Biological Trace Element Research (2019) 192:214–221
https://doi.org/10.1007/s12011-019-1649-2

Propolis and Its Combination with Boric Acid Protect Against Ischemia/Reperfusion-Induced Acute Kidney Injury by Inhibiting Oxidative Stress, Inflammation, DNA Damage, and Apoptosis in Rats

Fatime Geyikoglu[1] · Kubra Koc[1] · Suat Colak[2] · Huseyin Serkan Erol[3] · Salim Cerig[4] · Berna Kavakcioglu Yardimci[1,5] · Ozge Cakmak[1] · Muhammed Bahaeddin Dortbudak[6] · Gizem Eser[6] · Ferhunde Aysin[1,7] · Nihal Simsek Ozek[1,7] · Serkan Yildirim[5]

Received: 27 November 2018 / Accepted: 18 January 2019 / Published online: 19 February 2019

Abstract

Ischemia reperfusion (I/R) injury which causes kidney dysfunction is one of the most studied diseases directly linked to oxidative stress. In this regard, it is important to protect cells against damage by inducing antioxidant response. Herein, we aimed to evaluate the therapeutic roles and possible mechanisms of propolis and boric acid in kidney I/R injury based on relevant basic research and clinical studies. Sprague-Dawley rats were subjected to 50 min of ischemia followed by 3 h of reperfusion. Animals were randomly divided into a control group (the abdominal wall was just opened and closed), an I/R injury group, the propolis intervention group (200 mg/kg, intragastric administration, 1 h before ischemia), boric acid intervention group (14 mg/kg, intragastric administration 1 h before ischemia), and the propolis + boric acid intervention group (intragastric administration 1 h before ischemia). Kidney function, the antioxidant defensive system, and renal damage were assessed. In addition, the oxidative stress and inflammatory status were estimated in renal tissue. Furthermore, DNA damageand apoptosis were detected by immunohistochemistry. When compared with I/R group, propolis alone and especially propolis + boric acid groups significantly improved functional parameters. While the antioxidant response was increased, renal injury size and apoptosis were significantly decreased in both groups. Also, the MDA and TNF-α levels besides the 8-OHdG formation were downregulated. According to these outcomes, it can be said that especially propolis together with boric acid ameliorates kidney injury caused by I/R through acting as an antioxidant, anti-inflammatory, and antiapoptotic agent. In conclusion, propolis alone and its combination with boric acid could be developed as therapeutic agents against serious renal I/R injuries.

✉ Kubra Koc
kubrakc@hotmail.com

[1] Department of Biology, Faculty of Science, Ataturk University, Erzurum, Turkey

[2] Department of Biology, Uzumlu Vocational, Erzincan University, Erzincan, Turkey

[3] Department of Biochemistry, Faculty of Veterinary, Ataturk University, Erzurum, Turkey

[4] Department of Biotechnology, Faculty of Science, Bartin University, Bartin, Turkey

[5] Department of Chemistry, Faculty of Science, Dokuz Eylül University, Izmir, Turkey

[6] Department of Pathology, Faculty of Veterinary, Ataturk University, Erzurum, Turkey

[7] East Anatolian High Technology Research and Application Center (DAYTAM), Ataturk University, Erzurum, Turkey

Keywords Ischemia/reperfusion · Propolis · Boric acid · Kidney · Oxidative stress · Inflammation

Introduction

Despite many effective medical therapies at present, ischemia remains a major cause of morbidity and mortality worldwide [1]. Importantly, ischemic kidney disease is a leading cause of kidney dysfunction [2]. Therefore, significant clinical effort has been directed toward preventing acute renal dysfunction and reducing the size of dysfunction in individuals experiencing kidney disease. Although renal reperfusion therapies can relieve problems about kidney from following sustained ischemia, reperfusion per se paradoxically induces kidney injury, termed "reperfusion injury," which blunts the benefits of renal reperfusion [3]. Strategies to limit reperfusion injury and

nephrotic syndrome size have not been implemented well in clinical settings [4]. The increased generation of reactive oxygen species (ROS) is a major contributor to the pathophysiological mechanisms underlying reperfusion injury, hinting us to consider the decreases in ROS levels in the kidney for minimizing the damage associated with renal I/R [5].

Nowadays, there is a folk medicine branch called apitherapy that aims to treat diseases with bee products, including honey is quite popular as an alternative therapy[6]. Honey bee products (e.g., propolis) have medicinal and health-promoting properties in mouse/human model such as vasodilative, antiulcer, antibacterial, antiviral, anti-inflammatory, and antitumor activities [7]. In recent years, the propolis has been subjected to a range of pharmacological investigations and its value has been assessed in numerous studies [8]. However, little is known about the effects of this folk remedy on renal I/R injury. The composition of propolis represents high antioxidant activity [9], and thus can be investigated for the treatment of renal I/R injury.

Based on the clinical data available, the boron compounds appear to have therapeutic effects on systemic disorders. In medicine, boric acid (BA) is used for wound healing, bone mineralization, and inhibition of prostate cancer [10–12]. Boron compounds have been reported as a dietary supplement [13]. The studies on this compound indicated that they are rapidly absorbed from the gastrointestinal tract and exist primarily as undissociated boric acid in the body. Moreover, most of these compounds accumulated generally in bone and rarely in soft tissues [14]. They are primarily excreted via urine. Among boron compounds, only boric acid has been identified in urine since BA accounts for> 90% of the ingested boron dose within the body [15].

