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ORIGINAL ARTICLE

Antioxidant activity and protective effect of bee bread (honey and pollen) in aluminum-induced anemia, elevation of inflammatory makers and hepato-renal toxicity

Meryem Bakour[1] · Noori S. Al-Waili[2] · Nawal El Menyiy[1] · Hamada Imtara[1] · Anna Cristina Figuira[3] · Thia Al-Waili[2] · Badiaa Lyoussi[1]

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Abstract Aluminum toxicity might be related to oxidative stress, and the antioxidant activity and protective effect of bee bread, which contains pollen, honey and bees' enzymes, on aluminum induced blood and hepato-renal toxicity was investigated in rats. Chemical analysis and antioxidant capacity of bee bread were conducted. The animal experiment in rats included; group 1: received distilled water (10 ml/kg b.wt), group 2: received aluminum chloride (662.2 mg/kg b.wt), group 3: received aluminum chloride (662.2 mg/kg b.wt) and ethanolic extract of the bee bread (500 mg/kg b.wt), and group 4: received aluminum chloride (662.2 mg/kg b.wt) and ethanolic extract of the bee bread (750 mg/kg b.wt). Doses were given once daily via a gavage. C-reactive protein, transaminases, urea, creatinine, creatinine clearance, sodium and potassium and urine sodium and potassium were determined on day 28 of the experiment. Bee bread contained protein, fat, fiber, ash, carbohydrate, phenol and flavonoids and it exhibited antioxidant activity. Aluminum caused a significant elevation of blood urea, transaminase, C-reactive protein and monocyte count and significantly decreased hemoglobin. These changes were significantly ameliorated by the use of bee bread. Bee bread has an antioxidant property, and exhibited a protective effect on aluminum induced blood and hepato-renal toxicity and elevation of inflammatory markers C-reactive protein, leukocyte and monocyte counts.

Keywords Bee bread · Aluminum · Toxicity · Anemia · Antioxidant · C-reactive protein · Transaminase

Introduction

Aluminum is the most abundant metal in the earth's crust, and it is a non-essential trace element. The exposure to aluminum occurs as a result of inhalation of air, drinking water, preparation and storage of foods, cigarettes, antiperspirants, antacids, vaccines, and immunotherapy (Dzulfakar et al. 2011). Aluminum is a toxic metal and several studies showed adverse effects such as neurotoxicity, hepatotoxicity, disturbances in reproductive hormones level, Guamanian–Parkinsonian complex, amyotrophic lateral sclerosis, and Alzheimer's disease (Metwally and Mazhar 2007; Buraimoh et al. 2012). The mechanism of its toxicity is poorly understood, however, studies linked it to oxidative stress (OS) and reactive oxygen species (ROS) (Kumar and Gill 2014). Several natural products with antioxidant properties have been investigated in aluminum toxicity which showed protective effects such as gingerol, saffron, resveratrol, honey, quercetin, *Nigella sativa*, propolis, vitamin C, royal jelly, and melatonin (Shati and Alamri 2010; Al-Qayim et al. 2014; Oda 2016; Sharma et al. 2016).

Bee bread is one of the products of the hive, and bees prepared it by adding honey and bee secretions to pollen and stored it in brood cells. Therefore, it is a combination of honey, pollen and bee secretions. Bee bread provides

✉ Noori S. Al-Waili
Drnoori6@yahoo.com

[1] Laboratory of Physiology, Pharmacology and Environmental Health, Faculty of Sciences Dhar El Mehraz, University Sidi Mohamed Ben Abdallah, BP 1796 Atlas, 30 000 Fez, Morocco

[2] New York Medical Care for Nephrology, 87-40 134 Street, Richmond Hill, NY 11418, USA

[3] Department of Food Engineering, Institute of Engineering, University of the Algarve, 8005-139 Faro, Portugal

bees with proteins, lipids, microelements and vitamins. It also contains tocopherol, niacin, thiamine, biotin, folic acid, polyphenols, carotenoid pigments, phytosterols, enzymes and coenzymes.

