



Role of tamoxifen in Alzheimer Disease against Female Rats

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Abstract:

Objective: Selective estrogen Receptor modulators (SERM) are the class of drugs with mixed estrogen agonist-antagonist effects and have been proposed to be next generation of alternatives to traditional oestrogen replacement therapy, ideal SERM would exert estrogen agonist in the bone and in brain while simultaneously acting as an antagonist in tissue such as breast and uterus. This experiment was planned to explore the outcome of comparative effects of various estrogen receptor modulator Tamoxifen against Alzheimer disease in Wister female rats.

Materials and methods: Doses of Rivastigmine, Tamoxifen were selected based on literature survey, Alzheimer was induced by giving scopolamine 3 mg/kg (i.p). To assess the spatial memory and cognitive impairment in rats, behavioural models such as Morris Water Maze test are used.

Result: 30 days prior administration of different estrogen Receptor Modulators (ERM) i.e. Tamoxifen to female rats successfully reversed the oxidative stress, in increased the level of GSH induced by scopolamine and decreased the nitric oxide and acetylcholinesterase activity also increased the oestrogen level in serum of female rats. These effects associated enhancement of performance in behavioural models such as Morri's water maze and novel object recognition test.

Conclusion: The present study stresses the neuroprotective potential of estrogen receptor modulators; this finding contribute to the improvement of treatment in neurodegenerative disorder of Alzheimer type

Key word: Estrogen receptor modulators, Tamoxifen, Alzheimer, Scopolamine

1. Introduction

Alzheimer's disease (AD) is most commonly referred to as dementia a disease that's caused due to the damage of memory cells permanently, it causes the damage of nerve cells and finally death of nerve cells resulting in severe atrophy of frontal, temporal and parietal cortical areas which results in memory loss in AD. It Affects the routine life tasks such as reading, speaking, or writing. In last stages of Alzheimer's Disease face patient may cause more severe effects which include, respiratory system dysfunction and heart failure Which cause leading to death [1]. The main histopathological marks of AD include the extracellular accumulation of the amyloid-beta ($A\beta$) as core proteins in senile plaques and formation of neurofibrillary tangles (NFTs) by hyperphosphorylated tau protein.[2] Dementia is a syndrome which are characterized by the progressive

decline of mental functions. In addition to cognitive impairment, at least 90% of patients exhibit various neuropsychiatric or behavioural and psychological symptoms of dementia (BPSD) at any given point in the duration of their illness. BPSD can be defined as a wider range of non-cognitive symptoms involving perception (e.g., hallucinations), mood (e.g., depression, anxiety), behaviour (e.g., aggression, disinhibition), personality, and basic functioning [3]. The major risk factor included the psychosocial risk factors of Alzheimer's disease in Down syndrome such as cognitive status, educational level, social standing, employment, physical activity, psychosocial activity, ethnic group, sleep, and smoking habits, and other risk factors as age, sex, and genetic factors (APOE) which have a synergistic effect for the development of dementia.[4] major biochemical parameters include in AD are oxidative stress takes lead as the main trigger for AD leading to increased levels of Reactive Oxygen Species (ROS) and decreased energy supplement[5]. The most prevalent antioxidant in the brain, glutathione, is found in millimolar concentrations in most cells. The most abundant antioxidant, glutathione, plays a significant role in combating oxidative stress. The ratio of oxidized to reduced glutathione is utilized as a measure of intensity of oxidative stress level of glutathione. Glutathione level was reduced in AD [6]. In AD reduced acetylcholinesterase activity in plasma is observed.[7] In brain Although both men and women are able to develop AD, women have been proven to be more susceptible toward the AD, On the other hand, men begin exhibiting signs of lucidity before women. women are slower to show the side effects of AD, like mental decline, compared to men. the disease grows faster after menopause of AD in women. increasing age is also major factor for developing AD. [8] For the development and maintenance of neuron networks in the brain, epigenetic mechanisms are necessary, as indicated by recent findings. This includes some of the high-order brain processes, such as behaviour and cognitive functions. Epigenetic mechanisms could influence the pathophysiology or etiology of some neuronal diseases, altering disease susceptibility and therapy responses. Recent studies support epigenetic dysfunctions in neurodegenerative and psychiatric conditions, such as Alzheimer's disease (AD)[9] As the estrogen receptor present in the brain Recently, there has been a growing interest in the functions of selective estrogen receptor modulators (SERMs) Tamoxifen in the brain, particularly in whether they are neuroprotective for such neurodegenerative conditions as stroke, Alzheimer disease, and Parkinson disease. Estrogen receptor modulators antagonist activity in the breast and uterus and agonist activity in the cardiovascular system, bone, and brain. estrogen replacement therapy may also have beneficial neurotrophic and neuroprotective effects on the brain. the tamoxifen a estrogen receptor modulator the protective effect of tamoxifen was independent of cerebral blood flow changes, indicating a potential direct neuroprotective effect of this SERM in the brain. tamoxifen increased synaptic density in the hippocampus of OVX rats in vitro studies Several mechanisms have been proposed to explain how 17 β -E2 and SERMs may protect the brain. These proposals include 1) a genomic estrogen receptor (ER)-mediated mechanism. SERMs such as tamoxifen may have unrecognized but potentially clinically important neuroprotective and neurotrophic effects on the brain.[10] Hence, the goal of this study is to assess the state of the field of SERM neuroprotective action.

