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Protective Effects of A çai in Combination with Vitamin C against Aluminum-Induced Toxicity in Rat Liver

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Abstract

Aluminum is associated with the pathogenesis of several diseases. A çai has recently emerged as a natural source of antioxidants. The present study was conducted to evaluate the protective effect of A çai in combination with vitamin C against the aluminum chloride induced toxicity. Seventy rats were divided into 7 groups:- Group (GP) 1: control group, GP 2: treated with AlCl3, GP 3: treated with A çai, GP 4: treated with vitamin C, GP 5: treated with AlCl3 and A çai , GP 6: treated with AlCl3 and vitamin C, GP 7: treated with AlCl3, A çai and vitamin C. After 4 weeks, blood and liver specimens were collected to evaluate biochemical alterations and hepatic antioxidant and inflammatory parameters. AlCl3 treatment decreased liver enzymes (alanine aminotransferase, aspartate amino transferase, alkaline phosphatase), tumor necrosis factor-α and IL-6 while hepatic malondialdehyde was elevated. In contrast, hepatic glutathione, super oxide dismutase, catalase were decreased. A çai and vitamin C treatment improved the adverse effects induced by AlCl3, while co-administration with vitamin C promoted the action of a çai on hepatic damage and antioxidant parameters. A çai showed a protective effect against AlCl3 induced toxicity, particularly in combination with vitamin C.

Keywords: Aluminum chloride, A çai; Vitamin C, Oxidative stress, Inflammatory mediators.

1. Introduction

Aluminum is one of the most abundant metals in the earth's crust (Verstraeten et al., 2008), and is found in its ionic form in animal and plant tissues while, in nature it is found in combination with other elements such as oxygen, sulfate, chloride and other elements because of it is high reactivity (ATSDR, 2007). Humans are exposed to aluminum compounds from various sources as aluminum found in many food additives, toothpaste, medicines (such as aspirin), cheese, tea, cosmetics, drinking water, various household cookware and storage utensils; in addition aluminum is used to construct roofing and for many other industrial activities (Proudfoot, 2009). Exposure of humans to large quantity of aluminum leads to its accumulation in many organs, disturbing the pro-oxidant/antioxidant stability of tissues and causes lipid peroxidation (Exley, 2004). Aluminum inhibits enzymes such as hexokinase and, acid and alkaline phosphatases and, may bind to DNA and RNA (Kumar et al., 2009).

Fruits, particularly berries, have been shown to contain high levels of antioxidant compounds, such as polyphenols, phenolic acids, flavonoids, and carotenoids (Wang et al., 2008). These antioxidants are thought to scavenge free radicals and interact with reactive oxygen species (ROS) (Wang and Lin., 2000). Açai is a fruit that appears similar to blueberry in appearance and is widely distributed in northern South America. It is one of the main export products of the Amazon region and has been commercialized as frozen pulp or juice. In recent years, Açai has gained attention because of its potent antioxidant properties. Biochemical studies have demostrated that Açai is rich in phytochemicals, particularly polyphenols such as anthocyanins, proanthocyanidins and other flavonoids (Guerra et al., 2011; Xie et al., 2011)

Vitamin C (ascorbic acid) is an important nutrient obtained from food and, is needed for normal metabolic processes in the body such as the formation of bone, teeth and collagen as well as wound healing (Naziroğlu et al., 2011). Many studies have reported that vitamin C may be useful for treating chronic diseases such as cancer and cataract (Park; 2013; Ravindran et al., 2011). Vitamin C scavenges free radicals to regenerate other antioxidants such as vitamin E and prevents oxidative damages (Moser and Chun, 2016).

2.Materials and Methods

2.1 Experimental Animal

Seventy male albino rats (average body weight 150 - 180 g) were obtained from Pharos University (Animal House Unit). The animals were acclimatized under standard laboratory conditions for one week prior to dosing. Rats had free access to a standard diet and water *ad-libitum*.

