EVALUATION OF MEMORY ENHANCING ACTIVITY OF ALCOHOLIC EXTRACT OF CUSCUTA REFLEXA ON EXPERIMENTALLY INDUCED DEMENTIA (ALZHEIMER’S DISEASE) IN RODENTS

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ABSTRACT
The Cuscuta reflexa contains flavonoids and tannins which may responsible for its CNS actions. The recent findings on Cuscuta reflexa have demonstrated its antioxidant, antiepileptic, anxiolytic and hepatoprotective. The present study involves the effect of ethanolic extract of Cuscuta reflexa (EECR) was investigated for memory enhancing activity by elevated plus maze (EPM) using Scopolamine induced amnesic rats. EECR was administered orally with a dose of 200mg/kg, once a daily, for 14 consecutive days, where Piracetam (200mg/kg p.o), Nootropic was administered to the rats as a standard drug. Scopolamine (1mg/kg i.p) was administered on 14th day to rats to induce dementia and biochemical estimations were performed such as Acetylcholinesterase(AChE), Superoxide dismutase (SOD), Reduced glutathione (GSH), Lipid peroxidation (LPO) and Histopathological study. The transfer latency (TL) was observed in scopolamine induced rats using EPM, which showed significant increase in TL (p<0.001) in acquisition and retrieval phases as compared to normal control group. The Scopolamine induced rats showed significant increase in p<0.0001 AChE and LPO as compared to normal control, where as in EECR treated rats showed p<0.0001 decrease in AChE and LPO and increase in p<0.0001 SOD and GSH.

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INTRODUCTION

Dementia is a syndrome of progressive nature, characterized by impairment of memory and loss of intellectual ability. The dementing condition that has received the utmost attention in the past decade is Alzheimer’s disease (AD), which is the most common cause of dementia in the elderly, accounting for 60–70% of all cases. According to the World Health Organization (WHO), 5% of men and 6% of women aged above 60 years are suffering from dementia worldwide. AD is a progressive, neurodegenerative disease characterized by insoluble aggregates of beta-amyloid and neurofibrillary tangles. Loss of cholinergic neurons in nucleus basalis magnocellular of cortex is one of the prominent features of AD, accounting for memory loss. Clinical management of dementia is still a nightmare for the neurobiologist. Thus far, cholinesterase inhibitors like donepezil, rivastigmine, galantamine. But because of the adverse effects such as anxiety, insomnia, tremor, hepatotoxicity. Nootropic agents have limited use [2]. Anti-inflammatory agents, antioxidants, are the mainstay of AD therapy. The age-related changes include atrophy (shrinking) of certain parts of the brain, inflammation, the production of unstable molecules called free radicals, and mitochondrial dysfunction [1].

The aerial parts of Cuscuta Reflexa containing glycoside have been demonstrated to exert anti-aging effects [3] Chemical constituents like Cuscutin, amarbelin, cuscutalin, mangiferine, quersetic, kuskutin, lactone, reducing sugar, quercetin, resins and cucurbitine are slightly bitter and soluble in ether and chloroform [4]. The extract of Cuscuta reflexa has been reported to possess similar effect of acetylcholine when tested on isolated rabbit ileum and frog rectus abdomens and heart. These effects were blocked by atropine. Effect of Cuscuta reflexa extract on isolated frog rectus abdomen muscle was blocked by Pancuronium and potentiated by Neostigmine [5]. So, the objective of the present study is to evaluate the memory enhancement using suitable model.

MATERIAL AND METHODS

The whole plant of Cuscuta reflexa belonging to family Cuscutaceae was procured from the Sri Venkateshwara University Tirupati, AP., India. in the month of August 2015 and was authenticated by Dr. K Mahadeva Chetty, Plant Taxonomist, Assistant Professor, Dept of Botany (Voucher no: 1011).

Chemicals and drug used:

Acetyl thiocholine iodide (Himedia Lab Pvt Ltd, Mumbai), 5,5’-dithiobis-(2-nitrobenzoic acid) (Sisco research laboratory), Potassium dihydrogen phosphate (S. D. Fine Chemicals Ltd, Mumbai), Ethanol (Warehouse Distillery, Bangalore), Piracetam (Micro labs Pvt Ltd), Scopolamine (Sigma-Aldrich Pvt Ltd).

