EFFECT OF MENTAT AND ITS SELECTED INGREDIENTS ON 3-NITROPROPIONIC ACID INDUCED NEURONAL DAMAGE IN WISTAR RATS.

Preeti T, P.A. Patil*, Suneel.I.Majagi*

KLE College of Pharmacy, Belgaum-590010 and * Department of Pharmacology, J.N. Medical College, Nehru Nagar, Belgaum-590010. Karnataka- India.

Summary

‘Mentat’ is a commercial polyherbal preparation and is used as an adjuvant in the treatment of parkinsons disease, alzheimer disease etc. It contains over twentyingredients of which Withania somnifera, Acorus calamus, Asparagus racemosus are reported to possess beneficial effects on nervous tissue in ‘Ayurveda’. However no scientific data are available which states that which of these ingredients are effective in treating neurodegenerative disorders. Therefore we investigated the protective effect of Mentat and its selected ingredients on 3-nitropropionic acid (3-NPA) induced neuronal damage in Wistar rats. Basal values of locomotor activity were assessed after administration of 3-NPA and after 24 hours. The procedure was repeated for all the groups and on ninth day animals were sacrificed to assess parameters like creatine kinase and comet assay. In 3-NPA treated group the locomotor activity was significantly reduced, creatine kinase activity has increased significantly and there was also increase in comet length, tail moments, percentage DNA in tail while percentage DNA in head decreased. Pre treatment with Mentat, Withania somnifera, Acorus calamus, Asparagus racemosus prevented 3-NPA induced locomotor activity and increased creatine kinase activity. They also prevented increase in the comet length, tail moment, percentage DNA in tail and decrease the percentage DNA in head.

Mentat as a whole product appears to be most potent followed by Withania somnifera, Acorus calamus, Asparagus racemosus and all appear to be quite promising for the treatment of neurodegenerative disorder.

Key words: Acorus calamus, Asparagus racemosus, Mentat, Neurodegeneration,

3-Nitropropionic acid, Withania somnifera.

*Corresponding author: Dr. P.A.Patil.

Professor, Department of Pharmacology, J.N. Medical College,

Nehru Nagar, Belgaum-590010. Karnataka- India.

E-mail: drpapatil@yahoo.co.in

Phone: 0831-24091828,Fax: 08312470759.
Introduction

Neurodegeneration in various degenerative disorders of nervous system, is said to be a progressive and irreversible loss of neurons in specific regions of the brain [1]. The most consistent risk factor for developing neurodegenerative disorder is increasing age [2,3] and incidence of such disorders is likely to increase as life expectancy of man is increasing due to ever improving health care facilities.

The present drug therapy of neurodegenerative diseases such as parkinsons disease, alzheimers disease, Huntington disease and ALS etc is not satisfactory. Several approved drugs do alleviate symptoms but their chronic use is often associated with debilitating side effects and none seems to arrest the progressive pathology.

Ancient system of Indian medicine ‘Ayurveda’ mentions some herbs which are said to be effective in the management of nervous disorder. In an attempt to develop newer and effective agents for treating such disorder, several plant extracts have been tested for their neuroprotective activities e.g gingo biloba, piper methysticum (kava-kava)[4], ashwagandha [5], vacha [6], shatavari [5] etc.

Based on the ayurvedic principles one of the commercial herbal preparation, ‘Mentat’ containing Brahmi (Hydrocotyl asiatica), Ashwagandha (Withania somnifera), Vacha (Acorus calamus), Shatavari (Asparagus racemosus), Amla (Emblica officinalis), Shankhpushpi (Evolvulus alsinoides) and Triphala [7]; is reported to improve the mental quotient, memory span, concentration ability, stress threshold. It is also claimed to reduce anxiety, attention fluctuation, behavioural disorders and to improve articulation, speech disorders as well as parkinsonism activity [7,8,9,10].