2. Materials and method:

Procurement of Experimental Animals:

All the experiments were carried out with Wistar Albino Female Rats of 150–250 g approved by CPCSEA, registration No. and date of registration: 535/02/a/cpcsea/jan.2002. The animals were housed in polypropylene cages at a temperature of $24 \pm 2^\circ \text{C}$ with relative humidity of 40-60% and 12 hours light dark cycle. The Experiment was conducted in a noise free environment between 10:00 am and 02:00 noon. Animals were fed with a balanced diet and water *ad libitum* during the complete experimental period. All the animals were acclimatized for seven days before start of the experimental studies.

Chemicals:

Rivastigmine was procured from Ajanta Pharma, Pune.

tamoxifen was procured from Dexter Formulations, Chennai.

Scopolamine was procured from Sigma Aldrich, USA.

All other chemicals used for experimental purpose were of Analytical Grade.

Preparation of Doses:

- Dose of Tamoxifen were selected based on literature survey.
- Rivastigmine was dissolved in distilled water given per orally (p.o).
- Tamoxifen was dissolved in distilled water given per orally (p.o).
- Scopolamine (3 mg/kg) dissolved in distilled water, administered intraperitoneally (i.p).

Instruments and models:

Morris water Maze: The water maze was a black circular tank (136 cm) in diameter and 60 cm in height. The tank was filled with water ($20\pm 2^\circ\text{C}$) to a depth of 25 cm and water was made opaque with titanium dioxide. Apparatus was located in a room containing several extra maze cues. The escape platform used for the spatial task was submerged at a depth of 1 cm of water surface. Rats received one training block consisting of four trials in a day and were tested on four consecutive days. A trial was started by placing the rat in the pool facing the wall in one of four quadrants delineated by marks at the four cardinal directions. Rats were allowed to swim to the hidden platform and the escape latency (time to find the hidden platform) were recorded.[11] the rats were guided to the platform for 30 seconds if they did not find the platform within 120 seconds.[12] Two days prior to the commencement of training, rats were placed in the pool with no platform for 1 min habituation trial. For each rat, the platform position remained constant throughout the four training days. Escape latency was recorded at each trial using a stop watch, Moreover, the same groups of rats were subjected to retrieval or probe test at 24 hours and 30th day time points following acquisition protocol. Herein the platform was removed and the time spent by rats in the target quadrant, wherein the platform was previously placed, was measured.[13]

Determination of Reduced Glutathione:

Procedure:

The GSH levels were estimated by the method described by Moron *et al.*^[14] Briefly, 0.5 ml of plasma was precipitated with 5% TCA and the precipitate was removed by centrifugation. To an aliquot of the supernatant, 2 ml of 5-5'- Dithiobis, 2-nitrobenzoic acid (DTNB) reagent was added to make the final volume three millilitre. Absorbance was read at 412 nm against a blank containing TCA instead of the sample. The amount of reduced glutathione was expressed as $\mu\text{mol/ml}$ of plasma.

Determination of Nitric Oxide:

The Nitric oxide levels were estimated by the method given by Smita *et al.*^[15]

0.1 ml of plasma was precipitated with 5% TCA and the precipitate was removed by centrifugation. To the supernatant obtained, 100 μl of Griess Reagent, 300 μl of the nitrite-containing sample and 2.6 ml of deionized water were added. Incubated the mixture for 30 min at room temperature. Absorbance was taken at 548 nm against blank containing Griess reagent and deionized water.

