneration. Besides, Lornoxicam, Selegiline and co-administration of both significantly reversed Scopolamine administered increase in acetylcholine esterase activity. Present study indicates protective effect of Lornoxicam, Selegiline and co-administration of both drugs against Scopolamine induced cognitive impairment and oxidative damage. The memory enhancing capacity of the drugs was very significant when compared with disease control ( $P < 0.001$ ).

**Key Words:** Alzheimer, AChE, DPPH, GSH, Lornoxicam, Scopolamine, Selegiline, TBARS.

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### 1. Introduction

Alzheimer disease (AD) is characterized by progressive cognitive decline usually beginning with impairment in the ability to form recent memories, but inevitably affecting all intellectual functions and leading to complete dependence for basic functions of daily life and

premature death<sup>[1]</sup>. Neurodegenerative diseases, a local inflammatory reaction is sustained by activated Microglia and reactive astrocytes, as indicated by the presence of antigens associated with microglia/macrophage activation and inflammatory mediators, such as elements of the complement system, cytokines and free radicals<sup>[2]</sup>. It has been more than 10 years since it was first proposed that the neurodegeneration in AD may be caused by deposition of amyloid  $\beta$ -peptide ( $A\beta$ ) plaques in brain tissue. According to the amyloid hypothesis, accumulation of  $A\beta$  in the brain is the primary influence driving AD pathogenesis. The rest of the disease process, including formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between  $A\beta$  production and  $A\beta$  clearance<sup>[3]</sup>. Braak and Braak showed that neurofibrillary pathology follows a predictable progressive pattern which was placed into six stages (I–VI). The NFT start in the transentorhinal area (I) and spread into the entorhinal cortex and hippocampus (II). In stages III and IV, the neurofibrillary pathology increases in the entorhinal cortex, hippocampus, adjacent inferior temporal cortex and amygdala. Stages V and VI are characterized by spread to the neocortical association cortex and other brain regions<sup>[4]</sup>.

Alzheimer's disease International commissioned an international group of experts to reach a consensus on dementia prevalence and estimated incidence in 14 World Health Organization regions, based on epidemiological

data acquired over recent years. The results suggested that 24.2 million people lived with dementia at that time, with 4.6 million new cases arising every year<sup>[2]</sup>. Evidence from well-planned, representative epidemiological surveys is scarce in many regions. We estimate that 24.3 million people have dementia today, with 4.6 million new cases of dementia every year (one new case every 7 seconds). The number of people affected will double every 20 years to 81.1 million by 2040. Most people with dementia live in developing countries (60% in 2001, rising to 71% by 2040). Rates of increase are not uniform, numbers in developed countries are forecast to increase by 100% between 2001 and 2040, but by more than 300% in India, China and their south Asian and western Pacific neighbors<sup>[21]</sup>. AD is generally diagnosed after the age of 65 years, when it is referred to as late onset AD. The condition affects 5% of the population aged over 65 years and more than 20% of the population over 85 years. Only 10% of all persons diagnosed with AD develop symptoms before the age of 65 years. They are said to have early onset AD and approximately 10% of these early onset cases have a familial form of the condition, which is transmitted as an autosomal dominant trait<sup>[5]</sup>. There are three genes known to be important in the aetiology of the early-onset familial condition in which the APP gene on chromosome 21, the presenilin-1 (PS1) gene on chromosome 14 and the presenilin-2 (PS2) gene on chromosome 1.

Apolipoprotein E gene on chromosome 19 is an important risk factor for sporadic AD<sup>[6]</sup>.

One of the most fundamental and consistent features of AD is the severe degeneration of cholinergic neurons projecting from basal forebrain to cortical and hippocampus areas. A 90% loss of basal forebrain cholinergic neurons has been found in AD patients. In contrast to the marked reduction of acetylcholine content in cholinergic target areas in AD brains, other transmitters such as serotonin, norepinephrine and dopamine do not show a significant decrease. Moreover, the degree of neuronal loss in basal forebrain has a good correlation with the severity of AD symptoms before death, especially among presenile cases of AD<sup>[7]</sup>.

AChE inhibitors from general chemical classes such as physostigmine, tacrine, galantamine and physostigmine have been tested for the symptomatic treatment of AD. However, non-selectivity of these drugs, their limited efficacy and poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are among the several limitations to their therapeutic success. Therefore, it is worthwhile to explore the utility of other existing medicines for the treatment of various cognitive disorders<sup>[8]</sup>. Memory impairment in the scopolamine-induced animal model is associated with increased oxidative stress within rat brain. Moreover, strong evidence supporting the involvement of oxidative damage in

neurodegenerative disease has been suggested by various clinical studies<sup>[9]</sup>.