Animals used:

Albino Wistar rats of either sex weighing between (150-200g) were procured by Venkateshwara suppliers Bengaluru, and were maintained in the animal house of PES college of Pharmacy, Bengaluru CPCSEA Reg No (600/PO/Ere/02/CPCSEA). All the animals were acclimatized for seven days under standard husbanry conditions i.e. room temperature 27± 10°C, 45-55%, 12 h light/dark cycle. The animals had free access to standard rat pellets, with water supplied ad libitum under strict hygienic conditions. Protocol was presented in the IAEC meeting held on 13-12-2014. The approval for use of lab animals (Reference No. PESCP/IAEC/09/10-1-2015) was taken from the Institutional Animal Ethics Committee (IAEC), PES College of Pharmacy. All experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Extraction of plant material:

The whole plant was shade dried and reduced to a coarse powder. In the continuous hot extraction method, the plant powder of about 110g was packed in thimble and was extracted with 95% v/v of ethanol (700ml) for three regular days at temperature of 50-60°C. During the process of extraction, the alternate filling and emptying of the body of the extractor goes on continuously till the powder was exhausted and it was confirmed by discoloration of the solvent at the side tube of the extractor (Siphon). The extract obtained after 3 days was subsequently filtered and the extract is then concentrated by distilling solvent Evaporation (low temp). Extract is preserved in air tight container at room temperature.

Phytochemical investigation of extract:

The preliminary phytochemical investigations were carried out for whole plant extracts of Cuscuta reflexa for qualitative determination of chemical constituents. Test procedures were closely followed according to Treas and Evance [6].

Dose selection:

According to the literature survey it was found that the Cuscuta reflexa was safe to up to 2000mg/kg. Therefore, we have chosen 200mg/kg body weight as treatment protocol for memory enhancing activity [7].

Evaluation of memory and learning activity Cuscuta reflexa extracts in Scopolamine induced rats using Elevated plus maze.

Experimental design for Scopolamine induced dementia [8]. The animals were divided into four groups with 5 rats in each group. Group I animals received normal saline (p o) for 14 days, group II animals received 1mg/kg of Scopolamine (i p) on 14th day. Group III animals received Piracetam (p o) for 14days and Scopolamine (i p) on 14th day. Group IV animals were administered with 200mg/kg of the Cuscuta reflexa (p o) extract for 14 days and Scopolamine (i p) on 14th day. The animals was exposed to the training session (on 14th day) after the 45 min of Scopolamine administration, the retention was measured after 24hr (on 15th day) using elevated plus maze
EPM was originally designed to evaluate anti-anxiety agents and also currently extended to measure the cognitive performance, mainly spatial long term memory in rats and mice. The procedure and techniques and end point for testing memory was followed in accordance with standard literatures [9]. It consists of two open and closed arms which were based on the natural aversion of rodents to open and a high space was used to measure the anxiety state in animals. The animals tend to spend more time in enclosed arm than open arm, the aversive nature to open was not apparent until the animals enter them: Based on this parameter, several authors have demonstrated that Transfer Latency (TL), the time in which animal move from open arm to closed arm was markedly shortened if the animal had previously experienced entering the open arm and this shortened the TL has been shown to relate to memory process. TL on second day onwards was shorter than first day indicates the retention of the memory. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm), and the maze was elevated to a height of 25 cm from the floor.

Tissue preparation:
After the behavioral study, rats were decapitated and brain was excised and kept on a cooled Petri dish on crushed ice. Brain was washed superficially with isotonic saline to remove blood. Whole Brain was weighed in ACCULAB weighing balance and suspended in phosphate buffer individually. The tissue was homogenized (approximately 10 mg of tissue per ml of phosphate buffer (pH 8.0, 0.1 M) using homogenizer tubes and a motor-driven Teflon pestle (Remi motors) [10].

