NEUROPROTECTIVE EFFECT OF VANDA TESSELLATA AS “RASNA” SPECIES, ON ALUMINIUM CHLORIDE INDUCED ALZHEIMER’S IN RATS

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ABSTRACT

The present study focused on evaluation of neuroprotective effect of hydromethanolic extracts of Vanda tessellata (VT), also considered as Rasna. Aluminum Chloride (AlCl3) induces neuroinflammation in rats and finally the development of AD. PASS online and molecular docking insilico studies were conducted with PPAR-γ for β-sitosterol and AChE for gigantol. Total 36 trained Wistar rats were divided into VI groups 6 in each. Group I - normal control, Group II – Disease control, Group III - Rivastigmine (0.3mg/kg, p.o), Group IV and V – Hydromethanolic extract of VT (HMEVT, 150mg/kg, 300mg/kg, p.o) respectively. Group VI - Ayurvedic Formulation of Rasna (AFR) (1ml/kg, p.o). All the animals received Aluminum Chloride (AlCl3) (300mg/kg, p.o) except group I. The rats were treated for 20 days but mean time behavioural study, body weight changes were monitored on 0th, 7th, 14th & 20th day. On 21st day, rats were sacrificed, brains were isolated, then antioxidants enzymes levels, protein content and neurotransmitters levels were determined. Histopathology of cortex and hippocampus parts of the brain were studied. Group II animals showed reduction in locomotor activity, increased in the number of entries as well as time spent in closed arm and time taken to climb the pole was increased but it was reversed in groups treated with 150mg/kg, 300mg/kg doses of HMEVT and AFR. Increased level of protein content, malondialdehyde, reduction in body weight and antioxidants enzymes like superoxide dismutase, catalase, glutathione were observed in disease control group and it was due to free radicals generation and were corrected and restored in groups treated with HMEVT and AFR. Moreover, the histopathological report also showed cellular level protection efficacy found with HMEVT and AFR. The neuroprotective action of HMEVT was due to the active constituents and was proved in insilico study. The order of neuroprotective efficacy was HMEVT > AFR.

Keywords: Aluminum Chloride, Alzheimer’s disease, antioxidants, Rasna, Vanda tessellata; insilico

INTRODUCTION

Alzheimer’s disease (AD), a neurodegenerative disorder where neurofibrillary tangles (NFTs) and β-amyloid (Aβ) plaques are formed and gets accumulated in brain results in declining of cognitive function as well as memory (Borai et al., 2017). The etiology is multifactorial, factors such as genetic factors, oxidative stress, brain inflammation, head trauma and environmental factors including exposure to toxic aluminium (Zaky et al., 2017). Normal functions of neurons were interrupted upon the entry of aluminium via a specific transferrin receptor into blood-brain barrier result in memory loss (Singh et al., 2018) and development of AD is due to deposition of aluminium at hippocampus and frontal cortex where it causes toxic conformational change of cytoskeleton proteins in brain result in formation of Aβ and tau NFTs (Hesham and Mustafa, 2020).

Researchers reported that ‘nervines’ are a category of plants in Ayurveda are used to strengthen the central nervous system and their chemical constituents play vital role on restoration of memory (Dougue et al., 2018). One such Ayurvedic plants are “Rasna” - A controversial medicinal plant. Total 13 plants are listed under Rasna in which Pluchea lanceolata is an official name of Rasna. Vanda tessellata (VT) is called as Rasna in West Bengal and also in many parts of India and are traditionally used for diseases of the nervous system and neurological disorders (Palash et al., 2013). VT belongs to the family Orchidaceae, β-sitosterol and gigantol are the active constituents responsible for the neuroprotective effects (Kumar and Khanum, 2012) but not yet scientifically proven by animal experimental models. So, the current study was focused to evaluate the neuroprotective effect of V. tessellata where the hydromethanolic extract were taken to prove the potential effect on Aluminium Chloride (AlCl3) induced AD in rats.

MATERIAL AND METHODS

Plant material

The whole plant of VT was collected from Thiruvattar, Kanyakumari, Tamil Nadu, in the month of October 2019. Dr J Jameson, Plant taxonomist from Department of Botany, St Albert’s College (Autonomous), Ernakulam, identified and authenticated meantime herbarium specimen also prepared and deposited (voucher specimen number is 479) at Department of Botany, St Albert’s College (Autonomous), Ernakulam, Kerala, India.

Extraction procedure

Roots and leaves of VT were isolated and washed with water to free from soil particles and dried at room temperature (shade dry). Plant materials were powdered coarsely with a mechanical grinder in order to increase the contact between the plant materials with the solvent. The powdered materials (300g) were extracted by cold maceration using water and methanol (70:30) at room temperature for a period of 7 days with intermittent shaking until the soluble matter has dissolved or completion of extraction. After 7 days, the mixture was strained through muslin filter and then solvent was recovered by using rota evaporator under reduced pressure. The crude extract of VT was named as hydromethanolic extract of VT (HMEVT) and stored in refrigerator at 4°C in a well tight container for the further experimental purposes.

