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The Effect of Water Boiled in Aluminum Cookwares on Genomic Abnormalities in the Meristematic Cells of Onion Root

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ABSTRACT

Introduction: One of the ways of human exposure to aluminum (Al) is Al food packaging materials and cookwares. Although many studies have examined the biotic influence of nanoparticles or ionic form of heavy metals, there are limited studies conducted on the possible health risks of metals in the form of alloy used for making utensils. In this study, the effect of water boiled in Al cookwares with defined concentrations of Al on the genomic abnormalities and cell division of meristematic cells of onion root was evaluated using Allium cepa assay.

Materials and Methods: The onion roots were treated with water boiled in Al utensils (three pots) with different concentrations of Al (5 and 10 mg/l) for 42 to 43 hours and then analyzed for mitotic index (MI) and mitotic phase aberrations (MPA).

Results: The percent of MI in the study groups treated with 5 mg/l of Al from pot 1 and 10 mg/l from all pots increased significantly compared to the control group ($P < 0.05$). Also, the frequency of total MPA in all Al treated groups significantly increased compared to the control group ($P < 0.05$). The most significant results were derived by sticky chromosomes, anaphase bridge, going ahead chromosome and disturbed mitosis, respectively.

Conclusion: The result of this study confirmed the genotoxic effect of water boiled in Al cookwares containing the examined range of Al concentrations on the meristematic cells of onion root.

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Introduction

Heavy metals are natural components of the earth's crust. At proper concentration, some of the heavy metals, such as cobalt, iron, manganese, nickel, copper, zinc etc. are required for living things; however, high doses of them can induce harmful effects. Today, the term heavy metal is not restricted to the metals with high density or atomic weight, but also metals and metalloids with toxicity to human and environment. Because of natural and human activity, these elements move to the atmosphere and the hydrosphere, resulting in environmental pollution [1]. Aluminum (Al) is the third most plentiful element in the earth's crust and one of the toxic metals with broad distribution in daily life [1-2]. Humans are exposed to Al from different sources. Al can occur in the air as a result of industrial and natural activities, such as carbon burning, cement industry, and rocks erosion [3]. It is an important element of many cosmetics, such as antiperspirants and sunscreens and applied in some medications, including buffered aspirin, injection with allergen, using as an adjuvant in some vaccines and prostheses in dentistry [2]. The other source of Al is food. Packaging, cooking, and preserving of foodstuffs in Al foils and utensils as well as using Al containing food additives can concentrate Al in the diet [3]. Al absorption in the body takes place through skin,

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ingestion, and inhalation and this metal was found to be in different tissues, including lung, bone, kidney, liver, and brain [2]. Many studies have suggested toxic effect of Al on the central nervous system and its contribution to the neurodegenerative disorders [4]. It has been shown that Al administration leads to change in acetylcholinesterase activity and causes neurobehavioral, neuropathological, and neurochemical alterations, resulting in weakened learning skill [4]. Animal studies have shown that Al incorporates into the bone and induces calcium release and osteomalacia [4, 5]. Injection of Al to uremic rats led to a decrease in hematocrit and hemoglobin, resulting in microcytic anemia [6]. Prolonged administration of Al lactate to the rat increased Al concentration in the serum and renal tissue, which proposed to affect the balance water and sodium, impair the antioxidant defense system, and change renal tubular transport [7]. It is supposed that Al toxicity may exert trough membrane lipid peroxidation and oxidative stress; since increased lipid peroxidation and decreased catalase and superoxide dismutase activity was detected in the brain of mammals chronically exposed to Al [4, 8]. Also, examining the properties of mitochondria from the brain cell of the rats treated chronically with Al revealed impaired respiratory chain, ATP production, and antioxidant defense system, as well as loss of crista and increased production of reactive oxygen species [9].

