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ORIGINAL

Chia seed (*Salvia hispanica* L.) supplementation may contribute to raising the levels of vitamin B_{12}: An option for the vegan diet

Suplementação de semente de chia (Salvia hispanica L.*) pode contribuir para elevar os níveis de vitamina B_{12}: uma opção para a dieta vegana*

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ABSTRACT

Objective

The chia seed, an ancient pseudocereal, is rich in omega-3 fatty acids and polyphenols, and has been suggested to possess several health benefits. Although it has gained popularity among nutritionists, little is known about the systemic effects of chia and their interactions. Hence, hepatorenal indicators and plasma vitamin concentrations in chia-supplemented aluminum-exposed rats were investigated.

Methods

Wistar albino rats were either fed on a chia-rich- or standard-diet for 21 days and exposed to aluminum. Liver function tests (Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, Lactate Dehydrogenase), kidney function tests (Urea Nitrogen, Creatinine), and vitamin B_{12} and folic acid measurements were performed by using an automated analyzer.

Results

Aluminum exposure had no influence on renal function, as did chia supplementation. However, liver function was disturbed with the exposure to Aluminum and chia was of no use against it. Surprisingly, it was found that

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the animals fed on a chia-rich diet displayed higher concentrations of vitamin B_{12} which was not the case for folic acid.

Conclusion

It was deduced that a chia-rich diet has no effect on the renal function and is not able to reverse aluminum-induced hepatotoxicity; however, it may be of benefit against vitamin B_{12} insufficiency and thus, it may offer a novel treatment option which is particularly important in the vegan diet.

Keywords: Aluminum. Chia. Folic acid. Hepatorenal function. Vitamin B12.

RESUMO

Objetivo

A semente de chia, um antigo pseudocereal, é rica em ácidos graxos ômega-3 e polifenóis e tem sido sugerida como tendo vários benefícios para a saúde. Embora tenha ganhado popularidade entre os nutricionistas, na verdade, pouco se sabe sobre os efeitos e interações sistêmicas da chia. Assim, investigamos os indicadores hepatorrenais e as concentrações plasmáticas de vitamina em ratos expostos ao alumínio suplementados com chia.

Métodos

Ratos albinos Wistar foram alimentados com dieta rica em chia ou padrão por 21 dias e expostos ao alumínio. Testes de função hepática (Alanina Aminotransferase, Aspartato Aminotransferase, Fosfatase Alcalina, Lactato Desidrogenase), testes de função renal (ácido úrico, Creatinina) e medições de vitamina B_{12} e ácido fólico realizada usando um analisador automático.

Resultados

A exposição ao alumínio não influenciou a função renal, assim como a suplementação de chia. No entanto, a função hepática foi perturbada com a exposição e a chia foi inútil contra ela. Surpreendentemente, descobrimos que os animais que se alimentavam de uma dieta rica em chia apresentavam concentrações mais elevadas de vitamina B_{12}, o que não era o caso do ácido fólico.

Conclusão

Deduzimos que a dieta rica em chia não tem efeito sobre a função renal e não é capaz de reverter a hepatotoxicidade induzida pelo alumínio; no entanto, pode ser benéfico contra a insuficiência de vitamina B_{12} e, portanto, pode oferecer uma nova opção de tratamento que é particularmente importante na dieta vegana.

Palavras-chave: *Alumínio. Chia. Ácido fólico. Função hepato-renal. Vitamina B12.*

INTRODUCTION

Aluminum is the most abundant metal in the earth's crust and has been increasingly exposed to humans, correlated to the industrialization. The cumulative damage of aluminum, which has no known physiological role, has become a serious source of concern among physicians. It has been associated with several acute and chronic pathologies including Alzheimer's disease [1], multiple sclerosis [2], irritable bowel disease [3], osteodystrophy/osteomalacia [4], hepatotoxicity [5], and nephrotoxicity [6].