Others and we have found that bee products such as honey, propolis and pollen have active biological ingredients and antimicrobial, anti-inflammatory and antioxidant activities (Al-Waili 2004; Al-Waili and Haq 2004; Baltrusaityte et al. 2007; AL-Waili et al. 2013, 2015). Studies showed that propolis and honey have a protective effect in aluminum toxicity (Yousef and Salama 2009; Shati and Alamri 2010). Honey is widely used in human diseases and has been recently introduced to modern medicine as an important intervention in wound healing. It has various activities such as anti-inflammatory and antioxidant properties, and has hepato-renal protective activities (Al-Waili et al. 2006a, b, 2011; Kolayli et al. 2016). Recently we found that honey has anemia and hepato-renal protective activity in lead toxicity (Fihri et al. 2016). Honey has been mentioned in Holy books, the Talmud, both the old and new testaments of the Bible, and the Holy Quran as a healer of diseases. In the Surat Al-Nahel (The Bee) it says: And thy LORD taught the bee to build its cells in hills, on trees and in men's habitations, then to eat of all the fruits of the earth and find with skill the spacious paths of its LORD, there issues from within their bodies a drink of varying colors, wherein is healing for men, verily in this is a sign for those who give thought.

Recent review showed that bee pollen has anti-oxidant, anti-inflammatory, anti-cariogenic, hepatoprotective, and anti-atherosclerotic activities (Denisow and Denisow-Pietrzyk 2016). Bee pollen has antioxidants such as phenolic acids and flavonoids which have anti-inflammatory properties. It contains polyphenols, carotenoid pigments, phytosterols, tocopherol, vitamins, enzymes and co-enzymes which are attributed to its biological activities (Denisow and Denisow-Pietrzyk 2016). The composition of bee bread makes it an interesting intervention to be tested in many pathological conditions. Therefore, the aim of the present study is to investigate its protective effect in aluminum toxicity in rats.

Materials and methods

The analysis and experiments were conducted in Laboratory of Physiology, Pharmacology and Environmental Health, Faculty of Sciences Dhar El Mehraz, BP 1796 Atlas, University Sidi Mohamed Ben Abdallah, Fez 30 000, Morocco. The time of the research was during 2015.

Bee bread sample

Bee bread was obtained from professional beekeeper, Imouzer Marmoucha, Morocco, and was stored at −20 C°. For chemical analysis and antioxidant activity, bee bread was prepared as follows: one gram was macerated in 20 ml of ethanol (70%) for 1 week and then centrifuged for 10 min at 2000 g and 20 °C; the supernatant was collected for analysis. For animal's experiment, bee bread was macerated for a week in ethanol 70% under agitation, then filtered and passed in a rotary evaporator. Distilled water was added to obtain two concentrations (500 and 750 mg/kg b.wt).

Chemical analysis and antioxidant activity of bee bread

Using the AOAC method, the moisture content of bee bread was determined gravimetrically in a convection oven with drying at 105 °C to constant weight (AOAC Horwitz Wal 2005).The crude protein value was determined using the Kjeldahl's method (AOAC Horwitz Wal 2005). A conversion factor of 6.25 was used to alter the percentage of nitrogen into a percentage of crude protein. The crude fat was extracted using a soxhlet apparatus and diethyl ether, according to the AOAC recommended method no 27.006 (AOAC Williams 1984).The crude fiber content of the bee bread was analyzed according to AOAC (2005) method no 962.09 (AOAC Horwitz Wal 2005).The ash content of the samples was determined gravimetrically, according to the AOAC (2005) method no 942.05 with a minor modification, which consisted of samples being incinerated at 550 °C until constant weight (AOAC Horwitz Wal 2005). The total carbohydrate content was calculated according to the following expression: total carbohydrate = 100 − (% moisture + % protein + % fat + % ash).

Total phenolic content in the bee bread sample was determined by Folin–Ciocalteu reagent using method of Marghitas et al. 2009 (Singleton and Rossi 1965; Singleton et al. 1999; Marghitas et al. 2009). The concentration of total phenolic compounds was determined as mg of Gallic acid equivalent, using a calibration curve. Tests were carried out in triplicate.