Estimation of brain acetyl cholinesterase level of rats brain:
Thiocholine released because of the cleavage of Acetylthiocholine (ATC) by AChE is allowed to react with the -SH reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412 nm. The concentration of thionitro benzoic acid detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity. The extinction coefficient of the thionitro benzoic acid is $1.36 \times 10^4$ molar/centimeter. [11]

Biochemical Estimation of Markers of Oxidative stress:

Super oxide dismutase
Procedure: The assay mixture contained 1.3ml of solution A, (solution A is 0.1mM of EDTA and 50 mM of sodium carbonate), 0.5ml of solution B, (Solution B is 96mM of Nitro blue tetrazolium), to the above mixture 0.05ml of hydroxylamine hydrochloride and 0.05ml of Phenylazine methosulfate (PMS) was added, for the test sample 0.1ml of homogenised brain supernatant was added and mixture was allowed to stand for 10min. The colour intensity of the chromogen was measured at 560 nm against blank using semi autoanalyser and concentration of SOD was expressed as units /mg of protein [12].

Calculation:
The results were calculated as units/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 100%. Molar coefficient of NBT = 36 X 10^4 M^−1 cm^−1.

Lipid peroxidation
Procedure:
Aliquots of 0.5 ml distilled water and 1.0 ml 10% (Trichloacetic acid) TCA were added to a volume of 0.5 ml of tissue homogenate, mixed well and centrifuged at 3000 rpm for 10 min. To 0.2 ml supernatant, 0.1 ml 8%thiobarbituric acid (TBA) was added. The total solution was placed in a water bath at 80°C for 40 min and then cooled at room temperature. The absorbance of the clear supernatant was measured at 532 n m in spectrophotometer.

Calculation:
The values were calculated using molar extinction coefficient of TBA (1.56 × 10^5M^−1cm^−1) as follows: Concentration of MDA = absorbance/extinction coefficient

Reduced glutathione:
Procedure:
GSH was measured by its reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (Ellman reagent) to yield a yellow chromophore which was measured spectrophotometrically. The brain homogenate was mixed with an equal amount of 10 % trichloracetic acid (TCA) and centrifuge at 200 g for 10 min at 4°C. The supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2ml of phosphate buffer (pH 8.4), 0.5 ml of DTNB and 0.4ml of double-distilled water were added and the mixture was shaken vigorously on vortex. The absorbance was read at 412 nm with in 15 min [13].

Calculation:
The values were calculated using molar extinction coefficient of TNB (13.6 × 10^3M^−1cm^−1) as follows [14]. Concentration = absorbance/extinction coefficient.

Histopathological studies: After 14-day treatment, the brains of different groups were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and postfixed in the same fixative overnight at 48°C. The brains were then routinely embedded in paraffin and stained with Hematoxylin-Eosin. The hippocampal lesions were assessed microscopically at 40 magnification [15].
Statistical analysis

The values were expressed as Mean ± SEM from 6 animals. The results were subjected to Statistical analysis by using one-way ANOVA followed by Sidak’s test to calculate the significance. P<0.05 was considered as significant.

RESULTS

Extraction

The successive extraction of powdered whole plant of *Cuscuta reflexa* was carried out using ethanol by soxhlet extraction method. The ethanolic extract obtained was dark brown in color. The percentage yield of extract was 19 % w/w of powder weight. Total three batches of 330g used for the extraction process. The yield of three extracts was found to be 60g.

Preliminary phytochemical screening

The ethanolic extract obtained was subjected to preliminary phytochemical investigation to determine the presence of functional groups. The preliminary phytochemical screening confirms the presence of carbohydrates, proteins, steroids, alkaloids, glycoside, flavonoids, phenolic tannins, amino acids and saponins.

Evaluation of memory enhancing activity of *Cuscuta reflexa* in rats.

Learning and memory in Scopolamine induced animals using Elevated Plus Maze (EPM):

Table No 1: Effect of *Cuscuta reflexa* on TL against scopolamine induced dementia in rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENT</th>
<th>TRANSFER LATENCY in sec</th>
<th>% change in TL</th>
<th>Retrieval</th>
<th>% change in TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>27.80 ± 3.42</td>
<td>62.29</td>
<td>22.0 ± 2.16</td>
<td>66.76</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine</td>
<td>74.80± 10.57***a</td>
<td>59.62</td>
<td>20.80 ± 1.24***b</td>
<td>68</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam + Scopolamine</td>
<td>30.20 ± 1.65***b</td>
<td>59.62</td>
<td>20.80 ± 1.24***b</td>
<td>68</td>
</tr>
<tr>
<td>IV</td>
<td><em>Cuscuta reflexa</em> + Scopolamine</td>
<td>38.80 ± 4.29**b</td>
<td>47.59</td>
<td>27.0 ± 2.53**b</td>
<td>58.46</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. * P < 0.05, ** P < 0.01, *** P<0.001, ns= not significant

***a = p<0.001; when compared with normal control group.
***b = p<0.001, **b= p<0.001; compared with Scopolamine treated group (***)

The Statistical difference in mean was analyzed using one way ANOVA followed by Sidak’s multiple comparison tests.