PPAR $\gamma$  plays a critical role in regulating the inflammatory responses of microglia and monocytes to  $\beta$ -amyloid. We argue that the efficacy of NSAIDs in the treatment of AD may be a consequence of their actions on PPAR $\gamma$  rather than on their canonical targets the cyclooxygenases<sup>[10]</sup>. Deposition of  $\beta$ -amyloid fibrils within the brain subsequently, there is a phenotypic activation of microglial cells associated with the amyloid plaque. Microglia activation results in a complex local proinflammatory response and secretion of inflammatory products. Several epidemiological studies have indicated that patients taking NSAIDs for other diseases (e.g., rheumatoid arthritis) have a 50% lower risk of developing AD than those not taking NSAIDs<sup>[11]</sup>.

## 2. Materials and Methods

### 2.1. Animals

Wistar albino rats (150-175gms) are used to study AD activity and antioxidant activity. The animals were procured from Sanzyme Pharma Ltd, Hyderabad and housed into group of six animals per cage maintained at 24°C $\pm$ 1°C with relative humidity 45-55% and 12:12 hour's dark and light cycle. The animals had free access to food (standard chow pellets) and water *ad libitum*. All the experimental procedures were approved by IAEC (IAEC NO:

1047/ac/07/CPCSEA), Vaagdevi college of Pharmacy, Warangal, A.P., India.

## 2.2. Drugs

Lornoxicam was purchased from Sigma Aldrich Ltd, Selegiline is a gift sample from Intus pharmaceutical Ltd, Scopolamine purchased from Cadila health care Ltd, Donepezil purchased from Alkem laboratories Ltd.

## 2.3. Experimental Design

Animals (36) were weighed, kept in cages accordingly and randomly divided into 6 groups (n=6). Drugs were prepared freshly and given daily for 8 days. On day 1, the training sessions for all the animals were given. Drugs were administered, after 1 hr the retention was calculated. This is followed for consecutive 3, 5, 7, 9 days<sup>[8]</sup>.

Groups	Treatment Groups	Test Drugs
Group-I	Normal control	Vehicle (0.1% CMC)
Group-II	Disease control	Scopolamine(1.4mg/kg)i.p
Group-III	Standard	Donepezil(5mg/kg) <sup>[21]</sup> p.o + Scopolamine(1.4mg/kg)i.p
Group-IV	Test-I	Lornoxicam(1.3mg/kg) p.o+Scopolamine(1.4mg/kg)i.p
Group-V	Test-II	Selegiline(0.49mg/kg) p.o + Scopolamine(1.4mg/kg)i.p
Group-VI	Test-III	Lornoxicam(1.3mg/kg) p.o + Selegiline (0.49mg/kg) p.o + Scopolamine(1.4mg/kg)i.p

CMC (Carboxy Methyl Cellulose), i.p (Intraperitoneal), p.o (Per oral)

Memory impairment was induced by Scopolamine (1.4 mg/kg) i.p and 30min after administration of each drug. Scopolamine, Lornoxicam was dissolved in 0.1 % (w/v) CMC for injection. Donepezil, Selegiline were dissolved in 0.1 % (w/v) solution. All drugs were prepared fresh daily. Doses were given accordingly to the respective rat weight.

## 2.4. In vivo Methods (Behavioral Tests)

### 2.4.1. Rectangular Maze Test

Assessment of learning and memory can be effectively done by this method. The maze consists of completely enclosed rectangular box with an entry and reward chamber appended at

opposite ends. The box is partitioned with wooden slots into blind passages leaving just twisting corridor leading from the entry to the reward chamber. Animals were trained prior to the experiment by familiarizing with the rectangular maze for a period of 10 min for 2 h. Well-trained animals were taken for the experiment. Transfer latency (time taken to reach the reward chamber) was recorded. For each animal, four readings were taken and the average is taken as learning score (transfer latency) for that animal. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. The time taken by the animals to reach the reward chamber from the

entry chamber was noted on day 1, 3, 5, 7, and 9<sup>[8]</sup>.