Preparation of Griess reagent:

Mix together equal volumes of *N*-(1-naphthyl) ethylenediamine (Component A) and sulfanillic acid (Component B) to form the Griess Reagen

Determination of Acetylcholinesterase:

Procedure:

The Acetylcholinesterase levels were estimated as per the method described by Biradar *et al.*^[16] Briefly, to each test tube, 0.4 ml of supernatant (after plasma treatment), 2.6 ml phosphate buffer (0.1 M, pH 8) & 100 μ l of 5, 5'-dithiobis (2-nitro benzoic acid). The contents of the cuvette are mixed thoroughly absorbance is measured at 412 nm. When absorbance reaches a stable value, it is recorded as the basal reading. 20 μ l of substrate i.e., acetylthiocholine is added and change in absorbance is recorded for a period of 10 min at intervals of 2 min. Change in the absorbance per min is thus determined. Results expressed as nmol/min/g of plasma.

Experimental Design: The female rats were divided into different groups for employing behavioural memory model. Each group consist of six animals. In Normal animals received Distilled Water in the dose of 10 ml/kg per orally. Rivastigmine (1 mg/kg), Tamoxifen (3 mg/kg) were administered per orally for 30 successive days to different groups of female rats. After 90 minutes of the administration of the last dose (on 30th day), amnesia was induced in the female rats by injecting scopolamine (3 mg/kg, i.p). Female rats were exposed to the training session using Morris Water maze. Biochemical parameters estimation is done.

Short term memory was recorded after 24 h and long-term memory was recorded on 30th day in Morris Water Maze.

Table 1: Grouping of Female Rats.

Sr. No.	Groups	No of Animals	Dose
1.	Saline	6	10 ml/kg, p.o.
2.	Scopolamine only	6	3 mg/kg, i.p.
3.	Rivastigmine (Std)	6	1 mg/kg, p.o.
4.	17 β estradiol	6	0.1 mg/kg, p.o.
5.	Raloxifene	6	3 mg/kg, p.o.
6.	Tamoxifen	6	3 mg/kg, p.o.

Statistical Analysis:

Data were analysed using Graph Pad Prism 5 for Windows (version 6.01). Results were expressed as Mean \pm SEM. One-way analysis of variance (ANOVA) and Dunnett's T test were used to test the significance of the difference between the variables in various groups. The P values of less than 0.05 were considered to be statistically significant.

Result:

Morris Water Maze:

6.1.1 Escape latency in Morris Water Maze (After 24 Hours):

Escape latency in Morris Water Maze (After 24 Hours):

Table No. 2 Effect of ERM on escape latency after 24 Hours of Scopolamine in MWM

Sr. No	Groups	Dose	Escape latency (sec)				
			Day 1	Day 2	Day 3	Day 4	After Scopolamine
1	Saline	10 ml/kg p.o.	90.7 ±1.820	75.83 ±1.167	45.83 ±1.990	30.83 ±2.151	25.00 ±1.633
2	Control (Scopolamine Only)	3 mg/ kg i.p.	90.7 ±1.801	89.67 ±1.782	47.33 ±1.892	31.17 ±2.242	55.50 ±1.839 [#]
3	Rivastigmine + Scopolamine	1 mg/kg p.o	101.0 ±2.160	80.17 ±1.922	38.67 ±2.216	25.33 ±1.430	39.33 ±2.275 ***
4	Tamoxifen+ Scopolamine	3 mg/kg p.o.	91.5 ±1.668	89.67 ±3.403	68.67 ±1.892	23.50 ±1.432	42.17 ±3.331 **

Each group consists of 6 animals (n=6). Values are Mean ± S.E.M. [#]P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