INSILICO STUDY

PASS Online

PASS (Prediction of Biological Activity Spectra for Substance) an online tool used to predict biological activities like pharmacological effects, biochemical mechanism, toxic and adverse effects, enzymes interaction, metabolic and transporters link, influence on gene expression etc. β-sitosterol and gigantol were found to be important chemical constituents from VT. 2D structures of these compounds were chosen for the activity prediction. The value defining the likelihood for a given activity to be either revealed (Pa) or not revealed (Pi) (Raju et al., 2021; Mathew et al., 2021).
Molecular docking

The proteins (targets) selected for neuroprotective studies were PPAR-γ for fi-
sitosterol and AChE for gigantol. Ligands (β-sitosterol & gigantol) are an
important chemical constituents from VT. Autodock vina PyRx was used to find
binding energy expressed in Kcal/Mol. Number of hydrogen bonds between the
ligand and receptor and amino acid sequence of attachment of ligands on targets
were identified by using Pymol visualisation (Raju et al., 2021).

In vivo study

Experimental animals

Adult male and female Wistar rats with body weights of 150-250g were used for the
study. The animals were maintained under standard environmental conditions
(23-25°C, 12 hour light/12 hour dark cycle) and had free access to standard rodent
pellet and water ad libitum. The animals were acclimatized in laboratory condition
for a week before commencement of the study and were trained for behavioural
study. The method of study, treatment and handling of animals were presented
before IAEC and the committee approved the proposal number: SICP/IAEC/2019-
13/19 for the proceeding experiments of rats.

Treatment protocol

Treatment protocol was designed based on Singh et al., 2018, Somasekhar et al.,
2017. Total 36 well trained rats were grouped into six groups of six in each. All
the rats in the groups 2-6 were administered Aluminium Chloride (AlCl₃,
300mg/kg, p.o) daily for 20 days except group 1 which was taken as normal
control. Group I was considered as normal control, received only distilled water
(p.o). Group II was considered as disease control (AlCl₃, 300mg/kg, p.o). Group
III was considered as positive control and were treated with Rivastigmine
(0.3mg/kg, p.o). Group IV animals were treated with HMEVT (150mg/kg, p.o).
Group V animals were treated with HMEVT (300mg/kg, p.o) and group VI
animals were treated orally with 1ml/kg of Ayurvedic formulation Rasna (AFR)
for 20 days.

During treatment period, animals body weight and behavioural study were
assessed on 0, 7th, 14th and 20th day. Elevated plus maze was used to assess
memory, locomotor activity was assessed by using digital actophotometer, Cook’s
pole climbing apparatus was used to study of cognitive function, mainly a response to
conditioned stimuli during learning & its retention. On 21st day, bloods was
withdrawn immediately after euthanasia via retro orbital plexus to assess protein
content as well as serum antioxidants enzymes catalase (CAT), reduced glutathione
(GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) level. Brain
was isolated to measure aluminium content, neurotransmitter (dopamine, acetylcholine, noradrenaline and serotonin) estimation and histopathology of
cortex and hippocampus.

Behavioural Studies

Before the commencement of the experiments, all rats were trained in actophotometer, elevated plus maze test and pole climbing instruments according to the procedure of Cook and Weidley, 1957; Soman et al., 2004.

a) Actophotometer Test

Locomotor activity was assessed by using digital actophotometer, the apparatus
equipped with infrared light sensitive photocells. Each trained animals were kept
in digital actophotometer and motor activities were observed for a period of 5min.
When the beam of light falling on the photo cell is cut off by the animal that
considered as one count and were recorded, values were expressed as number of
counts per 5min. Locomotor activity assessment was made in all the groups on 0th
day and after drug treatment on 7th, 14th, 20th day.

b) Elevated Plus Maze Test

EPM (exteroceptive behavioral model) is widely employed for evaluating the learning, memory and anxiety in rodents. It has four arms; two open arms and two
closed arms are arranged opposite to central sheath which is elevated 50cm above
the ground floor. Under silent and dark condition each rat were placed at the centre
of the apparatus. The total number of entries as well as time spent in open and
closed arms was recorded. The elevated plus maze test were conducted for all the
group of trained rats on 0th day and after drug treatment on 7th, 14th, 20th day, on
each group of animals after drug treatment.

