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ORIGINAL CONTRIBUTION

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Both aluminum and polyphenols in green tea decoction (*Camellia sinensis*) affect iron status and hematological parameters in rats

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Abstract *Background* Green tea leaves naturally contain high levels of polyphenols and aluminum (Al). Polyphenols in green tea decoction are considered to be one of the major factors responsible of low iron status. However, the effects of Al from green tea decoction on iron status and hematological parameters remained unclear. *Aim of the study* The objective was to investigate the Al absorption from green tea decoction and studied its influence on iron status and hematological parameters in rats. *Methods* During the experiment period, rats were given the experimental diet + a simple dose of Al sulfate with or without graded doses of green tea decoction (25, 50 and 100 g/l). The Al absorption was evaluated in the serum; however, iron status was evaluated by the iron concentration in the liver, kidney, spleen and femur. In addition, the hemoglobin and hematocrit were evaluated. *Results* Our results showed that the serum Al significantly increased between 61.5 and 342%, as tea doses-dependant. The Al sulfate significantly decreased the reserve of iron in all studied organs between 21.7 and 17% ($P < 0.05$). In groups receiving green tea decoction alone or Al + graded doses of tea, the reserve of iron significantly decreased in all studied organs between 59.4 and 18.5% ($P < 0.01$). Al alone or associated with drinking doses of tea significantly decreased hemoglobin concentration between 23.6 and 9% ($P < 0.05$) and hematocrit between 12.7 and 7% ($P < 0.01$). *Conclusion* Our data showed that Al from green tea decoction was more absorbed in the serum than Al sulfate. Al absorption was associated with low iron status and reduction of hemoglobin and hematocrit. Considering that Al competes with iron in different stage of erythropoiesis including transferrin binding, so we could assume that the negative effect of tea on iron status arises not only from polyphenols iron complexes but also from Al released in tea decoction.

Key words green tea decoction – Al absorption – iron status – hematological parameters

Introduction

The dried tea leaves of plant *Camellia sinensis* constitute a popular beverage commonly consumed throughout the world. Green and black tea decoctions are a popular beverage in Tunisia and in the other North Africa countries. Decoction is obtained by cooking the dried tea leaves for a relatively long period of time which is different to infusion prepa-

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ration. The chemical composition of green and black tea is different. Briefly, most of the polyphenolic compounds in green tea are catechins:(−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC), while most of the polyphenolic compounds in black tea are thearubigens and teaflavins [40]. The widely recognized properties of the tea polyphenols are its influence on iron absorption and antioxidants status [41]. Therefore, tea decoction is considered to be one of the major causative factors of high prevalence of iron deficiency anemia [20, 32]. The mechanism may be explained by the forming of insoluble complexes polyphenols iron in the gastro intestinal lumen, making the iron less available for absorption [4]. On the other hand, chemical analysis demonstrated that green tea leaves, tea decoction or tea infusion contained a great amount of Al, arising from natural contamination or manufacturing [11]. Because Al competes with iron in different stage of erythropoiesis including transferrin binding, it could be responsible for the development of iron deficiency anemia [17, 26]. Therefore, we think that the negative effect of tea on iron status arises not only from polyphenols iron complexes but also from the Al naturally present in tea. Then, the investigation of the long-term effect of Al present in green tea decoction on iron status has a practical importance. The objective of our study was to investigate the Al absorption from green tea decoction and studied its influence on iron status and hematological parameters.