In spite of not having a physiological role, aluminum interacts with a wide range of enzymes and biological molecules. It alters the activity of glycolytic enzymes (inhibits hexokinase and phosphofructokinase [7,8], stimulates pyruvate kinase [9]), increases the production of proinflammatory mediators (tumor necrosis factor-alpha, macrophage inflammatory protein-1alpha [10] and nuclear factor kappa-B), activates proapoptotic caspase-12 [11], decreases the concentrations of choline acetyltransferase [12] and brain derived neurotrophic factor [10], provokes the release of excitotoxic

glutamate [13], and impairs long-term synaptic potentiation [12]. In addition to the prooxidant activity [14], the high reactivity of aluminum is necessarily responsible for its health impairing effects.

Chia (*Salvia hispanica* L.) seeds have become increasingly popular among nutritionists due to their high omega-3 fatty acid, protein and polyphenol content. This ancient pseudocereal has been suggested to bear anti-oxidant [15], anti-hyperglycemic [16], cardioprotective [17], and anti-hyperlipidemic [18] properties. In spite of its growing consumption and market value [19], the scarcity of knowledge about the systemic effects of chia seeds has raised safety concerns. Although the European Food Safety Authority [20] has stated that chia probably has no side effects, Garcia and this study's research team have found that it may indeed cause side effects; more recently, García-Jiménez *et al*. [21] reported a serious anaphylactic reaction induced by chia seeds, and this study's research team demonstrated an aggravation in the prognosis of experimental Alzheimer's disease with chia supplementation [22]. The aforementioned study served as an alert on possible unexpected consequences of the consumption of chia seeds. Therefore, the effects of this pseudocereal on basic biochemical blood parameters in aluminum-exposed rats were investigated.

METHODS

This study was carried out using rat blood samples, in accordance with the 3Rs principle of humane animal research, obtained during the execution of project number 2017/4-1 (rev.2018/6-3). All experimental procedures were approved by the local ethics committee at the Hatay Mustafa Kemal University (No.2018/5-1 rev.2018/8-4). The animals were housed under standard laboratory conditions (22±2°C temperature, 55±10% relative humidity, 12/12-h light/dark cycle). A total of 30 adult male *Wistar* albino rats (16-18-week old, 420-480g) were grouped as follows:

Control group (n=6): Received intraperitoneal physiological saline (the vehicle of aluminum chloride) for 21 days, and the standard diet.

Chia-Sim group (n=8): Simultaneously received intraperitoneal aluminum chloride (10mg/kg/day) and D-galactose (150mg/kg/day), and a chia-rich diet (36.2%[w/w]) for 21 days.

Chia-Post group (n=8): Received intraperitoneal aluminum chloride (10mg/kg/day) and D-galactose (150mg/kg/day) for 21 days followed by the administration of a chia-rich diet (36.2%[w/w]) for 21 days.

Exposure group (n=8): Received intraperitoneal aluminum chloride (10mg/kg/day) and D-galactose (150mg/kg/day) for 21 days, plus the standard diet.

The animals were fasted overnight and anesthetized with a combination of ketamine and xylazine (respectively, 80 and 12mg/kg), and cardiac blood was collected into tubes containing Ethylenediaminetetraacetic Acid (EDTA) anticoagulant. The tubes were centrifuged at 1500rpm for 15min and plasma was collected for the biochemical analysis. Kidney function tests (urea nitrogen and creatinine), liver function tests (Alanine Aminotransferase [ALT], Aspartate Aminotransferase [AST], and Alkaline Phosphatase [ALP]), Lactate Dehydrogenase (LDH), folic acid, and vitamin B_{12} were photometrically evaluated by using an automated analyzer (Advia Centaur XP/Dimension EXL, Siemens Diagnostics, *Erlangen*, Germany). Aluminum chloride and D-galactose were respectively imported from Merck (*Darmstadt*, Germany) and Sigma-Aldrich (*Steinheim*, Germany) whereas chia seeds were purchased from local suppliers.

Depending onto the parametricity of data, either one-way ANOVA and *post-hoc* Tukey's test or Kruskal-Wallis and *post-hoc* Dunn's test were used for statistical analyses. The calculations

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were carried out using the software Graphpad Prism v7.0 (La Jolla, California, United States). The significance level was set to $p<0.05$. The data were presented as Mean±Standard Error of the Mean (SEM) or interquartile range.