2.2 Experimental Design

The 70 male albino rats were randomly divided into seven equal groups. The groups were as follows:- Group 1(GP 1) served as a control and was given a normal distilled water; GP 2 was treated orally with 100 mg/kg body weight (BW) of $AlCl_3$ (Alamia Company for chemicals, Ramadan, Egypt); GP 3was treated orally with Açai (Sigma –Aldrich, St. Louis, MO, USA)

at a dose of 400 mg/kg BW; GP 4 was treated orally with vitamin C at a dose 100 mg/kg BW, GP 5 was treated orally with $AlCl_3$ at a dose 100 mg/kg BW, A çai at a dose 400 mg/kg BW; GP 6 was treated orally with $AlCl_3$ at a dose 100 mg/kg BW and vitamin C at dose 100 mg/kg BW ; GP 7was treated orally with $AlCl_3$ at a dose 100 mg/kg BW, A çai at a dose 400 mg/kg BW and vitamin C at a dose 100 mg/kg BW. Rats were treated daily for 4 weeks and, doses were selected based on those reported in the literature (Chaudhary et al., 2014 ; Mehmet and Meryem, 2008 ; Pereira et al,. 2016)

2.3 Blood Sampling

At the end of treatment, rats were fasted overnight with free access to water Under light anesthesia with diethyl ether, rats were sacrificed by cervical decapitation and the blood was collected into heparinized tubes. The collected blood was then centrifuged at 4000 r.p.m for 15 min. and, the obtained plasma was stored at 20 ℃ until analysis.

2.4 Preparation of Liver Homogenate

At the end the 4th week post treatment 1 g of liver tissues was collected from each rat. Liver tissue was washed with ice-cold 0.9% NaCl solution and homogenized in 9mL ice-cold phosphate-buffered saline (pH 7.5) using a homogenizer instrument. The homogenate was centrifuged for 15 min at 3000 r.p.m and the supernatant was collected used directly, or stored in Eppendorf tubes, and stored at –20 ℃ until use.

2.5 Biochemical Parameters

Liver function tests such of enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were conducted using a colorimetric method (Reitman and Frankel 1957). Alkaline phosphatase (ALP) was measured calorimetrically (Belfield and Golberg 1971).

2.6 Oxidative Stress Markers: Hepatic Malondialdehyde (MDA) and Antioxidant Markers

MDA, as a marker of lipid peroxidation (LPO) was measured calorimetrically in the liver homogenate as described by Ohkawa et al. (1979). The non-enzymatic antioxidant glutathione (GSH) in the liver was determined as described by Ellman (1959). Based on the method of Nishikimi et al. (1972), super oxide dismutase (SOD) activity was assayed. The catalase (CAT) activity in the liver was assayed as described by Aebi (1984). Total protein was assayed in the plasma and liver homogenate according to Lowry et al. (1951).

2.7 Inflammatory Mediators Assay

Levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in the liver homogenates were determined by enzyme-linked immuno sorbent assay (Cat no. ab46070, Abcam, Cambridge, UK and Cat no. ab100772, Abcam). Hepatic levels of TNF-α and IL-6 were expressed as pg/mg protein.

2.8 Histo-Pathological Analysis

Small pieces of liver from each animal in the control and treated groups were fixed in 10%

formal saline solution for 24 h. The samples were washed with tap water and then and then serially diluted with absolute ethyl alcohol for dehydration. After routine processing, paraffin bees wax tissue blocks were sectioned into 4 µm thick slices using a sliding microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin for histopathological examination under a light microscope (Lillie and Fullmer, 1976).

2.9 Statistical Analysis

Data were analyzed using statistical analysis program (SPSS, version 20, version 20, SPSS, Inc., Chicago, IL, USA) . Means and standard errors for each variable were estimated. Differences between means of different groups were analyzed by one - way analysis of variance with Duncan multiple comparison tests. In the tables, dissimilar superscript letters in same column indicate a significant difference ($P < 0.05$).

3. Result

3.1 Blood Biochemical Parameters

The data shown in Table 1 indicated that treatment with $AlCl_3$ resulted in a significant ($p < 0.05$) increase in the activities of AST, ALT, and ALP. Co-administration of A çai and/or vitamin C with $AlCl_3$ significantly ($p < 0.05$) decreased AST, ALT, and ALP activities indicating that both A çai and vitamin C and their combination alleviated the toxicity of $AlCl_3$. Notably, treatment with A çai or vitamin C caused no significant changes in the activities of AST, ALT, and ALP compared to control group.