Flavonoids were determined according to the method by Kong et al. 2012. The absorbance was read at 510 nm, using a calibration curve of quercétine. The result was expressed as mg of quercétine equivalent/g of the bee bread extract.

The antioxidant activity of the bee bread extract was evaluated by the phosphomolybdenum method according to the procedure by Zengin et al. (2012). The antioxidant capacity of the bee bread extract was evaluated as equivalents ascorbic acid (mg AA/g extract).

The scavenging activity of the bee bread extract for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Miguel et al. 2014 (Blois 1958; Hogg et al. 1961; Miguel et al. 2014). The absorbance was recorded at 517 nm. The tests were carried out in triplicate. Butyl hydroxy toluène (BHT) was used as a positive control and the scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation (Miguel et al. 2014):

$$IC_{50}\% = [(\text{control absorbance} - \text{sample absorbance})/(\text{control absorbance})] \times 100$$

ABTS radical scavenging activity was evaluated according to Silva et al. (2013). Each test was repeated three times. The capability to scavenge ABTS radical was calculated using the following equation: $IC_{50}\ \% = [(-A0 - A1/A0)*100]$, where A0 is the absorbance of a negative control (blank sample containing the same amount of solvent and ABTS solution) and A1 is the absorbance of the sample. Butyl hydroxy toluene (BHT) was used as a positive control (Silva et al. 2013).

The reducing power was determined according to Padmanabhan and Jangle (Padmanabhan and Jangle 2012). The absorbance of the mixture was measured at 700 nm. Ascorbic acid was used as a positive control.

Experimental animals

Male Wistar rats (body weight 198 ± 11.14 g) were used for experiment. The animals were housed in a standard environmental condition and fed with rodent rats and water ad libitum. The care and handling of the animals were in accordance with the internationally accepted standard guidelines for the use of animals, and the protocol was approved by the institutional committee on animal care following the French Technical Specifications for the Production, Care and Use of the Laboratory Animals.

Experimental design

Aluminum chloride hexahydrate ($AlCl_3$, $6H_2O$) solution was made fresh at the beginning of each experiment. For oral administration, aluminum chloride was dissolved in water and administered in a dose of 662.2 mg/kg b.wt (DL_{50} = 3311 mg/kg b.wt-oral rat) once daily via a gavage.

The rats were randomly allocated into four groups of four rats each: Group 1: was used as the control and received distal water (10 ml/kg b.wt), Group 2: received aluminum chloride hexahydrate (662.2 mg/kg b.wt), Group 3: received aluminum chloride (662.2 mg/kg b.wt) and the ethanolic extract of bee bread (500 mg/kg b.wt), and Group 4: received aluminum chloride hexahydrate (662.2 mg/kg b.wt) and the ethanolic extract of bee bread (750 mg/kg b.wt). Aluminum chloride hexahydrate and ethanolic extract of the bee bread were given daily, at the same time, to each rat for a total of 28 days. Doses of water, aluminum chloride hexahydrate and ethanolic extract of bee bread were given once daily via a gavage. Blood and urine samples were collected on day 28 of the experiment.

Biochemical methods

Blood sample was withdrawn from each rat by retro-orbital puncture under light diethyl ether anesthesia, and divided into two parts. The first part of the blood was mixed into EDTA in capillary tubes to analyze hematological parameters: hematocrit (Ht), hemoglobin (Hb), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLC), total leukocyte count, and lymphocyte and monocyte counts. The second part of the blood was centrifuged at 10 000×*g* for 10 min; plasma was obtained and stored at −20 °C to analyze C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (SCr), creatinine clearance, sodium, and potassium. Urine sample was collected from each rat on day 28 for analysis of sodium, potassium and creatinine.

Statistical analysis

The data were expressed as mean ± SD variables' reading in each group. Statistical comparisons between the groups were performed with one-way analysis of variance (ANOVA) followed by a Dunnett test to compare all columns with control column (Graph Pad Prism 5 software). The data followed normal distribution.