Materials and methods

Animals and diets

Forty-two male Wistar rats weighing between 125 and 135 g (aged 2 months) were used in this study. Upon arrival, rats were feeding a commercial semi-synthetic diet for 1 week to adapt them in our laboratory conditions and equilibrate their initial weights. The semi-synthetic diet is composed per g/100 g dry weight: Carbohydrates: 51; Proteins: 21.8; lipids: 5.7; linoleic acid 1.5; L-methionine: 0.4; L-cysteine: 0.6; Choline 0.2; vitamin mixture: 1.7 and mineral mixture: 3.2. This diet contained 0.190 g $FeSO_4.7H_2O$, corresponding to 40 mg iron/kg diet, which is roughly similar to the iron content of experimental diet (ED) figured in Table 1. Once the initial weights were equilibrated, the rats were randomly assigned into six groups of seven animals each. They were housed individually in stainless-steel wire cages. Room temperature was maintained at 25 ± 2°C, and lit daily from 0700 to 1900 hours. During the experimental period (8 weeks), the rats were given the ED ad libitum with or without green tea decoction as follows: the control group (CG) received the ED + ultra-pure water; the group 2 received the ED + 100 mg Al/kg diet (Al 100 group), the group 3 received the ED + green tea decoction prepared from 100 g/l (Tea 100 group), the group 4 received the ED + 100 mg Al/kg + green tea decoction prepared from 25 g/l (Al + Tea 25 group), the group 5 received the ED + 100 mg Al/kg + green tea decoction prepared from 50 g/l (Al + Tea 50 group) and group 6 received the ED + 100 mg Al/kg + green tea decoction prepared from 100 g/l (Al + Tea 100 group). Food intake was recorded throughout the experimental period and quantified by weighing the amount of food spilled and refused. The daily Al consumed was deduced from the intake diet by calculation. The volume of tea decoction consumed by each rat was measured daily. Before tea intake, each rat was given about 7 ml of ultra pure water to prevent dehydration. At the end of the experimental period, rats were weighed and then killed by decapitation. Blood was drawn in vacutainer tubes, centrifuged at 3,000 rpm for 10 min, and then the plasma was removed and frozen for the analysis of Al. Another aliquot of total blood was used for the determination of hemoglobin and hematocrit concentrations. In addition, liver, kidney, spleen and

Table 1 Composition of the experimental diet

Ingredients	Amounts g/kg diet
Powder skim milk[a]	400
Vegetable oil	55
Maize starch	299
Sucrose	180
L-cysteine	1
Choline	1.5
$FeSO_4$[b]	0.2
$CaCo_3$	20
Na_3Po_4	20
NaCl	5
KCl	5
$Al_2(SO_4)_3{\cdot}18H_2O$[c]	1.2
Vitamin mixture[d]	10
Mineral mixture[e]	2.5

[a]As source of protein (Inesfood, Tunisia) provides 140 g proteins/kg
[b]Amount of $FeSO_4$ added to the ED provides 40 mg iron/kg which is similar to that of the semi-synthetic diet. Protein and iron widely covered the requirements of rats
[c]Aluminum sulfate [1.2 g of Al_2 $(SO_4)_3{\cdot}18H_2O$], corresponds to 100 mg pure aluminum was mixed in the maize starch of diets of groups 2, 4, 5 and 6
[d]Vitamin mixture (per kilogram diet); synthetic vitamin A concentrate = 10,000 IU; cholecalciferol = 2,000 IU; α-tocopherol acetate = 4 mg; pyridoxine hydrochloride = 4 mg; riboflavin sodium phosphate = 3 mg; nicotinamide = 20 mg; ascorbic acid = 100 mg; dexpanthenol = 8 mg, and finely powdered sucrose to make 10 g
[e]Mineral mixture (grams per kilogram diet): $MgSO_4{\cdot}7H_2O$ = 1.85; $ZnSO_4{\cdot}7H_2O$ = 0.50; $MnSO_4{\cdot}4H_2O$ = 0.15; $CuSO_4{\cdot}5H_2O$ = 0.020; KIO_3 = 0.0015; $FeSO_4{\cdot}7H_2O$ = 0.200; Na_2SeO_3 = 0.022

femur were removed, weighed and frozen to determine the iron concentration.

Preparation of the ED

The ED was prepared by mixing ingredients and chemical compounds listed in Table 1 in a stainless blender. A no toxicity dose level of Al sulfate (1.2 g of $Al_2(SO_4)_3.18H_2O$), corresponds to 100 mg pure Al/kg diet was mixed with the maize starch of the ED. This amount of Al is comparable to that present in green tea decoction prepared from 100 g/l. We noted that the oral lethal dose of Al sulfate in mice and rats ranged from 200 to 1,000 mg of Al per kg of body weight. The mineral and the vitamin supplements of the experimental diet were prepared as recommended by the APRIA [2]. The homogeneous diet was transformed into a piece of cake, then dried at 45°C and stored at 4°C for a short time.