The animals were subjected to transfer latency (TL) to evaluate the acquisition and retrieval of memory in behavioral paradigm. TL of day 14 reflects acquisition (learning behavior) of the animals whereas; on day 15 reflects the retention of the information or memory.

During Acquisition Phase: The animals administered with Scopolamine (1mg/kg *i.p*) showed significant increase in TL (74.80±10.57) as compared to control group (27.80 ± 3.42). In the standard group, the animals treated with Piracetam (200 mg/kg p.o.) produced significant decrease in TL (30.20 ± 1.65) as compared to Scopolamine treated group (74.80±10.57). In the treatment group, the animals treated with *Cuscuta reflexa* at 200 mg/kg p.o produced significant decrease in TL (38.80 ± 4.29) as compared to Scopolamine treated group (74.80±10.57).

During Retrieval Phase: The animals administered with Scopolamine (1mg/kg *i.p*) showed significant increase in TL (63.20±11.29) as compared to control group (22.00 ± 2.16). In the standard group, the animals treated with Piracetam (200 mg/kg p.o.) produced significant decrease in TL (20.80 ± 1.24) as compared to Scopolamine treated group (63.20±11.29). In the treatment group, the animals treated with *Cuscuta reflexa* at 200 mg/kg p.o produced significant decrease in TL (27.00 ± 2.53) as compared to Scopolamine treated group (63.20±11.29) as shown in Table No.1 and graphically represented in Fig. No: 1a & 1b.
**Effect of Cuscuta reflexa on acquisition against Scopolamine induced Dementia using EPM.**

![Graph showing the effect of Cuscuta reflexa on TL in acquisition phase.]

**Figure No 1 a: Effect of Cuscuta reflexa on TL in acquisition phase.**

**Effect of Cuscuta Reflexa on Retrieval against Scopolamine induced dementia using EPM**

![Graph showing the effect of Cuscuta reflexa on TL in retrieval phase.]

**Figure No 1 b: Effect of Cuscuta reflexa on TL in retrieval phase.**

Estimation of brain acetyl cholinesterase level in scopolamine induced rats.

The animals treated with Scopolamine (1mg/kg i.p.) showed significant (P<0.0001) increase in AChE enzyme activity (0.146 ± 0.012) as compared to Control group (0.058 ± 0.001).

In the standard group, the animals treated with Piracetam at 200 mg/kg p.o produced significant (P<0.0001) reduction of AChE enzyme activity (0.056±0.004) as compared to Scopolamine treated group (0.146 ± 0.012).

In the treatment group, the animals treated with Cuscuta reflexa at 200 mg/kg p.o produced significant (P<0.001) reduction of AChE enzyme activity (0.084 ± 0.005) as compared to Scopolamine treated group (0.058 ± 0.001).

Percentage inhibition of AChE activity was 52.05 % in Cuscuta reflexa treated group and standard (61.64%). The activity and percentage inhibition of AChE is shown in Table No.3 and graphically represented in Fig. No 3.

Table No 2: Effect of Extract of Cuscuta reflexa on Acetyl cholinesterase estimation against Scopolamine induced Dementia in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Acetylcholinesterase Inhibition</th>
<th>Rate of degradation of M of Ach/gm of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.05</td>
<td>65.75</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine</td>
<td>0.146</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam + Scopolamine</td>
<td>0.056</td>
<td>61.64</td>
</tr>
<tr>
<td>IV</td>
<td>Cuscuta reflexa + Scopolamine</td>
<td>0.07</td>
<td>52.05</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. * P < 0.05, ** P < 0.01, *** P<0.001, **** P<0.0001, ns= not significant.