RESULTS

Kidney function tests

As illustrated in Figures 1A and B, neither the exposure to aluminum nor chia-rich diets altered the renal function parameters of BUN and creatinine, despite a non-significant increase of creatinine in the animals that fed on a chia-rich diet following the aluminum exposure (one-way ANOVA test; $F_{(3,26)}=1.6$, $p=0.213$). Being fed with the chia-rich diet during or after the aluminum exposure resulted in similar plasma BUN and creatinine concentrations ($p>0.05$). There was no significance in the BUN/Creatinine *Ratio* (BCR) (one-way ANOVA test; $F_{(3,26)}=0.504$, $p=0.683$).

Liver function tests

As depicted in Figure 2A-C, the activities of ALT, AST, and ALP were significantly different between the groups (Kruskal-Wallis test; respectively, $p=0.026$, $p=0.039$, and $p<0.001$). The *post-hoc* test revealed a significant increase in ALT and AST in aluminum-exposed standard diet-fed animals as compared to the control group (respectively, $p=0.011$ and $p=0.004$). Neither regimens of chia-rich diets were able to alter the increased levels of AST ($p>0.05$), but the ALT activity had a decrease in post-exposure chia-rich diet-fed animals although it was not as low as the levels measured in the control group ($p>0.05$). The ALP activity was similar in both control and exposure groups, but decreased in both chia-fed groups (Control *vs* Chia-Sim, $p=0.023$; Control *vs* Chia-Post, $p=0.007$; Exposure *vs* Chia-Sim, $p<0.001$; Exposure *vs* Chia-Post, $p<0.001$). There was no difference between the chia-rich diet regimens in regard to the ALP activity ($p>0.05$). As seen in Figure 2D, the lactate dehydrogenase activity was similar in all groups although the statistical analysis produced a probability value (*p*-value) which was very close to the significance threshold (alpha-value) (Kruskal-Wallis test; $p=0.058$).

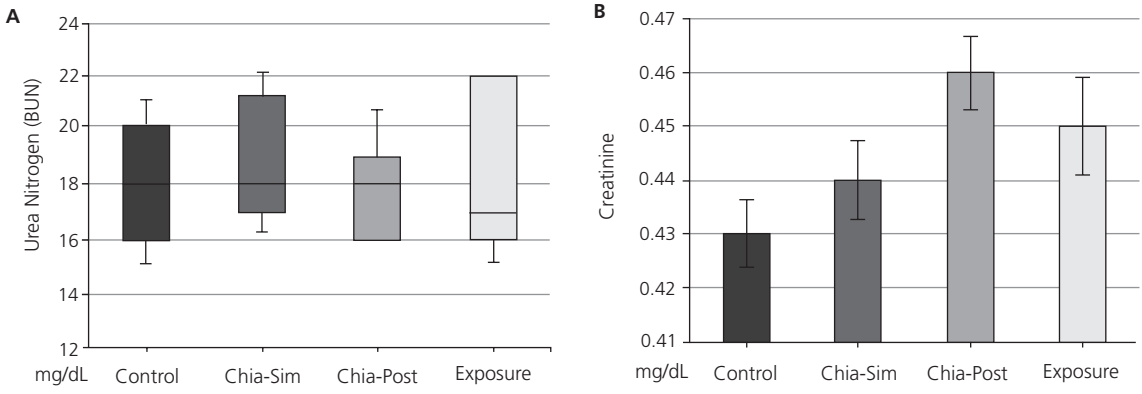

Figure 1. Plasma concentrations of (A) Urea Nitrogen and (B) creatinine.