Although many research studies have examined the biotic influence of nanoparticles or ionic form of heavy metals, limited studies investigated the possible health risks of metals in the form of alloy used for making utensils. Previously, in a pilot study, the cytogenotoxic effect of water boiled mildly in Al pots for about 2 h was examined on the meristematic cells of onion root and a marginal significant increase was found in the mean total prevalence of genomic abnormalities from treated groups compared to the control [10]. In that study, the Al concentration in the boiled water was not measured; however, it was shown that boiling water or neutral solutions in Al utensils did not induce much Al leaching. In the present study, to confirm the genotoxic effect of water boiled in the cookwares made of Al, the effect of these water samples with defined concentrations of Al was examined on the genomic abnormalities and cell division in the meristematic cells of onion root using *Allium cepa* assay.

Materials and Methods

In this study, the mitotic index (MI) (indicating the rate of cell division) and M phase aberrations (MPA) in the onion root meristematic cells exposed to water boiled in the cookwares made of Al were examined using *Allium cepa* assay. This assay is a low cost, short-term, and favorable bioassay to examine the cytotoxic and genotoxic effects of substances [11]. In the present study, using this assay, different types of MPA, such as sticky chromosomes, disturbed and C-mitosis, anaphase bridge, going ahead chromosome, vagrant chromosome, and chromosome breaks were evaluated. Three Al cookwares from different company were purchased and before using for the experiment, the water was boiled in them several times and discarded. Then they were used for boiling distilled water with high flame for a long time (more than 20 hours) to ensure that Al was concentrated in the remaining water. At the end of boiling, the Al concentration was measured by atomic absorption spectrometer and the water sample was diluted by adding distilled water to make the water containing 5 and 10 mg/l of Al. For each treatment, five bulbs were used.

Clean and healthy onions were prepared from fruit shops. For rooting, the onions were exposed to distilled water and kept in an incubator at 21 ± 1 °C for one day and then exposed to water boiled in Al cookwares with different concentration of Al (5 and 10 mg/l) for 42 to 43 hours. Distilled water was used as a control. The culture media was refreshed every day .At the end of the treatment, at 8:00 am, the tip of several healthy roots was separated from each onion bulb and subjected to staining with Schiff's reagent as described in the authors' previous study [10]. The slides were observed by the Olympus microscope under a total magnification of 400 ×. In total, more

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than 1200 cells were evaluated for each onion sample to determine the MI and the frequency of MPA using the following formulas [10]:

MI% = number of cells in mitosis × 100 / total cell number

MPA% = number of aberrant cells × 100 / total cell number

Statistical analysis

The results were presented as the mean and standard error of five repeats per each Al concentration from each pot. One-way ANOVA was performed to examine the difference between the study groups in respect of evaluated parameters. The post-hoc Duncan (in the case of equal variances) or Games- Howell (in the case of unequal variances) tests were used to assess the difference between the control and each of treated groups. Spearman's correlation test was applied to determine the correlation between Al concentration and the frequency of total MPA. Statistical analysis was performed using the SPSS version 22 and significance was considered at $P < 0.05$.

Ethical Issue

This study was authorized by Shiraz University ethics committee (ECBDE-SU-9-6177616).

Results

The result of MI in the root tip cells treated with water sample containing different concentrations of Al from each pot is demonstrated in Table 1. The main ANOVA result for pot 1 and 2 was significant (pot 1: $F(2, 12) = 5.49$, $p < 0.05$; pot 2: $F(2, 12) = 28.32$, $p < 0.0001$), and for pot 3 was not significant ($F(2, 12) = 2.89$, $p = 0.094$). According to the post hoc test, the percent of MI in the study groups treated with 5 mg/l of Al from pot 1 and 10 mg/l from all pots increased significantly compared to the control group ($P < 0.05$). However, the percentage of MI in the groups exposed to 5 mg/l of Al from pot 2 and 3 were not different from the control group.