C) Pole Climbing Test

Cognitive function of a rat was evaluated by using Cook’s Pole Climbing
Apparatus where learning & memory retention were evaluated under conditional
stimuli (CS). It was a wooden chamber (25 × 25 × 25cm) with stainless steel rods

grid floor, 6mA shock is delivered to the floor. At the top lid, a pole (2.5cm width)
was at the centre of the chamber. Each rats were placed for 45seconds to explore
inside the chamber. A buzzer signal followed by an unconditioned stimulus i.e
electric shock was supplied through steel rods grid floor for 45sec. Trained rats
were learned to associate the buzzer sound followed by foot shock and try to escape
from the foot shock by pole climbing after the buzzer signal. Cut off time to
climbing reaction is 10sec. The pole climbing test was conducted for all the group
of trained rats on 0th day and after drug treatment on 7th, 14th, 20th day.

Estimation of antioxidants

Preparation of brain homogenate

Animals were sacrificed after 20 days of treatment by Ketamine (80mg/Kg, i.p) +
Xylazine (10mg/Kg, i.p). Brains were removed carefully. Parts of brain such as
hippocampus and cortex were separated, weighed and homogenized with ice-cold
phosphate buffer of pH 7.4 to prepare brain homogenate. It was centrifuged at
800×g for 5min at 4°C to remove the nuclear debris. The supernatant was used for
the estimation of MDA content. The remaining supernatant was further centrifuged
at 10,000×g for 30min at 4°C to get the post-mitochondrial supernatant which
was used for the estimation of GSH, CAT and for SOD; the homogenate was
centrifuged at 12000rpm for 20min at 400°C (Raju and Sinchu, 2017) and were
estimated by using auto analyser with reagent kit.

Determination of total protein

In each tube were added 50μl of above homogenate, 2950μl of 0.9% NaCl and
3000μl of Biuret reagent and the total protein was estimated based on the procedure
of Mahere et al., 2018.

Estimation of brain neurotransmitters

Weighed brain tissue was homogenised in homogenizer with 5ml HCl butanol
solution for about 1min. Then it was centrifuged for 10min at 2000 rpm. Under
identical condition 1ml of supernatant was added in a centrifuge tube and shaken
with 2.5ml heptane and 0.3ml of 0.1M HCl for 10 min. Discard the organic layer
and take the aqueous phase (0.2ml) to estimate serotonin, dopamine and
noradrenaline (Raju and Sinchu, 2018; Schlumpf et al., 1974). This procedure
was carried out at 0°C and the estimation of AChE was performed according to
the procedure of Ellman (1959).

Estimation of aluminium content

Weighed (30mg) brain tissue was added with 0.05ml nitric acid, 0.2ml H₂O₂
and0.1ml of polytetrafluoroethylene and incubated at 120°C for 2h. Atomic
absorption spectrophotometer was used for the estimation of aluminium
(Arokiasamy et al., 2015).

Histopathology brain

Hippocampus and cortex were isolated, washed and placed in 10% formaldehyde.
Then they were stained with haematoxylin and eosin (H & E). Under light
microscope parts were examined by a pathologist.

Statistical analysis

All the Imvivo study data were expressed as the Mean ± SEM of six values. The
difference between treatment groups was compared to disease control by One Way
Analysis Of Variance (ANOVA) followed by Newman-Keul’s multiple
comparison test; where, p ≤ 0.05 implied significance calculation.

RESULTS

Calculation of percentage yield of extracts

The percentage yield was calculated with standard formula and found to be 8.5
(%w/w) from 300g of crude plant materials.

Insilico study

Figure 1 showed the predicted biological activity of the compound β-sitosterol
from VT. It has predicted 8 different types of CNS activity, out of which
Acetylcholine neuromuscular blocking agent activity scored Pa > 0.6, it means
that the ligand exhibits the CNS activity at acetylcholine pathway was confirmed.
Figure 2 showed the predicted biological activity of the compound Gigantom from VT. It has been predicted total 6 different types of CNS related biological activity, out of which Neurotransmitter antagonist agent scored Pa > 0.6, it means that the ligand revealed the CNS activity in experiment.

Table 1 Docking scores of Gigantol and β-Sitosterol on AChE, PPAR-γ receptor

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Ligand</th>
<th>Receptor</th>
<th>Binding score (Kcal/mol)</th>
<th>Hydrogen bonds</th>
<th>Binding site</th>
<th>PDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanda tessallata</td>
<td>Gigantol</td>
<td>AChE</td>
<td>-7.7</td>
<td>1</td>
<td></td>
<td>61 ASP</td>
</tr>
<tr>
<td></td>
<td>β-Sitosterol</td>
<td>PPAR-γ</td>
<td>-9.1</td>
<td>1</td>
<td></td>
<td>376 ALA</td>
</tr>
</tbody>
</table>

Figure 3 showed docking of β-Sitosterol at the active site region of PPAR-γ (PDB: 2 HWQ), with high binding affinity, as indicated by total docking scores of -9.1 and also showed strong molecular interactions formed between ALA376 residues of PPAR-γ.