Results

Chemical analysis and antioxidants

Chemical analysis showed that the bee bread samples contain moisture (9.85% ± 0.165), fat (2.31% ± 0.574), carbohydrate (28.46% ± 0.994), proteins (12.81% ± 0.167) and fiber (8.30% ± 1.351). The content of phenol was 14.88 ± 0.98 mg GAE/g, flavonoids was 1.67 ± 0.12 mg QE (quercetin equivalents)/g, and total antioxidant capacity was 143.78 ± 11.38 mg AAE (ascorbic acid equivalents)/g. The antioxidant capacity of bee bread was demonstrated by an IC50 of DPPH

(0.05 ± 0.01) mg/ml, ABTS (0.08 ± 0.05) mg/ml, and reducing power (0.05 ± 0. 04) mg/ml, while BHT was demonstrated by an IC50 of DPPH (0.009 ± 0.0001) mg/ml, and ABTS (0.003 ± 0.01) mg/ml. The antioxidant capacity of ascorbic acid was demonstrated by reducing power (0.003 ± 0.0001) mg/ml.

Effect of aluminum with and without bee bread on hematology parameters

Aluminum caused a significant lowering of Ht, Hb and RBC (Table 1). The administration of aluminum along with the bee bread extract at a dose of 750 mg/kg b.wt caused insignificant lowering of Ht, RBC and Hb (Table 1). Higher doses of the bee bread (750 mg/kg b.wt) caused a significant elevation of Hb as compared to the lower dose (500 mg/kg b.wt).

Similar results were obtained regarding other blood variables (MCH, MCV and HCHC) where aluminum alone or aluminum given with the bee bread at a dose of 500 mg/kg b.wt caused a significant lowering of the variables, while aluminum given along with the bee bread at a dose of 750 mg/kg b.wt did not cause significant changes in these variables as compared to the control except for MCV (Table 1). Higher doses of the bee bread extract (750 mg/kg b.wt) caused a significant elevation of RCB and MCH as compared to the lower dose of the bee bread extract (500 mg/kg b.wt).

Aluminum elevated platelet, monocyte, leukocyte and lymphocyte counts; the elevations were significant for monocyte, leukocyte and platelet as compared to the control (Table 1). The administration of aluminum along with either dose of the bee bread extract did not cause any significant changes in the platelet, leukocyte, monocyte, and lymphocyte counts as compared to the control (Table 1). Concomitant administration of the bee bread at both doses and aluminum caused a significant lowering of platelet, monocyte, lymphocyte and leukocyte counts as compared to the aluminum group.

Effects of aluminum with and without bee bread on liver enzymes and CRP

Regarding the liver enzymes and CRP, aluminum significantly increased CRP, AST and ALT as compared to the control group (Table 2). However, CRP and AST were not significantly changed in rats received aluminum along with the both doses of bee bread extract. As compared to aluminum group, the bee bread extract at a higher dose caused a significant lowering of CRP and AST. In case of ALT, Aluminum caused a significant elevation of ALT when administrated alone, and this elevation was less when aluminum was administered along with either dose of the bee bread extract (Table 2). Bee bread in both doses ameliorates the toxic effect of aluminum on liver enzymes.

Table 1 The effect of aluminum alone or in combination with the bee bread extract on Hematocrit, Hemoglobin, Red Blood Cells, MCH, MCV, MCHC concentration and blood cells counts