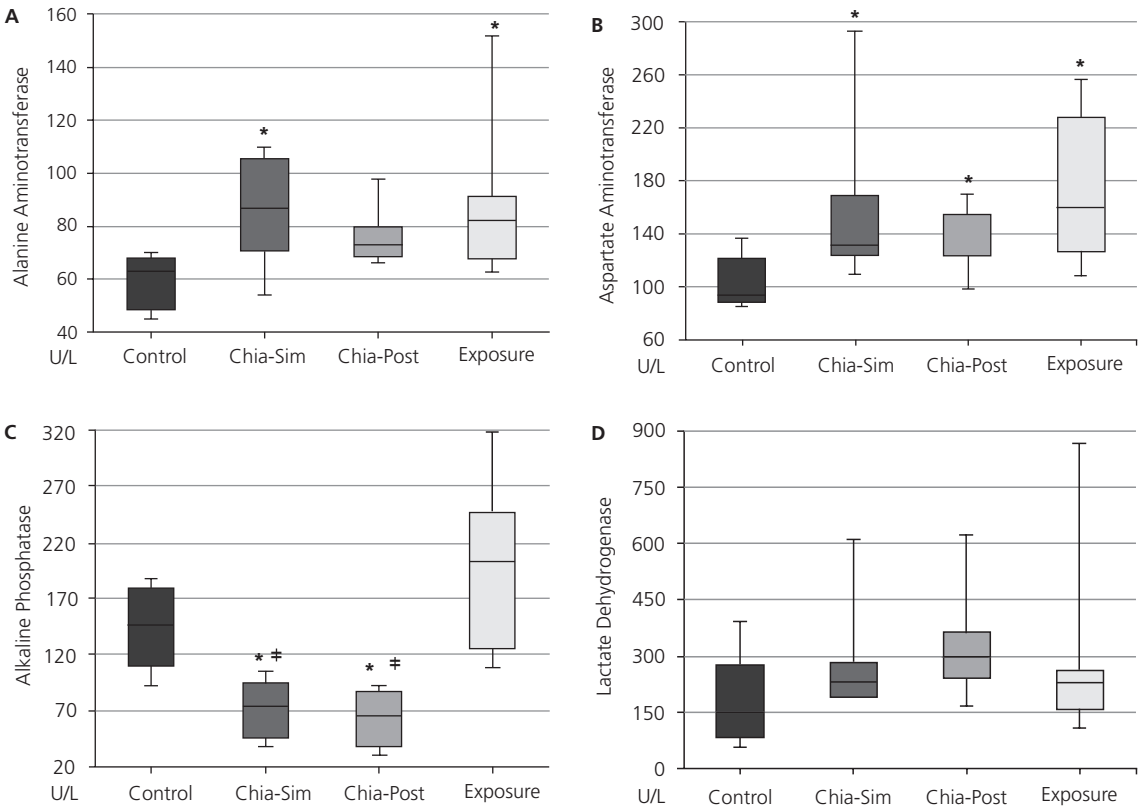

Figure 2. Activities of (A) Alanine Aminotransferase, (B) Aspartate Aminotransferase, (C) Alkaline Phosphatase, and (D) Lactate Dehydrogenase.

Asterisk (*****): significance (p<0.05) *versus* Control; Palatal click (ǂ): significance (p<0.05) *versus* Exposure.

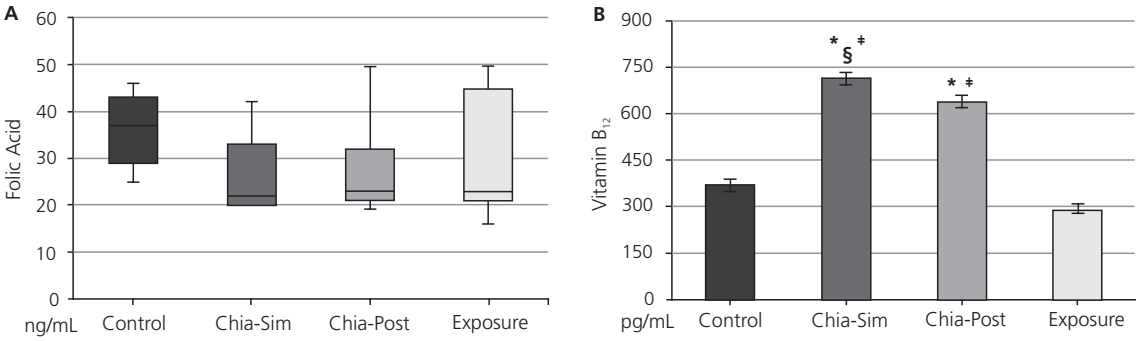

Figure 3. Plasma concentrations of (A) folic acid and (B) vitamin B_{12}.