On the other hand, acute exposure of the boron compounds has been reported to toxic effects on sperm quality and biochemical parameters in aquatic animals and rats [16]. BA has lower toxicity compared with other boron compounds. However, the boron compounds as the anti-renal ischemic agents have not been reported yet. Moreover, the mechanisms of both the beneficial and the toxic effects of boric acid are not fully understood. Therefore, the current study was firstly aimed to explore the protective ability of boric acid against renal I/R injury.In addition, it was secondly aimed to propose a new strategy to present the protective therapy of the combined boric acid with a natural compound, propolis against renal I/R injury.

Material and Methods

Animals

Adult Sprague-Dawley rats ($n = 35$), weighing 250–300 g, were purchased from Atatürk University Experimental Research Center (Erzurum/Turkey). The rats were kept under standard laboratory conditions, maintained in temperature- and humidity-controlled rooms on a 12-h/12-h light/dark cycle, and had free access to standard commercial rat pellets (purchased from Bayramoğlu Yem, Erzurum, Turkey, 1.5% fat, 7.5% carbohydrates, 23% protein, 1–2% vitamins and minerals; 3% trace elements, iron, selenium, manganese, zinc, cobalt, iodide, 270 kcal 100 g^{-1}) and water.

Propolis and Boric Acid Preparation and Experimental Design

The water-soluble propolis extract was purchased from Aksuvital Natural Products Food Industry Trade Inc. (Turkey) and boric acid was supplied by theSigma-Aldrich Chemical Company (St. Louis, MO, USA).They were separately dissolved in 100 ml distilled water. Then, the propolis solution was diluted to 200 mg/kg and the boric acid solution was diluted at 14 mg/kg concentrations. Their doses were selected according to the literature data [17, 18]and our preliminary studies. The solutions were given to rats as 1 cc by gavage 1 h before ischemia onset. The rats were randomly divided into fivegroups ($n = 7$ per group): I) Control-sham group (the abdominal wall was only opened and closed), II) I/R group (the rats were subjected to 50 min of ischemia followed by 3 h of reperfusion), III) Propolis intervention group (200 mg/kg, intragastric administration, 1 h before ischemia), IV) Boric acid intervention group (14 mg/kg, intragastric administration 1 h before ischemia), and V) Propolis + boric acid intervention group (intragastric administration 1 h before ischemia).

I/R injuries were created as described previously [19]. Briefly, each rat was anesthetized via intraperitoneal injection of a combination of ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). An abdominal incision was created, and unilateral ischemia was induced by clamping the left renal artery. After 50 min of ischemia, the clamp was removed to allow reperfusion. Body temperature was maintained at 36 °C using heating pads throughout the experiment. At 3 h of reperfusion, rats were killed and kidney tissues were collected. Kidney samples were stored at − 20 °C for a period before they were used in later analysis; kidney slices were also processed for histological studies.

Measurement of Biochemical Parameters

The kidney samples were homogenized, and the supernatants were used to determine the antioxidant enzyme profile, oxidative status, and cytokine levels. The levels of superoxide dismutase (SOD), glutathione (GSH), lipid peroxidation (LPO), and tumor necrosis factor (TNF-α) in the rat renal tissue were measured with the Clinical Automatic Biochemistry Analyzer 7600 (Hitachi, Japan) employing ELISA kits (R&D Systems, Minneapolis, MN) according to

the manufacturer's instructions. All experiments were performed with triplicate samples and repeated three times.

Immunohistochemical andHistopathological Assessments

The kidney tissues were fixed in 10% neutral buffered formalin overnight, dehydrated, and embedded in paraffin for hematoxylin and eosin (H&E) staining or immunostaining. The kidney tissues were sectioned at 5 μm. Sections were stained with H&E and analyzed using a light microscope (Leica DM 1000, Germany). Analysis of the sections was performed by the same pathologist blindly. 8-hydroxylo-2′-deoxyguanosin (8-OHdG) and Bax were detected by specific monoclonal antibodies. The tissues were cut into 4-μm sections and the sections were deparaffinized. After Diaminobenzidine (DAB) was applied as chromogen, slides were counterstained with hematoxylin, dehydrated, and covered by coverslips. Immunohistochemical staining of Bax was performed using Bax-Antibody (B-9) (Santa Cruz), following the manufacturer's instructions. Immunohistochemical staining of 8-OHdG was performed using anti-8-OHdG antibody (Santa Cruz; 1:2500 dilution) with a Novolink Polymer Detection kit (Leica Microsystems Pte Ltd., Taipei, Taiwan), following the manufacturer's instructions. The pathologists continuously observed at least tenhigh-power fields (× 200) for each slice, counted the number of positive cells in each high-power field, and calculated the average number of positive cells to reflect the intensity of positive expression. The sections were evaluated as none (−), mild (+), moderate (++), and severe (+++) according to their immunity positivity [20].

Statistical Analysis

The differences in variance were analyzed statistically using a one-way analysis of variance (ANOVA) test by Graphpad prism 5.0 statistics software (GraphPad, La Jolla, CA, USA). Tukey's test was used as a post hoc.