Table 1. The activity of liver enzymes (ALT, AST, and ALP) in plasma of rats

Parameters	Experimental groups						
	Control	$AlCL_3$	A çai	Vit C	$AlCL_3$+A çai	$AlCL_3$+ Vit C	$AlCL_3$+ Vit C +A çai
ALT (U/L)	45.07±1.49^{a}	103.98±4.12^{b}	44.76±1.66^{a}	44.59±1.34^{a}	80.67±2.09^{c}	84.96±2.24^{c}	66.98±2.48^{d}
AST (U/L)	70.07±2.60^{a}	139.32±2.56^{b}	69.61±1.24^{a}	71.38±1.67^{a}	94.80±2.89^{c}	95.47±3.71^{c}	82.52±3.25^{d}
ALP (U/L)	157.73±2.22^{a}	242.31±2.74^{b}	155.81±2.73^{a}	156.51±2.54^{a}	185.80±2.43^{c}	187.63±3.31^{c}	170.61±2.54^{d}

Note : All values are expressed as means ± SE, *n*=10 for each experimental group.

*Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p < 0.05$.

3.2 Hepatic MDA Level and GSH Content

Table 2 showed that the level of MDA, an end-product of LPO, was significantly ($p < 0.05$) increased in $AlCl_3$-treated rats as compared to in the control group. In contrast, hepatic GSH content was significantly ($p < 0.05$) reduced in rats treated with $AlCl_3$ compared to in the control group. Administration of A çai and/or vitamin C in combination with $AlCl_3$ restored the values of MDA and GSH to the value of the control.

Table 2. Changes in the level of MDA, GSH, SOD, CAT in liver homogenate

Parameters	Experimental groups						
	Control	**$AlCl_3$**	**A çai**	**Vit C**	**$AlCL_3$+ A çai**	**$AlCl_3$+ Vit C**	**$AlCl_3$+ Vit C+ A çai**
MDA nmol/mg protein	0.45 ±0.23[a]	2.42 ±0.18[b]	0.45 ±0.04 [a]	0.46 ±0.02 [a]	1.82 ±0.08[cd]	2.01 ±0.06 [c]	1.61 ±0.48[d]
GSH nmol/mg protein	44.87 ±0.66 [a]	21.63 ±0.87 [b]	45.88 ±1.53[a]	46.04 ±1.77 [a]	35.28 ±0.92[c]	31.66 ±0.92[d]	38.51 ±0.75[e]
SOD U/mg protein	60.94 ±1.13[a]	33.77 ±1.37[b]	59.72 ±1.39 [a]	57.80 ±1.99[a]	44.45 ±1.38[c]	40.55 ±0.83[c]	51.23 ±2.27[d]
CAT U/mg protein	52.96 ±1.02 [a]	28.63 ±0.80 [b]	55.79 ±1.07 [ac]	56.51 ±1.65 [c]	43.88 ±0.96 [d]	37.21 ±1.11[e]	48.17 ±1.55 [f]
Total Protein mg/g tissue	157.86 ±1.50 [a]	112.36 ±2.30 [b]	154.58 ±1.11 [a]	153.46 ±0.82 [a]	143.89 ±1.33 [c]	144.66 ±1.5 [c]	146.01 ±2.22 [c]

Note: Values are expressed as means ± SE; n=10 for each group

[*]Mean values within a row not sharing a common superscript letters (a, b, c, d, e, f) were significantly different, $p < 0.05$.

3.3 Activity of SOD and CAT in the Liver

Changes in the activities of enzymatic antioxidants, particularly SOD and CAT, in the liver of control and experimental animals are shown in Table 2. Oral administration of $AlCl_3$ was associated with a significant ($p < 0.05$) decrease in the activities of these radical scavenging enzymes by 57.5%, 50.2%, and 56.6%, respectively, compared to the control. Administration of A çai and/or vitamin C prior to $AlCl_3$ administration enhanced the enzymatic antioxidative status as demonstrated by the significant ($p < 0.05$) increase in the activities of these enzymes in the liver compared to in the $AlCl_3$ group.

3.4 Inflammatory Cytokines

Table 3 show that TNF-α and IL-6 were significantly increased by 3-fold in $AlCl_3$-treated animals compared to in control rats ($p < 0.05$). However, concomitant administration of A çai and/or vitamin C with $AlCl_3$ resulted in significant decreases ($p < 0.05$) in the levels of both cytokines compared to in $AlCl_3$-treated animals.