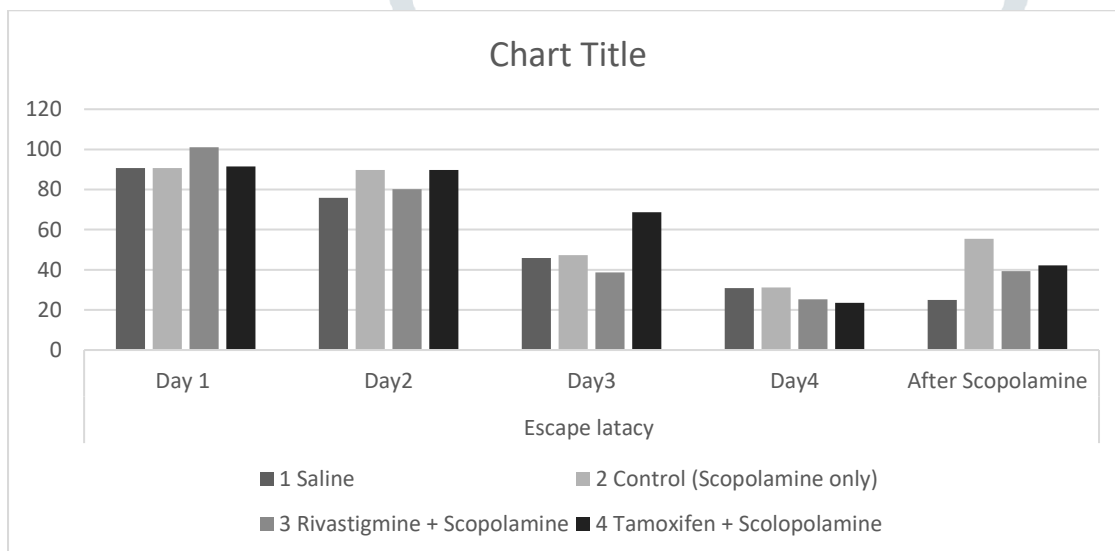


Fig. No. 1; 2 Effect of ERM on escape latency after 24 Hours of Scopolamine in MWM

Each group consists of 6 animals (n=6). Values are Mean ± S.E.M. [#]P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

Time spent in target quadrant in Morris Water Maze (After 24 Hours):

Table No. 3 Effect of ERM on retrieval memory after 24 Hours of Scopolamine in MWM

Sr. No	Groups	Dose	Time Spent in Target Quadrant (sec)
1	Saline	10 ml/kg p.o.	35.17 ± 1.633
2	Control (Scopolamine Only)	3 mg/ kg i.p.	22.67 ± 1.128 [#]

3	Rivastigmine + Scopolamine	1 mg/kg p.o	33.08 ± 1.177***
4	Tamoxifen + Scopolamine	3 mg/kg p.o.	30.40 ± 1.083 **

Each group consists of 6 animals (n=6). Values are Mean ± S.E.M. #P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

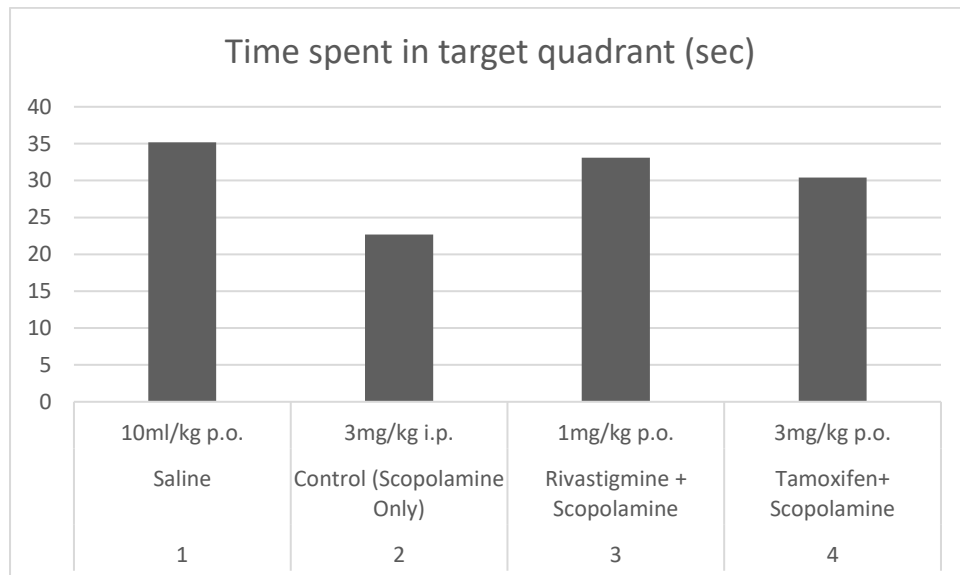


Fig. No. 2; Effect of ERM on retrieval memory after 24 Hours of Scopolamine in MWM