Results

Effect of Propolis and Boric Acid on Biochemical Parameters in Renal I/R

As shown in Fig. 1, comparedwith the sham-operated rats, the renal I/R ones showed significantly lower levels of the SOD and GSH (respectively, $p < 0.05$, $p < 0.0001$) but significant higher levels of LPO and TNF-α ($p < 0.0001$). However, pretreatment with Propolis (200 mg/kg) significantly increased levels of the SOD and GSH (respectively, $p < 0.05$, $p < 0.001$) but decreased in levels of the LPO and TNF-α in kidney compared with I/R group (respectively, $p < 0.0001$, $p < 0.01$). On the other hand, pretreatment with boric acid (14 mg/kg) significantly increased levels of the SOD ($p < 0.05$), while did not affect the level of GSH in kidney compared with the I/R group. Moreover, boric acid decreased in levels of the LPO and TNF-α in kidney compared with I/R group (respectively, $p < 0.0001$, $p < 0.01$). Particularly, the levels of LPO ($p < 0.001$) and TNF-α in pretreatment of the combination with propolis and boric acid group were lower than those of the other experimental groups in I/R model ($p < 0.0001$). In the combined treatment group, the levels of antioxidant enzyme were higher than I/R group.

Effect of Propolis and Boric Acid on 8-OHdG and Bax Immunoreactivity in Renal I/R

The representative images of 8-OHdG formation, expression of Bax are depicted inFig. 2 in rat kidney treated with propolis, boric acid, or combination of them prior to I/R and sham-irradiated control. I/R markedly stimulated the formation of 8-OHdG and expression of Bax in rat kidney cells in comparison with a control (Fig. 2). Pretreatment with propolis to kidney with I/R led to a moderate decrease in the 8-OHdG formation. On the other hand, boric acid slightly attenuated expression of8-OHdG in the renal cells with I/R. However, pretreatment with combination of them showed mild differences in 8-OHdG expression in rat kidney cells. Similar to 8-OHdG, the pretreatment of propolis significantly decrease the number of Bax-positive cells as compared to sham samples. The boric acid resulted also in slight decreaseof Bax expression in rat kidney when compared to in sham. In addition, the pretreatment with Propolis and boric acid significantly protected the renal cells from apoptosis (Table 1). The strongest protective effects were observed in combination with Propolis and boric acid in kidney with I/R.

Histopathological Assessments

Histopathological examination by H&E staining of tissue sections revealed normal histology of kidney in the control group (Fig. 3). The renal I/R caused severe congestion,hemorrhage, hydropic degeneration, and necrosis of tubules. On the contrary, when the propolis and boric acid were applied on rat kidney with I/R, the reduction in the number of histopathological change was observed especially for combination of propolis and boric acid and in a lesser reduction only for pretreatment with propolis (Fig. 3). In the combination group, necrosis in tubules and very little degeneration in tubular epithelium with nearly normal histological structures were determined. The protection was strongly pronounced when renal tissue was treated with propolis and boric acid prior I/R reduction.

