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RESEARCH ARTICLE

Effect of aluminum toxicity and *Bacopa monnieri* on plasma cortisol level in Wistar albino rats

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ABSTRACT

Background: Aluminum is used as cooking utensil, and aluminum foil is used in covering and packing the food. It was known that aluminum is highly reactive, particularly during high temperature. Aluminum accumulates in the brain and other vital organs and causes damage to the tissues. **Aims and Objectives:** The aim is to study whether aluminum exposure can alter the plasma cortisol level and also to study if aluminum exposure induced alterations in cortisol level could be normalized by the treatment of *Bacopa monnieri.* **Materials and Methods:** A total of 24 animals were divided into four groups. Group 1 is control receiving saline, Group 2 receives aluminum, Group 3 receives *Bacopa*, and Group 4 receives aluminum and *Bacopa*. After 30 days, blood sample was collected and plasma cortisol level was estimated. **Results:** Aluminum-treated animals showed a significant increase (df 3, 23; f = 9) when compared with control animals. Whereas, *Bacopa* alone treated group as well as aluminum- and *Bacopa*-treated group showed no significant changes compared with control groups animals. Moreover, aluminum-treated animals which received *Bacopa* showed a significant decrease in cortisol level from aluminum alone treated animals. **Conclusion:** Aluminum exposure-induced raise in blood cortisol level for 30 days indicates that aluminum may act as a chemical stressor. The aluminum-treated animals receiving *Bacopa* showed a significant decrease in the cortisol level.

KEY WORDS: Aluminum; *Bacopa*; Cortisol; Stress

INTRODUCTION

The increasing use for preparation and storage of food in Al vessels, cans, and foils may increase the Al content, particularly in food that is is salty, acidic, and alkaline.[1] Humans consume an average of 7600 g/day of aluminum from drinking water and food.[2] However, other aluminum sources such as cosmetics, drugs, or special foods increase the levels of exposure in some subgroups of population. Animal studies indicate that oral exposure to Al leads to accumulation in the brain, bone, muscle, kidney, and other organs. Levine *et al.* found that Al administered by intraperitoneal injection to rats produced a local toxic myopathy.[3] Garbossa *et al*. studied the effect of oral Al administration on erythropoiesis in male Wistar rats.[4] Their results demonstrate that Al can impair erythropoiesis at low doses *in vivo* and at higher doses exhibit toxicity to both CFU-E and mature erythrocytes. Aluminum compounds are not thought to be mutagenic or otherwise genotoxic.[5] However, aluminum has been reported to interact with DNA and possibly alter gene expression. This is strengthen by the Roy *et al*., who reported that inhibition of bone marrow cells and increased chromosome aberrations in male rats were observed after administrating the increased doses of aluminum sulfate in drinking water for 1–3 weeks.[6]

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Bielarczyk *et al*. studied the ability of $AlCl_3$ to affect cholinergic transmission on synaptosomal fractions of rat brain *in vitro*.[7] It has been reported that Al is involved in the etiology of dialysis dementia,[8] amyotrophic lateral sclerosis,[9] and senile dementia of Alzheimer disease type.[10] Aluminum was also shown to exert a pro-oxidant activity and promotes biological oxidation both *in vitro* and *in vivo*.[11] Furthermore, Yousef *et al*. showed that $AlCl_3$ induced free radicals and inhibited antioxidant enzymes.[12] It is very difficult to prevent the consumption of aluminum in the present lifestyle. Herbal drugs may prevent the toxic effect of aluminum which is entering in our body.

Bacopa monnieri is also called Brahmi, a name derived from Brahma, the creator god of the Hindu pantheon of deities. Brahmi has been an important constituent of the Ayurvedic Materia Medica and is frequently mentioned in Charaka Samhita (compilation of *Charaka* around 6th century AD). It is used in traditional Indian medicinal system Ayurveda as a nerve tonic and believes to Vatahara (which Calms vata) and Anuloma (that redirects the flow of vata downward). The Bhavprakasa Varg-Prakarana (drug classification of Bhavprakasa) of the 16th century states that Brahmi is sweet in taste, produces coolness, and increases vigor. It acts as a brain tonic and promotes longevity.[13] *B. monnieri* has been reported to possess anxiolytic, antidepressant, and memory enhancing activity.[14,15] Based on this, the herb *B. monnieri* (Brahmi) was selected.

Therefore, we studied whether aluminum exposure can alter the plasma cortisol level and also to study if aluminum exposure induced alterations in cortisol level could be normalized by the treatment of *B. monnieri.*

MATERIALS AND METHODS

Fresh, whole *B. monnieri* plants were washed, dried in the shade, and weighed before preparation of the extract. Leaves, roots, and stems of the plant were finely powdered and given to the rats orally at the dose of 300 mg/kg body weight. Healthy adult Wistar strain male albino rats, weighing between 160 and 180 g (3 months old), were used in this study. The rats were kept in the animal room with controlled ambient temperature (24 ± 2°C), humidity, and light (14 h/10 h light/dark cycle, lights on 07:00 am) with food and water *ad libitum*.

The Study Consist of Four Major Groups as Follows

- Group 1: Controls received saline for 30 days
- Group 2: Received aluminum chloride (320 mg/kg body weight) for 30 days
- Group 3: Received *B. monnieri* for 30 days
- Group 4: Received aluminum and *B. monnieri* for 30 days.

