Effect of wheat grass powder on aluminum induced Alzheimer’s disease in Wistar rats

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ABSTRACT

Objective: To find out the effect of wheat grass on aluminum induced Alzheimer’s disease in Wistar rats.
Methods: Memory impairment was induced by aluminum chloride (4.2 mg/kg, i.p.) for 28 d. Memory function was assessed by Morris water maze test. To study the activity of wheat grass (100 mg/kg, p.o.), Wistar rats were administered it for 28 d along with aluminum chloride. Biochemical parameters of oxidative stress were estimated in brain after the treatment.
Results: The major finding of this study is that aluminum enhanced oxidative stress. Wheat grass showed a significant improvement in reduction of this oxidative stress by reduction of malondialdehyde levels and enhancement of superoxide dismutase and catalase levels.
Conclusions: The present study clearly demonstrated the beneficial effects of wheat grass that shows good antioxidant properties, and this remarkable effect of wheat grass may act as a key to treat Alzheimer’s disease.

1. Introduction

Metals play a major catalytic role in the production of free radicals, and attention has centered on the role of many metals in Alzheimer’s disease, including iron, aluminum, mercury, copper, and zinc. Iron is involved in the formation of the free hydroxyl radical, which has recognized deleterious effects as described in Fenton’s and Haber–Weiss’s classical reactions. Many observations provide proof that the metabolism of iron is involved in Alzheimer’s disease[1–3].

Aluminum has been suggested as a causal factor in Alzheimer’s disease, in part because of reports showing the toxicity of aluminum, the elevation of aluminum concentrations in the brains of patients with Alzheimer’s disease, and an association between aluminum concentrations in water and the prevalence of Alzheimer’s disease[4].

The possibility of copper’s involvement in Alzheimer’s disease is supported by the fact that copper can act as a catalyst in the production of reactive oxygen species and by the data showing that the amyloid precursor protein (APP) molecule contains a copper—binding site[5]. The binding of Cu(II) leads to the modification of APP via the oxidation of cysteines 144 and 158, which leads to the formation of cystine and Cu(0). In this respect, APP serves in the electron transfer to Cu(II) in vitro.

The latest metal cited as a possible factor in Alzheimer’s disease is zinc. Zinc induces a rapid amyloid formation in humans, which perhaps explains the scarcity of cerebral β–amyloid in animals[6]. In addition, APP binds Zn(II), which are directly related to Alzheimer’s disease, there is increasing evidence suggesting that zinc accumulation can mediate neuronal death associated with other brain injuries, including ischemia[7].

Natural products derived from diets are known to exhibit a variety of biological effects including antioxidant, anticarcinogenic, antimutagenic and antiaging activities. Epidemiological, clinical and laboratory studies demonstrating the association between nutrition and
cognition highlight the importance of dietary intake in preventing or delaying Alzheimer’s disease[8,9]. Due to their relatively low side-effects and a long history of human use, edible phytochemicals may be good candidates for therapeutic and/or preventive application in Alzheimer’s disease.

The effectiveness and safety of herbal medicines for treating dementia were studied by comparing the herbal medicine with placebo and with pharmaceutical intervention[10]. Meta-analyses were performed on common cognitive performance outcome measures. All studies reported herbal medicine had significant effects in improving symptoms. In studies that employed active controls, herbal medicine was at least as effective as the pharmaceutical intervention. Meta-analyses found herbal medicines are more effective than no treatment or placebo and at least equivalent to control interventions, even though the overall effect was small. No severe adverse events were reported. These trials provide overall positive evidence for the effectiveness and safety of certain herbal medicines for Alzheimer’s management.

To date, there was no effective treatment for Alzheimer’s disease. Much research effort has been focused on developing new drugs from nutritional supplements which have multifunctional properties. Novel plant extracts and their major or bioactive components including alkaloids, flavonoids, glycosides and saponins with promising therapeutic and/or preventive application in Alzheimer’s disease, glutaminergic, serotonergic, catecholaminergic and histaminergic systems, Aβ–peptide Tau protein inhibition, enhancement of cerebral blood flow and elevation of RNA as well as protein levels have been studied. To develop new drugs from plant sources for the treatment of Alzheimer’s disease is a hopeful attempt to meet the unmet medical needs. Therefore, this study was conducted to evaluate the effect of wheat grass on aluminum induced Alzheimer’s disease in Wistar rats.