Literatures in ‘Ayurveda’ mentions that shatavari, vacha and ashwagandha are beneficial in nervous disorders[5,6]. Information regarding protection against neurodegeneration by ‘Mentat’ could be traced in available literature. Moreover, what is the contribution of individual ingredients of mentat towards neuroprotection provided by mentat as whole, if at all it arrests neurodegeneration, is also not well documented.

Hence the present study was undertaken to investigate the efficacy of ‘Mentat’ and its selected individual ingredients in 3-nitropropionic acid induced model of neurodegeneration, in male Wistar rats.

Materials and methods

Animals: The experiments were carried out using healthy male, adult rats of Wistar strain, weighing between 100-150 grams. The animals were acclimatized to laboratory conditions with 12:12 hr natural light-dark cycle and were maintained on standard laboratory diet with free access to tap water.

Drugs used and their doses: The animals were divided in to six groups (n=6, in each) to receive various treatments. Normal saline alone was administered to a group of normal rats which served as negative control and other group received neurotoxin 3-Nitropropionic acid (3-NPA) alone (positive control). Each of remaining four groups were pretreated with ‘mentat’ 200 mg/kg alcoholic extracts of (mg/kg) Withania somnifera-100[11], Acorus calamus-200[12] and Asparagus racemosus-100[13] before challenging with two i.p. doses (50 mg/kg) of 3-NPA.
The treatments were continued once every 24 hr for seven days in all the groups, except negative control all the groups received neurotoxin (3-NPA) on eighth day to induce neurodegeneration. All the treatments were administered by oral route. Before administering 3-NPA, the basal level of locomotor activity was recorded for 10 min using actophotometer. Neurodegeneration was induced, according to the method described previously [14]. This method selectively induces the striatal lesions, by irreversibly inhibiting succinic dehydrogenase activity. The animals received two intraperitoneal injection of 3-nitropropionic acid (25 mg/ml, pH 7.4, 50 mg/kg body wt) at 0 and 90 min and sacrificed after 24 hours for measuring creatine kinase activity and comet assay.

Sample preparation:

**Preparation of the brain tissue:** The animals were anaesthetised by putting them in a foam box containing dry ice for 2 min, then they were decapitated and the brain was dissected out, immediately and was immersed in ice cold phosphate buffered saline (PBS contains NaCl 8.01 g/L; KCl 0.20 g/L; Na₂HPO₄ 1.15 g/L; KH₂PO₄ 0.20 g/L, pH 7.4) [15].

The striatum was isolated with the help of stereotaxic guidelines described previously [16,17] and was divided into two parts, one part was utilised to carry out the comet assay and the other was homogenised with a Teflon glass homogeniser in 20 volumes of ice-cold TRIS-sucrose buffer, pH 7.5 (250 mM sucrose, 10 mM Trizma). The homogenate was centrifuged at 800 g for 10 min, the pellet was discarded, and the supernatant was centrifuged at 10,000 g for 15 min, the supernatant of second centrifugation, containing cytosol, and other cellular components such as endoplasmic reticulum, was collected for determination of cytosolic creatine kinase activity. Homogenate and cytosolic fractions were stored at -70°C when the assay was not carried out immediately [18]. Creatine kinase activity was measured in striatal tissue homogenates using spectrophotometer (Erba chem 5 plus).

**Protein determination:** The protein content of the striatal tissue homogenates was determined by bicinchoninic acid method as described previously [19]. The total protein was expressed in mg/ml.

**Creatine kinase assay:** Creatine kinase assay was carried out using standard creatine kinase assay kit (Techo Diagnostics, California).