#### 2.4 .2. Locomotor Activity Test

In order to detect the association of decreased activity in actophotometer with changes in motor activity, the locomotor activity was recorded for a period of 5 min using actophotometer on 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 9<sup>th</sup> day. Each animal was observed in a square (30 × 30 cm) closed arena equipped with infrared light sensitive photocells using digital actophotometer and locomotor activity was then expressed in terms of total photo beam counts for 5 min per animal. Animals were placed individually in the activity chamber for a 3 min habituation period before actual start of activity tasks. The apparatus was placed in a dark, light, sound attenuated and ventilated testing room, the activity is carried out after 30min of the drug administration<sup>[12]</sup>.

#### 2.5. In Vitro Methods

On day 9<sup>th</sup> following the behavioral testing, animals were sacrificed and the brain tissues were quickly removed, cleaned with ice cold saline and stored at -20°C for bio chemical estimations.

##### 2.5.1. Preparation of Brain Homogenate

Brain tissue samples were thawed homogenized 10 times (w/v) with ice cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates from rat brains were separated, centrifuged at 3000 rpm for 30min and the

supernatant was then used for biochemical estimation.

##### 2.5.2. Estimation of Cholinergic Status in the Rat Brain

Inhibition of acetyl cholinesterase activity of samples was measured by the micro plate assay. AChE inhibitory activity was measured by slightly modifying the spectrophotometric method from the literature which was initially developed by Ellman. Briefly, in this method, 100 µL of 0.1 mM sodium phosphate buffer (pH = 8.0), 20 µL of DTNB, 20 µL of test solution and 2 µL of AChE solution were added using a multichannel automatic pipette into a 96-well micro plate and incubated for 15 min at 25 °C. After incubation, acetylthiocholine iodide (100 µL of 0.05 mM water solution) was added as a substrate and AChE activity was determined by UV spectrophotometry from the absorbance changes at 412 nm for 3.0 min at 25 °C. The concentration of the compounds which caused 50% inhibition of the AChE activity (IC50) was calculated via nonlinear regression analysis<sup>[13]</sup>.

##### 2.5.3. DPPH Radical Scavenging Assay

DPPH radical scavenging effect was carried out according to the method first employed by Blois. Compounds of different concentration were prepared in distilled ethanol (1ml of each solutions have different concentrations of distilled ethanol) 1ml of each compound solutions having different concentrations were taken in different test tubes, 4 ml of a 0.1m

ethanol Solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration and percent quenching of DPPH was calculated on the basis of the observed decrease in absorbance of the radical. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the

Formula: Radical scavenging activity (%) =  $[(A_0 - A_1 / A_0) \times 100]$

Where  $A_0$  is the absorbance of the control (blank, without compound) and  $A_1$  is the Absorbance of compound. The radical scavenging activity of ascorbic acid was also measured and compared with that of the different synthesized compound. For all the Compounds and standards half inhibition concentration ( $IC_{50}$ ) was calculated<sup>[14]</sup>.

#### 2.5.4. Hydrogen Peroxide Scavenging Assays (Catalase Activity):

Catalase activity was assessed by the method of Luck, wherein the breakdown of hydrogen peroxide is measured. Briefly, the assay mixture consisted of 3mL of  $H_2O_2$  phosphate buffer and 0.05mL of the supernatant of the tissue homogenate. The change in absorbance was

recorded for 2 minutes at 30-second interval at 240nm using UV spectrophotometer. The results were expressed as micromoles of  $H_2O_2$  decomposed per minute per mg protein<sup>[15]</sup>.

Formula: Scavenged percentage hydrogen peroxide =  $(A_0 - A_1 / A_0) \times 100$

Here,  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the presence of samples<sup>[22]</sup>.

#### 2.5.5. TBARS (Thiobarbituric Acid Reactive Substances) Assay

The tissue homogenate (0.5ml) was supplemented with 0.5ml of phosphate buffer and 1ml of 10% Trichloroacetic acid. The mixture was centrifuged at 3000rpm at 4°C for 10 min. the supernatants of the tissue homogenates were incubated with 1 ml of 0.8% w/v of the thiobarbituric acid at 100°C for 15 min. After a cooling period, TBARS concentration was spectrophotometrically determined at 532nm. The levels of lipid peroxides were expressed as nM of TBARS. Standard graph was plotted using TEP (1, 1, 3, 3-Tetra Ethoxy Propane)<sup>[8]</sup>.