2. Materials and methods

2.1. Animals

Wistar albino rats of either sex were procured from Mahaveer Enterprises, Hyderabad, India. The animals were maintained on light for 12 h and dark for 12 h. They were fed, ad libitum regular grain chow (Rayans Biotechnologies Pvt. Ltd., Hyderabad). Diet containing 56.00% grain derived carbohydrate, 21.00% protein, 6.70% moisture, 3.58% total oil, 2.58% dietary fiber, 5.50% cellulose, 0.80% calcium, 0.60% phosphorus, 0.30% sodium chloride. The animal housing and handling were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines. The prior permission for the study was obtained from our Institutional Animal Ethics Committee.

2.2. Aluminum chloride

Aluminum chloride (CDH, India) was purchased from local chemical suppliers for the induction of Alzheimer’s disease.

2.3. Wheat grass powder

Wheat grass powder was bought from Arogya Raksha, Visakhapatnam, India. The beneficial effects of wheat grass include antioxidant, anti-inflammatory, cardiovascular protection, anticancer activity, anti–ulcer effects, anti–allergic activity and antiviral activity.

2.4. Induction of Alzheimer’s disease

Albino Wistar rats were selected. Aluminum chloride was dissolved in water and injected to rats through intraperitoneal route at a dose of 4.2 mg/kg body weight lightly anaesthetized with ether. Aluminum chloride was given for 28 d and Alzheimer’s disease was confirmed by measuring nootropic activity weekly till the 28th d. Following 28 d of Alzheimer’s disease, rats were subjected to surgical procedure for the estimation of various parameters.

2.5. Treatment schedule

The animals were divided into five groups (n=6) and assigned to different treatments. Group 1 was labeled normal control rats. Group 2 was normal rats treated with 1% sodium and 1 mL carboxymethyl cellulose orally. Rats in Group 3 were administered wheat grass 100 mg/kg orally. Normal rats in Group 4 were administered wheat grass 100 mg/kg orally. Normal rats in Group 4 were treated with aluminum chloride intraperitoneally (4.2 mg/kg). Rats in Group 5 were injected with aluminum chloride intraperitoneally (4.2 mg/kg) and treated with wheat grass 100 mg/kg orally.

2.6. Assessment of cognitive performance

Animals were tested in a spatial version of Morris water maze test[11,12]. The Morris water maze (MWM) is a test of spatial learning in rodents. Since its first application in 1981, the MWM has become one of the most frequently used tools for analyzing spatial learning and memory. It has been used widely in investigations of different aspects of learning and memory in rodents and in investigations of the variables that may affect the animal’s behavior in the task. It has also been used as a tool to investigate chemically induced effects on learning and memory. MWM test explains the nootropic activity of the wheat grass in terms of latency period (seconds). The time taken for the animal to reach the hidden platform is recorded as latency period.

2.7. Biochemical assessment

Biochemical tests were conducted after the last behavioral
test. Animals were sacrificed by cervical dislocation. Brains were removed and homogenized, the obtained supernatant was used for biochemical estimations. Oxidative parameters in brain tissue, such as malondialdehyde (MDA), superoxide dismutase (SOD) and catalase levels were measured.

2.8. Statistical analysis

All the values were expressed as mean±SEM. The data were analysed using One-way repeated measure ANOVA. A level of \( P<0.05 \) was considered as statistically significant. Tukey’s test was performed to find the significant difference at \( P<0.05 \).

3. Results

Aluminium chloride treated animals showed an increase in escape latency initially, which continued during the training for spatial navigation task. From the results obtained, Table 1 explains that there was a significant difference in the mean escape latency period of aluminium treated group when compared to the control group. The percentage degree of variation was more for Alzheimer’s treated rats with wheat grass powder when compared to the normal rats treated with only wheat grass powder this was clearly represented in Table 1.

### Table 1
Effect of wheat grass on nootropic effect in terms of latency period (seconds) in normal and Alzheimer’s group rats.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group 1</th>
<th>Group 2</th>
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<td>25.90</td>
<td>24.60</td>
<td>16.30</td>
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</table>

The values given in the parentheses represent percent variation.

There was significant biochemical alterations in the oxidative stress parameters such as MDA, SOD and brain catalase levels. In Alzheimer’s group rats there was a significant increase in brain MDA levels and a significant decrease in brain SOD and catalase levels when compared to vehicle treated rats (Group 2), the results were shown in Tables 2, 3 and 4.