Each group consists of 6 animals (n=6). Values are Mean ± S.E.M. #P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

Table No. 4 Effect of ERM on retrieval memory in on 30th Day after Scopolamine in MWM

Sr. No	Groups	Dose	Time Spent in Target Quadrant (sec)
1	Saline	10 ml/kg p.o.	24.78 ± 0.873
2	Control (Scopolamine Only)	3 mg/ kg i.p.	16.65 ± 1.115#
3	Rivastigmine + Scopolamine	1 mg/kg p.o.	21.22 ± 0.663*
4	Tamoxifen + Scopolamine	3 mg/kg p.o.	18.83 ± 0.991**

Each group consists of 6 animals (n=6). Values are Mean ± S.E.M. #P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

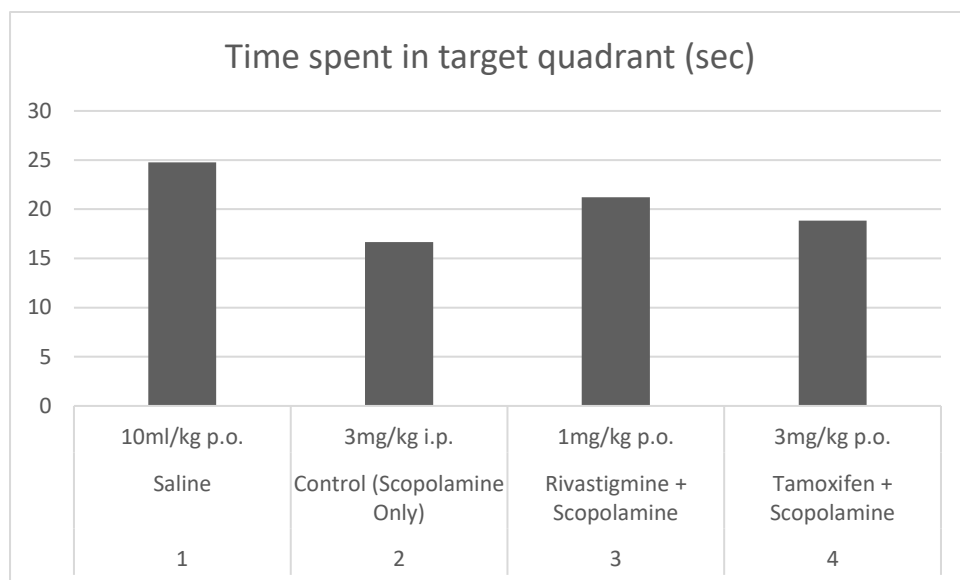


Fig. No. 3; Effect of ERM on retrieval memory in on 30th Day after Scopolamine in MWM

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P < 0.001 as compared to Saline group. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to Scopolamine group.

Biochemical Parameters Estimation:

Determination of Reduced Glutathione (GSH) in rats.

Table No. 5 Effect of ERM Treatment on the Level of Reduced Glutathione in plasma of Scopolamine treated Groups

Sr. No	Groups	Dose	GSH μ mol/ml of plasma
1.	Saline	10 ml/kg, p.o.	1.259 \pm 0.097
2.	Control (Scopolamine)	3 mg/kg, i.p	0.640 \pm 0.093 [#]
3.	Scopolamine + Rivastigmine	1 mg/kg, p.o.	1.471 \pm 0.080***
6.	Scopolamine + Tamoxifen	3 mg/kg, p.o.	1.100 \pm 0.052**

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P < 0.001 as compared to Saline group. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to Scopolamine group.