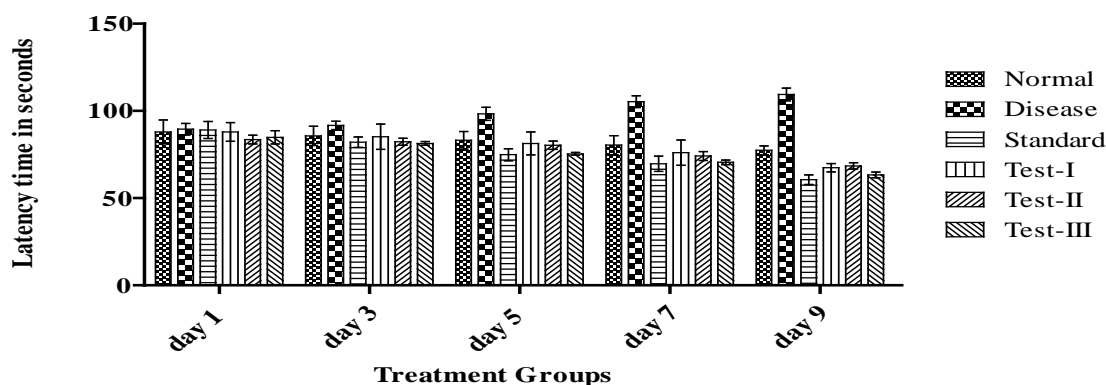
#### 2.5.6. Measurement of Reduced GSH

To measure the reduced glutathione (GSH) level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was added to equal volume of 20% TCA containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 2000 rpm. The supernatant was then

**Table 1.**Effect of test drugs on AchE levels in Scopolamine induced cognitive impaired rats.

S. No	Groups	% Inhibition of Enzyme
1	Normal	13.00±2.1 <sup>***</sup>
2	Disease	8.11±2.13
3	Standard	33.41±5.8 <sup>***</sup>
4	Test-I	24.53±6.9 <sup>***</sup>
5	Test-II	22.34±9.0 <sup>**</sup>
6	Test-III	28.91±6.33 <sup>***</sup>

Values are Mean ±SD, n=6 in each group. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  when compared with disease control group (Dunnet's test for multiple comparisons).

**Figure 1.** Effect of Test drugs on Neuroprotective activity by using Rectangular maze.

transferred to a new set of test tubes and 1.8 ml of the Ellman's reagent (DTNB), 0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution was added. The volume was made up to 2 ml in all the tubes. The absorbance was measured at 412 nm against blank. Absorbance values were determined from a standard curve of GSH<sup>[23]</sup>.

### 3. Results and Discussion

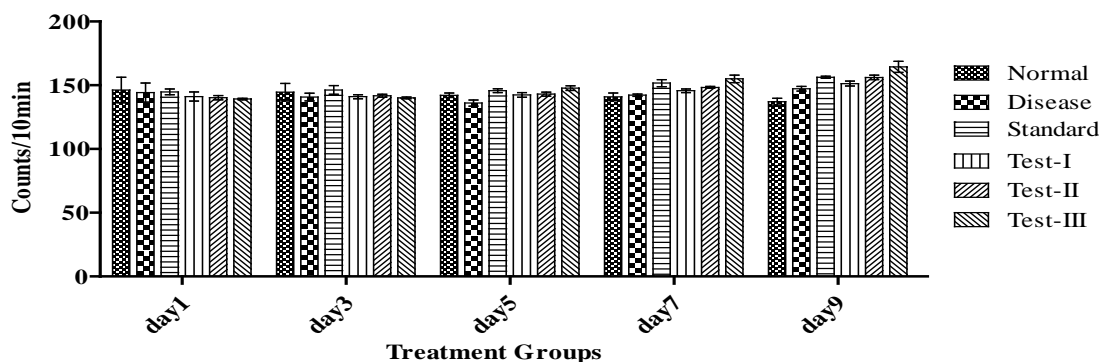
#### 3.1. Rectangular Maze

Lornoxicam, Selegiline and co-administration therapy showed significant ( $p < 0.01$ ) and standard shown significant ( $p < 0.001$ ) decrease in latency time on day 7 and on day 9, Lornoxicam, Selegiline, standard and co-administration therapy shown significant ( $p < 0.001$ ) decrease in latency time induced by scopolamine was significantly reversed by treatment group compared with scopolamine

**Table 2.**Effect of Test drugs on DPPH activity in Scopolamine induced Cognitive impaired rats.

S. No	Groups	% Inhibition of DPPH
1	Normal	65.32±0.01 <sup>***</sup>
2	Disease	20.00±0.025
3	Standard	72.00±0.035 <sup>***</sup>
4	Test-I	40.60±0.11 <sup>***</sup>
5	Test-II	42.30±0.1 <sup>***</sup>
6	Test-III	51.00±0.024 <sup>***</sup>

Values are Mean ±SD, n=6 in each group. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 when compared with disease control group (Dunnet’s test for multiple comparisons).