**Comet assay:**

**Preparation of slides:** The procedures involved are sample preparation, making microgel, lysing, electrophoresis, neutralising, drying and staining [14,15,20,21]. In brief single cell suspension of the striatal tissue is used to perform comet assay. A small piece of striatal tissue was placed in 1 ml cold phosphate buffered saline (PBS) containing 10% DMSO and tissue was minced in to fine pieces with the scissor, and allowed to settle for a minute, 10 µl of cell suspension was removed and mixed with 200 µl LMPA (low melting point agarose [14,15], and processed according to the method described earlier [21]. This sample cell preparation (10 µl) was mixed with 220 µl of 0.7% agarose in an eppendorff tube to place an agarose precoated slides. The slides were kept in coplin jar containing lysing solution, before subjecting for electrophoresis and then staining.

**Preparation of micro gel electrophoresis (MGE) slides:** Scoring [22,23]: The slides were analysed using a florescent microscope with a blue filter (used for FITC excitation 490 nm, emission 510 nm and dichroic 500 nm) at magnification of 400X. A minimum of 50 cells per slide were counted. Comet length, tail moment (pixels), % DNA in head and % DNA in tail were assessed as indices of DNA double-strand breaks, with the help of software (Autocomet-II,
Triteck Corporation, Japan). The study was approved by IAEC, constituted in accordance with CPCSEA guidelines and the study was approved by IAEC.

**Statistical Analysis:** Data were expressed as Mean ± SEM and analysed by ANOVA followed by Dunnet’s test and p≤0.05 was considered as significant.

**Results**

**Locomotor activity:** 3-NPA significantly (p<0.001) reduced the locomotor activity. Where as treatments with *Withania somnifera*-100, *Acorus calamus*-200, *Asparagus racemosus*-100 as well as Mentat-200 restored locomotor activity in 3-NPA treated animals (Table I).

**Creatine Kinase activity:** 3-NPA significantly (p<0.01) increased creatine kinase values. Where as treatments with *Withania somnifera*-100, *Acorus calamus*-200, *Asparagus racemosus*-100 as well as Mentat-200 showed significant decrease in creatine kinase activity in 3-NPA treated animals as compared with the values of 3-NPA alone treated group (Table I).

**Table I: Effect of various treatments on locomotor activity and creatine kinase in animals challenged with 3-NPA.**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Groups (n=6)</th>
<th>Locomotor activity (Mean±S.E.M)</th>
<th>Creatinine Kinase (n mol/sec/mg) of protein (Mean±S.E.M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drugs and Dose mg/kg</td>
<td>8th day</td>
<td>9th day</td>
</tr>
<tr>
<td>1</td>
<td>Normal Saline (Negative Control)</td>
<td>194.2±7.16</td>
<td>197.8±9.93</td>
</tr>
<tr>
<td>2</td>
<td>3-NPA + Normal Saline (Positive Control)</td>
<td>213.2±1.94</td>
<td>18.5±1.95**</td>
</tr>
<tr>
<td>3</td>
<td>W.Somnifera 100</td>
<td>200.2±7.33</td>
<td>225.5±6.73</td>
</tr>
<tr>
<td>4</td>
<td>A.Calamus 200</td>
<td>210.3±6.95</td>
<td>224.0±6.56</td>
</tr>
<tr>
<td>5</td>
<td>A.Racemosus 100</td>
<td>135.7±18.5</td>
<td>210.3±13.57</td>
</tr>
<tr>
<td>6</td>
<td>Mentat 200</td>
<td>206.5±7.88</td>
<td>214.2±6.62</td>
</tr>
</tbody>
</table>

ANOVA followed by Dunnet’s test, p<0.01*, p<0.001**.

Note: a) 3-NPA significantly reduced the locomotor activity of rats.

b) All the treatment groups restored locomotor activity in 3-NPA treated animals.

c) 3-NPA significantly increased creatine kinase activity of rats.

d) Significant decrease in creatine kinase activity in all the treated groups as compared to 3-NPA treated group.
DNA damage: 3-NPA caused significant (p<0.05, p<0.01, p<0.001) DNA damage. While treatments with *Withania somnifera*-100, *Acorus calamus*-200, *Asparagus racemosus*-100 as well as Mentat-200 showed significant (p<0.01) decrease in DNA damage induced by 3-NPA as compared to that of control group (Table II) and (Fig I).