Preparation of tea decoction

Tea decoction was freshly prepared throughout the experimental period. Amounts of 25, 50 or 100 g green tea leaves (*C. sinensis*) were soaked in hot water and boiled in 1,000 ml of ultra pure water for 20 min in a Pyrex glass (Duran), then cooled to room temperature before distribution.

Analysis of samples

Al concentration in green tea leaves and green tea decoction

Al concentration in green tea leaves and green tea decoction was analyzed by modified method of Marco et al. [27]. The determination was performed in two steps.

Extraction of Al from tea leaves and decoctions

Green tea leaves of 5 g or 5 ml or green tea decoction prepared from 100 g/l were placed in platinum dishes and 3 ml of concentrated H_2SO_4 plus 3 ml of concentrated HNO_3 were added, after evaporation to dryness on a sand-bath, the solid residue in the dish was dissolved with 15 ml of nitric acid solution (0.2 ml HNO_3 in 100 ml ultra-pure water).

Analysis of Al concentration

Al concentrations were analyzed by direct electro thermal atomic absorption spectrophotometer (Shimadzu 680, Japan) equipped with a pyrolitic graphite furnace (Shimadzu GFA-4B, Japan). The instrumental conditions were 309.2 nm wavelength, 4 mA lamp current, 2.00 nm slit width, and deuterium background correction.

Serum Al concentration

Al was determined after dilution of 0.5 ml serum in 0.450 ml of ultra-pure water, completed with 50 µl of a graded standard Al solution 0, 20, 40 and 60 µg/l. The solutions were analyzed by the same methods as described before. Then, we traced the curve of absorbance according to the concentration of standard Al concentration and we deduced the Al concentration from the intersection of the curve with abscissa axis.

Evaluation of iron status and hematological parameters

The iron status was evaluated by the iron concentration in liver, kidney, spleen and femur. The hematological parameters were evaluated by hemoglobin and hematocrit levels.

Iron in organs

The studied organs were ashed between 480 and 550°C in a muffle (STUART) for 48 h to obtain white ash. After being cooled to room temperature, the ashes were recovered by 2.5 ml concentrated HCl and taken up to 25 ml with ultra-pure water. Iron was determined by atomic absorption spectrophotometry (Perkin-Elmer 305 B) using titrisol standard solutions (Merck, Darmstadt, Germany)

Evaluation of hematological parameters

Measurement of hemoglobin and hematocrit

The hemoglobin was determined by the standard method [5]. The hematocrit was determined after micro centrifugation of blood.

Statistical analysis

The results are expressed as mean ± SEM and were analyzed with one-way analysis of variance (ANOVA) and "*t*" test of STUDENT. Differences were considered significant at $P < 0.05$.

Results

Al concentrations in green tea leaves and decoction were 583 mg/kg and 219 mg/l, respectively. The mean

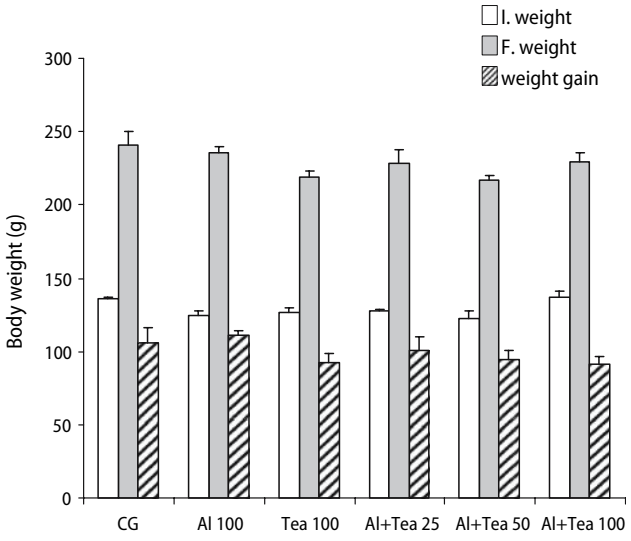

Fig. 1 Initials, finals and gains weight of different groups

volume of tea decoction consumed by each rat was 17 ml/day. The food intake ranged between 14 and 16 g/day. The Al sulfate did not significantly influence the food intake; however, green tea alone (100 g/l) had significantly reduced the daily food intake ($P < 0.05$). The initial, final and gain weights are presented in Fig. 1. At the end of the experimental period, the body weight gains did not significantly varied among groups. The profile of Al absorption and its influence on iron status are presented in Figs. 2, 3 and Table 2. The serum Al significantly increased as green tea doses-dependant, compared to the control group. The levels of serum Al significantly