The food intake was monitored along with the bodyweight changes and observed no marked change between the aluminum- and *Bacopa*-treated animals.

Specimen Preparation

Rats were anesthetized by ether and blood sample was collected from the jugular vein and immediately stored in heparin-coated tube. Plasma cortisol was estimated by spectrophotofluorimeter method.[16] It is a fluorometric assay, in which cortisol stock solution 100 mg/dL was prepared and diluted to produce a range of concentrations 10–100 µg/dL. Samples and cortisol standards were then mixed in 7.5 mL of dichloromethane. From this, 5 mL of the resulting dichloromethane extract phase was then transferred and mixed with 2.5 mL of fluorescent reagent containing concentrated sulfuric acid:absolute ethanol (70:30) and the tubes were thoroughly mixed. An aliquot of the lower phase was removed and the fluorescence measured at excitation 470 nm and emission 530 nm with a Hitachi-650-10M spectrophotofluorimeter. The results were expressed as µg/dL of plasma. The following formula was used to calculate the cortisol level, cortisol level (µg/dL) = (reading of unknown – Blank)/(reading of standard – Blank) ×100.

RESULTS

All the data were expressed as Mean±SD analyzed using the analysis of variance (ANOVA) and when there was a significant F test ratio it was followed by Tukey's multiple comparisons using SPSS version 11.0 (SPSS, Cary, NC, USA) . For statistical significance the significance fixed was $P<0.05$. Aluminum-treated animals showed a significant increase in plasma cortisol level (df 3, 23; $f = 9$) when compared with control animals, whereas *Bacopa* alone treated group as well as aluminum- and *Bacopa*-treated group showed no significant changes compared with control groups animals. Moreover, aluminum-treated animals which received *Bacopa* showed a significant decrease in cortisol level from aluminum alone treated animals [Table 1].

DISCUSSION

In this study, it was found that aluminum increases the plasma cortisol level suggesting that aluminum may act as a chemical stressor. Whereas, treating with Bacopa decreases the plasma cortisol level shows that Bacopa may reduce the toxic effects of aluminum. Aluminum has the potential to be toxic in humans and animals. The toxic effects of aluminum have been suggested to be due to the generation of reactive oxygen species, which results in the oxidative deterioration of cellular lipids, proteins, and DNA.[17] Hashem in 2009 suggested that the aluminum chloride-induced free radicals may inhibit the enzymes involved in antioxidant defense

Table 1: Effect of 30 days treated with aluminum and ***B. monnieri*** on plasma cortisol level

Plasma cortisol	Control	Aluminum	*Bacopa*	Aluminum+*Bacopa*
	42.32±2.05	56.9±8.41*	42.9±4.7#	48.8±3.6*

Data are expressed as mean±SD. *P<0.05 - difference between control and aluminum as well as aluminum and *Bacopa* received groups, #P<0.05 - difference between difference between *Bacopa* and aluminum received groups

such as SOD and CAT.[18] Further, he observed a significant decrease in the enzymes in the kidney and liver of treated rats. Increase in free radicals levels causes poor cognition and neuronal degenerative changes.[19] Aluminum might facilitate membrane peroxidation by increasing their susceptibility to free radical-induced damage.[20] In this study, there is an increase in the cortisol level. It has been well established that the cortisol level is an indicator of stress intensity and greater HPA axis activation in response to stress.[21] Activation of the HPA axis leads to a rapid secretion of ACTH in the anterior pituitary and to an increase in circulating glucocorticoids.[22] The corticosterone is a stress marker which indicates that the aluminum may be a chemical stressor. Vitamin C content in *Emblica officinalis*, an important antioxidant reported to reduce the corticotrophin releasing hormone levels in the bloodstream[23] The aluminum-treated animals receiving *Bacopa* showed a normal cortisol level indicating that the stress level has reduced and some of the constituents in *Bacopa* may be responsible for this.

Role of *Bacopa*

In this study, the *Bacopa*-treated normal animals did not show any variation from controls indicating its adaptogenic activity. The herbal constituents are known to decrease the free radicals directly by quenching them. Antioxidant principle constituents can interfere with the oxidation process by reacting with the free radicals, chelating free catalytic metals, and also by acting as oxygen scavengers.[24] Antioxidants are also reported to interrupt the free-radical chain of oxidation and to donate hydrogen from phenolic hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids. The tannoid principles of *E. officinalis* had been reported to show an antioxidant activity in chronic stress-induced changes in rat brain.[25] Strong evidence indicated that the flavonoids had potent antioxidant-dependent and Vitamin C-sparing activity.[26] The analysis of *B. monnieri* reported to contain phenolic, flavonoids, and carotinoids. 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.[27]

CONCLUSION

Aluminum exposure for 30 days raise blood cortisol level indicates that aluminum may act as a chemical stressor. The aluminum-treated animals receiving *Bacopa* showed a significant decrease in the cortisol level showing that *Bacopa* prevented the changes induced by aluminum toxicity and indicating that some of the constituents in *Bacopa* have reduced the stress level. Moreover, this study suggests not to use aluminum utensils, foils, and aluminum-based cosmetics.

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