**Table II: Effect of various treatments on 3-NPA induced DNA damage.**

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Drugs and Dose mg/kg</th>
<th>Comet Length</th>
<th>% DNA in Head</th>
<th>% DNA in Tail</th>
<th>Tail moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>26.64±0.787</td>
<td>95.77±0.508</td>
<td>4.22±0.508</td>
<td>0.418±0.079</td>
</tr>
<tr>
<td>2</td>
<td>3-NPA</td>
<td>42.00±0.549*</td>
<td>76.41±0.663*</td>
<td>23.59±0.663***</td>
<td>0.91±0.081**</td>
</tr>
<tr>
<td>3</td>
<td>*W.*Somnifera 100</td>
<td>23.94±0.938</td>
<td>95.33±0.683</td>
<td>4.665±0.683</td>
<td>0.423±0.064</td>
</tr>
<tr>
<td>4</td>
<td>*A.*Calamus 200</td>
<td>24.54±1.007</td>
<td>93.79±1.245</td>
<td>6.209±1.245</td>
<td>0.477±0.141</td>
</tr>
<tr>
<td>5</td>
<td>*A.*Racemosus 100</td>
<td>25.58±0.743</td>
<td>95.41±0.581</td>
<td>4.593±0.581</td>
<td>0.3177±0.079</td>
</tr>
<tr>
<td>6</td>
<td>Mentat 200</td>
<td>22.66±0.748</td>
<td>97.19±0.500</td>
<td>2.809±0.500</td>
<td>0.2024±0.047</td>
</tr>
</tbody>
</table>

ANOVA followed by Dunnet's test, p<0.05*, p<0.01** and p<0.001***.

Note: a) 3-NPA treated groups showed significant increase in DNA damage as compared to controls.

b) No significant change in DNA damage in all the treated groups as compared to control groups.

**Fig I: Comet images in various treated groups.**

a. Control (Saline)  
b. 3-Nitropropionic acid (3-NPA)
c. Withania somnifera - 100  
d. Acorus calamus - 200  

e. Asparagus racemosus - 100  
f. Mentat - 200  

Note: 3-NPA treated group (b) showing increase DNA damage (increase in comet length, tail and decrease in head) where as all treated groups (c to f) decreased the DNA damage by 3-NPA.

Discussion

The mitochondrial toxin 3-nitropropionic acid (3-NPA) inhibits complex II of the electron transport system and causes neurodegeneration that resembles Huntington’s Disease in the striatum of human and experimental animals [24]. Other mechanisms like mitochondrial DNA (mt-DNA) damage and induction of Zn2+ release in hippocampus slices have also been suggested [24,25]. In the present study Mentat, Withania somnifera, Acorus calamus significantly decreased creatine kinase activity and also restored SMA to normal indicating their efficacy as neuroprotectives. These findings of Withania somnifera, Acorus calamus agree with earlier reports though they carried out in stress induced [26,27] or ischaemic brain injury [28]. The other agent Asparagus racemosus also showed significant protection in the present study and there are no similar reports about it. It is obvious that the neuroprotective activity of Withania somnifera, Acorus calamus is reported due its antioxidants property [28,29]. It has been reported that Withania somnifera by down regulating nNOS and by neurochemical alterations of specific neurotransmitter systems produces neuroprotection [26]. Asparagus racemosus has not been studied widely against neuroprotective activity. The new proactivity of Asparagus racemosus in the present study could be due its antioxidant constituents. The neuroprotective activity of mentat thus appears to be due to its constituents which individually have shown neuroprotective activity. Superiority of mentat as neuroprotective amongst the other plants tested is obviously due to
augmentation of antioxidant ability by its contents. However its efficacy against various neurodegenerative disorders need to be confirmed clinically.

References