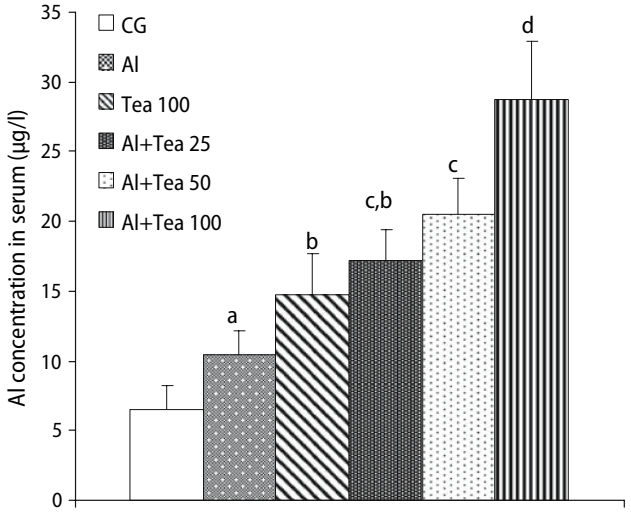

Fig. 2 Effect of green tea decoction, aluminum sulfate or aluminum sulfate + doses of green tea decoction on aluminum concentration in serum. *a*, *b* Significantly different from the control group $P < 0.05$. *c* Significantly different from the control group $P < 0.01$. *d* Significantly different from the control group $P < 0.001$

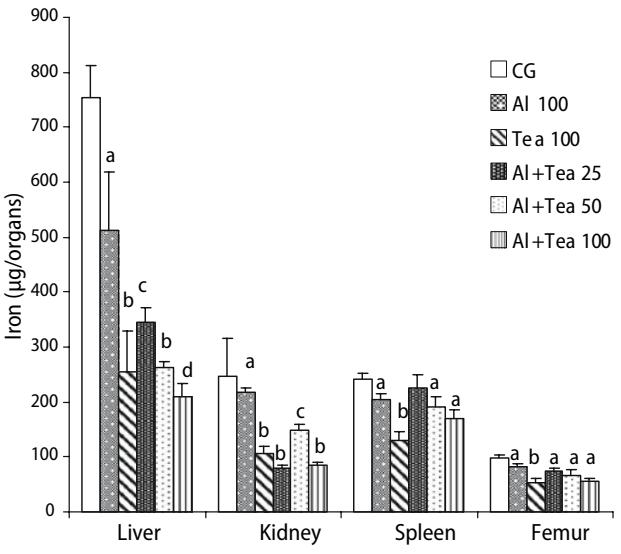

Fig. 3 Effect of green tea decoction alone, aluminum sulfate or aluminum sulfate + doses of green tea decoction on iron concentration in organs. *Fe liver*: *a* significantly different from the control group $P < 0.05$. *b*, *c* Significantly different from the control group $P < 1 \times 10^{-5}$. *d* Significantly different from the control group $P < 1 \times 10^{-6}$. *Fe kidney*: *a* significantly different from the control group $P < 0.01$. *b*, *c* Significantly different from the control group $P < 0.001$. *Fe spleen*: *a* significantly different from the control group $P < 0.05$. *b* Significantly different from the control group $P < 1 \times 10^{-5}$. *Fe femur*: *a* significantly different from the control group $P < 0.001$. *b* Significantly different from the control group $P < 1 \times 10^{-5}$

increased from 6.5 ± 1.7 µg in the CG to 10.5 ± 1.7, 14.7 ± 3, 17 ± 2, 20.5 ± 2.6 and 28.7 ± 4 µg/l, corresponding to 61.5, 126, 161.5, 215.4 and 341.5% in Al 100, Tea 100, Al + Tea 25, Al + Tea 50 and Al + Tea 100 groups, respectively. On the other hand, Al sulfate, green tea decoction or Al sulfate + graded doses of green tea decoction significantly decreased the reserve of iron stored in studied organs, especially in kidney and liver (Fig. 3). The decreases of iron concentrations were between 72 and 32% in liver, 65.5 and 12% in kidney, 46.5 and 15.4% in spleen, 45 and 17% in femur. In addition, they significantly decreased hemoglobin concentration between 23.6 and 9% and hematocrit between 12.7 and 7%.