Figure 4 showed docking of Gigantom at the active site region of AChE (PDB: 4PQE), shown high binding affinity, as indicated by total docking scores of -7.7 and also showed strong molecular interactions formed between ASP61 residues of AChE.

The locomotor activity of trained rats which were treated with HMEVT and AFR on AlCl3 induced Alzheimer’s disease was monitored on 0th, 7th, 14th and 20th day of study were illustrated in Tab 2. The locomotor activity on 0th day was not significant (p>0.05) in between the trained rats in each group but on 7th day, the locomotor activity of 300mg/kg HMEVT and 1ml/kg of AFR treated rats showed significant (p<0.01) effect when compared with disease control group whereas 150mg/kg HMEVT treated rats showed non-significant (p>0.05) effect. After 14 days of continuous treatment, 300mg/kg HMEVT showed more significant (p<0.001) effect than 150mg/kg HMEVT (p<0.05) and 1ml/kg of AFR (p<0.01) treated rats when compared with disease control group but on 20th day of treatment the locomotor activity was more significantly (p<0.001) increased rats which were simultaneously treated with 300mg/kg HMEVT than 150mg/kg HMEVT (P<0.05) and 1ml/kg of AFR (p<0.01) treated rats when compared with disease control group.

Table 2 Effect of HMEVT and AFR on locomotor activity of AlCl3-induced Alzheimer’s disease in trained rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of counts / 5min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
</tr>
<tr>
<td>Normal Control</td>
<td>206.50 ± 2.29</td>
</tr>
<tr>
<td>Disease Control (AlCl3 300mg/kg)</td>
<td>201.33 ± 1.35</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl3 (300mg/kg)</td>
<td>204.50 ± 1.72</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl3 (300mg/kg)</td>
<td>207.33 ± 2.04</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl3 (300mg/kg)</td>
<td>205.00 ± 1.52</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl3(300mg/kg)</td>
<td>208.16 ± 1.53</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SEM, n = 6. The data were analysed by One Way Analysis of Variance (ANOVA) followed by Newman-Keuls’s multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05.

The table 3 illustrated the learning, memory ability and anxiety of trained rats which were received with HMEVT and AFR were recorded as number of entries in open and closed arms within 5min on AlCl3 induced Alzheimer’s disease. On 0th day, it was not significant (p>0.05) in extract and AFR treated rats when...
compared with disease control rats. After 7 days of continuous treatment, 300mg/kg HMEVT and 1ml/kg of AFR treated rats showed significant (p<0.01) effect on the number of entries in open and closed arm but it was progressively improved upon continuous 20 days treatment. At the end of the study, 300mg/kg HMEVT and 1ml/kg of AFR showed highly significant (P<0.001) whereas 150mg/kg of HMEVT showed less significant (p<0.05) on time spent in open and closed arms but it was progressively improved upon continuous 20 days treatment. At the end of the study, administration of 300mg/kg HMEVT (p<0.001), 1ml/kg of AFR (p<0.01) and 150mg/kg of HMEVT (p<0.05) showed drastic change on learning, memory ability and the disease control group animals.

The Table 3 Effect of HMEVT and AFR on learning, memory and anxiety of AlCl3 induced Alzheimer’s disease in trained rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of entries in open arm &amp; closed arm / 5min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Days 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>4.50±0.76</td>
</tr>
<tr>
<td>Disease Control (AlCl3, 300mg/kg)</td>
<td>3.83±0.60</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl3 (300mg/kg)</td>
<td>6.33±1.14</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl3 (300mg/kg)</td>
<td>2.83±0.60</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl3 (300mg/kg)</td>
<td>6.11±1.19</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl3 (300mg/kg)</td>
<td>2.3±0.49</td>
</tr>
</tbody>
</table>

The Table 4 Effect of HMEVT and AFR on learning, memory ability and anxiety (time spent in open and closed arm) of AlCl3 induced Alzheimer’s disease in trained rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time spent in open arm &amp; closed arm / 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Days 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>0.90±0.15</td>
</tr>
<tr>
<td>Disease Control (AlCl3, 300mg/kg)</td>
<td>0.45±0.047</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl3 (300mg/kg)</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl3 (300mg/kg)</td>
<td>0.48±0.041</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl3 (300mg/kg)</td>
<td>1.62±0.058</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl3 (300mg/kg)</td>
<td>0.65±0.033</td>
</tr>
</tbody>
</table>

The Table 5 Effect of HMEVT and AFR on conditioned avoidance response test of AlCl3 induced Alzheimer’s disease in trained rats.