Table 3. Levels of TNF-α and IL-6 in liver homogenate

Parameters	Experimental groups						
	Control	$AlCl_3$	A çai	Vit C	$AlCl_3$+ A çai	$AlCl_3$+ Vit C	$AlCl_3$+ Vit C+ A çai
TNF-α pg/mg protein	2.32 ±0.09[a]	11.81 ±1.07[b]	2.13 ±0.10 [a]	2.27 ±0.17 [a]	6.36 ±0.31[c]	7.40 ±0.32 [c]	4.33 ±0.42[d]
IL-6 pg/mg protein	9.68 ±0.17[a]	34.05 ±1.56[b]	10.75 ±0.36 [a]	9.96 ±0.27[a]	27.16 ±0.66[c]	29.01 ±0.77[c]	21.63 ±1.02[d]

Note: Values are expressed as means ± SE; n = 10 for each group.

[*]Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p < 0.05$.

3.5 Histology Findings of Liver

Histology of the liver in control rats or those treated with A çai or vitamin C showed normal structure, hepatocytes were arranged in plates radiating from the central vein to the peripheral lobule, and irregular spaces between hepatic plates were occupied by liver sinusoids lined with fenestrated endothelial cells (Figures 2 A, C, and D). While liver sections from the $AlCl_3$ group (Figure 2 B) showed disruption of the normal architecture of hepatocytes with degenerative changes and vacuolated cytoplasm, some cells showed eosinophilic cytoplasm and pyknotic nuclei surrounded by clear halos. The hepatocytic plate was disheveled, central veins were congested, spaces between hepatocytes were widened, and sinusoids were distorted and congested. Livers from rats treated with A çai + $AlCl_3$, Vit. C + $AlCl_3$, and A çai + Vit. C + $AlCl_3$ (Figure E, F, and G) revealed that most histological alterations induced in the $AlCl_3$-treated group were markedly reduced; histological analysis of the rat livers revealed that changes following $AlCl_3$ treatment were attenuated from severe to moderate after treatment with A çai and vitamin C.