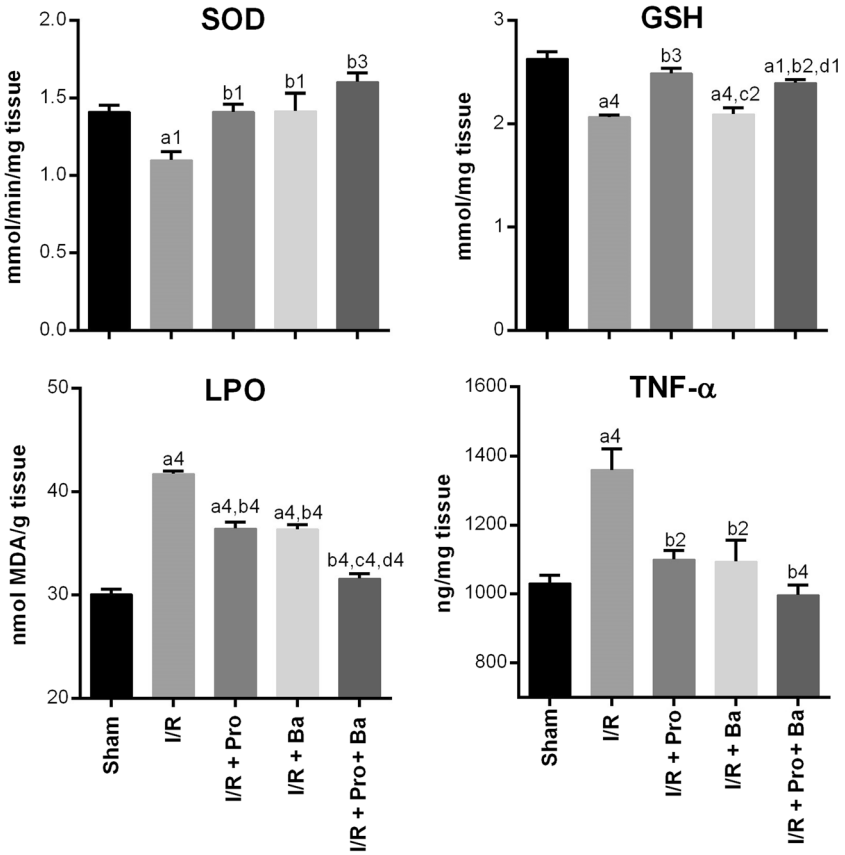

Fig. 1 The effects of propolis and boric acid on kidney SOD, GSH, LPO, and TNF-αlevels after I/R. Data are presented as mean ± SEM ($n = 7$). [a] denotes significant differences between other studied groups and sham ($^{a1}p < 0.05$, $^{a4}p < 0.0001$), [b] denotes significant differences between other studied groups and I/R group ($^{b1}p < 0.05$, $^{b2}p < 0.01$, $^{b3}p < .0.001$, $^{b4}p < 0.0001$), [c] denotes significant differences between other studied groups and I/R + Pro group ($^{c2}p < 0.01$, $^{c4}p < 0.0001$), [d] denotes significant differences between other studied groups and I/R + BA group ($^{d1}p < 0.05$, $^{d4}p < 0.0001$) by Tukey's multiple range tests. Abbreviation used: I/R:ischemia/reperfusion, Pro: propolis, BA: boric acid

Discussion

Oxidative-stress-induced free radicals have been found to be key players in the pathophysiology of I/R [21]. In view of the use of the antioxidants in combination with bee products for treatments of disorders and promotion of health, we think that these pharmacological materials may represent a remedy to be used in severe I/R damages now. According to our literature survey,a combined therapy with propolis in I/R injury hasnot been identified so far.Thus, this study provides, for the first time, evidence of the use of propolis and boric acid combination against renal I/R injury.

Preliminary clinical trials demonstrated that propolis had anti-diabetic activity in humans by improving glycemic control in subjects with insulin resistance [22]. Besides, the application of topical propolis extract gel to traumatic ulcer caused by diabetes mellitus (DM) ulcers accelerates wound healing [23]. However, the application of this folk remedy as a means to prevent renal I/R injury has yet to be completely evaluated. Our study supported that this natural product can be promising

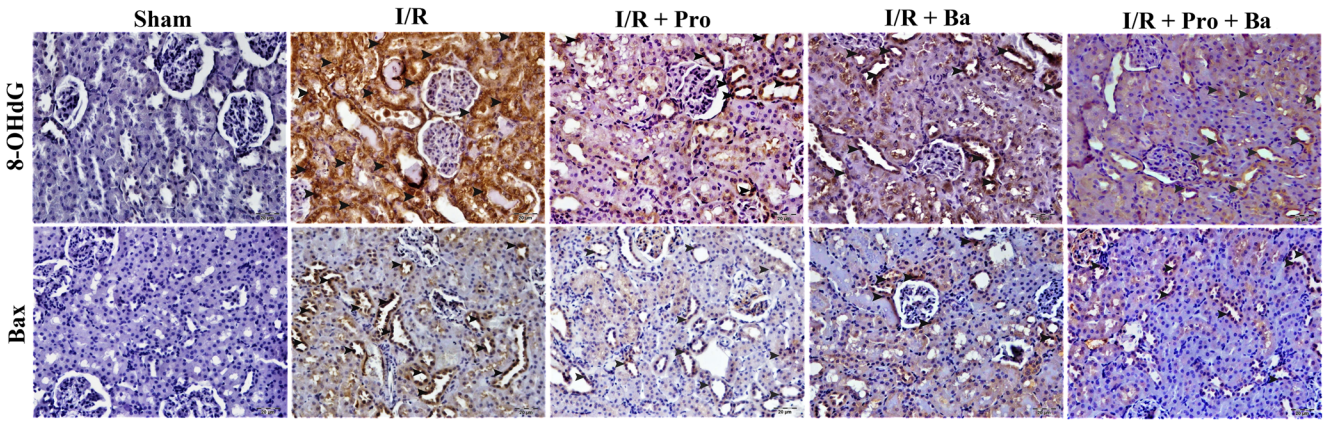

Fig. 2 Immunohistochemical staining of the 8-OHdG and Bax in the kidney tissue. Sham; 8-OHdG and Bax negative, I/R; severe8-OHdG and Bax expression, I/R + Pro; moderate 8-OHdG and Baxexpression I/R + Ba; slightly decreased 8-OHdG and Baxexpression, I/R + Pro + Ba; mild 8-OHdG and Bax expression(Bar: 20 μm). Abbreviation used: I/R:ischemia/reperfusion, Pro: propolis, Ba: boric acid

Table1 Immunohistochemical findings and their scores in kidney tissue

Groups	8-OhDG	Bax
Sham	–	–
I/R	+++	+++
I/R +Pro	++	++
I/R + Ba	++	++
I/R + Pro + Ba	+	+