****a = p<0.0001; when compared with normal control group.

****b = p<0.0001, ***b = p<0.001; compared with Scopolamine treated group (****a = p<0.0001)

The Statistical difference in mean was analyzed using one way ANOVA followed by Sidak’s multiple comparison tests.
Effect of *Cuscuta reflexa* on Brain Acetylcholinesterase estimation against Scopolamine induced Dementia

Assessment of antioxidant activity by estimation of reduced glutathione (GSH), SOD and LPO in Scopolamine induced dementia in rats.

Table No 3: Effect of *Cuscuta reflexa* on GSH, SOD and LPO.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENT</th>
<th>GSH (Units/mg of protein)</th>
<th>SOD (Units/mg/protein)</th>
<th>LPO (µ moles/MDA/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>3.35 ± 0.19</td>
<td>31.62±3.99</td>
<td>2.09 ± 0.42</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine</td>
<td>1.62 ± 0.19****&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.05 ± 0.18****&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.21 ± 0.17****&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Scopolamine + Piracetam</td>
<td>4.17 ± 0.30****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.96±0.21****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.90±0.47****&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td><em>Cuscuta reflexa</em> + Scopolamine</td>
<td>4.070 ± 0.43***&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.61±2.57****&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.35 ± 0.18***&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. * P < 0.05, ** P < 0.01, *** P<0.001, **** P<0.0001. ns= not significant

****<sup>a</sup> = p<0.0001; when compared with normal control group.

****<sup>b</sup> = p<0.0001, ***<sup>b</sup> = p<0.001; compared with Scopolamine treated group (**<sup>a</sup> = p<0.0001)

The statistical difference in mean was analyzed using one way ANOVA followed by Sidak’s multiple comparison tests.

**Effect of *Cuscuta reflexa* on GSH:**

The animals treated with scopolamine (1mg/kg i.p.) showed significant (p<0.0001) decrease in GSH level (1.62± 0.19) as compared to Control group (3.35 ± 0.19). In the standard group, the animals treated with Piracetam (200 mg/kg p.o.) produced significant (p<0.0001) increase in GSH level (4.17 ± 0.30) as compared to Scopolamine treated group (1.62 ± 0.19). In the treatment group, the animals treated with *Cuscuta reflexa* at 200 mg/kg p.o produced significant (p<0.001) increase in GSH level (4.07± 0.43) as compared to Scopolamine treated group (1.62±0.195).

**Effect of *Cuscuta reflexa* on SOD:**

The animals treated with scopolamine (1 mg/kg i.p.) showed significant decrease (p<0.0001) in SOD activity (7.05 ± 0.18) as compared to Control group (31.62±3.99). In the treatment group, the animals treated with *Cuscuta reflexa* at 200 mg/kg p.o produced significant increase (p<0.0001) in SOD activity (22.61 ± 2.57) as compared to Scopolamine treated group (7.05 ± 0.18). In the standard group, the animals treated with Piracetam (200 mg/kg p.o.) produced significant increase p<0.0001 in SOD activity (30.96±0.21) as compared to Scopolamine treated group (7.05 ± 0.18).

**Effect of *Cuscuta reflexa* on LPO**

The animals of treated with Scopolamine (1mg/kg i.p.) showed significant p<0.0001 increase in LPO activity (5.21 ± 0.17) as compared to Control group (2.09 ± 0.42). The animals treated with *Cuscuta reflexa* at 200 mg/kg p.o produced significant (p<0.001) reduction of LPO activity (3.35 ± 0.18) as compared to Scopolamine treated group (5.21 ± 0.17). In the standard group, the animals treated with Piracetam (200 mg/kg p.o.) produced significant (p<0.001) reduction of LPO activity (2.90±0.47) as compared to Scopolamine treated group (5.21 ± 0.17) as shown in Table No. 2 and graphically represented in Fig. No: 3,4,5.

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**Figure No 2: Acetyl cholinesterase estimation.**
Effect of GSH ON Cuscuta Reflexa

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Protein U/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Piracetam</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Cuscuta reflexa</td>
<td>6.0 ± 0.5</td>
</tr>
</tbody>
</table>

Effect of SOD on Cuscuta Reflexa

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Protein U/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>Piracetam</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>Cuscuta reflexa</td>
<td>40 ± 4</td>
</tr>
</tbody>
</table>

Effect of on LPO Cuscuta Reflexa

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>LPO µmoles/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>Piracetam</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Cuscuta reflexa</td>
<td>6.5 ± 0.9</td>
</tr>
</tbody>
</table>

Figure No 3: Effect of Cuscuta reflexa on GSH.