Variables	Control	Aluminum	Aluminum + bee bread (500 mg/kg b.wt)	Aluminum + bee bread (750 mg/kg b.wt)
Hematocrit (%)	39.87 ± 0.53	27 ± 3.53^{a*}	34.25 ± 2.58^{a*b*}	37.7 ± 1.07^{c*}
Hemoglobin (g/dl)	14.75 ± 0.81	8.5 ± 1.1a^{a*}	9.5 ± 1.21^{a*}	13.02 ± 0.64^{c*d*}
Red Blood Cells (M/mm3)	4.12 ± 0.78	1.37 ± 0.14^{a*}	1.77 ± 0.43^{a*}	3.17 ± 0.95^{c*d*}
MCH (pg)	32 ± 1.07	17.13 ± 2.09^{a*}	18.55 ± 1.09	26.65 ± 1.34^{c*d*}
MCV (fl)	69.60 ± 7.95	52.2 ± 7.75^{a*}	54.75 ± 2.94^{a*}	55.32 ± 4.76^{a*}**a***
MCHC (g/dl)	34.15 ± 0.74	30 ± 1.22^{a*}	31.75 ± 1.47^{a*}	34.62 ± 0.32
Platelets × 10^9/L	400 ± 23.58	497 ± 15.08^{a*}	427.5 ± 55.39^{b*}	430 ± 162^{c*}
Monocytes × 10^9/L	0.7 ± 0.072	1.37 ± 0.13^{a*}	0.6 ± 0.13^{b*}	0.825 ± 0.20^{c*}
Lymphocytes × 10^9/L	3.75 ± 0.31	4.17 ± 0.26	3.55 ± 0.33^{b*}	3.57 ± 0.7^{c*}
Leukocytes × 10^9/L	5.5 ± 0.33	8.85 ± 0.5^{a*}	6.5 ± 0.36^{b*}	7.67 ± 3.25

Data presented as mean ± SD. (* $p < 0.05$)

[a]Comparison between normal group and all groups

[b]Comparison between aluminum group and aluminum +bee bread (500 mg/kg b.wt)

[c]Comparison between aluminum group and aluminum +bee bread (750 mg/kg b.wt)

[d]Comparison between aluminum + bee bread (500 mg/kg b.wt) and aluminum + bee bread (750 mg/kg b.wt)

Table 2 The effect of aluminum alone or in combination with the bee bread extract on transaminase (ALT and AST) and C-reactive protein (CRP)

Variables	Control	Aluminum	Aluminum + bee bread (500 mg/kg b.wt)	Aluminum + bee bread (750 mg/kg b.wt)
ALT (IU/L)	34.75 ± 4.95	83.5 ± 6.72[a*]	78.33 ± 21.78[a*]	68.66 ± 16.24[a*]
AST (IU/L)	105 ± 3.06	182.25 ± 29.79[a*]	163.75 ± 21.3	146.75 ± 43.99[c*]
CRP (mg/ml)	6.75 ± 0.81	19 ± 8.52[a*]	10.5 ± 1.8[b*]	9.25 ± 4.48[c*]

Data presented as mean ± SD. (* $p < 0.05$)

[a]Comparison between normal group and all groups

[b]Comparison between Aluminum group and Aluminum +Bee bread (500 mg/kg b.wt)

[c]Comparison between aluminum group and aluminum +bee bread (750 mg/kg b.wt)

[d]Comparison between aluminum + bee bread (500 mg/kg b.wt) and aluminum + bee bread (750 mg/kg b.wt)

Effects of aluminum with and without bee bread on kidney function and plasma and urine electrolytes

Regarding serum electrolytes and kidney function test, aluminum did not cause any significant changes in the serum level of potassium, sodium and SCr (Table 3). However, it causes a significant elevation of BUN and lowering of creatinine clearance, which were significantly reversed toward normal level with use of bee bread extract (500 and 750 mg/kg b.wt) (Table 3). Aluminum administration combined with administration of the bee bread extract in both doses did not cause significant changes in urine potassium level and renal clearance as compared to the control group (Table 3). Higher dose of bee bread extract caused significant increase in urine sodium excretion, which was a dose dependent.

Discussion

The result of this study showed that the bee bread extract has a protective effect on aluminum induced anemia and it significantly ameliorates hepato-renal toxicity and reduces the elevated CRP levels and monocyte and leukocyte counts encountered after aluminum exposure. Aluminum alone caused significant anemia, elevation of BUN, CRP, leukocyte and monocyte counts and liver enzymes, ALT and AST, and decreased creatinine clearance.