*I/R*ischemia/reperfusion, *Pro*propolis, *Ba*boric acid

According to immunohistochemical findings: none (−), mild (+), moderate (++) and severe (+++)

and a pharmacological active compound to serious renal I/R injury. In the study conducted by da Costa et al., it was shown that Red propolis (150 mg/kg/day) administration by gastric gavage 3 days before the procedure improved oxidative stress status (GSH, MDA, heme-oxygenase,and eNOS) when compared with the I/R group [24]. On the other hand, with this paper, we firstly reported the effects of the propolis on the activities of the antioxidant enzymes in renal I/R injury. In our study, propolis (200 mg/kg) was administered to rats as 1 cc by gastric gavage 1 h before ischemia onset and association between oxidative stress and antioxidative response was determined. It is well known that oxidative stress is increased by a system in which the rate of free radical production is enhanced and endogenous antioxidant mechanisms are impaired in I/R [25]. According to the results of this study, GSH level, SOD, and CAT activities in renal cells were significantly higher in the propolis pretreatment group when compared with I/R. These data could be results of the antioxidative system response induced by propolis against free radical production in the case of I/R. Additionally, to assess the extent of oxidative DNA damage [26] we analyzed 8-OHdG-immunopositive cells in a rat model of renal I/R injury. The results showed that propolis significantly decreased the 8-OHdG formation in kidney cells. Supportingly, previous studies have showed that propolis has great antioxidant effects. Lungkaphin et al. found that propolis could decrease MDA levels and oxidative stress effects in myocardial I/R injury [27]. Propolis also exerted antioxidant abilities in concanavalin A-induced liver injury, cancer development, gentamicin-induced renal damage, and so forth [28, 29].

Here, we also provide evidence for the first time the effects of boric acid against renal I/R injury. Boron supplement was shown to induce SOD activity in hepatotoxicity [30] and enterocolitis [31]. In our experiments, the increased SOD activity in kidney supported the hypothesis that SOD is tightly controlled in response to I/R injury in boric acid group [32]. Conversely, the decrease in the SOD activity could lead to an excess of superoxide anion ($O_2^{\cdot}-$) and hydrogen peroxide (H_2O_2) (which in turn generated hydroxyl radicals) in biological systems, resulting in the initiation and propagation of lipid peroxidation [33]. It was also reported that the blood flow during reperfusion phase of I/R injury could produce oxygen free radicals which lead to the MDA formations to destroy the antioxidant defense system [34, 35]. Based on our in vivo findings, the anti-oxidant activity of boric acid decreased MDA level in kidney cells when compared with I/R group. On the other hand, the level of reduced glutathione (GSH), the most important intracellular antioxidant molecule, is a sensitive index of the efficiency of cellular antioxidant defenses and its decrease during focal cerebral ischemic in rats leads to the development of severe complications [36]. Boron can cause change in the structure and function of proteins by interacting with them [37]. In the present study, as a result of the increasing free radical concentration, one of the consequences of I/R, the renal cells may not have allowed GSH synthesis to increase in order to protect themselves from the effects of these reactive species. In our experimental model, boric acid is believed to scavenge free radicals by working together with GSH, thereby leading a decrease in the level of GSH. Additionally, our results showed the positive effect of boric acid on DNA against its oxidation in a rat model of renal I/R injury which might supportthe antioxidant activities of the compound withdepletion of free radicalgeneration. Similar to our findings, Yılmaz et al. demonstrated the capability of boric acid to decrease significantly the H_2O_2-induced oxidative DNA damage in Chinese hamster lung fibroblast V79 cell lines [38]. By the sister chromatidexchanges (SCEs) and micronucleus (MN test) results, Türkez et al. reported that antioxidant effective boric acid decreased DNA damage against heavy metal toxicity in human blood culture [39]. By using comet assay in rat

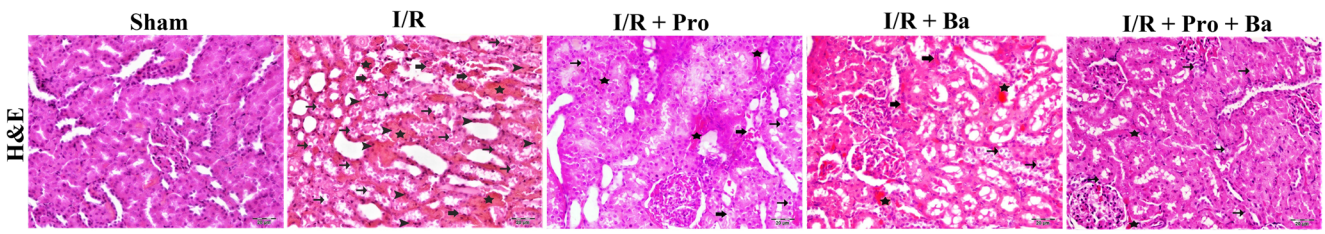

Fig. 3 Rat kidney (H&E), the control group with normal kidney histology. The kidney histology in I/R group; Arrow heads: Necrosis, Thin arrows: Hydropic degeneration Asterisk: Congestion, Thick arrows: Hemorrhage. Bar: 20 μm. Abbreviation used: I/R: ischemia/reperfusion, Pro: propolis, Ba: boric acid

mononuclear leukocytes, Ince et al. found that boron supplementation in the form of boric acid to rats was able to reduce the intracellular ROS generated by cyclophosphamide and protected cellular components against DNA damage and membrane lipid peroxidation [40].