**Figure 2.** Effect of Test drugs on locomotor activity using Actophotometer.

group and co-administration therapy showed significantly higher efficacy than standard donepezil which was used as positive control (Figure 1).

### 3.2. Locomotor Activity

Lornoxicam and Selegiline co-administration therapy showed significant \*\*\**p*<0.001, \*\**p*<0.01, \**p*<0.05 as compared to disease control group. On day 7 and 9, shows significant increase in locomotor activity compared to disease control in Lornoxicam alone and co-administration with Selegiline (Figure 2).

### 3.3. AChE Estimation

Antioxidant activity has been expressed in percentage inhibition of AchE Enzyme activity. Scopolamine treatment decreased brain antioxidant activity significantly. Improvement of antioxidant activity was observed in standard and test groups compared to scopolamine treated groups. Co-administration of Lornoxicam and Selegiline has shown synergistic action in increasing antioxidant activity than Lornoxicam and Selegiline individually see Table 1.



**Table 3.** Effect of Test drugs on H<sub>2</sub>O<sub>2</sub> scavenging activity in Scopolamine induced Cognitive impaired rats.

S. No	Groups	%H <sub>2</sub> O <sub>2</sub> scavenging activity
1	Normal	77.9±0.043 <sup>***</sup>
2	Disease	33.14±0.056
3	Standard	78.78±0.10 <sup>***</sup>
4	Test-I	45.23±0.06 <sup>***</sup>
5	Test-II	50.23±0.07 <sup>***</sup>
6	Test-III	74.90±0.10 <sup>***</sup>

Values are Mean ±SD, n=6 in each group. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 when compared with disease control group (Dunnet's test for multiple comparisons)

**Table 4.** Effect of Test drugs on MDA levels(nm/mg of tissue)in Scopolamine induced cognitive impaired rats.

S. No	Groups	MDA Levels(nm/mg of tissue)
1	Normal	7.15±1.1 <sup>***</sup>
2	Disease	9.43±0.96
3	Standard	6.46±0.56 <sup>***</sup>
4	Test-I	7.88±0.76 <sup>*</sup>
5	Test-II	7.53±1.21 <sup>**</sup>
6	Test-III	6.99±0.67 <sup>***</sup>

Values are Mean ±SD, n=6 in each group. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 when compared with disease control group (Dunnet's test for multiple comparisons).

### 3.4. DPPH Assay

Antioxidant activity has been expressed in percentage inhibition of DPPH activity. Scopolamine treatment decreased brain antioxidant activity significantly. Improvement of antioxidant activity was observed in standard and test groups compared to scopolamine treated groups. Co-administration of Lornoxicam and

Selegiline has shown synergistic action in increasing antioxidant activity than Lornoxicam and Selegiline individually see Table 2.

### 3.5. Catalase Activity

Catalase activity has been expressed in percentage hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity. Scopolamine treatment decreased brain

catalase activity significantly. Improvement of catalase activity was observed in standard and test drugs treated groups compared to scopolamine treated group. Co-administration of Lornoxicam and Selegiline has shown synergistic action in increasing catalase activity than Lornoxicam and Selegiline individually see Table 3.

3.6. TBARS Assay

Scopolamine treatment significantly increased the brain malondialdehyde (MDA) levels compared to control group. Standard drug donepezil and test drug (Lornoxicam and Selegiline) treatment significantly decreased brain MDA levels compared to scopolamine treated group. Co-administration of Lornoxicam and Selegiline has shown synergistic action in reducing MDA levels than Lornoxicam and Selegiline individually see Table 4.

3.7. Estimation of GSH

Scopolamine treatment significantly

decreased the brain glutathione (GSH) levels compared to control group. Standard drug donepezil and test drug (Lornoxicam and Selegiline) treatment significantly decreased brain GSH levels compared to scopolamine treated group. Co-administration of Lornoxicam and Selegiline has shown synergistic action in increasing GSH levels than Lornoxicam and Selegiline individually see Table 5.