<table>
<thead>
<tr>
<th>Parameters &amp; Treatment</th>
<th>Time taken to climb the pole (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Days 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>2.56±0.05</td>
</tr>
<tr>
<td>Disease Control (AlCl3, 300mg/kg)</td>
<td>2.51±0.04</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl3 (300mg/kg)</td>
<td>2.43±0.02</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl3 (300mg/kg)</td>
<td>2.57±0.07</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl3 (300mg/kg)</td>
<td>2.62±0.03</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl3 (300mg/kg)</td>
<td>2.52±0.02</td>
</tr>
</tbody>
</table>

The Table 6 Effect of HMEVT and AFR on change in body weight of AlCl3 induced Alzheimer’s disease in rats.

<table>
<thead>
<tr>
<th>Parameters &amp; Treatment</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Days 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>160.33±9.00</td>
</tr>
<tr>
<td>Disease Control (AlCl3, 300mg/kg)</td>
<td>157.16±6.45</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl3 (300mg/kg)</td>
<td>160.50±9.73</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl3 (300mg/kg)</td>
<td>170.00±4.85</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl3 (300mg/kg)</td>
<td>172.00±8.27</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl3 (300mg/kg)</td>
<td>169.16±5.40</td>
</tr>
</tbody>
</table>

The Table 7 showed the effect of HMEVT and AFR on protein content as well as aluminium concentration in brain of AlCl3 induced Alzheimer’s disease in rats. There was increased level of protein content and aluminium concentration in brain with disease control rats. Protein content in brain was significantly (p<0.001) reduced after 20 days of continuous administration of 150mg/kg and 300mg/kg HMEVT. Administration of 1ml/kg of AFR also showed significant effect whereas 150mg/kg of HMEVT showed less significant (p<0.05) on time spent in open and closed arms but it was progressively improved upon continuous 20 days treatment. At the end of the study, administration of 300mg/kg HMEVT (p<0.001), 1ml/kg of AFR (p<0.01) and 150mg/kg of HMEVT (p<0.05) showed drastic change on learning, memory ability and the disease control group animals.
(p<0.01) reduction in aluminum concentration and protein content of AlCl₃ induced Alzheimer’s disease in rats.

**Table 7** Effect of HMEVT and AFR on total protein and Aluminium content in brain of AlCl₃ induced Alzheimer's disease in rats.

<table>
<thead>
<tr>
<th>Parameters &amp; Treatment</th>
<th>Total protein content (mg/g)</th>
<th>Concentration of Aluminium (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>36.76 ± 0.70</td>
<td>-</td>
</tr>
<tr>
<td>Disease Control (AlCl₃ 300mg/kg)</td>
<td>53.70 ± 0.74</td>
<td>9.1 ± 0.15</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl₃ (300mg/kg)</td>
<td>35.3 ± 0.68</td>
<td>2.3 ± 0.17</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl₃ (300mg/kg)</td>
<td>29.89 ± 0.70*</td>
<td>3.1 ± 0.29*</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl₃ (300mg/kg)</td>
<td>31.74 ± 0.72*</td>
<td>2.9 ± 0.19*</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl₃ (300mg/kg)</td>
<td>34.27 ± 0.91*</td>
<td>3.6 ± 0.20*</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SEM, n = 6. The data were analysed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul’s multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05.

Table 8 showed the effect of HMEVT and AFR on antioxidant status in brain of AlCl₃ induced Alzheimer’s disease in rats. The CAT, SOD and GSH level were decreased in disease control group but they were significantly increased and restored in animals treated with 300mg/kg (p<0.001) and 150mg/kg of HMEVT (p<0.01). Like 150mg/kg of HMEVT, 1ml/kg of AFR also restored the antioxidant enzyme level in the brain of AlCl₃ induced Alzheimer’s disease in rats. There was an increased level of MDA found in brain of disease control animals, 20 days continuous treatment with 300mg/kg (p<0.001) and 150mg/kg of HMEVT as well as 1ml/kg of AFR (p<0.01) reduced the content significantly as that of normal in the brain of AlCl₃ induced Alzheimer’s disease in rats.