### Table 2
Effect of wheat grass on brain MDA levels (nmol/mg wet tissue) in normal and Alzheimer’s group rats (n=10).

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<tr>
<th>S. No.</th>
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<td>23.90</td>
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<td>10.40</td>
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### Table 3
Effect of wheat grass on brain SOD levels (units/mg protein) in normal and Alzheimer’s group rats (n=10).

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<tr>
<th>S. No.</th>
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<td>26.63±1.12</td>
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<td>22.02±0.87</td>
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There was a significant decrease in elevated MDA levels (Table 2) and a significant increase in brain SOD (Table 3) and catalase levels (Table 4) in wheat grass powder treated rats for 28 d indicating the antilipid peroxidation properties and antioxidant properties of wheat grass.

The results indicated that the degree of reduction in MDA levels was more with Alzheimer’s group when compared to normal rats treated with wheat grass. Similarly a significant increase in SOD and catalase levels were observed more in Alzheimer’s group when compared to normal rats. The results from all the tables revealed the significant antioxidant activity of wheat grass powder in diseased animals when compared to the normal animals.

### Table 4
Effect of wheat grass on brain catalase levels (μmol of H₂O₂ decomposed/mg protein/min) in normal and Alzheimer’s group rats (n=6).

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There was a significant decrease in elevated MDA levels (Table 2) and a significant increase in brain SOD (Table 3) and catalase levels (Table 4) in wheat grass powder treated rats for 28 d indicating the antilipid peroxidation properties and antioxidant properties of wheat grass.

4. Discussion

Aluminum is a potent neurotoxin involved in the initiation and progression of various cognitive disorders like Alzheimer’s disease. Chronic aluminum exposure induces oxidative stress which might be the mechanism responsible for the role of aluminum in Alzheimer’s disease[13,14]. In this study we investigated the behavioral changes caused by aluminum exposure and the possible effect of wheat grass treatment using behavioral and biochemical tests. In Morris water maze, aluminum exposure was associated with decrease spatial memory and this was evidenced by the results.

Oxidative stress caused by the formation of reactive oxygen species, such as hydrogen peroxide, superoxide radical (O₂⁻). The free radical production caused cell damage by damaging the DNA, cytosolic and membrane–bound macromolecules. The present study also demonstrated that increased oxidative stress was observed in rats treated with aluminum for 28 d as
it was evidenced by increased MDA levels, decreased SOD and catalase levels in rats treated with aluminum when compared to vehicle control rats.

Wheat grass is an excellent source of minerals like calcium, cobalt, iron, phosphorus, potassium, sulfur, zinc, antioxidant vitamins such as A, B, C, E and antioxidant enzymes such as SOD, cytochrome oxidase and other enzymes[15,16]. The antioxidant enzymes present in wheat grass helps rid of free radicals thus improving memory. Wheat grass containing antioxidant properties is anticipated to exert neuroprotective effects via the regulation of cellular homeostasis and augmentation of self-defense to oxidative stress[17]. This study investigated the possible protective effects of wheat grass on Aβ–induced apoptosis and the cholinergic memory deficits caused by scopolamine in rats. Pery et al., (1992) reported that antioxidant activity of wheat grass was by scavenging superoxide anion and hydroxyl radical in vitro.

In the present study, treatment with wheat grass powder demonstrated a significant decrease in MDA levels and a significant increase in SOD and catalase levels in both normal and Alzheimer’s group rats. The degree of variation in oxidative stress was more in Alzheimer’s group rats when compared to normal rats. Based on the oxidative hypothesis in Alzheimer’s disease, it is important to maintain a balance between antioxidants and oxidants in living organisms, and increased intake of dietary antioxidants may help in maintaining an adequate antioxidant status. Since last three decades, the association between aluminum and Alzheimer’s disease had gained much interest and it has been shown that aluminum accumulates in all the regions of the brain maximum being in hippocampus, which is the key site of memory and learning.

In conclusion, our study demonstrated that aluminum exposure was associated with impaired memory and cognitive functions in Wistar rats. Such situation was reversed by wheat grass, the results suggested that wheat grass may have preventive and/or therapeutic potential in the management of Alzheimer’s disease.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgements
We thank Andhra University College of Pharmaceutical Sciences for providing good laboratory services and our study was supported by University Grants Commission/Rajiv Gandhi National Fellowship, New Delhi, Grant No.: (RGNF=4878).

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