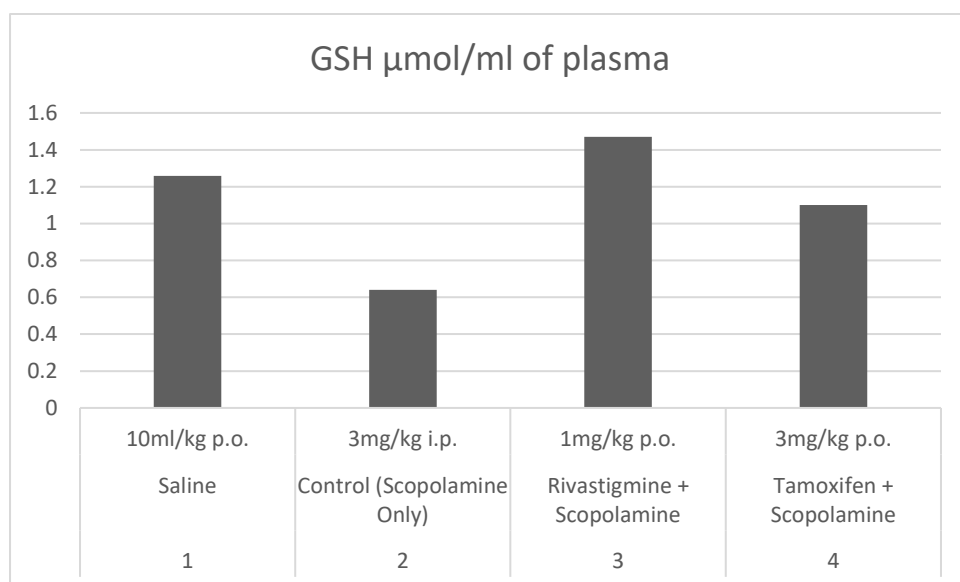


Fig. No. 4; Effect of ERM Treatment on the Level of Reduced Glutathione in plasma of Scopolamine treated Groups

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

Determination of Nitric oxide (NO) activity in rats:

Table No. 6 Effect of ERM Treatment on the Level of Nitric oxide (NO) in plasma of Scopolamine treated Groups

Sr. No.	Groups	Dose	NO $\mu\text{mol/ml}$ of plasma
1.	Saline	10 ml/kg, p.o.	0.1418 \pm 0.0055
2.	Control (Scopolamine)	3 mg/kg, i.p	0.1778 \pm 0.0050 [#]
3.	Scopolamine + Rivastigmine	1 mg/kg, p.o.	0.1342 \pm 0.0050 ^{**}
4.	Scopolamine + Tamoxifen	3 mg/kg, p.o.	0.1300 \pm 0.0039 [*]

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

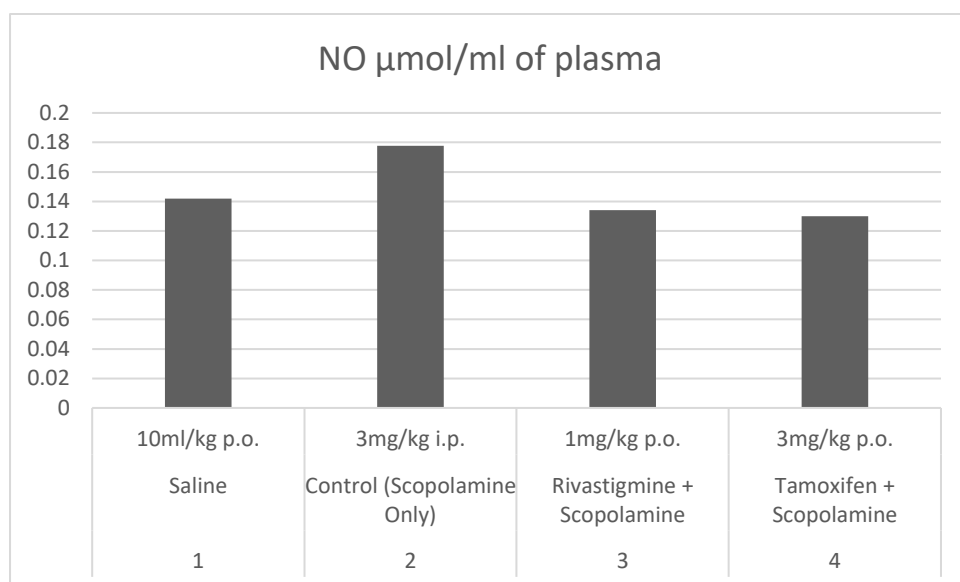


Fig. No. 5; Effect of ERM Treatment on the Level of Nitric oxide (NO) in plasma of Scopolamine treated Groups

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P < 0.001 as compared to Saline group. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to Scopolamine group.