**Table 8** Effect of HMEVT and AFR on antioxidant status in brain of AlCl₃ induced Alzheimer's disease in rats.

<table>
<thead>
<tr>
<th>Treatment &amp; Parameters</th>
<th>CAT (U/Mol)</th>
<th>GSH (Millimol/gm)</th>
<th>MDA (nmoles of MDA/mg wet tissue)</th>
<th>SOD (U/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>33.05 ± 0.71</td>
<td>53.67 ± 0.75</td>
<td>43.11 ± 0.68</td>
<td>71.34 ± 0.61</td>
</tr>
<tr>
<td>Disease Control (AlCl₃ 300mg/kg)</td>
<td>10.48 ± 0.84</td>
<td>16.87 ± 0.72</td>
<td>75.09 ± 0.72</td>
<td>31.58 ± 0.74</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl₃ (300mg/kg)</td>
<td>27.38 ± 0.60</td>
<td>50.00 ± 0.70</td>
<td>46.51 ± 0.76</td>
<td>66.91 ± 0.57</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl₃ (300mg/kg)</td>
<td>18.44 ± 0.77b</td>
<td>35.35 ± 0.77b</td>
<td>44.85 ± 0.65b</td>
<td>41.54 ±0.81b</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl₃ (300mg/kg)</td>
<td>29.05 ± 0.69b</td>
<td>38.97 ± 0.71b</td>
<td>38.82 ± 0.82b</td>
<td>54.71 ± 0.66b</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl₃ (300mg/kg)</td>
<td>22.37 ± 0.78b</td>
<td>32.64 ± 0.77b</td>
<td>45.61 ± 0.67b</td>
<td>55.36 ± 0.70b</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SEM, n = 6. The data were analysed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul’s multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05.

Table 9 showed the effect of HMEVT and AFR on brain neurotransmitter level in AlCl₃ induced Alzheimer’s disease in rats. The quantity of dopamine, noradrenaline and serotonin were reduced in disease control group, but they were more significantly (p<0.01) raised in rats which were treated with 300mg/kg of HMEVT and 1ml/kg of AFR (p<0.01) whereas 150mg/kg of HMEVT was not restored the dopamine level after twenty days of treatment moreover it showed less significant (p<0.01) effect on normalization of serotonin and noradrenaline level.

**Table 9** Effect of HMEVT and AFR on brain neurotransmitter level in AlCl₃ induced Alzheimer's disease in rats.

<table>
<thead>
<tr>
<th>Parameters &amp; Treatment</th>
<th>Dopamine (µg/mg tissue)</th>
<th>Noradrenaline (µg/mg tissue)</th>
<th>Serotonin (µg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.974±0.001</td>
<td>0.615±0.0006</td>
<td>0.475±0.0009</td>
</tr>
<tr>
<td>Disease Control (AlCl₃ 300mg/kg)</td>
<td>0.627±0.0009</td>
<td>0.378±0.0007</td>
<td>0.253±0.0010</td>
</tr>
<tr>
<td>Rivastigmine (0.3mg/kg) + AlCl₃ (300mg/kg)</td>
<td>0.954±0.001</td>
<td>0.601±0.001</td>
<td>0.459±0.005</td>
</tr>
<tr>
<td>HMEVT (150mg/kg) + AlCl₃ (300mg/kg)</td>
<td>0.708±0.0004*</td>
<td>0.499±0.0005</td>
<td>0.367±0.0007*</td>
</tr>
<tr>
<td>HMEVT (300mg/kg) + AlCl₃ (300mg/kg)</td>
<td>0.763±0.0005*</td>
<td>0.538±0.0007</td>
<td>0.403±0.0006*</td>
</tr>
<tr>
<td>AFR (1ml/kg) + AlCl₃ (300mg/kg)</td>
<td>0.936±0.0008*</td>
<td>0.586±0.0012</td>
<td>0.423±0.0007*</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SEM, n = 6. The data were analysed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul’s multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05.

Table 10 showed histopathological reports of the cortex and hippocampus of the brain of Aluminum Chloride induced Alzheimer’s disease with different treatment groups (n=6).

**Table 10** Histopathology reports of cerebral cortex and hippocampus of brain of AD-induced rats and treated groups with HMEVT and AFR.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parts</th>
<th>Histopathology image</th>
<th>Report (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>Cortex</td>
<td>Section showed that all the cellular functional units were within normal limits</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Section showed that all the cellular functional units were within normal limits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Disease Control (AlCl₃ 300mg/kg)

- Cortex: Section showed vacuolation, neuropil, multifocal, marked vacuolation, neuronal, hippocampus, diffuse, marked congestion/haemorrhage, multifocal, marked
- Hippocampus: Section showed vacuolation, neuropil, multifocal, moderate congestion/haemorrhage, multifocal, moderate

Rivastigmine (0.3mg/kg) + AlCl₃ (300mg/kg)

- Cortex: Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion/haemorrhage, multifocal, minimal
- Hippocampus: Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion/haemorrhage, multifocal, minimal

HMEVT (150mg/kg) + AlCl₃ (300mg/kg)

- Cortex: Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion/haemorrhage, multifocal, minimal
- Hippocampus: Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion/haemorrhage, multifocal, minimal

HMEVT (300mg/kg) + AlCl₃ (300mg/kg)