The results of the hematological parameters of the present study showed that aluminum decreases significantly Ht, Hb, RBC, MCV, MCH, and MCHC. Other studies showed similar results (Bazzoni et al. 2005; Al-Qayim et al. 2014; Ghorbel et al. 2015). Previously it has been found that aluminum chloride administration for 60 days significantly decreased total RBC, Hb concentration,

Table 3 Effect of aluminum alone or aluminum and the bee bread on serum and urine sodium and potassium, blood urea, serum creatinine and creatinine Clarence

Variables	Control	Aluminum	Aluminum + bee bread (500 mg/ml)	Aluminum + bee bread (750 mg/ml)
Blood urea (mg/dl)	48.5 ± 2.2	134.5 ± 19[a*]	119.5 ± 29[a*]	91.2 ± 12[a*c*]
Serum creatinine (mg/dl)	0.5 ± 0.069	0.45 ± 0.11	0.52 ± 0.04	0.60 ± 0.06
Serum potassium (mEq/L)	5.6 ± 0.2	5.1 ± 0.17	4.9 ± 0.34	5.3 ± 0.31
Serum sodium (mg/dl)	143 ± 2.42	144 ± 4.33	146 ± 2.94	145.2 ± 2.42
Urine potassium (mEq/L)	105.75 ± 3.34	96.35 ± 7.86	96.82 ± 3.04	110.55 ± 9.83
Urine sodium (mg/L)	67.82 ± 15.05	64.55 ± 17	64.9 ± 10.68	89.3 ± 14.75[a*c*d*]
Creatinine clearance (ml/kg/min)	2.03 ± 0.08	1.46 ± 0.32[a*]	1.95 ± 0.17	2.10 ± 0.17[c*]

Data presented as mean ± SD. (* $p < 0.05$)

[a]Comparison between normal group and all groups

[b]Comparison between aluminum group and aluminum + bee bread (500 mg/kg b.wt)

[c]Comparison between aluminum group and aluminum + bee bread (750 mg/kg b.wt)

[d]Comparison between aluminum + bee bread (500 mg/kg b.wt) and aluminum + bee bread (750 mg/kg b.wt)

PCV%, MCV, MCH, MCHC, RBCs osmotic fragility, serum catalase activity, serum iron, total iron binding capacity, and serum ferritin, and increased reticulocyte count (Al-Qayim et al. 2014). Interestingly, the use of bee product, propolis, normalized these parameters when were used with aluminum chloride (Al-Qayim et al. 2014). Another study showed that periodical injections of aluminum to rats over a period of 3 months caused significant decreases in Ht and Hb concentration, erythrocyte aggregate size and aggregation rate, and it disorganized erythrocyte membrane by altering its mechanical properties (Bazzoni et al. 2005). Regarding mechanism of action, it has been proposed that aluminum's toxicity—induced anemia is due to hemolysis caused by the generation of free radicals leading to the degradation of the cell membrane or due to alteration in iron level caused by aluminum (Farina et al. 2016). Furthermore, aluminum causes an increase in reticulocytes which is another feature of hemolysis (Al-Qayim et al. 2014). Recently, another study showed that aluminum decreases iron level and can interfere with iron in the erythrocytes because it shares several characteristics such as trivalency and it can share the same mechanism of absorption, distribution and metabolism (Farina et al. 2016). Our previous study showed that honey, part of bee bread, increased antioxidants and caused a significant elevation of iron level, Hb, and PCV in normal individual (Al-Waili 2003). Therefore, in the present study honey and bee pollen increased Hb and PCV, partly due to an increase in iron level in aluminum exposure. However, further studies are needed. Also honey and bee pollen have an antioxidant activity which might help restoring hematological parameters changes induced by aluminum exposure.