Determination of acetylcholinesterase (AChE) activity in rats:

Table No. 7 Effect of ERM Treatment on the acetylcholinesterase (AChE) activity in plasma of Scopolamine treated Groups

Sr. No.	Groups	Dose	AChE $\mu\text{mol/ml}$ of plasma
1.	Saline	10 ml/kg, p.o.	21.75 \pm 1.245
2.	Control (Scopolamine)	3 mg/kg, i.p	38.83 \pm 1.089 [#]
3.	Scopolamine + Raloxifene	3 mg/kg, p.o.	33.03 \pm 1.147*
4.	Scopolamine + Tamoxifen	3 mg/kg, p.o.	30.28 \pm 1.131 ^{ns}

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P < 0.001 as compared to Saline group. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to Scopolamine group.

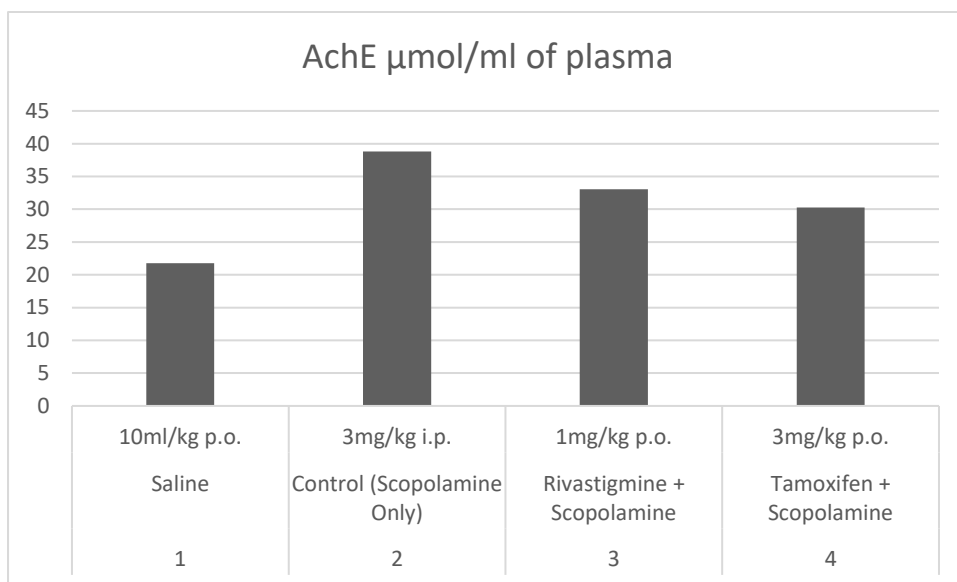


Fig. No. 6; Effect of ERM Treatment on the acetylcholinesterase (AchE) activity in plasma of Scopolamine treated Groups

Each group consists of 6 animals (n=6). Values are Mean \pm S.E.M. #P< 0.001 as compared to Saline group. *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Scopolamine group.

Result:

Table 2 shows the effect on time to find the hidden platform (escape latency) in Morris Water Maze during training sessions (Day 1-4) and 24 hours after scopolamine. Scopolamine treatment significantly increased the escape latency (#P<0.001) as compared to Saline after 24 hours of training in Morris Water Maze. There was significant decrease in escape latency by treatment with Rivastigmine (***P<0.001), tamoxifen (**P<0.01) as compared to scopolamine group.

Table 3 shows the effect of Tamoxifen on time spent in target quadrant without platform (retrieval memory) in Morris Water Maze after 24 hours of scopolamine. Scopolamine treatment significantly decreased the time spent in target quadrant (#P<0.001) as compared to Saline after 24 hours of training in Morris Water Maze. There was significant increase in time spent in target quadrant by treatment with Rivastigmine (***P<0.001), Tamoxifen (**P<0.01) as compared to scopolamine group.

Table 4 shows the effect of Tamoxifen on time spent in target quadrant without platform (retrieval memory) in Morris Water Maze after 30 Days of scopolamine. Scopolamine treatment significantly decreased the time spent in target quadrant (#P<0.001) as compared to Saline after 30 days of scopolamine in Morris Water Maze. There was significant increase in time spent in target quadrant by treatment with Rivastigmine (*P<0.05) as compared to scopolamine group. There was no significant increase in time spent in target quadrant by Tamoxifen as compared to scopolamine group.