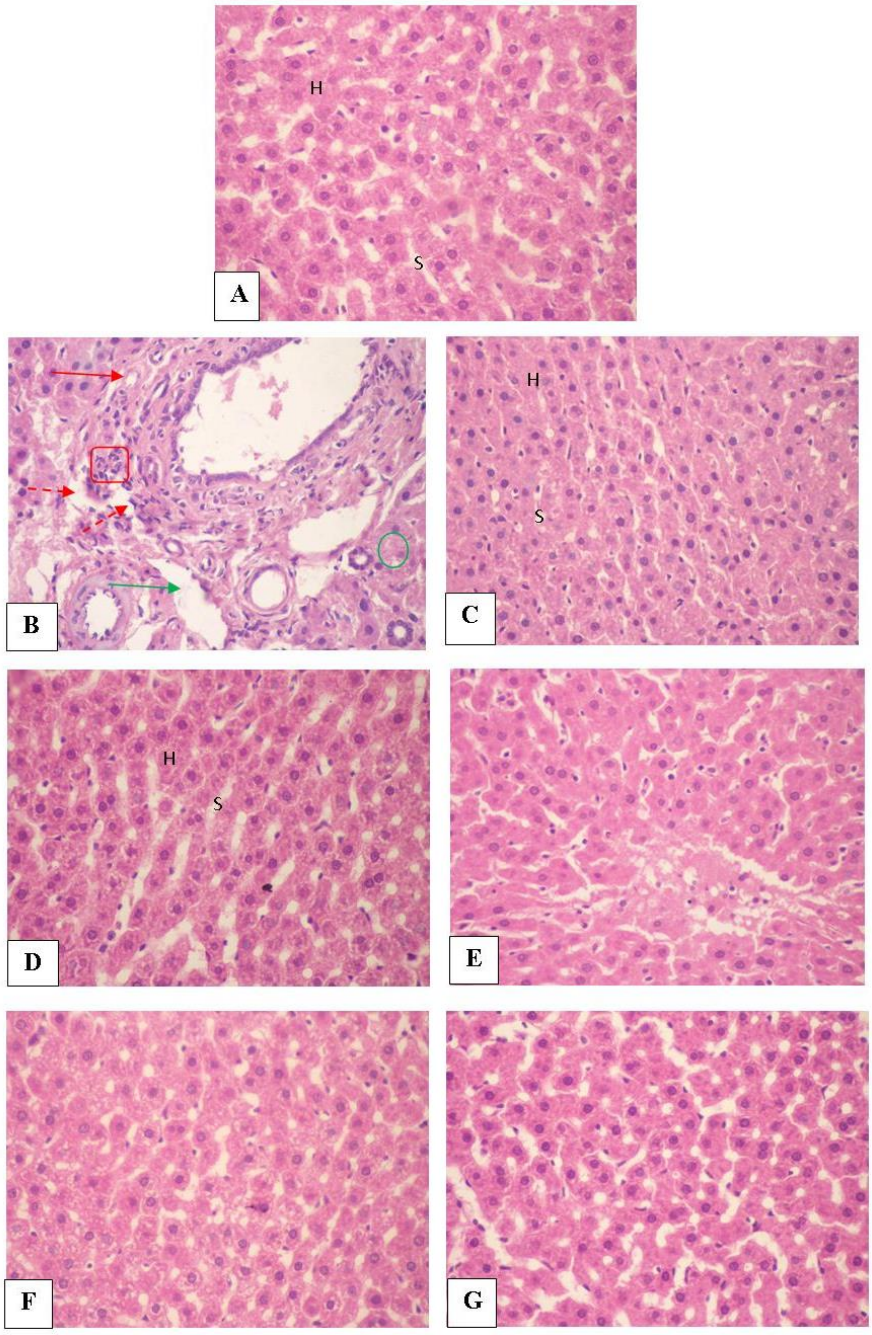

Fig. 1: :Light photomicrograph of liver sections: (A) control group, (C) A çai-trated group and (D) Vit.C-treated group; showing normal hepatocytes architecture, central vein, normal blood sinusoids (S) and hepatocytes (H).(B) $AlCl_3$-treated rats showing disruption of normal archeticture of hepatocytes, distended and hemorrhage in the central and portal vein (red arrow and green arrow), inflammatory infiltrate (red square), degenerated hepatocytes with eosinophillic cytoplasm and pyknotic nuclei (green circle) surrounded with clear haloes and vacuolated cytoplasm (red dotted arrow) .Histological alterations induced after $AlCl_3$treatment. (E) A çai, Vit.C and AlCl3 treated rats showing hepatocytes architecture similar to the control group.

4. Discussion

Aluminum has the potential to be toxic to humans and animals. Liver damage was assessed by biochemical methods to evaluate the liver enzymes transaminases (AST and ALT) and ALP, which are known as cholestatic liver enzymes. Our results (Table 1) showed that oral administration of aluminum chloride for 4 weeks significantly increased plasma transaminases (AST and ALT). Activities of AST, ALT, and ALP were increased after $AlCl_3$ administration, possibly because of leakage of these enzymes from the liver cytosol into the bloodstream. The increase in plasma AST and ALT of animals treated with $AlCl_3$ agreed with the findings of Türkez et al., (2010) and Yeh et al., (2009), who observed increased aminotransferase activities upon oral administration of aluminum in rats. ALP is a sensitive biomarker of metallic salts because it is a membrane-bound enzyme related to the transport of various metabolites. The activity of ALP is related to energy metabolic activities and processes in the body, and decreased ALP activity indicate impaired energy processing in the cell (Dera, 2016). In our study, $AlCl_3$ treatment increased ALP, contributing to the increased permeability of the plasma membrane or cellular necrosis of hepatic cells (Gaskill et al., 2005). Our results agree with those of El-Demerdash (2004) and Türkez et al., (2010) who found that treatment with $AlCl_3$ in rats at mg/kg BW orally for 30 days resulted in a significant increase in the plasma activities of ALT, AST, and ALP.

It is very important to search for protective substances that minimize the toxic effects of different chemicals. Açai (*Euterpe oleracea* Mart.) has gained attention in recent few years for having a wide range of health-promoting and therapeutic benefits. Açai treatment *in vitro* has anti-proliferative, anti-inflammatory, antioxidant, and cardio-protective effects, but *in vivo* studies are lacking. Here, we employed an *in vivo* approach to investigate the protective effect of Açai. Açai berries significantly reduced plasma ALT, AST, and ALP ($p < 0.05$) in rats compared to in group 2 ($p < 0.05$) (Table 1). Decreased plasma activity of AST, ALT, and ALP in rats treated with Açai indicates that this fruit protects against aluminum toxicity. Our findings agree with those of Pereira et al. (2016), who found a 30% reduction in serum ALT in rats with nonalcoholic fatty liver disease after administration of Açai for 6 weeks.

