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RESEARCH ARTICLE

Effect of aluminum toxicity and *Bacopa monnieri* on hexokinase enzyme activity in Wistar albino rats

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ABSTRACT

Background: Aluminum enters human body through food and drinking, cosmetics, and occupational exposures. Aluminum (Al) is a highly neurotoxic element causes neuronal degeneration in humans as well as in animals. Studies reported that *Bacopa monnieri* has anxiolytic, antidepressant, antioxidant, and memory-enhancing property. Hexokinase is a very important glycolytic enzyme and also rate-limiting enzyme determines the rate of glucose utilization by the cells. **Aims and Objectives:** The study was planned to investigate whether Al exposure affects the hexokinase activity and protective effect of *Bacopa* on hexokinase activity in discrete regions of brain and other vital organs. **Materials and Methods:** Wistar albino rats were divided into four groups, each group consists of six. Group 1 as control received distilled water, Group 2 received Al chloride, Group 3 received crude extract of *Bacopa*, and Group 4 received Al as well as *Bacopa*. After 30 days of Al and *Bacopa* administration, rats were sacrificed, tissues were homogenized and hexokinase was assayed. **Results:** In all the tissues (liver, muscle, kidney, cerebral cortex, hippocampus, and cerebellum), Al treated as well as Al + *Bacopa*-treated animals showed significant decrease in hexokinase activity when compared with control animals. However, the *Bacopa* and Al-treated animals showed a marked increase in the hexokinase activity from Al alone treated animals. Moreover, no change in hexokinase activity was observed between control and *Bacopa*-treated animals. **Conclusion:** Al affects the hexokinase activity whereas, Bacopa enhances the hexokinase activity by inhibiting the toxic effects of Al.

KEY WORDS: Aluminum; *Bacopa monnieri*; Hexokinase

INTRODUCTION

In India, aluminum (Al) is widely used as chief cooking utensil. Al is a lighter metal used for aircraft, electric cables, and other machineries. It is having low melting point of 660°C. Since early 20th century, Al was widely used as cooking utensils. During cooking process, lot of Al will mix with the food because of its low melting point. Al has no known biological and biochemical functions.[1] Al enters human body through food, water, cosmetics, and occupational exposures.[2,3]

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Animal studies reported that oral administration of Al results in accumulation in discrete regions of brain, bones, muscles, kidney, and other organs. Al is proved as neurotoxic element can cause neuronal degeneration in brain of humans. Perl *et al*. reported that Al accumulates predominantly within nuclear region and cytoplasm of neurons in patients with sclerosis and Parkinsonism.[4] It has been reported that Al is one of the risk factors and involved in pathogenesis of dialysis dementia[5] and senile dementia of Alzheimer disease type.[6] Al changes glutamate and c-aminobutyrate levels and also activities of

the associated enzymes with regional specificity.[7] Al also inhibits glutamate dehydrogenase (GDH), an important enzyme in glutamate metabolism.[8] GDH can stimulate the reversible deamination of L-glutamate to a-ketoglutarate which is directly connected to the Krebs cycle.[9] Hexokinase is a very important glycolytic enzyme and also rate-limiting enzyme, determines the rate of glucose utilization by the cells. Since the detoxification process is normally done by the liver, this study focuses the hexokinase activity.

Choice of the Herb as Antidote

Bacopa monnieri (brahmi) was widely used by ayurvedic medical practitioners in India since 3000 years and is classified as a Medhya rasayana, a drug that improves memory and intellect (Medhya) and used as nerve tonic and believe to Vatahara (which Calms vata) and Anuloma (that redirects the flow of vata downward). Various studies of *B. monnieri* reported to have anxiolytic, antidepressant, and memory-enhancing activity.[10] *Bacopa* reduces stress which was induced by Al toxicity and also it possesses antistressor activity.[11] Based on this, the herb *B. monnieri* (Brahmi) was selected.

This study evaluated whether 30 days of Al administration cause any effect on hexokinase activity in liver, kidney, muscle, and discrete areas of brain and also studied whether the effect of *B. monnieri* could prevent the Al-induced toxicity in the discrete brain regions.

MATERIALS AND METHODS

Ethical consent was obtained from the Institutional Animal Ethical Committee (IAEC) University of Madras, Chennai, India. Fresh *B. monnieri* plants were washed, dried under the shade and finely powdered. The preparation was mixed with water and given orally to rats at the dose of 300 mg/kg body weight. Adult male Wistar albino rats, weighing 160–180 g (3 months), were utilized in this study. The rats were maintained as per the guidelines of IAEC.

The study consists of four major groups are as follows:
Group 1: Controls treated with distilled water for 30 days.
Group 2: Oral Al chloride (320 mg/kg) for 30 days.
Group 3: Received *B. monnieri* (300 mg/kg) for 30 days.
Group 4: Al-treated animals received *Bacopa* for 30 days.