Figure No 4: Effect of Cuscuta reflexa on SOD.

Figure No 5: Effect of Cuscuta reflexa on LPO.

Figure No:6

Group I: Normal control

Figure No:8

Group II: Scopolamine

Figure No: 9
Group I: Blue arrows indicate the H & E Section of the brain reveals the intactness of neurons spread across as shown in figure No 6. Group II: Red arrows indicate the presence of senile plaques. Yellow arrows indicate the presence of neurofibrillary tangles as shown in figure No: 7. Section of the brain reveals the degenerative lesion like clumping of cytoplasmic materials to dark basophilic aggregates, plaques and tangled microfilaments. Group III: White arrows indicate regeneration of neurons as shown in figure No: 8. Group IV: Black indicated the intact but crowded neurons spread across. There are stray capillaries with erythrocytes seen as shown in figure No: 9.

DISCUSSION

There are number of parasitic plants which are medicinally important and one among them is *Cuscuta reflexa* (*Cuscutaceae*) [16]. *Cuscuta reflexa* reported to have in-vitro anti-inflammatory activity which was analyzed by Reverse transcriptase polymerase chain reaction (RTPCR). Results showed that inhibition of cox-2, TNF α genes and NFκB signaling [17] Talma et al. have reported that compounds possessing anticholinesterase activity reduces TNF α activity in a brain which is inflammatory mediator in AD [18].

Administration of Scopolamine in animals affects several aspects of short term memory and attention leading to cognitive deficits as observed (Table No 1) during acquisition and retrieval phase which is similar to aging and dementia patient. Scopolamine is muscarinic receptor antagonist produces the reversible amnesic affect [19]. Use of EPM in our study as an extroceptive behavioral in which TL was used as the parameter to screen the extract of *Cuscuta reflexa*. On treatment with alcoholic effect, significantly reversed Scopolamine induced dementia during learning and retrieval phase and also reduced the degradation of acetylcholine by inhibiting AChE was supports the assumption. (Table No 2) this could be due to presence of flavonoids, alkaloids or tannins in these extracts. Ach degradation by *Acetylcholinesterase* is responsible for the duration and the efficacy of cholinergic neurotransmission. In present study *Cuscuta reflexa* was able to reverse the scopolamine induced dementia, by reducing the acetylcholine degradation by inhibiting *Acetylcholinesterase*.

Many clinical studies have strong evidence that oxidative stress is involved in the pathogenesis of Alzheimer's disease. The oxygen-free radicals are implicated in the process of age related decline in the cognitive performance may be responsible for the development of Alzheimer's disease in elderly persons [20]. The *Cuscuta reflexa* also showed in vitro antioxidant property [21]. Memory impairment in the scopolamine-induced animal model is associated with the increased oxidative stress within rat brain. An increased oxidation of lipids, proteins and deoxyribonucleic acid, 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. Moreover, strong evidence supporting the involvement of oxidative damage in neurodegenerative disease has been suggested by various clinical studies. The drugs with antioxidant effects might be beneficial for preserving brain function. Antioxidant enzymes such as superoxide dismutase (SOD), lipid peroxidation (LPO) as well as glutathione reductase (GSH) are involved in the reduction of oxidative stress. Antioxidant enzymes display the reduced activities in the affected brain region of patients of Alzheimer's disease [23]. SOD and GSH levels were significantly reduced in the scopolamine treated rats and increased level of LPO (Table no 1). *Cuscuta reflexa* treated rats showed increased level of SOD and GSH and reduced the level of LPO (Table No 3).

Puchchakayala Goverdhan et al reported the Scopolamine induced neurodegeneration in brain of rats, which was evaluated by histopathological studies. There was presence of senile plaque, neurofibrially tangles which confirmed the neurodegeneration [15]. In our present study *Cuscuta reflexa* treated brain of rats reversed the scopolamine induced neurodegeneration which showed Hematoxylin-Eosin (H & E) section of the brain having intact but crowded neurons spread across.

CONCLUSION

This study has shown that the ethanolic extracts of *Cuscuta reflexa* possess Memory enhancing activity. Further research could be done to determine its specific mechanism of action.
REFERENCES


