Molecular Biology Reports
https://doi.org/10.1007/s11033-020-05888-4

ORIGINAL ARTICLE

Honey protects against chronic unpredictable mild stress induced-intestinal barrier disintegration and hepatic inflammation

Nasrin Mehranfard[1] · Azadeh Yazdi[2] · Asiye Rafiee Sardooi[2] · Zeinab Shakerin[3] · Maedeh Ghasemi[2]

Received: 18 July 2020 / Accepted: 30 September 2020

Abstract

Chronic stress is linked to liver injury by increasing intestinal permeability to lipopolysaccharide (LPS), which in turn can result in systemic and liver inflammation and damage. Beneficial effect of honey in the prevention of liver injury has been shown in previous studies, but mechanisms underlying are still less known. Here, we examined the therapeutic impacts of honey on intestinal nuclear factor-κB (NF-κB; an important regulator of stress-induced immune and inflammatory responses) and ileal tight junction (TJ) proteins of claudin-1 and ZO-1, serum LPS, liver inflammation and oxidative markers of malondialdehyde (MDA), nitric oxide (NO), (erythroid-derived 2)-like 2 (Nrf2), tumor necrosis factor (TNF)-α and total antioxidant capacity (TAC) following chronic unpredictable mild stress (CUMS) using Western blotting, ELISA kit and spectrophotometry. Male rats were subjected to CUMS for 28 consecutive days. Honey (0.2 and 2 g/kg/day, by gavage) was administered pretreatment (10 days) and during stress. Honey reduced stress-induced LPS elevation by preventing reduction in the intestinal TJ proteins of claudin-1 and ZO-1, while did not affect NF-kB levels. In liver, honey significantly suppressed stress-induced increase in MDA, NO, TNF-α and Nrf2 expression and normalized TAC. Noteworthy, honey high-dose provoked a greater decrease in TNF-α, Nrf2 and LPS levels than honey low-dose. Together, our study indicated that honey protects against stress-induced liver damage by modulating at least two pathways; intestinal barrier protection via increased TJ protein complex expression, and hepatic TAC protection that may be involved in the inhibition of MDA, NO, TNF-α and Nrf2 expression.

Keywords Honey · Hepatic injury · Intestinal permeability · Intestinal TJ proteins · LPS · NF-kB · Nrf2 · TNF-α

Introduction

Previous studies have indicated that stress can induce liver injury both directly and indirectly. The liver is the organ that filters and removes foreign substances from the body, and thereby maintains immunological tolerance under physiological states. Under stress states, however, immunological tolerance is perturbed and leads to liver inflammation [1]. Several factors have been suggested to contribute to this process including high levels of stress hormones, increased sympathetic nervous system activity, hypoxia/reoxygenation injury, over-influx of gut-derived lipopolysaccharide (LPS), hyperactivity of Kupffer cells and oxidative stress. Over-influx of gut-derived LPS and other antigens into liver has particularly been proposed to play an important role in liver inflammation [2]. Multiple factors including corticotropin-releasing factor, acetylcholine, glucocorticoids, gut microbiota, pro-inflammatory cytokines and neuroactive chemicals such as histamine and serotonin have been recognized to increase intestinal permeability and facilitate the influx of LPS into liver under stress conditions [3–5]. This in turn can induce a wide array of gastrointestinal disorders including liver injury [6]. Results obtained from human and animal studies suggest that the stress-induced enhancement of intestinal permeability can be a consequence of weakening of the tight junction (TJ) proteins and increased bacterial translocation into the intestinal wall. In animal studies, the

✉ Maedeh Ghasemi
ghasemi.m@med.mui.ac.ir

1 Neurophysiology Research Center, Cellular and Molecular Medicine Institute, Urmia University of Medical Sciences, Urmia, Iran

2 Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

3 PhD of Anatomical Science, Department of Anatomy, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Published online: 12 October 2020

degree of increase in intestinal permeability has been shown to be determined by the intensity of stress [7, 8].

A role of gut LPS in liver inflammation has been demonstrated by previous studies that indicated over-influx of LPS into the liver activates intracellular molecular pathways, resulting in the polarization of hepatic Kupffer cells into M1 macrophages and the production of pro-inflammatory cytokines (tumor necrosis factor-α (TNF-α) and interleukins), the activation of nuclear factor kappa B (NF-κB), CXC chemokines (CXCLs) and CC chemokine ligands (CCLs), vasoactive agents like nitric oxide (NO) and reactive oxygen species (ROS) that lead to oxidative stress, inflammation, and the development and progression of liver diseases [9, 10]. Previous studies also indicated that antioxidant and anti-inflammatory therapy is beneficial in mitigating liver injury [11–13] .For example, Adachi et al. demonstrated that ethanol-induced enhancement of intestinal permeability and liver injury are improved by administration of antibiotics depleting gut microbiota [11]. In addition, co-administration of antioxidants or zinc injection (an inhibitor of intestinal permeability) with ethanol in rodents was not associated with an increase of LPS in the blood as well as liver injury [14–16]. These data suggest that preventing intestinal leakiness and endotoxemia may be beneficial in the liver protection from the toxic effects of chronic stress.