Table 1: The effect of water boiled in Al cookwares with different concentrations of Al on the MI of onion root tip cells

	Treatment mg/l Al	MI ± SE (%)
Pot 1	5	10.20 ± 0.41**
	10	9.57 ± 0.79*
Pot 2	5	7.70 ± 0.34
	10	10.48 ± 0.22**
Pot 3	5	8.90 ± 0.60
	10	9.29 ± 0.46*
Control		7.74 ± 0.32

*: $P < 0.05$ and **: $P < 0.01$ expresses the significance level compared to the control.

The data of abnormalities in the M phase are presented in Table 2. The main ANOVA results from all three pots for the total aberrations were significant (pot 1: $F(2, 12) = 19.72$, $p < 0.001$; pot 2: $F(2, 12) = 22.63$, $p < 0.0001$; pot 3: $F(2, 12) = 33.69$, $p < 0.0001$). According to the post hoc test, the total MPA percentage in all Al treated study groups increased significantly compared to the control group ($P < 0.05$). Analyzing each type of abnormality showed that among the abnormalities in the M phase, the most significant results were derived by sticky chromosomes, anaphase bridge, going ahead chromosome, and disturbed mitosis, respectively. The frequency of sticky chromosomes significantly increased in all Al treated groups, except for concentration of 10 mg/l from pot 2, compared to the control group. The frequency of anaphase bridge showed a significant increase in the groups treated with concentration of 5 mg/l from pot 2 and 10 mg/l from pot 2 and 3 compared to the control. At the concentration of 10 mg/l, the frequency of disturbed mitosis from pot 1 and going ahead chromosome from pot 2 significantly increased compared to the control group. In the other abnormalities, no significant difference was observed between the control and treated groups.

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Table 2: The effect of water boiled in Al cookwares with different concentrations of Al on the MPA

Treatment	mg/l Al	Abnormalities percentage							
		SCH	AB	GCH	CM	CHB	VCH	DM	Total
Pot 1	5	1.35 ± 0.11**	0.22 ± 0.07	0.09 ± 0.03	0.0 ± 0.0	0.03 ± 0.02	0.03 ± 0.03	0.20 ± 0.05	1.92 ± 0.13**
	10	2.09 ± 0.20**	0.26 ± 0.06	0.20 ± 0.09	0.03 ± 0.02	0.0 ± 0.0	0.0 ± 0.0	0.51 ± 0.12*	3.09 ± 0.35**
Pot 2	5	1.24 ± 0.08*	0.45 ± 0.04**	0.07 ± 0.04	0.0 ± 0.0	0.01 ± 0.01	0.04 ± 0.03	0.25 ± 0.05	2.07 ± 0.11**
	10	1.99 ± 0.37	0.79 ± 0.11**	0.12 ± 0.02**	0.01 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.37 ± 0.08	3.34 ± 0.38**
Pot 3	5	2.31 ± 0.25**	0.48 ± 0.11	0.10 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.25 ± 0.07	3.19 ± 0.42*
	10	2.52 ± 0.12**	0.98 ± 0.17**	0.23 ± 0.08	0.0 ± 0.0	0.10 ± 0.03	0.03 ± 0.02	0.25 ± 0.06	4.11 ± 0.17**
Control		0.72 ± 0.06	0.12 ± 0.04	0.0 ± 0.0	0.01 ± 0.01	0.0 ± 0.0	0.0 ± 0.0	0.18 ± 0.06	1.03 ± 0.13

*: P < 0.05 and **: P < 0.01 expresses the significance level compared to the control in the same column. SCH: sticky chromosomes, AB: anaphase bridge, GCH: going ahead chromosome, CM: c-mitosis, CHB: chromosome breaks, VCH: vagrant chromosomes, DM: disturbed mitosis, Total: total aberration

Also, there was a significant positive correlation between Al concentration and the frequency of total MPA (Table 3). Higher percentages of total MPA were detected at higher concentrations of Al.

