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The Impact of Aluminum Utensils on the Morphology of the Nucleolus in the Meristematic Cells of Onion Root

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ABSTRACT

Background: Aluminum (Al) and its compounds are naturally present in the air, water, and many food sources. Excessive exposure to Al could adversely affect respiratory, hematological, nervous, immune, and skeletal systems. Considering the fact that under stress, the nucleoli number or size might change, this study aimed to investigate the influence of boiled water in Al containers on the nucleolus size and number in the root meristem cells of *Allium cepa* L.

Methods: Onion roots were exposed to water boiled in an Al container at concentrations of 5, 10, and 15 mg/l of Al for 42 to 43 h. Subsequently, the meristematic zone of the root tip cells was examined for nucleolus number, as well as the area of the nucleus and nucleolus.

Results: According to the results, the mean nucleolus number and area in the study groups treated with any concentration of Al were not significantly different from that in the control group.

Conclusion: The result of this study does not support the effect of aluminum cookware on the nucleolus number and area. Further investigations are needed to confirm the impact of Al on nucleolar morphology.

1. Introduction

Aluminum is a widespread element, abundantly found in the Earth′ s crust. It is naturally present in the air, water, and many foodstuffs such as vegetables and fruits. Additional exposure pathways for humans include food additives, packaging materials, cooking devices, cleaning agents, and pharmaceutical products, such as vaccines (Affourtit et al., 2020; Mahmud et al., 2020). Excessive exposure to Al could adversely affect respiratory, hematological, nervous, immune, and skeletal systems (Willhite et al., 2014). The nucleolus is a distinct subnuclear region responsible for ribosome biogenesis and other critical cellular functions, such as controlling the cell cycle, assembling signal recognition particles, sensing cellular stress, and modifying small RNAs (Corman et al., 2023; Ma et al., 2016). Given its diverse functions, nucleolar alterations have been associated with cellular stressors and pathologies, including neurodegeneration, cancer, and aging. Morphological changes of nucleolus in response to cellular disturbances are stressor-specific and can include alterations in nucleolar size or number, generation of other structures such as nucleolar caps, and moving of nucleolar components to the cytosol and nucleoplasm (Corman et al., 2023). Nucleolar size serves as a critical indicator of cellular division and growth; specifically, there is a positive correlation between nucleolar size and number and the level of growth and proliferation of cancerous cells (Stępiński, 2018). Moreover, a decrease in the production of ribosomal RNA and nucleoli size, which happens during aging, is considered a risk factor for neurodegenerative abnormalities (Parlato & Kreiner, 2013). Also, nucleoli have been reported to be the target of some viral proteins in viral infection (Yang et al., 2018). In our previous studies, the cytogenotoxic impact of water boiled in

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Al pots was examined on the onion root cells of the meristematic zone using *Allium cepa* assay and found that the percent of the mitotic index and mitotic phase aberrations in some study groups raised compared to that in the control group (Zendehboodi, 2018; Ekhlasi & Zendehboodi, 2021). Considering the fact that nucleolar size and number may change under stress, this investigation aims to evaluate the influence of boiled water from Al containers, at defined concentrations of Al, on nucleolus size and number in the root meristem cells of *Allium cepa* L.

2. Materials and Methods

A single Al pot was applied for boiling distilled water for over 20 h at a high flame to ensure sufficient leaching of Al. The Al concentration in distilled water and the boiled water sample was then evaluated by atomic absorption spectrometer. Next, the boiled water was diluted with distilled water to prepare samples with Al concentrations of 5, 10, and 15 mg/L. It should be mentioned that the Al concentration in the distilled water was zero. Healthy and clean onions were purchased from grocery shops. Discs of onion were placed in distilled water and incubated at 21 ± 1 °C for one day to facilitate root development. Following this, the root discs were treated with boiled water samples containing 5, 10, and 15 mg/L of Al for 42 to 43 h. Since after boiling the pH of water increased, the pH of the boiled water was adjusted to 7 using HCl. The onions exposed to the distilled water with the same temperature and duration as other study groups were used as a control group. At the end of the treatment, five roots from each bulb were subjected to Feulgen staining, and slides were prepared from the meristematic area. These slides were examined using an Olympus microscope equipped with a digital camera. Fifty randomly selected cells from each bulb (ten cells from each of the five root photos) were analyzed for nucleolar number, as well as nuclear and nucleolar areas, using Image J software (Figure 1). In the case of more than one nucleolus per nucleus, the total area of nucleoli was considered. Three onions were included in each study group. To inspect the distinction between the study groups regarding the examined parameters, one-way ANOVA was applied. The SPSS version 22 was used to analyze the data and $P < 0.01$ was accepted as a significant level.

