Withania somnifera and Eclipta alba Ameliorate Oxidative Stress Induced Mitochondrial Dysfunction in an Animal Model of Alzheimer’s Disease

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ABSTRACT

Objective: To investigate the protective effects of the methanolic extract of Withania somnifera roots and Eclipta alba whole plant inameliorating oxidative damage and mitochondrial dysfunction in the rat brain.

Methods: The methanolic extracts of W. somnifera and E. alba were analyzed for their total phenolics and flavonoid content. In vitro antioxidant activity was evaluated by employing DPPH and ABTS radical scavenging assays. Anti-amnesic activity of methanolic extracts of W. somnifera and E. alba (50, 100 and 200 mg/kg, p. o.) after 8 days dosing was studied in comparison with the standard drug Donepezil hydrochloride and Piracetam treatment. On 8th day, 90 minutes after the administration of last dose, elevated plus maze was carried out and subsequently animals were sacrificed and brain homogenate was prepared to estimate lipid peroxidation and MTT reduction as current markers of antioxidant status and cell viability, respectively.

Results: The methanolic extracts showed high phenolic and flavonoid content and also showed comparable antioxidant activity with standard reference. Results of elevated plus maze demonstrated protection from memory deficit. MEW Sand MEEA at doses 100 and 200 mg/kg showed significant decrease in the transfer latency as compared to the toxicant and control group. MEWS and MEEA at 200mg/kg produced a reduction MDA content of 51.49±0.15 nmol/g tissue and 50.23±0.50 nmol/g tissue, respectively comparable to 47.96±0.06 nmol/g tissue of Donepezil hydrochloride 3 mg/kg. 200mg/kg dose of MEWS and MEEA were effective in increasing the reduction of MTT 72.01% and 71.59%, respectively comparable to 66.33% of Piracetam 200mg/kg.

Conclusion: Withania somnifera and Eclipta alba could be potential candidates demonstrating neuroprotective activity in oxidative stress.
INTRODUCTION

Oxidative stress has been implicated as an important factor in the process of neuro degeneration. Brain cells are particularly vulnerable to oxidative damage due to their high energy expenditure and oxygen demand. Cellular depletion of ATP in the CNS represents a physiopathologic event leading to a series of morphologic, biochemical and physiologic derangements involving energy deficit, reactive oxygen species (ROS) formation and oxidative stress. Indeed, ROS generation is an important mechanism accounting for cellular injury in many neurodegenerative disorders, affecting the structure and function of macromolecules such as membrane lipids, proteins and nucleic acids. Although cellular defense against ROS-mediated injury is provided by enzymatic (catalase, superoxide dismutase, glutathione peroxidase, etc.) and nonenzymatic (GSH, α-tocopherol, vitamin C, urate, etc.) radical scavenging systems, these systems are commonly susceptible of being affected and diminished under pathologic conditions. Recent overwhelming attention to plant products and alternative medicine has encouraged plant chemists, pharmacologists, biochemists, and molecular biologists to combine their efforts in search for natural agents that can limit ROS-mediated injuries.

Withania somnifera (DUN) (family Solanaceae) is highly reputed as “Indian ginseng” in Ayurvedic medicine, is noted for its beneficial effects on the nervous system. It is also used as a general energy-enhancing tonic known as Medharasayana, which means ‘that which promotes learning and a good memory.’ The roots have been reported to contain various chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannin.

The plant Eclipta alba (Linn) Hassk (family Asteraceae) has been mentioned in ancient texts to be a nervine tonic in addition to possessing hepatoprotective, hair growth promoting and anti-aging properties. The plant is reported to contain the phytoconstituent secalbatin, alpha-amyrin, ursolic acid, oleanolic acid, ecliptasaponin, daucosterol, stigmasterol-3-O-glucoside and coumestans as main active principles.