The correlation between the decrease in cholinergic markers and the cognitive decline in dementia may not be clear as assumed. Taking into account the results of the different fields of research, Ach plays a pivotal role in learning and memory processes seems to be overstated. Even when the role of other neurotransmitter systems in learning memory is taken into consideration, it is unlikely that Ach has a specific role in these processes. On basis of the available data, Ach seems to be more specifically involved in attentional processes than in learning and memory processes<sup>[16]</sup>.

Central cholinergic muscarinic receptors

**Table 5.** Effect of Test drugs on GSH Levels (nm/mg of tissue)in Scopolamine induced Cognitive impaired rats.

S. No	Groups	Increased GSH levels
1	Normal	1.50±0.08***
2	Disease	0.34±0.12
3	Standard	1.05±0.09***
4	Test-I	0.95±0.03***
5	Test-II	0.93±0.05***
6	Test-III	1.04±0.025***

blockade produces profound cognitive impairments in human and animal subjects. Loss of cholinergic neurons and subsequent deficits in cholinergic neurotransmission in the hippocampus and cerebral cortex is strongly correlated with clinical signs of cognitive impairment and dementia in AD patients. The effects of cholinergic drugs and cholinergic receptor antagonists on learning and memory tasks have been investigated. The most commonly used model is based on the finding that scopolamine, a muscarinic receptor antagonist, induces amnesia in healthy subjects comparable with that in old, untreated subjects. These deficits may be reversed by AchE inhibitors. Compounds that reverse this scopolamine induced deficits in experimental animals, may be considered as potential drugs to treat cognitive impairment<sup>[17]</sup>. In animals exposed to conditioned fear, scopolamine treatment (1.4 mg/kg) elevated brain MDA while reducing GSH levels<sup>[18]</sup>. Therefore, scopolamine was considered as reliable tool to study Neuroprotective effect of candidate molecule. The present study investigated the effect of Lornoxicam and Selegiline drugs in the prevention of cognitive impairment and dementia Alzheimer's type using scopolamine induced rats.

Deprenyl (Selegiline) is an irreversible monoamine-oxidase B (MAO-B) inhibitor which has antioxidant and Neuroprotective effects. Deprenyl is known to upregulate activities of antioxidant enzymes in

rat brain. Deprenyl is used for treatment of Parkinson's disease because of these effects and can attenuate the progressive degeneration of nigro-striatal dopaminergic neurons during aging and neurodegenerative disorders. Deprenyl may also improve age-related cognitive deficits in aged rats<sup>[19]</sup>. This may characterize the scopolamine effects of progressive deterioration of learning and memory, oxidative stress and decreased acetylcholine turnover.

Current treatment approaches in AD are primarily symptomatic, with the major therapeutic strategy based on acetylcholine esterase inhibition. AD research should advance over ensuing decade(s) to yield better symptomatic therapies, drugs designed to slow the rate of progression, and disease preventing agents. The next generation cholinergic agents will include long acting cholinesterase inhibitors with a good safety profile and brain specific muscarinic agonists. The most critical advances in AD treatment, however, will target slowing of disease progression and prevention of dementia. Therapeutic agents are being developed that interfere with the synthesis, deposition and aggregation of  $\beta$ -amyloid protein. In addition, estrogen, anti-inflammatory agents (e.g. cyclooxygenase inhibitors) and antioxidant approaches (e.g. vitamin E) are currently being proposed or utilized in disease prevention trials<sup>[20]</sup>.