Discussion

Tea (*C. sinensis*) is one of a few plants accumulating Al, making it a major source of dietary Al intake. The amount of Al found in green tea leaves used in our study (583 mg/kg) was similar to those reported by Pennington et al. [29] who found levels ranged between 400 and 600 mg/kg green tea leaves. It was also in accordance with those of Fimreite et al. [12], who found 515 mg/kg Al in tea Lipton and 526 mg/kg in tea twining. Moreover, we observed a better leaching

Table 2 Effect of green tea decoction, aluminum sulfate or aluminum sulfate + doses of green tea decoction on hemoglobin and hematocrit concentrations

Parameters	CG	Al 100	Tea 100	Al + Tea 25	Al + Tea 50	Al + Tea 100
Hemoglobin (g/dl)	11 ± 0.1	10 ± 0.1*	10 ± 0.1*	11 ± 0.2	10 ± 0.1*	8.4 ± 0.2***
Hematocrit (%)	43 ± 0.4	40 ± 1.3**	40 ± 0.3**	40 ± 0.5**	39 ± 0.4**	37.5 ± 0.7***

* Significantly different from the control group: $P < 0.05$
*** Significantly different from the control group: $P < 0.001$
** Significantly different from the control group: $P < 0.01$

of Al in the decoction preparation (219 mg/l), which is in agreement with values of different tea preparations reported in previous review [14]. Al sulfate has been widely used as a reference because of its high degree of solubility [19, 25]. Our results showed that Al leaching in green tea decoction is more absorbable in serum than Al sulfate, which is in agreement with those found by Fujii et al. [16]. These authors reported that among three types of tea infusions, the serum Al levels in rats receiving green tea infusion were high compared to those of black or oolong tea groups. With few exception [8], most of the animal studies demonstrated that Al concentrations in the serum or organs were significantly more important in rats or mice consumed tea infusion than Al from other dietary sources [14]. In human, the profile of Al absorption and its metabolism remained controversially because it is poorly absorbed and with no known biological function [31]. Some studies on Al speciation reported that serum Al concentrations and its urinary excretion in volunteers drinking tea infusion were significantly more important than Al diluted in water or present in diets [5, 18, 30]. Powell et al. [30] observed a little difference in the concentrations of Al in urine in two 24-h collection periods in volunteers drinking tea or water. Gardner and Gunn [18] observed in four healthy volunteers drinking tea containing 4 mg Al/l, or a soft mineral water that the mean Al excretion rate was about three times higher after drinking tea than water. However, another investigation have found that Al serum levels in men receiving tea or tea in the presence of milk or lemon did not significantly differ from the control group [5]. At present, it is difficult to give a satisfactory explanation of the high Al absorption from green tea decoction than Al sulfate observed in our study. However, when taking into consideration that more than 90% of the Al of tea are bound to organic matter, accordingly it could facilitate its absorption [15]. Studies using size exclusion chromatography technique have found that up to 28–33% of Al were in the form of polyphenolic complexes, 10–19% in cationic forms and approximately 10% formed a complex with fluoride in tea infusion [6, 7]. Furthermore, Al and Fe share the same proteins for transport and storage and they both bind to transferrin, entering to the cell via transferrin receptors [10, 26]. As a result, we think that by forming insoluble complexes iron-polyphenols, catechins of green tea prevent the binding of nonheme iron to the intestinal carrier protein, making freer ion sites, which could bind more Al ions and facilitate its mucosal uptake. Another possibility is that decoction preparation from green tea leaves may release some other organic compounds such as simple phenols, caffeine, amino acids, peptides, proteins and carbohydrate compounds which have the potential to increase the solubility and facilitate Al absorption [22]. The most common organic acids present in green tea leaves are oxalic and malic acids, followed by citric, isocitric, succinic acids and maltol [22]. Citric acid and maltol are able to form complexes citrate-Al or Al-maltolate enough stable to pass through the gastrointestinal tract, which stimulate Al absorption [13, 39].