Scopolamine hydrobromide has been proved to impair memory acquisition and retrieval in humans and rodents. As cognitive dysfunction is a central feature of Alzheimer's disease, scopolamine induced memory impairment model in rats is considered to be a good model for Alzheimer's disease. It is reported that memory impairment in the scopolamine (1mg/kg, i.p.) induced animal model is associated with the increased oxidative stress within rat and mice brain. Scopolamine is known to affect the CNS by disrupting mitochondrial electron transport through the inhibition of succinate dehydrogenase, thus leading to deficit in cellular energy and neurodegenerative events in mammals. Lipid peroxidation is an important indicator of neuro degeneration in the brain. The subsequent peroxidation of the lipid in the mitochondrial membrane can further destroy the mitochondrial membrane and impair the functions of respiratory chain enzymes that reduced cellular ATP concentration. All these toxic features serve to propose scopolamine hydrobromide as a suitable model for investigating oxidative stress in the brain.
such as lipid peroxidation and mitochondrial dysfunction.

**MATERIALS AND METHODS**

**Plant material**

With aniasomnifera (DUN) dried roots and Ecliptaalba (Linn.) Hassk dried whole plant were collected from local market in Mumbai. The dried roots *withania somnifera* was identified and authenticated by Plant Science Division, Agharkar Research Institute, Pune where a voucher specimen (R-136) was deposited for reference to Plant Sciences Division. The dried whole plant of *Ecliptaalba* was identified and authenticated by Department of Life Science, RamnarainRuia College, Matunga, and Mumbai.

**Preparation of extracts**

The dried and powdered roots of *W. somnifera* (300g) were successively extracted with 1.5L of petroleum ether and methanol, in a soxhlet apparatus at 60-70°C each for 10-12h consecutively. Methanol was removed from the extract under vacuum and a semisolid mass was obtained. The yield of methanolic extract was (MEWS) was 10.55% (w/w).

The dried whole plant of *E. Alba* was powdered using a mechanical grinder and passed through 40-mesh sieve. Powder (300g) was successively extracted with 1.5L of petroleum ether and methanol, in a soxhlet apparatus at 60-70°C each for 10-12h consecutively. Solvents used were of analytical grade. Methanol was removed from the extract under vacuum and a semisolid mass was obtained. The yield of methanolic extract was (MEEA) was 11.75% (w/w). Both the extracts were stored in sterile amber colored storage vials in refrigerator until used for experimentation.

**Drug and Chemicals**

1, 1-diphenyl-2-picryl hydrazyl, ascorbic acid, azinobis (3-ethylbenzothiozoline-6-sulfonic acid) disodium salt, potassium persulphate, gallic acid, rutin, and [3,-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-tetrazolium bromide] (MTT) were obtained from Himedia (Mumbai, India), Sigma (St. Louis, USA). The marketed preparations of standard drugs Donepezil hydrochloride and Piracetam; Aricept and Cerecetam used to compare the activity of extracts were procured. All other chemicals and reagents used were of analytical grade.

**Selection of Animals**

Albino male rats (Wistar) weighing 150 to 200g were obtained from Haffkine Research Institute, Mumbai. All procedures were carried out in adherence to the guidelines of Institutional Animal Ethics Committee (CPCSEA/ IAEC/SPTM/P-47/2013). The animals were kept for at least 1 week before the experiment at optimum temperature (23±2°C) and humidity controlled (50-60%) animal rooms under 12:12h light/dark cycle with free access to food and water ad libitum.

**Determination of total phenolics and flavonoids content**

Drug and Chemicals

The total phenolic content was determined according to the method described by Siddhuraju and Becker14and the results were expressed as gallic acid equivalents (GAE). Total flavonoids in the extracts were estimated as rutin equivalent according to the method of Zhishen et al.15
In vitro antioxidant assays

Free radical scavenging activity on diphenyl-picrylhydrazyl radical (DPPH•)

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al.\textsuperscript{16} Plant extract 0.1 ml (concentration range 20-200 µg/ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated as [(A0–A1)/A0] × 100, Where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard. A blank is the absorbance of the control reaction (containing all reagents except the test compound). A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