Specimen Preparation

Animals were anesthetized by ether and sacrificed by cervical dislocation, and then, liver, muscle, and brain were removed quickly and placed in a cold saline. Various regions of brain - cortex (CO), hippocampus (HI), and cerebellum (CE) were dissected out in cold (4°C) condition. 10% homogenate was prepared using Tris-HCl buffer (0.1 M, pH 7.4) and homogenate was centrifuged at 2000 rpm, 4°C for 15 min. The supernatant was used for the following analysis. Similarly, a part of liver, kidney, and skeletal muscle was weighed and homogenized with Tris-HCl buffer (0.1 M, pH 7.4), a 10% homogenate was prepared. Hexokinase was assayed.[12]

RESULTS

All the data were expressed in mean ± standard deviation [Table 1] and analyzed by analysis of variance (ANOVA) and when there was a significant, *F*-test ratio was followed by Tukey's multiple comparisons using SPSS version 11.0. $P < 0.05$ was considered statistically significant.

In all the tissues (liver, muscle, kidney, cerebral CO, HI, and CE), Al treated as well as Al-treated animals with *Bacopa* showed a significant decrease in hexokinase activity compared with control animals. However, the *Bacopa* received Al-treated animals showed a significant increase in hexokinase activity from Al alone treated animals. Moreover, no change in hexokinase activity was observed between control and *Bacopa*-treated animals.

DISCUSSION

Al is toxic to humans as well as animals when it is consumed. Hexokinase is the enzyme involved in phosphorylation of glucose and converts them into glucose-6-phosphate. This conversion is essential for retaining the glucose within the cell. This study shows that Al decreases the activity of hexokinase in muscle, brain, as well as in kidney of the rat. Lai *et al*., in 2006, reported that Al inhibits hexokinase activities in rat brain.[13] This may be due to its accumulation of Al in these tissues. Anand *et al*. reported that Al accumulates in many tissues such as in brain, kidney, muscles, and liver.[14] Al deposition in kidney causes nephritic degeneration.[15] A study by Van der Voet *et al*. observed that Al accumulated in rat liver when $AlCl_3$ is administered intraperitonea.[16] Al was observed to accumulate in almost all the regions of brain, especially in hippocampal region.[17]

Table 1: Effect of 30 days treated with Al and *B. monnieri* on hexokinase enzyme activity

Organs	Control	Al	*Bacopa*	Al+*Bacopa*
Liver	14.25±0.90	7.99±0.50*	14.12±0.94$^{\#}$	11.56±1.16*
Muscle	11.25±1.62	5.90±0.69*	11.04±1.70$^{\#}$	9.24±1.08*
Kidney	10.18±1.43	4.94±1.00*	10.08±1.20$^{\#}$	8.28±0.69*
Cerebral CO	9.24±0.62	4.81±0.46*	9.26±1.18$^{\#}$	7.25±1.30*
HI	12.73±1.60	5.99±0.96*	12.19±1.43$^{\#}$	10.47±1.50*
CE	10.53±1.03	5.97±0.82*	12.19±1.43$^{\#}$	8.60±1.13*

Data are expressed as mean±SD. *P<0.05 - difference between control and Al as well as Al and *Bacopa* received groups, $^{\#}P$<0.05 - difference between *Bacopa* and aluminum received groups. CO: Cortex, HI: Hippocampus, CE: Cerebellum, SD: Standard deviation

Possible Mechanism Behind

Al salts can bind to DNA and RNA results in decreased expression of enzymes such as hexokinase, phosphatases, and phosphodiesterase.[18] Glycolysis was inhibited by Al shows that the enzyme involved in glycolysis may be vulnerable to Al toxicity.[19] The toxic effects of Al may be due to production, and accumulation of more reactive oxygen free radicals causes oxidation of lipids, proteins, DNA, and RNA.[20,21] It was reported that Al-induced oxygen free radicals may inhibit superoxide dismutase and catalase.[22] Al enhances free radical-induced cellular damage.[23]

Role of *Bacopa*

In this study, there is no significant difference between *Bacopa*-treated animals and controls indicating the adaptogenic property of *Bacopa*. Moreover, the Al-treated animals receiving *Bacopa* showed marked increase in the hexokinase activity. The herbal constituents are known to decrease the free radicals directly by quenching them. Antioxidant property of *Bacopa* can inhibit the oxidation process by reacting with the free radicals, chelating free catalytic metals, or by acting as oxygen scavengers.[24] Antioxidants are also reported to inhibit free radical generation and to donate hydrogen from phenolic hydroxyl groups, thereby forming stable free radicals, which inhibits further propagation of lipid oxidation. Strong evidence indicated that flavonoids and alkaloids have potent antioxidant- and Vitamin C-sparing activity.[25]

The analysis of *B. monnieri* reported to contain phenolic, flavonoids, and carotenoids. Moreover, animal researchers also reported that *Bacopa* extracts could modulate the expression of certain enzymes involved in generation and scavenging of reactive oxygen species in the brain.[26]

CONCLUSION

The 30 days of Al administration decreases the hexokinase activity in discrete area of brain, liver, kidney, and muscle. The Al-treated animals receiving *Bacopa* showed a significant increase in the hexokinase activity showing that *Bacopa* prevented the changes induced by Al toxicity, indicating some of the constituents in Bacopa have reduced the stress level. To study, if Al exposure induced alterations in these parameters could be normalized by the treatment of a herb, *B. monnieri*. Moreover, this study suggests to prevent the usage of Al utensil, foils, and cosmetics.

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