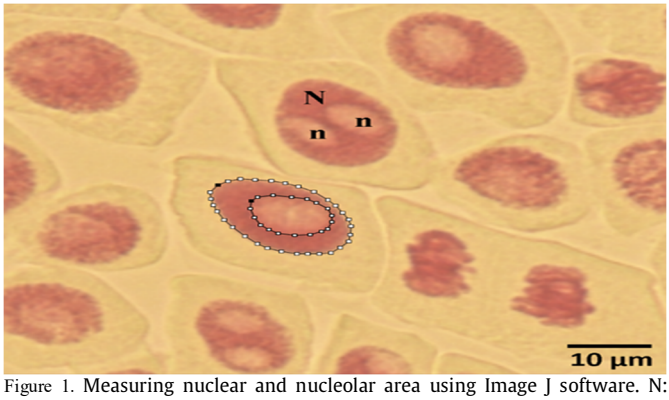


Figure 1. Measuring nuclear and nucleolar area using Image J software. N: Nucleus, n: Nucleolus

3. Results and Discussion

The mean values for nucleolus number, nucleus, and nucleolus area, and the ratio of nucleolus to nucleus area in root tip cells exposed to water samples with various Al concentrations are depicted in Table 1. The data derived from the main ANOVA are as follows: mean nucleolus number: $F(3, 8) = 0.782$, $p = 0.537$; mean nucleus area: $F(3, 8) = 1.183$, $p = 0.376$; mean nucleolus area: $F(3, 8) = 1.391$, $p = 0.314$, and mean nucleolus to nucleus area ratio; $F(3, 8) = 2.898$, $p = 0.102$. Nucleoli play a critical role in numerous cellular processes, particularly in ribosome production which is essential for efficient protein biosynthesis necessary for cell growth and division (Boulon et al., 2010). The morphology of the nucleolus is very sensitive to different cellular disturbances, such as the reduction of nucleolar factors, heat shock, cytotoxic agents, hypoxia, nutrient starvation, viral infection, and UV radiation (Corman et al., 2023). A growing body of studies indicates the responsiveness of nucleoli to heavy metals and toxic agents. For instance, a study investigating the neurotoxic effects of Platinum-based drugs reported that carboplatin, *R,R*-ormaplatin, oxaliplatin, *S,S*-ormaplatin, and cisplatin decreased the nucleolar diameter in rat neurons (McKeage et al., 2001). In addition, cadmium and atrazine have been shown to increase the number of nucleoli while decreasing their area in the meristematic cells of *Allium cepa* root (Lima et al., 2019). Under lead stress, alterations in *Allium cepa* root cells have been noted, with certain nuclear proteins being overexpressed and relocating from nucleoli to the nucleoplasm or cytoplasm (Jiang et al., 2014). Because of the repetitive nature of ribosomal genes and their intensive transcription, ribosomal DNA is very vulnerable to damage. It is suggested that DNA damages such as double-strand breaks probably interfere with the transcription process and lead to nucleolar stress (Hua et al., 2022). Consistently, previous studies showed that Al has genotoxic effects such as inducing micronuclei, nuclear bud, and different chromosomal aberrations including breaks and gaps (Lima et al., 2007; Lima et al., 2011; Francisco et al., 2021).

Table 1. The effect of water boiled in Al cookware on the morphology of nucleoli in onion root tip cells

	Al concentration (mg/l)	Number of nucleolus/ Nucleus	Nucleus area (µm²)	Nucleolus area (µm²)	Nucleolus area/Nucleus area
water boiled in Al cookware	5	1.21 ± 0.05	150.58 ± 8.40	33.06 ± 1.09	0.22 ± 0.01
	10	1.15 ± 0.02	139.57 ± 9.09	28.55 ± 2.66	0.21 ± 0.01
	15	1.22 ± 0.09	132.55 ± 6.00	31.00 ± 1.72	0.24 ± 0.00
	Control	1.27 ± 0.01	135.95 ± 4.23	28.58 ± 1.53	0.21 ± 0.01

All measurements are presented as the mean ± SE based on three independent replicates for each water sample.

4. Conclusion

In conclusion, the findings of the current study do not support the effect of aluminum cookware on the nucleolus number and area. This preliminary study is the first of its kind to assess the influence of water boiled in Al cookware on nucleolus features. It should be noted that in addition to Al, other metals such as Cd, As, Cu, Ni, and Pb have been shown to leach from these pots, with the leaching rate of some metals increasing with the age of the cookware (Alabi et al., 2020). Further investigations examining more utensils from different sources (new, old, made of scrap metals) with larger biological samples are needed to confirm the impact of Al utensils on the nucleolus morphology and their possible toxic consequences.

Authors' Contributions

Zahra Zendehboodi: Study concept and design; Drafting of the manuscript; Critical revision of the manuscript for important intellectual content; Study supervision; Analysis and interpretation of the data. Fathiyeh Ekhlasi: Data collection and experiments conduction; Analysis and interpretation of the data.

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Conflicts of Interest

No conflicts of interest are declared by the authors.

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No item.

Ethical considerations

This study was authorized by the Shiraz University Ethics Committee (Grant No. 97GCU2M256246).

Using artificial intelligence

No AI tools were used.

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