Diet-induced oxidative stress and inflammation in renal cells are proposed mechanisms linking diet and kidney function [41]. The dietary supplementation of propolis reversed the consumption of GSH [42] and ameliorated metabolic disorders in a mouse model through the resolution of adipose tissue inflammation [43]. Frión-Herrera et al. suggested further investigation of the apoptotic mechanism induced by propolis to develop novel insights for preventing or treating apoptosis-related diseases such as cancer [44]. In our study, propolis (200 mg/kg) significantly ameliorated the content of MDA, the expressions of TNF-α, and also pro-apoptotic protein (Bax) in the kidney of rats. Our results showed that TNF-α production significantly decreased in pretreatment group with boric acid (14 mg/kg) in comparison to I/R group.TNF-α, an endogenous danger signal, is produced by activated immune cells upon inflammation [45]. Because oxidants affect all stages of the inflammatory response, it can be said that I/R injury contributes to increase the cross-talk between oxidative stress and inflammation in kidney. From this point of view, we concluded that boric acid is an important influencing factor on inflammation and oxidative stress after renal I/R injury in rats. In a study conducted by Durick et al., it was found that boron prevented the activation of pro-inflammatory cytokines through NF-κB transcription factor, and therefore, it blocked the inflammation process [46].In addition, boron supplementation reduced inflammatory response against phyto-hemagglutinin (PHA-P) in gilts [47], enhanced immunity by increasing serum levels of TNF-α and interferon-gamma (IFN-γ) in pigs [48], and increased serum levels of TNF-α in steers inoculated with bovine herpes virus type-1 [49].

Lastly, our combination study showed a significant interaction of boric acid with propolis, meaning that the combinedtreatment of propolis and boric acid can provide an additive antioxidant, anti-inflammatory, and anti-apoptotic effects against kidney I/R injury. Moreover, our findings demonstrated that boric acid and propolis together significantly improved renal function and also reduced genetic and histological alterations. The prevention of necrosis represents a major unmet clinical need [50]. In our study, propolis and boric acid could be considered as a potential therapeutic target to ameliorate renal necrosis in I/R injury. Based on the previous reports, propolis has high phenolic and flavonoid contents, which contribute to its antioxidant and anti-inflammatory activities [51]. In a similar manner, the biological functions, including antioxidant properties of boric acid are mainly associated with its composition. It was reported that boric acid contains electron-withdrawing groups, indicating that it may play important role in maintaining protective effects against tissue damage [52, 53].

Conclusion

Our data revealed that propolis and boric acid together could modulate oxidative stress, inflammation, apoptosis, and DNA damage, suggesting the potential of the combined therapy for I/R of kidney. Thus, propolis+ boric acid combination could be a clinically useful strategy to combat renal I/R injury and dysfunction.

Compliance with Ethical Standards

Experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All experimental procedures in this study were approved by the Atatürk University Local Ethics Committee for Animal Experiments (No. 66, 22.03.2018).

Conflict of Interest The authors declare that they have no conflict of interest.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Ito R, Kumada Y, Ishii H, Kamoi D, Sakakibara T, Umemoto N, Takahashi H, Murohara T (2018) Clinical outcomes after isolated infrapopliteal revascularization in hemodialysis patients with critical limb ischemia: endovascular therapy versus bypass surgery. J Atheroscler Thromb 25:799–807
2. Xu Z, Dai C (2017) Ablation of FGFR2 in fibroblasts ameliorates kidney fibrosis after ischemia/reperfusion injury in mice. Kidney Dis 3:160–170
3. Goetz L, Laskowski J, Renner B, Pickering MC, Kulik L, Klawitter J, Stites E, Christians U, van derVlag J, Ravichandran K, Holers VM, Thurman JM (2018) Complement factor H protects mice from ischemic acute kidney injury but is not critical for controlling complement activation by glomerular IgM. Eur J Immunol 1
4. Cheungpasitporn W, Khoury NJ, Thongprayoon C, Craici IM (2017) Is remote ischemic conditioning of benefit to patients undergoing kidney transplantation? J Investig Surg 12:1–3
5. Song Y, Liu W, Ding Y, Jia YY, Zhao J, Wang F, Bai J, Cheng L, Gao K, Liu M, Yao M, Li L, Zhang Y, Wen A, He L (2018) Salvianolic acid A ameliorates renal ischemia/reperfusion injury by activating Akt/mTOR/4EBP1 signaling pathway. Am J Physiol Renal Physiol 315:F254–F262
6. Martinotti S, Bucekova M, Majtan J, Ranzato E (2018) Honey: an effective regenerative medicine product in wound management. Curr Med Chem. https://doi.org/10.2174/0929867325666180510141824
7. Farooqui T, Farooqui AA (2012) Beneficial effects of propolis on human health and neurological diseases. Front Biosci (Elite Ed) 4: 779–793
8. Bankova V, Popova M, Trusheva B (2018) The phytochemistry of the honeybee. Phytochemistry 24(155):1–11