- Cortex: Section showed vacuolation, neuropil, diffuse, severe, congestion/haemorrhage, multifocal, minimal
DISCUSSION

“Rasna” is one of the most significant medicinal plants in the indigenous system of medicine. It is a controversial medicinal plant and has a wide application in the health care system. There are 13 plants which are currently being identified and used as Rasna in different parts of India. VT is called as Rasna in West bengal and also in many parts of India and are traditionally used for diseases of the nervous system. It is also used in gastro-intestinal complications like dyspepsia, flatulence and neurological disorders (Palash et al., 2013). Many chemicals are used for the induction of Alzheimer’s disease including aluminium, scopalamine, colchicine, streptozocin, sodium azide and ethanol. Aluminium is considered as one of the frequently used heavy metals for induction of cognitive impairment (Akroram and Nawaz, 2017). It also causes anemia, osteomalacia, and hepatic and neurological disorders. Oral administration of high amount of 300 mg/kg body weight of aluminium has been reported that, induction of AD and associated oxidative stress, cholinergic deficit and accumulation of Aβ & NFTs in the brain of rats (Mahdi et al., 2019).

This neurotoxicity effect is due to free radical productions which make damage to lipids and proteins in brain. aluminium crosses the blood-brain barrier through a specific transferrin receptor and induces profound memory loss via disruption of various normal neuronal functions. It also causes progressive apoptotic neuronal loss, ultrastructural alterations of neurons present in cortex and hippocampus region of brain, protein misfolding, plaques deposition and biochemical modifications followed by changes in genes expression in the brain (Zaky et al., 2017; Balgoon et al., 2015)

Aggressive behaviour was more frequent in patients with AD. Therefore behavioural and psychological symptoms were more likely to be observed closer to the time of diagnosis AD because it has severe dementia, neurodegeneration and loss of functional independence which leads to early death. Different neuropsychiatric instruments were used to assess the behavioural change in AD. Long term administration of aluminium has been reported to change in behavioural pattern (Kangtao and Sourav, 2018; Li et al., 2014). Hence in the present study, behavioural changes also investigated on aluminium exposure for 20 days and the possible effect of HMEVT and AFR were assessed on restoration of behavioural changes. Actophotometer test, elevated plus maze test and pole climbing apparatus were used to monitor the behavioural changes.

By using actophotometer, locomotor activity was assessed to check the CNS stimulant or depressant effect on rats. Administration of aluminium for 35 days, there was a decline in locomotor activity in aluminium treated rats which was indicated that the CNS depressant effect on chronic aluminium exposure (Lakshmi et al., 2015). In the present study also treatment with HMEVT and AFR corrected the locomotor incoordination caused by AlCl3. EPM is extensively employed for assessing the learning, retaining memory ability and anxiety in rodents. Animals which were received only Aluminium decreased in the number of entries in open arm, decreased percentage of time spent in open arms and increased in the number of entries in closed arm (Murugaiyan and Bhragavan, 2020; Rabiee et al., 2018). Researchers reported that the percentage of time spent in open arms and the number of entry to open arms are related the anxiety indicator parameters in the EPM and are related to GABAṣ receptor complex (Jafarian et al., 2019). However the treatment with HMEVT and AFR reverse effects on AlCl3 induced Alzheimer’s disease in rats.

From Cooks pole climbing apparatus, memory retrieval capacity was determined as the ability of an animal to retention the acquire memory process. It was indicated by increasing number of avoidance response (Reddy et al., 2020). Ganga Raju et al., 2020 reported that the time taken to climb the pole was increased in the AlCl3 exposure group and was due to dementia. In the present study, taken to climb the pole was noted where the time taken to escape from the electric shock field was reduced as that of normal trained rats after continuous 20 days treatment of HMEVT and AFR.

Weight loss is the common problem found in AD which leads to weaker in muscle mass, hard to maintain physical balance and more susceptible to get systemic infection. The reason behind in weight loss is change in olfactory system which contains neurotransmitter such as acetylcholine which was deficient in AD. Changes in food consumption and behavioral disturbances are also occur in AD leading to decreased energy intake but increased energy expenditure is not the cause of weight loss in AD (Tamura, 2007). In the present study, AlCl3 administration significantly diminished the body weight in the disease control group of animals. It was because of less desired to water and food intake, transient diarrhoea and reduced efficacy in converting feed which leads to reduction in body mass (Mathiyazahan and Arokiasamy, 2019). Gain in body weight was observed among other groups treated with HMEVT and AFR.