Asterisk (*****): significance (p<0.05) *versus* Control; Palatal click (ǂ): significance (p<0.05) *versus* Exposure; Section sign (§): significance (p<0.05) *versus* Chia-Post.

Vitamin levels

The plasma concentrations of folic acid were not different between the groups (Kruskal-Wallis test; p=0.137) (Figure 3A). However, there was a significance in vitamin B_{12} levels (one-way ANOVA test; $F_{(3,26)}$=131, p<0.001). As shown in Figure 2B, the concentrations of vitamin B_{12}

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were increased in both simultaneously chia-rich fed animals or post-exposure chia-rich diet-fed animals as compared to the control groups (*vs* Chia-Sim, $p<0.001$; *vs* Chia-Post, $p<0.001$) and the exposure group (*vs* Chia-Sim, $p<0.001$; *vs* Chia-Post, $p<0.001$). Furthermore, the increment in vitamin B_{12} levels were significantly higher in the Chia-Sim group as compared to Chia-Post animals ($p=0.025$). No significance was found between the control and exposure groups ($p>0.05$).

DISCUSSION

In the industrialized modern world, aluminum exposure is not a rare phenomenon anymore. According to the World Health Organization [23], neglecting other routes such as water, air and skin, the exposure through food alone can be as high as 13mg/day, which exceeds the recommended tolerable limit of 1mg/kg body weight/week [24]. The desire for a healthy life and protection against personally unavoidable hazards drives people to using supplementations including chia seeds, even though there is no adequate amount of scientific evidence of their benefits (or harms). This issue triggered the researchers of this study to investigate the interaction between aluminum exposure and chia supplementation by means of routinely ordered conventional biochemical tests in the clinical practice. To this aim, kidney and liver function tests were performed, and folic acid and vitamin B_{12} levels were estimated in aluminum-exposed rats which were fed either with a standard or a chia-rich diet.

Urea is a water-soluble side product of protein catabolism, mainly produced by the liver and excreted by the kidneys. The nitrogen content of BUN is more commonly evaluated by clinicians. Although BUN does not have high sensitivity and specificity due to being influenced by non-renal pathologies, adding creatinine, which is constantly produced from phosphocreatine, to the test panel provides a better insight into renal function. Creatinine superiorly reflects the glomerular filtration rate, and hence, renal functionality. However, it also has some drawbacks such as the inability of detecting mild/moderate dysfunction and being altered by muscular pathologies, traumas, the individual's diet, etc. [25]. Even so, BUN and creatinine are the first-order tests to evaluate renal function [26]. Aluminum accumulates the second-most in kidneys [27,28]. An extended exposure to aluminum results in renal tubular fibrosis and peritubular cell infiltration in rats [29,30]. Also, there are some reports that suggest only a single bout of aluminum exposure is capable of disturbing renal function [31,32], even though there are some other contradicting studies which used a dose which was more than twice the dose of the former ones [33,34]. Nevertheless, the results demonstrated that neither the exposure to aluminum nor a chia-rich diet can alter renal function which was evaluated by measuring BUN, creatinine, and BCR. The controversy may be originated from the differences between species, age, weight, and even the amount of water ingested daily. Noteworthy, the glomerular filtration rate was not measured in this study. Although urea and creatinine are valuable indicators, the gold standard for assessing renal function is the glomerular filtration rate [35].

In contrast to the renal function, there seems to be a consensus regarding the devastating effects of aluminum exposure on liver function [36-39]. Liver aminotransferases, ALT and AST, catalyze the transfer of the amino group that they are named after to alpha-ketoglutarate to form pyruvate, the critical substrate of the Krebs cycle. The elevation in ALT activity is more specific to the liver than AST [40], but as a practical intuition, both aminotransferases are routinely ordered to assess liver function. The results of the present study demonstrated an increase in these aminotransferases with aluminum exposure. The animals that simultaneously fed on a chia-rich diet and exposed to aluminum had a significant (when compared to the control group elevation in AST activity and a

non-significant elevation in ALT activity. Besides, their counterparts that were fed on a chia-rich diet after the exposure showed a significant elevation in both ALT and AST activities. Heretofore, there are no internationally accepted reference values for liver aminotransferases in rats. The researchers are hence unable to claim whether a non-significantly elevated ALT is above or within the normal range. Nevertheless, centrilobular (Acinar Zone III) injury is represented by a greater elevation in AST than in ALT [41]. This zone is the main center of detoxification [42] and thus, these results can be interpreted as the chia-rich diet having no benefits to treating aluminum-induced hepatotoxicity.