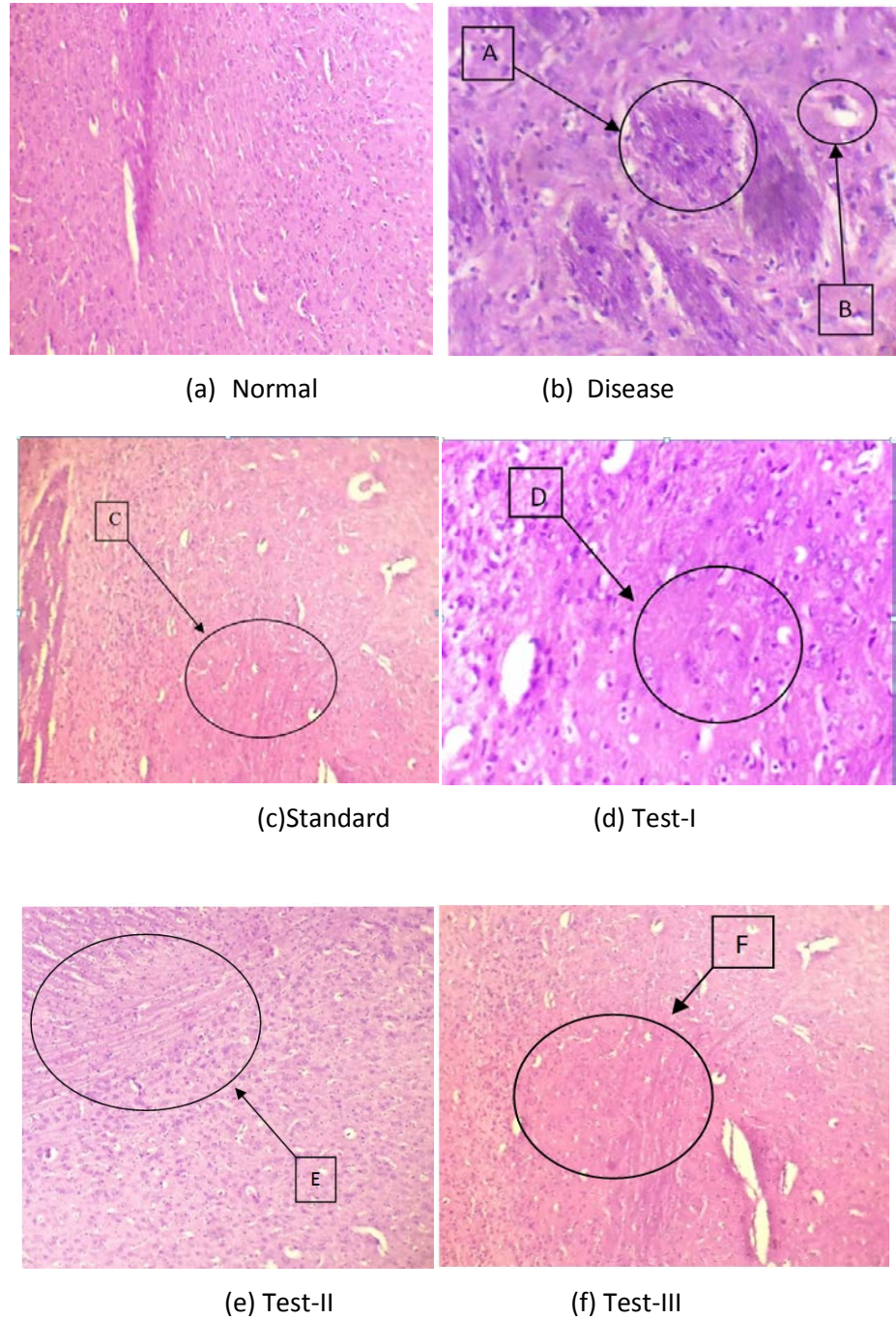
COX-1 is distributed in neurons throughout the brain, but it is most prevalent in forebrain, where PGs may be involved in complex

integrative functions, such as modulation of the autonomic nervous system and sensory processing. COX-2 is expressed constitutively in only a few organs and one of those is the brain. This expression is restricted to certain parts of the CNS, notably the cortex, hippocampus, hypothalamus and spinal cord. It is the predominant isoform in the brains of neonate pigs and in the spinal cord of the rat, while human brain tissues contain equal amounts of mRNA for COX-1 and COX-2. The most interesting feature of COX-2 in the CNS is that the enzyme is upregulated by normal or by abnormal (convulsive) nerve activity. The major PGs in the CNS of most mammalian species including humans, monkeys and rats are PGE<sub>2</sub> and PGD<sub>2</sub><sup>[21]</sup>.

The possible implication of COX-1 in AD is further substantiated by the Alzheimer's disease Cooperative Study (ADCS). Multicenter clinical trial found that a repressor of COX-2 expression, prednisone neither prevented nor accelerated cognitive decline in AD, although interpretation of these data is complex because glucocorticoids are fairly nonspecific and affect many other pathways. Nevertheless, the ADCS has initiated a trial to compare a nonselective NSAID and a selective COX-2 inhibitor for effectiveness in slowing the rate of cognitive decline in AD. Indomethacin showed promising results in a pilot clinical trial. Whether COX-2 inhibitors will be more effective is uncertain, since the enzyme is constitutively expressed in neurons and may play some role in normal brain

function. Animal experiments suggest that COX-2 may be responsible for the regulation of adaptive functions associated with normal neurons and protective functions associated with stressed neurons. Other mechanisms for NSAID Neuroprotective potency unrelated to their ability to inhibit COX-1 or COX-2, such as inhibition of monocytes cytotoxicity, have been suggested based on in vitro neurotoxicity assays<sup>[9]</sup>.