Several health benefits of honey including wound healing and tissue regeneration (improvement of ulcers, wounds and other infections) have been demonstrated in previous studies [17, 18]. Moreover, honey has been shown to significantly enhance the regenerative capacity of cells and promote re-epithelialization [19]. These health-beneficial impacts of honey are attributed to its anti-bacterial, anti-inflammatory, antioxidant, antitumoral, and other properties [18, 20–23]. Accumulating data suggest an essential role of intestinal epithelial barrier dysfunction in the perturbation of liver hemostasis. This raises the question whether honey can protect the liver in part by preventing intestinal leakiness and endotoxemia. In the present study, we assessed impacts of honey on intestinal leakiness by evaluating the expression of intestinal TJ proteins of claudin-1 and ZO-1, as well as on liver defense system by evaluating liver inflammation and oxidative markers of malondialdehyde (MDA), NO, Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), TNF-α and total antioxidant capacity (TAC) following chronic stress.

Material and methods

Male Wistar rats (n = 48, BW: 200–250 g) were housed in groups of 4 in polyethylene cages and kept under controlled temperature conditions (22 ± 3 °C) with a 12 h-light/dark cycle and free access to water and food. The experimental procedures were approved by the Ethics Committee of the Isfahan University of Medical Sciences in accordance with ethical guidelines for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA).

The animals were randomly assigned into the following six experimental groups of eight rats each; control, two positive groups that received just honey (0.2 and 2 g/kg) for 38 days, chronic unpredictable mild stress (CUMS) group and 2 CUMS + Honey groups that received honey (0.2 and 2 g/kg/day) before (10 days) and during CUMS induction (28 days). Animals were exposed to CUMS with or without honey treatments for 4 weeks. Honey was given by intra-gastric gavage (0.2 and 2 g/kg, once a day) for consecutive 38 days. The doses of honey were chosen based on previous studies [24–27]. Honey and vehicles were administered at 30 min prior to stress exposure. The control rats were housed in separate rooms and had no contact with rats in the stressed groups. All animals were fed standard diet during the experimental period of 4 weeks.

Experimental procedures

A schematic depiction of timeline for the experimental procedures was illustrated in Fig. 1. The treatment groups received intra-gastric thymus honey (0.2 and 2 g/kg/day, Organization of agriculture- Jahad-Alborz (Dehdaz), Iran) or vehicle (saline) for 38 consecutive days from 10 days before CUMS induction. The quality of honey was analyzed by Isfahan Hourtash Lab.

Induction of chronic unpredictable mild stress

Based on the modified method of Wei et al. 2019 [28], the stressed groups were subjected to the following different mild stressors for 28 days: 40°cage tilt (24 h), food deprivation (18 h), water deprivation (10–12 h), tail pinch (1 min, 1 cm from the distal portion of the tail), physical restraint (45 min), moist

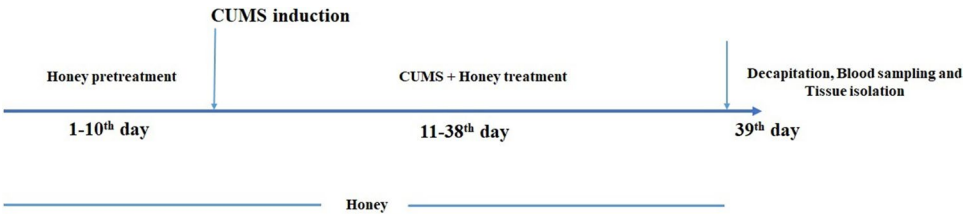

Fig. 1 A schematic representation of timeline for the experimental procedure.

bedding (300 ml of water was added to 300 g sawdust bedding) and overnight lighting. Each experimental week consisted of 7 days with random stressors. Control animals were left undisturbed in their home cages in a separated room throughout the 28-day period. To eliminate the effect of bacterial contamination, at the beginning of the experiment and between bedding change, all cages were cleaned according to the SOP protocol. Because one of mentioned stressors was moist bedding, we usually cleaned the bed twice a week and used wood shavings for bedding.

Honey was given orally once a day between 8:00 and 9:00 a.m. Termination procedure, blood and tissue sampling for molecular and chemical tests were performed after the 4-week period of CUMS exposure at final day (between 09:00 and 11:00 a.m). During sacrifice, all surfaces were disinfected with a disinfectant of peroxide hydrogen to clean and mask any body odor from the previously sacrificed rat. Then, the rat was taken from its cage and euthanasia applied as soon as possible. Rats still in line for sacrifice were kept in separated room and protected from any sound or smell that could induce any stress about to what is happening.

A pilot study of sucrose intake test (as described by Liu et al. 2014, [29]) was performed to verify the modified method of CUMS procedure. The pilot testing results are presented as supplemental data.

Biochemical assessment

Serum LPS assay

Blood samples from the animals were drawn via decapitation under deep anesthesia at the end of the experiment. Sera were collected via centrifugation and stored at − 80°C for later use. The levels of LPS were measured using General LPS ELISA Kit (Catalog No: MBS452438) with intra-assay precision: CV< 10%.