Moreover, our data showed that Al absorbed from green tea decoction significantly reduced iron status by affecting the iron stored in organs, in particular liver and kidney. Our findings are in agreement with those of Han et al. [21] who found that dietary Al intake reduced iron storage in liver, intestine and kidney. The slightly reduction of iron storage in the control group may be related to the fast growing [37]. However, in treat groups, two mainly factors could be responsible for the low iron status. First, catechin compounds from green tea are known to inhibit iron absorption, and then it reduced its bioavailability. Secondly Al which is for 80% transported in serum by transferrin, it results a competition for iron uptake which could influence its storage in organs [10, 17, 28].

As indicated before, we have used iron concentration in liver, kidney, spleen, femur, hemoglobin and hematocrit as the most relevant of iron status parameters because red blood cells and these organs constitute the most important sites of iron metabolism and storage [23, 36]. When iron is absorbed, it is transported by serum transferrin to the cells or to the bone marrow for erythropoiesis [36]. An excess of absorbed iron is stored as ferritin or hemosiderin, particularly in the liver, intestine, spleen and bone marrow. As in humans, absorbed iron is also found in the plasma bound to transferrin in rats [38]. The iron

concentration in the spleen is also a good indicator of the iron metabolism because it indicates the level of erythrocyte degradation, which gave a rapid iron release in the spleen [23]. Al, tea decoction or Al + graded doses of tea decoction consumptions significantly reduced the hemoglobin and hematocrit levels. These findings are in accordance with those of Mahieu et al. [26] who demonstrated that chronically administered Al to rats significantly decreased the hemoglobin and hematocrit levels, serum iron and transferrin saturation. In the same way, Ganchev et al. [17] showed that after 7-days treatment with Al sulfate, Al significantly decrease the level of hemoglobin, hematocrit, plasma iron, TIBC and ^{59}Fe absorption, suggesting that Al is present at transferrin sites. Farina et al. [10] showed alterations in hemoglobin and hematocrit levels in rats after 18 months of Al exposure. The reduction of hemoglobin and hematocrit levels suggests that Al sulfate or more potentially Al from green tea decoction induced not only low iron status but also iron deficiency anemia in rats. The iron deficiency anemia may be the consequence of Al accumulation in organs, which exerts toxic effects on different stage of erythropoiesis [26]. Besides, reduction of hemoglobin and hematocrit levels may be also related to a direct alteration of circulating erythrocytes associated to membrane alterations due to lipid peroxidation or inhibitory effect of Al concentrations on ô-aminolevulinic acid dehydratase activities [1, 9]. This enzyme catalyzes the second step in the heme biosynthetic pathway [34].

We concluded that the negative effect of green tea decoction, commonly consumed in Tunisia as well as in other North Africa countries, arises not only from polyphenols, mainly catechins of green tea but also from the high absorption of Al released in the decoction. Comparison of physiological pathways in rat and human indicated some differences between the two species [33], so it is difficult to extrapolate the present data to humans. Nevertheless, if some analogies in the competition mechanism between Al and Fe will be also obtain in human nutritional conditions, the regular green tea decoction consumption could constitutes an important additional source of dietary Al. Then it could have in a long term, a negative consequence on iron status and erythropoiesis toxicity, particularly in patients with high iron requirements or with chronic renal failure like hemodialysis [24]. However, this negative consequence must not mask the beneficial antioxidant properties of green tea decoction, which had been widely described elsewhere [20]. In this way, although the green tea polyphenols have a negative effect on iron status, evidence suggests that the reduction of iron absorption, especially in patients with low iron requirements, may protect tissues against damage caused by oxygen free radicals and lipid peroxidation. The cytoprotective effects of tea polyphenols against lipid peroxidation arise, not only from their antioxidant properties, including the scavenging of oxygen and lipid radicals, but also from their iron-chelating activity [20].

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