Vitamin C was reported to attenuate hepatic damage induced by various chemical agents, particularly in animals. This is supported by the results of Bashandy and Alwasel (2011) who reported that vitamin C normalized the levels of ALT, ASP, and ALP in the liver of carbon tetrachloride-intoxicated rats. Additionally, Mongi et al. (2011) found similar results when Wistar rats were administered deltamethrin (1.28 mg/kg). ALT, ASP, ALP, and gamma glutamyl transpeptidase were significantly increased. Pretreatment with vitamin C (200 mg/kg) normalized the above-mentioned parameters. Mahmoud and Elsoadaa (2013) reported that oral administration of vitamin C for 8 weeks in rats treated with $AlCl_3$ at a dose of 34 mg/ kg BW/daily down regulated ALT, AST, and ALP activities. Vitamin C stimulated the protein synthesis mechanism by disrupting the binding of aluminum with DNA and RNA.

Aluminum accumulates in all tissues in mammals, such as the kidneys, liver, heart, blood, bones, and brain (Al Kahtani 2010). $AlCl_3$ caused significant increases in the levels of pro-inflammatory cytokines, including TNF-α and IL-6 (Table 3), which agrees with previous

reports (Mannaa et al., 2013). These findings agree with findings of Dera (2016) who found 3-fold increases in TNF-α and IL-6 in the kidneys of rats treated with $AlCl_3$ for 40 days. In this study, administration of Açai or vitamin C reduced the increases in inflammatory mediators (TNF-α and IL-6). Additionally, the main mechanism by which Açai functions to ameliorate $AlCl_3$-induced liver damage may be related to its potent and anti-inflammatory effects.

It has been reported that in various inflammation models, Açai berry extract exhibits anti-inflammatory activities by regulating protein enzymes expressed by pro-inflammatory cytokines. Xie et al., (2012) showed that compounds in Açai such as flavonoids, particularly velutin, a unique flavone isolated from the pulp of Açaí fruit, effectively inhibited the expression of TNF-α and IL-6 by inhibiting nuclear factor-κB activation and p38 and JNK phosphorylation. They also found lower serum levels of TNF-α and IL-6 in apo E-deficient mice fed with 5% freeze-dried Açai juice powder for 20 weeks. In a mouse experiment, Açai extract also reduced inflammatory and oxidant markers such as superoxide dismutase, catalase, gluthathione peroxidase, TNF-α, and nitrites, which are increased by cigarette smoking (Moura et al., 2012). Additionally, they demonstrated that *Açai* reduces acute lung inflammation in mice by decreasing the numbers of alveolar macrophages and neutrophils in lung sections and decreasing TNF-α expression in lung homogenates. Another important function of Açai in controlling the inflammatory process is inhibiting nitric oxide production by reducing the expression of inducible nitric oxide synthase.

It has also been suggested that the toxic effects associated with aluminum are related to the generation of ROS, which results in oxidative deterioration of cellular lipids, proteins, and DNA (Mansour et al., 2006). One of the probable mechanisms by which aluminum ions can disturb cellular metabolism is stimulation of oxidative stress and interruption of the intracellular redox system. Lipid peroxidation of biological membranes leads to a loss of membrane fluidity, changes in membrane potential, an increase in membrane permeability, and alterations in receptor functions (Turkez et al., 2010). Our data indicate that oral administration of $AlCl_3$ in rats significantly increased the hepatic MDA level ($p < 0.05$) (Table 2) compared to in control rats, suggesting the participation of free radical-induced oxidative cell injury in mediating the toxicity of $AlCl_3$ (Wen et al., 2012). $AlCl_3$ also decreases antioxidant parameters such as GSH, SOD, and CAT in the liver of rats. These observations are similar to those of previous studies demonstrating that aluminum intake promoted oxidative stress (Nehru and Anand 2005; Yousef et al., 2007). Another study reported that exposure to aluminum disrupted the mineral balance, resulting in aluminum ions replacing iron and magnesium and leading to reduced Fe^{2+} binding to ferritin (Ward et al., 2001). The primary effects of aluminum on liver functions are thought to be mediated via damage to cell membranes. Lipid peroxidation of biological membranes leads to a loss of membrane fluidity, changes in membrane potential, an increase in membrane permeability, and alterations in receptor functions. The increased lipid peroxidation is due to inhibition or a change in the activity of non-enzymatic and enzymatic components of the oxidative system (GSH, SOD, and CAT) in the liver.