Recently, many studies reported that memory impairment in the scopolamine-induced animal model is associated with increased oxidative stress within the brain. Oxidative stress is the cytotoxic consequence of oxyradical and oxidant formation and the reaction with cellular constituents. Reactive oxidative species (ROS) are generated continuously in nervous system during normal metabolism and neuronal activity. Because the brain has a high consumption of oxygen, large amount of polyunsaturated fatty acids (PUFAs), high contents of free ions and low levels of antioxidants defense were compared to other organs. Increased MDA level as one of the ROS has been shown to be an important marker for in vivo lipid peroxidation. From the behavioral test, rectangular maze test it is clearly seen that there was a decrease in the transfer latency in all treated groups compared to the scopolamine treated group. The memory loss due to scopolamine is more prominent when compared with the control group. In comparison with Donepezil, the drugtreated groups had shown almost equal performance which



**Figure 3.** Histopathological analysis of Neuroprotective effect of Lornoxicam, Selegiline and co- administration in Wistar rats brain.

indicates synergistic effect of Lornoxicam and Selegiline against memory loss. Meanwhile locomotor activity test is done which also

indicates the leaning ability. The major antioxidant and oxidative free radical scavenging enzymes like DPPH,GSH and

Catalase play an important role to reduce oxidative stress in brain. In this study, from the rugs. In the present study rats after scopolamine treatment showed a significant increase in the brain levels of malondialdehyde, which is the measure of lipid peroxidation and free radical generation. In the drug treated groups, there is a significant decrease in the levels of malondialdehyde which is nearly equal to the standard group. From the results, it is clear that the anti-inflammatory activity of Lornoxicam decreases the disease progression. The antioxidant activity of Selegiline is clear from

the biochemical tests, which includes the estimation of antioxidant enzymes<sup>[8]</sup>DPPH assay antioxidant levels were estimated in which the enzyme levels are decreased in the scopolamine treated group when compared with the control group. The enzyme levels are almost equal in combination group and the standard group. It supports the antioxidant action of

### 3.8. Histopathology

Histopathological analysis of Neuroprotective effect of Lornoxicam, Selegiline and co administration of Selegiline and Lornoxicam on Wistar albino rat's brain, A) Amyloid plaques B) Lewy bodies D) Protective area of Donepezil E) Protective area of Selegiline E) Protective area of Lornoxicam F) Protective area of co administration of Selegiline and Lornoxicam.

Histopathological analysis of Neuroprotective effect of Lornoxicam,

Selegiline and co- administration in Wistar rats brain, (a) Negative control. The, neuronal cells were found that normal architecture without any damage due to normal conditions. (b) Disease control, the neuronal cells were found that abnormal damaged due to induction of Neurodegeneration with Scopolamine (A) Neurofibrillary Tangles (B) Amyloid plaques (c) Standard, (C) the neuronal cells were found that normal cell structure without any damage, infiltration and inflamed cells also, it may be reduced the Neurodegeneration of scopolamine due to the retaining of damaged neuronal cells to normal cells. (d) Test-I, (D) Neuronal cells were found with less damaged, it may be reduced the Neurodegeneration of scopolamine due to the retaining of damaged neuronal cells to normal cells. (e) Test-II, (E) Neuronal cells were found with less damaged, it may be reduced the Neurodegeneration of scopolamine due to the retaining of damaged neuronal cells to normal cells. (f) Test-III, (F) Neuronal cells were found without any damage, infiltration and inflamed cells also, it may be reduced the Neurodegeneration of Scopolamine due to retaining of damaged Neuronal cells to normal cells see Figure 3.

### 4. Conclusion

The present study showed reduction in TBARS levels, AchE levels and GSH and Catalase levels were elevated. From the present study, it is evident that administration of Lornoxicam, Selegiline and co administration of

Lornoxicam and Selegiline proved Neuroprotective activity and free radical scavenging activity.

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