9. Patel S (2016) Emerging adjuvant therapy for cancer: propolis and its constituents. J Diet Suppl 13(3):245–268
10. Nzietchueng RM, Dousset B, Franck P, Benderdour M, Nabet P, Hess K (2002) Mechanisms implicated in the effects of boron on wound healing. J Trace Elem Med Biol 16(4):239–244
11. Barranco WT, Eckhert CD (2006) Cellular changes in boric acid-treated DU-145 prostate cancer cells. Br J Cancer 94:884–890
12. Hakki SS, Bozkurt BS, Hakki EE (2010) Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). J Trace Elem Med Biol 24:243–250
13. Vijay Bhasker T, Gowda NKS, Krishnamoorthy P, Pal DT, Sejian V, Awachat VB, Verma AK (2017) Boron supplementation provides hepato-protective effect and improves performance in Wistar rats fed calcium deficit diet. Indian J Anim Sci 87(10):1213–1218
14. Moseman RF (1994) Chemical disposition of boron in animals and humans. Environ Health Perspect 102:113–117
15. WHO; Environ HealthCriteria Document No. 204 (1998): Boron (7740-42-8) p.53
16. Acar Ü, İnanan BE, Zemheri F, Kesbiç OS, Yılmaz S (2018) Acute exposure to boron in Nile tilapia (Oreochromis niloticus): median-lethal concentration (LC50), blood parameters, DNA fragmentation of blood and sperm cells. Chemosphere 213:345–350
17. Gheisari A, Shahrvand S, Landy N (2017) Effect of ethanolic extract of propolis as an alternative to antibiotics as a growth promoter on broiler performance, serum biochemistry, and immune responses. Vet World 10:249–254
18. Bahadoran H, Naghii MR, Mofid M, Asadi MH, Ahmadi K, Sarveazad A (2016) Protective effects of boron and vitamin E on ethylene glycol-induced renal crystal calcium deposition in rat. Endocr Regul 50(4):194–206
19. Choi DE, Jeong JY, Lim BJ, Chung S, Chang YK, Lee SJ, Na KR, Kim SY, Shin YT, Lee KW (2009) Pretreatment of sildenafil attenuates ischemia-reperfusion renal injury in rats. Am J Physiol Renal Physiol 297(2):F362–F370
20. Yıldırım S (2017) Investigation by histopathological and TUNEL method of the protective effect of resveratrol on carbon tetrachloride induced liver toxicity in rats. Ataturk Univ J Vet Sci 12:178–186
21. Bovo E, Mazurek SR, Zima AV (2018) Oxidation of ryanodine receptor following ischemia/reperfusion increases propensity of Ca^{2+} waves during β-adrenergic receptor stimulation. Am J Physiol Heart Circ Physiol 315(4):H1032–H1040
22. Cao H, Ou J, Chen L, Zhang Y, Szkudelski T, Delmas D, Daglia M, Xiao J (2018) Dietary polyphenols and type 2 diabetes: human study and clinical trials. Crit Rev Food Sci Nutr 11:1–19
23. Puspasari A, Harijanti K, Soebadi B, Hendarti HT, Radithia D, Ernawati DS (2018) Effects of topical application of propolis extract on fibroblast growth factor-2 and fibroblast expression in the traumatic ulcers of diabetic Rattus norvegicus. J Oral Maxillofac Pathol 22(1):54–58
24. da Costa MF, Libório AB, Teles F, Martins Cda S, Soares PM, Meneses GC, Rodrigues FA, Leal LK, Miron D, Silva AH, Martins AM (2015) Red propolis ameliorates ischemic-reperfusion acute kidney injury. Phytomedicine 22(9):787–795
25. El-Marasy SA, Abdel-Rahman RF, Abd-Elsalam RM (2018) Neuroprotective effect of vildagliptin against cerebral ischemia in rats. Naunyn Schmiedeberg's Arch Pharmacol 391(10):1133–1145
26. Tanaka A, Ito Y, Kawasaki H, Kitabayashi C, Nishioka R, Yamazato M, Ishizawa K, Nagai T, Hirayama M, Takahashi K, Yamamoto T, Araki N (2018) Effects of memantine on nitric oxide production and hydroxyl radical metabolism during cerebral ischemia and reperfusion in mice. J Stroke Cerebrovasc Dis 27(6):1609–1615
27. Lungkaphin A, Pongchaidecha A, Palee S, Arjinajarn P, Pompimon W, Chattipakorn N (2015) Pinocembrin reduces cardiac arrhythmia and infarct size in rats subjected to acute myocardial ischemia/reperfusion. Appl Physiol Nutr Metab 40(10):1031–1037
28. Aldahmash BA, El-Nagar DM, Ibrahim KE (2016) Reno-protective effects of propolis on gentamicin-induced acute renal toxicity in swiss albino mice. Nefrologia 36(6):643–652
29. Mounieb F, Ramadan L, Akool ES, Balah A (2017) Propolis alleviates concanavalin A-induced hepatitis by modulating cytokine secretion and inhibition of reactive oxygen species. Naunyn Schmiedeberg's Arch Pharmacol 390(11):1105–1115
30. Ince S, Keles H, Erdogan M, Hazman O, Kucukkurt I (2011) Protective effect of boric acid against carbon tetrachloride-induced hepatotoxicity in mice. Drug Chem Toxicol 15:1–8
31. Yazıcı S, Akşit H, Korkut O, Sunay B, Çelik T (2014) Effects of boric acid and 2-aminoethoxydiphenyl borate on necrotizing enterocolitis. J Pediatr Gastroenterol Nutr 58(1):61–67
32. Hu X, Ma R, Lu J, Zhang K, Xu W, Jiang H, Da Y (2016) IL-23 promotes myocardial I/R injury by increasing the inflammatory responses and oxidative stress reactions. Cell Physiol Biochem 38(6):2163–2172
33. Tanko Y, Kabiru A, Abdulrasak A, Mohammed KA, Salisu AI, Jimoh A, Gidado NM, Sada NM (2017) Effects of fermented ginger rhizome (Zingiber officinale) and Fenu Greek (Trigonella foenum-graceum) supplements on oxidative stress and lipid peroxidation biomarkers in Poloxamer-407 induced -hyperlipidemic Wistar rats. Niger J Physiol Sci 32(2):137–143
34. Mahalakshmi A, Kurian GA (2018) Evaluating the impact of diabetes and diabetic cardiomyopathy rat heart on the outcome of ischemia-reperfusion associated oxidative stress. Free Radic Biol Med 118:35–43
35. Cakir S, Eren M, Senturk M, Sarica ZS (2017) The effect of boron on some biochemical parameters in experimental diabetic rats. Biol Trace Elem Res 184(1):165–172
36. Luo YP, Zhang H, Hu HF, Cao ZY, Zhang XZ, Cao L, Wang ZZ, Xiao W (2017) Protective effects of Ginkgo terpene lactones meglumine injection on focal cerebral ischemia in rats. Zhongguo Zhong Yao Za Zhi 42(24):4733–4737
37. Cao J, Jiang L, Zhang X, Yao X, Geng C, Xue X, Zhong L (2008) Boric acid inhibits LPS-induced TNF-alpha formation through a thiol-dependent mechanism in THP-1 cells. J Trace Elem Med Biol 22(3):189–195
38. Yılmaz S, Ustundag A, Cemiloglu Ulker O, Duydu Y (2016) Protective effect of boric acid on oxidative DNA damage in Chinese hamster lung fibroblast V79 cell lines. Cell J 17(4):748–754
39. Türkez H, Geyikoğlu F, Tatar A, Keles MS, Kaplan I (2012) The effects of some boron compounds against heavy metal toxicity in human blood. Exp Toxicol Pathol 64:93–101
40. Ince S, Kücükkurt I, Demirel HH, Acaröz DA, Akbel E, Ciğerci IH (2014) Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. Chemosphere 108:197–204
41. Jiang Y, Liu J, Zhou Z, Liu K, Liu C (2018) Fangchinoline protects against renal injury in diabetic nephropathy by modulating the MAPK signaling pathway. Exp Clin Endocrinol Diabetes 126:1–7
42. Castaldo S, Capasso F (2002) Propolis, an old remedy used in modern medicine. Fitoterapia 73:1–6
43. Kitamura H, Saito N, Fujimoto J, Nakashima KI, Fujikura D (2018) Brazilian propolis ethanol extract and its component kaempferol induce myeloid-derived suppressor cells from macrophages of mice in vivo and in vitro. BMC Complement Altern Med 18(1):138
44. Frión-Herrera Y, Díaz-García A, Ruiz-Fuentes J, Rodríguez-Sánchez H, Maurício Sforcin J (2018) Mechanisms underlying the cytotoxic effect of propolis on human laryngeal epidermoid carcinoma cells. Nat Prod Res 32(17):2085–2091
45. Rauch J, Kolch W, Laurent S, Mahmoudi M (2013) Big signals from small particles: regulation of cell signaling pathways by nanoparticles. Chem Rev 113(5):3391–3406
46. Durick KA, Tomita M, Hunt C, Bradley D (2005) Evidence that boron down-regulates inflammation through the NF-KB pathway. J Federation Am Soc Exp Biol 19(5):A1705