ABTS radical cation scavenging activity

ABTS forms a relatively stable free radical, which decolorizes in its non-radical form\textsuperscript{17}. The spectrophotometric analysis of ABTS•+ radical scavenging capacity was determined according to the method of Re et al.\textsuperscript{18} ABTS•+ was produced by reacting 2 mM ABTS in H2O with 2.45 mM potassium persulfate (K2S2O8), stored in the dark at room temperature for 12 h. The ABTS•+ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer (pH 7.4). Then, 1 ml of ABTS•+ solution was added to 3 ml of extracts in ethanol at 20-200 µg/ml concentrations. The absorbance was recorded 30 min after mixing and the percentage of radical scavenging was calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance. For preparation of a standard curve, different concentrations of ABTS•+ were used. The scavenging capability of test compounds was calculated using the following equation:

\[
\text{% Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100,
\]

Where A0 is the absorbance of the control, and A1 is the absorbance in the presence of the sample of extracts or standards.

Animal groups and induction of oxidative stress

A total of 60 rats were randomly divided into ten groups: control group, toxicant group, two standard drug treated groups, three groups treated with HAWS and three groups treated with MEEA. Rats in the control and toxicant group were given 0.5% CMC (carboxymethyl cellulose) p.o.; the two standard drug treated groups were given Donepezil hydrochloride (3mg/kg, p.o.) and Piracetam (200mg/kg, p.o.), respectively; six groups treated with MEWS and MEEA were given the extracts at three doses 50, 100 and 200mg/kg, p.o., respectively. All the groups received the respective treatments for a period of 8 days. On 8th day, 90 minutes after the last dose, oxidative stress was induced in the animals by scopolamine hydrobromide (1mg/kg, i.p.). Scopolamine treatment was given to all the animals except the control group.

Alzheimer’s disease model (Evaluation of short term memory using Elevated plus maze)

It is an exteroceptive behavioural model to evaluate short term memory in mice/rats. Retention of the learned task (memory) was examined 24 hours after the first day trial (i.e., ninth day, 24 hours after the last dose) by recording the transfer latency values. Significant reduction in TL
value of retention indicated improvement in memory.\textsuperscript{19,20} The animal activities were tracked and recorded via an overhead video camera linked to a monitor with computer software Smart Version 2.5 (Panlab Co., USA). The apparatus was thoroughly cleaned with 70\% ethanol after each trial.

Preparation of rat brain tissue homogenate

Animals were sacrificed by decapitation on 9th day after EPM. The rat brains were removed and striata was isolated from the brain to prepare striatal synaptosomes. The pooled striata were homogenized in sucrose (0.32 M). Homogenates were centrifuged at 1,073 x g for 10 min, and supernatants re-centrifuged at 17,172 x g for 15 min. The resulting pellets were re-suspended in HEPES buffer (pH 7.4). The resulting synaptosomal fractions were then immediately used for estimation MTT reduction.\textsuperscript{21} The remaining parts of rat brain tissue were homogenized with 10 times (w/v) ice – cold 0.1 M phosphate buffer pH (7.4).\textsuperscript{22} Aliquots of the homogenate were used to determine lipid peroxidation. Total protein content in the homogenate was also estimated by Folin-Lowry method.\textsuperscript{23}

Estimation of mitochondrial function (MTT assay)

Mitochondrial function was estimated by the MTT reduction assay. It was carried out by the method of Mosmann.\textsuperscript{24} This method is currently employed as an index of the functional status of the respiratory chain as the formation of formazan salts occurs through the action of mitochondrial dehydrogenases in viable cells or cell fractions. The formation of formazan was quantitated spectrophotometrically at 570 nm using Biotek ELx800 Microplate Reader (Bio-Tek Instruments, Inc). Results were expressed as the percentage of MTT reduction with respect to control values.

Estimation of lipid peroxidation

The assay for lipid peroxidation was carried out following the method of Iqbal \textit{et al}. The amount of TBARS formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank. The results were expressed as MDA content (nmol/g tissue) at 37\textdegree C.\textsuperscript{25,26}

Statistical analysis

Results were expressed as mean±SEM. The data was analyzed by one-way ANOVA followed by Bonferroni Multiple Comparison post-hoc test for comparing control and various groups, using Graph-Pad software. P values <0.001 were considered as significant.