The elevation of liver enzymes might be a result of hepatic cellular injury. Similar results have been reported by other studies (Yousef and Salama 2009; Türkez et al. 2010; Shati and Alamri 2010). In this regards, aluminum chloride caused a significant increase in GGT, ALT, AST, ALP, and lipid peroxidation and these changes were alleviated by saffron, a spice derived from the flower of *Crocus sativus*, and honey (Shati and Alamri 2010). Another study revealed that aluminum chloride causes a significant increase in alkaline phosphatase, AST, ALT and lactate dehydrogenase, and causes sinusoidal dilatation, congestion of central vein, lipid accumulation and lymphocyte infiltration in the liver (Türkez et al. 2010). Our previous studies showed that honey, part of bee bread, causes amelioration of elevated liver enzymes during acute blood loss and food restriction (Al-Waili et al. 2006a, b). Furthermore, we have found that honey feeding ameliorates the elevation in AST and ALT obtained following CCl_4 administration in rats (Al-Waili et al. 2006a, b). In the present study, we know that the preventive effect against liver injury might be due to honey effect and probably bee pollen. Synergistic effect may be considered if future studies will show preventive effect of bee pollen too. Moreover, the effect of honey and bee pollen most likely is due to their antioxidant activity. Further studies would be useful to explore this possibility.

Regarding kidney parameters, aluminum causes a significant increase in BUN and a significant decrease in creatinine clearance in the present study, however, the result showed that aluminum has no effect on SCr. The elevation of BUN might be due to renal injury or other mechanisms. Liver synthesizes urea and during liver injury low BUN is encountered. Therefore, the real mechanism of increasing BUN in the setting of liver injury is not clearly identified. However, other studies showed that aluminum chloride increases both BUN and SCr (Ghorbel et al. 2015; Al Dera 2016). In the present study aluminum chloride was used for 28 days while the other studies use aluminum for longer period of time. This might explain why there was no change in SCr in the present study. Recently, it was found that aluminum chloride administered for 40 days increases BUN and SCr and decreases urine volume and creatinine clearance (Al Dera 2016). This was accompanied by increased renal OS and inflammation. This is in agreement with our present finding that aluminum decreases creatinine clearance. Previously we have found that honey has a reno-protective effect on acute blood loss, CCI4 toxicity and lead toxicity (Al-Waili et al. 2006a, b, Fihri et al. 2016). Furthermore, it was found that honey improved renal function in normal individuals which has been suggested due to increasing nitric oxide and decreasing prostaglandins (Al-Waili 2005). Study to explore the effect of bee pollen on renal function will be interesting to see if the combination of honey and bee pollen will result in synergetic preventive effect. Regarding mechanism of action, the antioxidant properties of honey and bee pollen might play a role in their favorable effect of kidney function.

Interestingly, the present data showed that aluminum increases leukocyte, monocyte and lymphocyte counts and CRP level. These are markers of inflammation. Another study showed that aluminum increases hepatic pro-inflammatory cytokine expression including interleukin-1β, tumor necrosis factor-α, and interleukin-6 (Ghorbel et al. 2015). CRP is a highly sensitive biomarker for systemic inflammation. Other studies have demonstrated that albumin exposure caused OS and inflammation, and it decreased activity of the antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Ghorbel et al. 2015; Oda 2016). The use of antioxidants ameliorates aluminum—induced hepato-renal toxicity and restores antioxidant system (Oda 2016; Sharma et al. 2016). Therefore, bee bread might encounter or ameliorate albumin toxicity by its antioxidant activity. Furthermore, honey and bee pollen might have a synergistic effect. This

needs to evaluate the effect of each and in combination in aluminum toxicity.

Conclusion

Bee bread, a composition of pollen, honey and bees' enzymes, exhibited an antioxidant activity and has a considerable protective effect on aluminum induced toxicity in rats. It ameliorated the reduction of Hb and the elevation of CRP, monocyte and leukocyte counts, and reversed the elevation of liver enzymes and BUN toward normal level. The mechanism of action might be related to the antioxidant and anti-inflammatory activity of the bee bread extract. More studies are needed to verify this possibility. Furthermore, study of the effect of honey and bee pollen as well as combined honey and bee pollen would be useful to explore the possible synergistic effect. In addition, this study will pave the way for further studies including preclinical and clinical investigations to use bee bread as a potential intervention in aluminum toxicity.

Compliance with ethical standards

Conflict interest The authors declare that there are no conflict of interest.

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