Our results showed that treatment with $AlCl_3$ and Açai or vitamin C significantly decreased

MDA and increased all anti-oxidative parameters compared to in the $AlCl_3$ group. This result agrees with the findings of Periera et al. (2016) who found that consumption of Açai (2 g/day) for 6 weeks by rats had health benefits and antioxidant properties, supporting that dietary antioxidants are a promising approach for enhancing defensive systems against oxidative stress.

Açai contains various bioactive secondary metabolites, mostly phenolic acids and flavonoids, which have been identified as potential antioxidants (Odendaal et al., 2014). Açai pulp contained high levels of total phenolic compounds, particularly anthocyanins, which may explain its high antioxidant capacity (Honzel et al., 2008). In addition to polyphenols, other nutrient fractions in Açai pulp may exert beneficial biological effects. Dietary polyphenols such as those found in Açai may play an important role in improving antioxidant status, as they neutralize ROS, chelate metal ions, and modulate the activity of enzymes, including paraoxonase (Rock et al., 2008). A recent study of women showed that the daily intake of 200 g of Açai pulp for 4 weeks improved the antioxidant status by increasing the activity of the enzyme CAT and total antioxidant capacity in polymorphonuclear cells and decreasing the production of ROS (Barbosa et al., 2016). Administration of Açai prevented these abnormalities by stabilizing the cell membrane and protecting the tissue from free radical-mediated toxicity (Udani et al., 2011).

Vitamin C efficiently prevents oxidative stress-induced cytotoxicity by aluminum. Vitamin C can diminish the production of ROS to inhibit continuous lipid peroxidation (Padayatty et al., 2003). The primary role of vitamin C is to nullify free radicals and prevent them from causing damage. Free radicals seek out an electron to regain their stability, and vitamin C is an excellent source of electrons; therefore, it can "donate electrons to free radicals such as hydroxyl and superoxide radicals to quench their activity" (Bindhumol et al., 2003). EL-Gendy et al. (2010) found that administration of vitamin C to an imidacloprid-intoxicated group normalized antioxidant enzyme activities and LPO and GSH contents. Furthermore, Saravanan et al. (2002) revealed that vitamin C or/and silymarin had hepatoprotective and antioxidant effects against ethanol intoxication. Vitamin C was also reported to scavenge aqueous ROS by rapid election transfer to inhibit lipid peroxidation (Harrison et al., 2003).

4.1 Histopathological Examination

After oral administration of aluminum chloride for 4 weeks, Al accumulated in the liver and by using an electronic microscope, it was found that Al accumulated in lysosomes and vesicles of hepatocytes. According to Galle et al. (1987), Al does not have toxic effects in the liver because, together with lysosomes, it is eliminated from hepatocytes into the bile. In contrast, Abubakar et al. (2003) found that Al in hepatocytes, even in small quantities, is associated with increased ROS and lipid peroxidation. Gonzales et al. (2004) found hepatocyte dysfunction in terms of reduced organic molecule transport over the sinusoidal and bile canalicular membrane, which leads to unbalanced bilirubin and bile acid. Our study showed that Al concentration was significantly increased in the liver tissues of exposed rats. This suggests that aluminum was absorbed and deposited in the liver. It penetrated the liver through the Glisson's capsule and lymph. (Fig.1 B) Furthermore, histochemical staining

showed that aluminum accumulated in phagocytes of the portal area (macrophagocytes and Kupffer cells) as well as in macrophagocytes of the subcapsular area. Aluminum was not observed in either the cytoplasm or the organelles of hepatocytes. Even if aluminum accumulated in organelles, particularly lysosomes, its quantities were too small to be stained by aluminon. In the capsular and subcapsular area, where aluminum was detected, the granulomatous reaction was significant. In addition to changes in Glisson's capsule and subcapsular area in exposed rats, specific changes were observed in the portal area and acini, which were not observed in the liver of control animals. These changes included slight microvascular fatty changes, Kupffer cell hyperplasia, and multiplication of bile canalicules, showing that aluminum reached all liver structures. This suggests that some metal particles passed the macrophagocyte barrier.

5. Conclusion

Aluminum has adverse effects on human health. The present study demonstrated that Açai or vitamin C administered in combination with aluminum minimized its hazards. In addition, Açai or vitamin C alone decreased the levels of free radicals and liver enzymes and increased GSH, CAT, and SOD. Exposure to aluminum should be reduced and attention paid to sources of aluminum in foods, water, and personal-care products. Furthermore, diets rich in Açai and vitamin C may be beneficial for alleviating the toxic effects of aluminum.

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