Table 5 shows the effect of Tamoxifen on the level of Reduced Glutathione in plasma of rats. Scopolamine treatment significantly decreased the level of reduced glutathione in plasma as compared to Saline. There was significant decrease in the level of reduced glutathione by treatment with Rivastigmine ($***P<0.001$), and Tamoxifen ($**P<0.01$).

Table 6 shows the effect of Tamoxifen on the Nitric oxide activity in plasma of rats. Scopolamine treatment significantly increased in the Nitric oxide activity ($\#P<0.001$) as compared to Saline. Treatment with Tamoxifen ($**P<0.01$) and Rivastigmine ($**P<0.01$) significantly decreased the level of Nitric oxide activity in plasma as compared to scopolamine group.

Table 7 shows the effect of different Estrogen Receptor Modulators (ERM) on the Nitric oxide activity in plasma of rats. Scopolamine treatment significantly decreased in the Acetylcholinesterase activity ($\#P<0.001$) as compared to Saline. Treatment with Rivastigmine ($***P<0.001$), Tamoxifen ($*P<0.05$) significantly increased the level of acetylcholinesterase activity in plasma as compared to scopolamine group.

Discussion:

Alzheimer's disease is a severe neuron disease that damages brain cells which lead to permanent loss of memory also called dementia. Many people die due to this disease every year because this is not curable but early detection of this disease can help restrain the spread. Alzheimer's is most

common in elderly people in the age bracket of 65 and above [17] In AD, the dysregulation of the amyloid-beta ($A\beta$) level leads to the appearance of senile plaques which contain $A\beta$ depositions. $A\beta$ is a complex biological molecule which interacts with many types of receptors and/or forms insoluble assemblies and, eventually, its nonphysiologically depositions alternate with the normal neuronal conditions.[18] Oxidative stress has been associated with the onset and progression of mild cognitive impairment (MCI) and Alzheimer disease (AD). AD and MCI brain and plasma display extensive oxidative stress as indexed by protein oxidation, lipid peroxidation, free radical formation, DNA oxidation, and decreased antioxidants. The most abundant endogenous antioxidant, glutathione, plays a significant role in combating oxidative stress. The ratio of oxidized to reduced glutathione is utilized as a measure of intensity of oxidative stress. Antioxidants have long been considered as an approach to slow down AD progression. The most prevalent antioxidant in the brain, glutathione, is found in millimolar concentrations in most cells. Level of glutathione is reduced in AD. [19] Scopolamine model was used to memory impairment induced by a single injection of scopolamine at the dose of 3 mg/kg i.p. The behavioural tasks used to evaluate by Morris water maze apparatus and biochemical parameter estimation. Scopolamine which impairs short-term and long term-memory in animals and humans Through the interference with acetylcholine in the brain, scopolamine can cause oxidative stress leading to cognitive impairment Thus, scopolamine-induced memory impairment is a valid model for the evaluation of anti – amnesic effects of new drugs.[20] Many studies reveals that selective estrogen receptor modulators having neuroprotective potential, Tamoxifen was also recently shown to protect the striatum against 1-methyl-4-phenylpyridine-induced toxicity, suggesting that its protective abilities may extend to regions of the brain that are known to be affected in Parkinson disease. . Many studies suggest that SERMs can exert agonist effects in the brain and that clinically relevant SERMs such as tamoxifen and raloxifene may have heretofore unrecognized but potentially clinically important neuroprotective and neurotrophic effects on the brain.[21] In Present study 30 days prior treatment of tamoxifen in Wistar albino female rats show protective action by increasing reduced glutathione level reducing oxidative stress , significantly increased the level of acetylcholinesterase activity .also same effect in morris water maze test by reducing time escape latency and increasing time in target quadrant .hence tamoxifen may provide advance treatment against AD.

Conclusion:

In conclusion, 30 days prior administration of Estrogen Receptor Modulators (ERMs) i.e. Tamoxifen to female rats successfully reversed the oxidative stress, increased the level of GSH induced by scopolamine and decreased the nitric oxide and acetylcholinesterase activity. These effects were associated with the enhancement of performance in behavioural models such as Morris Water Maze (MWM).

Thus the present study stresses the neuroprotective potential of ERM with potent activity by antioxidant and anti-inflammatory mechanisms. These findings contribute to the improvement of treatment in conditions associated with neurodegenerative disorder of Alzheimer's type.

Thus Tamoxifen could be considered as supplementary agent in AD treatment.

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