Table 3: Correlation of Al concentration and the total MPA

Treatment	Al concentration	
	r	P value
Pot 1	0.907	< 0.001
Pot 2	0.926	< 0.001
Pot 3	0.869	< 0.001

Discussion

One of the ways of human exposure to Al is Al food packaging materials and cookwares. Evidence has shown that Al cookwares and foils increase the Al content of the foods cooked or preserved in [12-15] and many factors, such as pH, food composition, heating temperature, the presence of salt, and other ions have been suggested to affect the Al leaching. Al seems to deposit for a long time in different tissues of humans and animals, affecting many aspects of body functions in experimental animals [16].

In the present study, the results of onion root meristematic cells exposed to the water boiled in Al cookwares showed that the water sample containing 5 mg/l of Al from pot 1 and 10 mg/l of Al from all pots significantly increased the MI. Increase in the MI was also detected in the authors' previous study when the mean MI from the Al treated groups was compared with the control group. The stimulatory effect of low concentrations of Al on the cell proliferation was reported in the cultured human blood cells, while higher concentrations showed to have repression effects [17]. The repression effect of Al on cell division was also observed with barley root cells exposed to $AlCl_3$ [18] as well as *Zea mays* and *Allium cepa* root treated with Al_2SO_4 [19]. Since making alteration in the rate of cell division could be an indicative of being cytotoxic or tumorigenesis of a substance [11], it is suggested to investigate the effect of Al on the MI with more precisely monitoring experiments.

Evaluation of the MPA in the root tip cells indicated that the frequency of total aberrations in each treated group significantly increased compared to the control and there was a significant positive correlation between Al concentration and the frequency of total aberrations. Among the examined type of abnormalities, sticky chromosomes, anaphase bridge, going ahead chromosome, and disturbed mitosis showed the most significant results respectively. However, there was no significant difference between the control and the treated groups in respect of the other abnormalities. It should be noted that in the authors' previous study, no significant difference was observed in the total mitotic and interphase aberrations or in any specific aberration, in each treated cell group compared to the control group[10]. The result confirmed the adverse effect of water boiled in Al cookwares with stronger leaching condition on genomic abnormalities. In line with the present study, Alabi et al. reported an increased frequency of mitotic aberrations in the onion root tip cells treated with the water boiled in Al cookwares, especially the old ones. It is

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noteworthy that in that study, Al concentrations of the water samples were very low (0.02 to 0.05 mg/l) compared to the present study. In other words, they boiled water for 1 hour; this condition of water boiling is somewhat similar to that of the authors' previous study, in which no significant increase was observed in the total mitotic and interphase aberrations. This difference and also the variation observed in the present study between different pots regarding MI and MPA, might be due to various compositions of Al cookwares from different sources, in line with Alabi et al. who detected other heavy metals in addition to Al in the water samples which may damage the cell [20]. Several studies have reported that Al promotes cell and DNA damage, apoptosis, and cell division impairment [21-27]. It has been shown that in onion root meristematic cells treated with Al_2O_3 nanoparticles, the MI decreased while chromosomal aberrations increased [21]. Human lymphocytes exposed to $AlCl_3$ showed increased levels of micronuclei, DNA damage, and apoptosis [22, 23].Increased levels of DNA damage and apoptosis were also observed in the lymphocyte cells of carp living in the environment containing Al [24]. In a study conducted on the mammalian BJ and L929 cells, it was shown that in spite of the transfer of Al oxide nanoparticles to the cells, no increase in the apoptosis was detected. Moreover a significant decrease in the cell viability was observed only at the highest concentration of Al_2O_3 [25]. However, reduction of cell viability, decreased activity of antioxidant enzymes, increased lipid peroxidation, and morphological changes were observed in the chinook salmon cell line exposed to Al_2O_3 nanoparticles [26]. Al chloride was also reported to damage lymphocytes and thymocytes separated from mice in a time - and dose –dependent manner [27].

Conclusion

The results of the study confirmed the genotoxic effect of water boiled in Al cookwares containing the examined range of Al concentrations on the meristematic cells of onion root. Further research especially human epidemiological studies concerning Al exposure may more disclose the consequences of Al pollution and its possible threat to the public health.

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Conflict of interest

No conflicts of interests are declared by the authors.

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