RESULTS

Total phenolic and flavonoid content

Plant phenolics and flavanoids form a large group of natural compounds, ubiquitous in the plant kingdom. It is known that these secondary metabolites display a remarkable array of biochemical interactions, probably due to their antioxidant properties. The total phenolic content expressed in terms of GAE of MEWS and MEEA extracts were found to be 20.31±1.79 mg of GA/g and 28.69±1.46 mg of GA/g, respectively. The flavanoid contents were calculated using the following linear equation based on the calibration curve of gallic acid; A=0.008X+0.0727, \textit{R}=0.9967. The flavanoid contents were expressed in terms of rutin equivalent of MEWS and MEEA extracts were found to be 22.51±1.38 mg of R/g and 24.69±1.26, respectively. The flavanoid contents were calculated using the following linear equation
based on the calibration curve of rutin; $A=0.081X+0.0637$, $R^2=0.9927$.

**In vitro antioxidant assays**

**Free radical scavenging activity on diphenylpicrylhydrazyl radical (DPPH•)**

The extracts of MEWS and MEEA& ascorbic acid exhibited 50% scavenging effect at 65.84µg/ml, 69.34µg/ml and 57.64 µg/ml computed as per Graph Pad Prism 5.0. The highest (%) scavenging effect of the extracts was 79.84 and 74.92, respectively and for ascorbic acid 87.41 at the concentration 200 µg/ml.

**ABTS radical scavenging activity**

The extracts of MEWS and MEEA and ascorbic acid exhibited 50% scavenging effect at 4.63µg/ml, 6.91µg/ml, and 5.35µg/ml computed as per Graph Pad Prism 5.0. The highest antioxidant activity (%) for the extracts were 81.35, 73.13, respectively and for ascorbic acid 86.89 at 20µg/ml.

**Effect of MEWS and MEEA on Transfer latency using Elevated Plus-maze**

Transfer latency on day 9 reflected retention of learned task or memory. The rats treated with MEWS and MEEA (100 and 200mg/kg, p.o.) showed dose dependent reduction in TL of ninth day by 22.70±0.36s and 15.37±0.28s; 21.75±0.37s and 19.94±0.53s for the respective doses of the extracts indicating significant improvement in memory, when compared with the disease control group. Scopolamine hydrobromide (1mg/kg, i.p.) injected before training significantly ($P<0.001$) increased the TL by 50.17±1.45s on day 9 indicating impairment in memory (amnesia). The rats treated with MEWS and MEEA (100 and 200mg/kg, p.o.) for eight days reversed successfully scopolamine-induced amnesia. Donepezil hydrochloride and Piracetam (used as the positive controls) at the dose of 3mg/kg, p.o. and 200mg/kg, p.o. respectively, improved memory ($P<0.001$) of rats and reversed amnesia induced by scopolamine [Fig 3].

**Effect of MEWS and MEEA on lipid peroxidation**

TBARS concentrations (expressed as MDA) in the brain tissue homogenates of all the experimental animals are shown in Figure 4. After scopolamine hydrobromide administration, the MDA levels increased significantly ($P<0.001$) to 74.68±0.32 nmol/g brain tissue homogenate. However pretreatment of hydromethanolic extract of *W. somnifera* and methanolic extract of *E. alba* at 200mg/kg for 8 days decreased the MDA levels to 51.49±0.15 nmol/g tissue and 50.23±0.50 nmol/g tissue, respectively in a dose dependent manner. Donepezil hydrochloride and Piracetam treated animals also showed significant ($P<0.001$) decrease in the MDA levels as compared to scopolamine treated animals.

**Effect of MEWS and MEEA on Mitochondrial function**

Synapotosomal fractions of scopolamine hydrobromidetreated rats exhibited a significant ($P<0.001$) decrease in MTT reduction with respect to control values. In contrast, MEWS and MEEA at 200mg/kg prevented the decreased mitochondrial capability of MTT reduction induced by scopolamine hydrobromide by 72.01% and 71.59%, respectively. The activity of the extracts was comparable to that of Donepezil hydrochloride 3mg/kg and Piracetam 200mg/kg, which increased the reduction of MTT by 77.99% and 66.33%, respectively [Fig. 5].