47. Armstrong TA, Spears JW, Lloyd KE (2001) Inflammatory response, growth, andthyroid hormone concentrations are affected by long-term boron supplementation in gilts. J Anim Sci 79: 1549–1556
48. Armstrong TA, Spears JW (2003) Effect of boron supplementation of pig diets on theproduction of tumor necrosis factor-α and interferon-γ. J Anim Sci 81:2552–2561
49. Fry RS, Brown TT, Lloyd KE, Hansen SL, Legleiter LR, Robarge WP et al (2011) Effect of dietary boron on physiological responses in growing steersinoculated with bovine herpesvirus type-1. Res Vet Sci 90:78–83
50. Schumer M, Colombel MC, Sawczuk IS, Gobé G, Connor J, O'Toole KM, Olsson CA, Wise GJ, Buttyan R (1992) Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. Am J Pathol 140(4):831–838
51. Cornara L, Biagi M, Xiao J, Burlando B (2017) Therapeutic properties of bioactive compounds from different honeybee products. Front Pharmacol 28(8):412
52. Pérez-Rodríguez M, García-Mendoza E, Farfán-García ED, Das BC, Ciprés-Flores FJ, Trujillo-Ferrara JG, Tamay-Cach F, Soriano-Ursúa MA (2017) Not all boronic acids with a five-membered cycle induce tremor, neuronal damage and decreased dopamine. Neurotoxicology 62:92–99
53. Ugwu DI, Okoro UC, Ahmad H (2017) New carboxamide derivatives bearing benzenesulphonamide as a selective COX-II inhibitor: design, synthesis and structure-activity relationship. PLoS One 12(9):e018380