Alkaline Phosphatase is a hydrolytic enzyme targeting phosphate monoesters at alkaline pH. Although ALP is not specific to the liver and isoenzymes are produced by bone tissue, spleen, intestines and kidneys, it is of great use to detect secretory function of the liver when evaluated together with liver aminotransferases [43]. Zinc is the cofactor of ALP and thus, zinc deficiency leads to a decreased ALP activity. However perplexing it may appear, zinc supplementation also results in a decrease in ALP activity in individuals who have normal zinc levels [44]. Probably since zinc is abundantly present in chia seeds [45], chia-rich diet-fed animals, regardless of timing of the diet regimen, displayed a lower level of ALP in this study. The highest ALP activity was measured in the exposure group, which were fed with the standard diet. Even so, the difference versus control group was statistically non-significant which might be related to the sample size.

Lactate Dehydrogenase is the enzyme responsible for the conversion of pyruvate to lactate and vice versa. Lactate is the end-product of anaerobic glycolysis and its conversion generates Nicotine Adenine Dinucleotide (NAD^+) which is required for maintaining glycolysis. LDH exists in abundance, probably in all cells. Because of its abundance, LDH is indeed recommended not to be used in the evaluation of the liver function [46]. Nevertheless, it provides a general perspective of tissue injury and hence, can be a valuable and informative indicator in severe liver injury if assessed in conjunction with other liver function tests. In the present study, LDH activity was non-significant between all groups. When considered together with the results of the aminotransferases, this may suggest a mild/moderate liver injury to which the chia-rich diet has no benefit.

Vitamin B_{12} (cobalamin) is an animal-derived essential vitamin which plays a role in nucleotide biosynthesis in coordination with folic acid. The absorption of vitamin B_{12} depends on the presence of haptocorrin (R-protein), gastric intrinsic factor, cubilin, and transcobalamin-II. Haptocorrin is secreted mainly from the salivary glands and protects vitamin B_{12} from gastric acid degradation. The intrinsic factor, which is secreted by the gastric parietal cells, binds vitamin B_{12} in the duodenum and the vitamin-bound complex attaches to its receptor, cubilin, in the ileum. Following the receptor-mediated endocytosis, vitamin B_{12} dissociates and binds to transcobalamin-II which carries it to the liver. Any obstacle on this route can result in vitamin B_{12} deficiency, but also, increased expressions of these proteins can augment the absorption of the vitamin and elevate its plasma concentrations. In this study, it was found that folic acid concentrations were similar between groups, but the animals that were fed the chia-rich diet displayed higher plasma vitamin B_{12} levels although solely aluminum exposure did not alter either folic acid or vitamin B_{12} levels. One may inquire if this finding indicates a liver injury in which falsely elevated vitamin B_{12} levels can be seen [47]; however, this possibility was rejected on the grounds of the unsupported liver function test results. Hence, it is speculated that the chia supplementation may reinforce the bioavailability of vitamin B_{12} and may particularly be of benefit against vitamin B_{12} insufficiency. Given that a vegan diet strictly excludes any kind of animal products, chia supplementation can naturally improve vitamin concentrations in vegan diet followers.

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CONCLUSION

The present study demonstrated that aluminum exposure does not alter kidney functions (in the employed dosage and duration), but results in liver injury. Furthermore, chia supplementation cannot reverse liver injuries. The most interesting finding of this study was the increment in vitamin B_{12} levels with the chia supplementation which was independent of the aluminum exposure. This result implies a potential medicinal use of chia seeds against vitamin B_{12} insufficiency. Conclusively, the researchers believe that even if chia seeds may bear a therapeutic potential, vast expectations should not be created until more data are gathered about this novel food's metabolic properties and interactions.

CONTRIBUTORS

EA DEMIR conceived and supervised the study, analyzed the data and drafted the manuscript. Y BILGIC conducted the experiments and arranged the data for analyses.

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