**DISCUSSION**

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. The nervous system is particularly vulnerable to the deleterious effects of ROS. El-Sherbiny et al. reported that memory impairment in the scopolamine-induced animal model is associated with the increased oxidative stress within rat brain. Recently, many other studies also reported that memory impairment in the scopolamine-induced animal model is associated with increased oxidative stress within the brain. An increased oxidation of lipids and alterations in mitochondrial function and a possible role of amyloid beta and its precursor protein in oxidative reaction in experimental models of Alzheimer’s disease are demonstrated. Antioxidants are thought to exert a potential neuroprotective effect. These biomolecules contribute to prevention of neurodegenerative damage and improve cognition by inhibiting oxidative reactions. Due to the presence of wide variety of antioxidant compounds, several assay methods have been applied to screen, quantitate and evaluate the total antioxidant activity of such samples. In the present work the quantitative estimation of phenolic and flavonoids were performed since these phytoconstituents contribute towards neuroprotective activity. DPPH and ABTS assay were used to evaluate the total antioxidant activity of the extracts. The present study also investigated the neuroprotective effects of hydromethanolic extract of *Withania somnifera* and methanolic extract of *Eclipta alba* on scopolamine hydrobromide induced oxidative stress induced mitochondrial dysfunction in the brain of male Wistar rats treated with the extracts at a dose regimen of 50, 100 and 200mg/kg for a period of eight days. The results demonstrated that treatment with MEWS and MEEA attenuated memory impairment induced by scopolamine in behavior paradigm. Furthermore, in biochemical investigations, MEWS and MEEA significantly decreased the lipid peroxidation activity and restored the functional status of mitochondrion.

The quantitative estimation of phytochemicals revealed the presence of phenolics and flavonoids in both MEWS and MEEA. DPPH is a stable free radical that possesses a characteristic absorption maximum at 517 nm, which is diminished in the presence of a compound capable of reducing it to its hydrazine form by hydrogen/electron donation. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. In this assay, results are expressed as the ratio percentage of the absorbance decrease of DPPH radical solution in the presence of extract at 517 nm to the absorbance of DPPH radical solution at the same wavelength. MEWS and MEEA exhibited significant free radical scavenging activity on DPPH assay. The ABTS assay is based on the inhibition by antioxidants of the absorbance of the radical cation of ABTS, which has a characteristic long-wavelength absorption spectrum showing maxima at 660, 734 and 820 nm. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals. Higher concentrations of the MEWS and MEEA were more effective in quenching free radicals in the system. The results obtained clearly imply that the methanolic extracts of plant species inhibit the radical or scavenge the radical in a concentration dependent manner.

The ameliorative effects of MEWS and MEEA on learning and memory were studied using elevated plus maze method. The elevated plus maze served as the exteroceptive behavioral model to evaluate learning and memory in rodents. Normal aging is associated with the impairments in
learning and memory. Oxygen free radicals are implicated in the process of aging and may be responsible for development of AD. Using sodium nitrite hypoxia, a model of aging brain, Bhattacharya studied the effects of an herbal formulation (Mentat) on learning acquisition by the elevated plus maze and step-down tests and showed facilitating effect. The time consumed by the animal to move from the open to the closed arm in EPM is recorded as transfer latency. The cognitive processing of spatial information takes place when the animal navigates the maze at intervals following the first exposure. Re-exposure to the maze would enable the animal to recall places and things reflecting explicit memory. In this experiment, scopolamine-treated mice exhibited significantly longer transfer latencies. MEWS and MEEA (50, 100 and 200 mg/kg) treatment revealed a significant increase in TL in the animals. Thus, MEWS and MEEA significantly reversed the deficit produced by scopolamine. Donepezil hydrochloride (3 mg/kg) and Piracetam (200 mg/kg) used as positive control, also increased the TL, which is consistent with previous reports.