Toxic beta-amyloid plaques proteins are formed in Alzheimer’s and collects between neurons which affect the cell function. Similarly, neurofibrillary tangles (tau) are formed and get accumulated inside neurons. In brain, one type of glial cell called microglia which engulfs and remove waste and toxins from the healthy brain. In AD, microglia fails to clear the waste debris and protein including beta-amyloid plaques. Sometimes oxidative modified proteins (carbonyl protein) also formed in brain (hippocampus) due to oxidative stress in AD (Aksenov, 2001). The declined level of protein was found in of Aluminium treated group and was reported by Yokel and McNamara, 1989. This was due to less intake of food, increased catabolism of proteins and formation of reactive oxygen species (ROS) in which hydroxyl radicals responsible for the oxidation of the side chains of some amino acids resulting in proteins hydrolysis (Mahre et al., 2018). However, in the present study also decreased level of protein content was found with after the administration of HMEVT and AFR.

Crapper et al., 1973 stated that long term exposure of AlCl3 is associated with high aluminium concentration in brain. It enters into the brain via the specific high affinity receptors for transferrin (TR) expressed in the blood brain barrier (BBB) and get accumulated in all the regions of rats with AD. In the present study also high Al concentration was found with disease control group rats, but treatment with HMEVT and AFR reduced the aluminium level in rats. Neurotoxicity caused by aluminium is mediated mainly by increasing cellular oxidative stress which gets accumulate and enhance reactive oxygen species (ROS) formation, which depletes the normal antioxidant defense mechanism, thereby further enhancing oxidative stress and lipid peroxidation processes. It also causes changes in iron homeostasis, causing excessive free iron ions leading to oxidative damage, finally culminating in neurodegeneration (Lakshmi et al., 2015). Long term exposure with AlCl3 resulted in marked oxidative stress, which is indicated by increased lipid peroxidation result in increased level of MDA as well as decreased in reduced GSH, CAT and SOD activity. This activity may be due to the reduced axonal mitochondria turnover, disruption of the golgi or reduction of synaptic vesicles induced by aluminium exposure (Prema et al., 2017). The present study also found that decreased level of CAT, GSH and SOD as well as
CONCLUSION

The present study was focused on few traditional uses of Rasna, were experimentally proven with animal models. The neuroprotective effect and the mechanism of VT were studied by using PPARγ and AChE receptor with flutamide and guggulsterone. Oral administration of HMEVT and AFR reverses the effects given by AICl3, in a dose-dependent manner. Therefore VT can be used as a remedy for the treatment of AD and neurotoxicity.

REFERENCES


Aksenov, M. Y., Aksenova, M. V., Butterfield, D. A., Geddes, J. W., & Markesbery, W. R. (2001). Protein oxidation in the brain in Alzheimer's disease, significant decline of antioxidant defense system is consistent with oxidative processing. In present study, reduced level of acetylcholine, dopamine, noradrenaline and serotonin levels were observed in only AICl3 treated animals. However, the treatment with HMEVT and AFR raised the neurotransmitters level as that of normal rats.

Histopathology of hippocampus and cortex samples were analysed from neuroprotective study inorder to know the structural changes and organ toxicity. The three major structural changes occurring in the brain include neuronal loss, formation and accumulation of hyperphosphorylated tau protein called (NFTs) and aggregation of β-amyloid (Aβ) peptide termed senile or amyloid plaques. These changes are most prominent in the cholinergic system, particularly in hippocampus and cortex, which is closely associated with memory loss and cognitive dysfunction in AD. So cortex and hippocampus of brain were selected for the histopathology (Vecchio et al., 2018). Researchers found that the AICl3 induced cellular damage in organs such as brain were analysed by histopathology. In the present study also it was confirmed by the pathologist. It’s being a comparative neuroprotective study, the order of potency was HMEVT > AFR.

increased level of MDA content was found with disease control rats but re-established in rats which were treated with HMEVT and AFR.

Neurotransmitters play an important role in maintaining synaptic and cognitive functions by sending signals across synapses. They also have a major role in causing oxidative stress, which is known to be involved in AD pathogenesis (Reddy, 2017). Neurotoxic effect of aluminium significantly increases AChE activity, the key enzyme which is responsible for acetylcholine hydrolysis thereby reduction in acetylcholine level found in brain (Ramachandran et al., 2019). Similarly AICl3 causes depletion of dopaminergic transmitters in the central nervous system and induced neurotoxicity (Zhong and Liang, 1999). Moreover it also causes neuronal loss in the brain region. This neuronal loss and the resultant compensatory mechanisms lead to changes in the level of norepinephrine available in the brain, which consequently affect cognitive functions (Gannon et al., 2015). Reynolds et al., 1995 also reported that in Alzheimers disease, significant decline of acetylcholine level in hippocampus of brain as compared to normal rat as similar to our results. A recent study on Neurotransmitter and its drugs in Alzheimers disease in Wistar rats. Neurochemical research, 4(11), 767–776.