An elevated brain oxidative status in amnesic rats resembles the clinical situation in patients suffering from dementia, which have been reported to have elevated oxidative stress and membrane lipid peroxidation levels. In addition; overall peroxidation activity in the brains of AD patients has been reported to be significantly elevated. More specifically, showed that the entire AD brain is subjected to oxidative challenges. Lipid peroxidation is initiated by free radical attack on membrane polyunsaturated fatty acids leading to their transformation and fragmentation to alkanes and reactive aldehyde compounds. The measurement of lipid peroxidation is a convenient method to monitor oxidative damage, the enzymatic antioxidant defence systems are natural protectors against lipid peroxidation, these enzymes prevent generation of hydroxyl radicals and protect cellular constituents from oxidative damage. Treatment of naive animals with amnestic dose of scopolamine (1 mg/kg) elevated brain MDA. This association of oxidative stress with amnesia could be substantiated by the findings of other studies. Pretreatment of the animals with 50, 100 and 200 mg/kg W. somnifera and E. alba extracts for eight days prior to scopolamine injection about 90 minutes after the last dose administration on eighth day of dosing schedule resulted in dose-dependent antioxidant activity through affecting brain MDA levels. Donepezil hydrochloride (3 mg/kg) and Piracetam (200 mg/kg) used as positive control, also decreased the MDA levels significantly. These results suggest that W. somnifera and E. alba extracts demonstrate antioxidant properties by protecting rat brain from elevated oxidative status due to administration of scopolamine.

Mitochondrial metabolic function was assessed by the conversion of the dye methyl thiazole tetrazolium (MTT) to formazan. MTT reduction is attributed to mitochondrial activity, although it is related both to non–mitochondrial enzymes and to lysosomes and endosomes. The level of MTT reduction was significantly lower in scopolamine treated group, compared to Donepezil hydrochloride, MEWS 200mg/kg, MEEA 200mg/kg and Piracetam pre-treated groups. This difference is most likely due to lower activity of succinate dehydrogenase (SDH), the enzyme primarily responsible for reduction of MTT, in the mitochondria of extract treated rats. Our finding that scopolamine reduces mitochondrial metabolic function in the MTT assay is in agreement with results of study on brain synaptosomal preparation reported in literature, where exposure to 3-nitropropionic acid (20mg/kg/day, i.p.) for 3 days reduced MTT metabolism to approximately 84% of control levels.
The memory improving activity of *Withania somnifera* and *Eclipta alba* may be attributed to its antioxidant, neuroprotective and memory enhancing activity, suggesting that both the species might have chemical constituents which possess nootropic activity. It has been known that neuroprotective compounds showed the significant anti-amnesic activity in memory deficit induced by age and scopolamine. The above behavioral and biochemical results suggest that HAWS and MEEA has the ability to improve or ameliorate short term memory loss by the regulation of the antioxidant system. The observed beneficial effects of *Withania somnifera* and *Eclipta alba* may be attributed to its diversified chemical components namely glycosides, flavonoids, tannins, saponins, coumestans. The overall neuroprotective activity of the extracts were found to be more than 50% when compared to the protection offered by standard drugs, indicating the potential benefits of indigenous plants in ameliorating oxidative stress induced mitochondrial dysfunction.

**CONCLUSION**

In conclusion, this study demonstrated that *Withania somnifera* and *eclipta alba* decreases scopolamine-induced lipid peroxidation and increases mitochondrial activity in the rat brain.

**REFERENCES**


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**Figure 1.** DPPH radical scavenging activity of the methanolic extracts of the plant and ascorbic acid.
Figure 2. ABTS radical scavenging activity of the metabolic extracts of the plant and ascorbic acid

Figure 3. Effect of Scopolamine, Donepezil hydrochloride, Piracetam, HAWS and MEEA in Elevated plus Maze
Figure 4. Effect of Scopolamine, Donepezil hydrochloride, Piracetam, HAWS and MEEA on Lipid peroxidation (MDA content)

Figure 5. Effect of Donepezil hydrochloride, Piracetam, HAWS and MEEA on scopolamine induced mitochondrial dysfunction