Total antioxidant capacity in the liver

TAC was determined according to the company's instructions using (Kiazist, Iran). TAC was measured by the cupric reducing (Cu^{+2} to Cu^{+1}) into cuprous in the presence of antioxidants and produced color in the presence of chromogen. The absorbance of the color was read at 450 nm. The TAC concentrations were determined by the standard curve and reported as nmol/g tissue. CUPRAC assay can measure antioxidants such as thiol, which cannot be identified by methods that work with the ferric reducing capacity.

Lipid peroxidation measurements in the liver as oxidative stress marker

To determine the levels of lipid peroxidation, malondialdehyde (MDA) contents were measured spectrophotometrically based on the reaction with thiobarbituric acid (TBA) to produce a pink-colored pigment with a maximum absorption of 532 nm (TBA assay kits, Kiazist, Iran). The content of MDA was expressed as nmol of MDA per gram of tissue.

Nitrite content determination in the liver as nitrosative stress marker

The level of nitrite was determined with an enzyme-linked immunosorbent assay kit (Elabscience Biotechnology Inc.). NO is easily oxidized to form $NO2^-$ in aqueous solution, and a reddish azo compound is formed with the color developing agent, and the concentration of the azo day is linearly related to the concentration of NO. The concentration of NO was calculated indirectly by measuring the OD value at 550 nm. The concentration of NO was expressed as μmol/L.

Western blot analysis

To examine alterations in claudine-1, ZO-1, NF-κB, TNFα, Nrf2 levels in the intestine (ileum) and liver between groups, Western blotting was used (n = 5 for each group). After the 28-day CUMS exposure, rats were sacrificed under deep anesthesia and then liver and ileum tissues were dissected out. The dissected ileum segments were washed with cold PBS. Then, liver and ileum tissues were stored at – 80 °C for later use. Extraction of total protein was performed with cold lysis buffer, protease inhibitor and phosphatase inhibitor cocktail. Equal amounts of proteins were separated by 8% or 10% SDS-polyacrylamide gels and then transblotted to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% skim milk prepared in tris-buffered saline with Tween (TBST) and incubated with primary antibodies for claudine-1, ZO-1, NF-κB, TNFα, Nrf2 (Abcam, Cambridge, MA, USA) and β-actin (Sigma-Aldrich) at 4 °C, overnight, followed by the appropriate HRP-conjugated secondary antibodies for 1 hour at room temperature and then visualized through increased chemiluminescence detection. Western blot analysis was performed with two repetitions. Protein expression was quantified by densitometric analysis using Image J software (National Institutes of Health, Bethesda, MD, USA). The expression of protein was normalized with that of β-actin (internal control protein), and then to control group.

Histopathological analysis

The liver was dissected out and fixed in 10% neutral formalin solution and the tissue was processed. The slides (4 to 5-micron thick paraffin sections) were stained with Haematoxylene and Eosin stain and examined histologically under light microscope by a specialized pathologist.

Statistical analysis

The results are representative of at least six independent experiments and are expressed as the mean ± S.E.M. One-way analysis of variance (ANOVA) with an appropriate post hoc Tukey's comparison was used to determine the significance of the differences among the groups. $p < 0.05$ was considered statistically significant.

Results

Physicochemical properties of honey

The physicochemical and antioxidant properties of honey were presented in Table 1. The honey used in this study contained proline amino acid 569.4 mg/100 g, free acidity 13.2 meq/kg, electrical conductivity of 0.37 mS/cm, glucose 33.92 g%, fructose 36.9 g%, sucrose < 0.02 g%, F/G 1.09, turanose 1.4 g%, maltose 1.06g%, melezitose 0.25 g% and total phenolic content 0.04 mg/ml.

Honey promotes TJ proteins expression and improves intestinal permeability

A 'leaky' barrier is attributable to TJ disruption, which alters paracellular permeability. To confirm whether honey can protect barrier integrity, we used honey at doses of 0.2 and 2 g/kg/day to treat CUMS rats. The results are as follows:

TJ proteins expression

Image j densitometries analysis showed a significant reduction in TJ transmembrane protein of claudin-1 expression in ileal tissue following CUMS ($p < 0.001$), while the reduction was not significant in TJ peripheral membrane protein of ZO-1 (Fig. 2a and b). Treatment with honey (0.2g and 2 g/kg) could significantly improve the TJ proteins expression compared to CUMS group ($p < 0.01$ and $p < 0.001$), even there was a significant difference between the two concentrations in claudin-1 expression (Fig. 2a). Attractively, as shown in Fig.2, honey treatment also dose-dependently promoted claudin-1 and ZO-1 proteins expression in non-stressed rats compared to controls (up to 2 fold for ZO-1 protein, $p < 0.001$), which in turn could protect the animals well under stress conditions.

Honey inhibits CUMS-induced endotoxemia in rats via reducing serum LPS levels

Intestinal permeability alterations were assessed by measuring serum LPS levels in the animals at end of the experiment. As illustrated in Fig. 3, CUMS exposure caused a 2.7-fold increase in the LPS of serum compared to controls ($p < 0.001$), indicating an increase in intestinal permeability. Honey treatment markedly ($p < 0.001$)

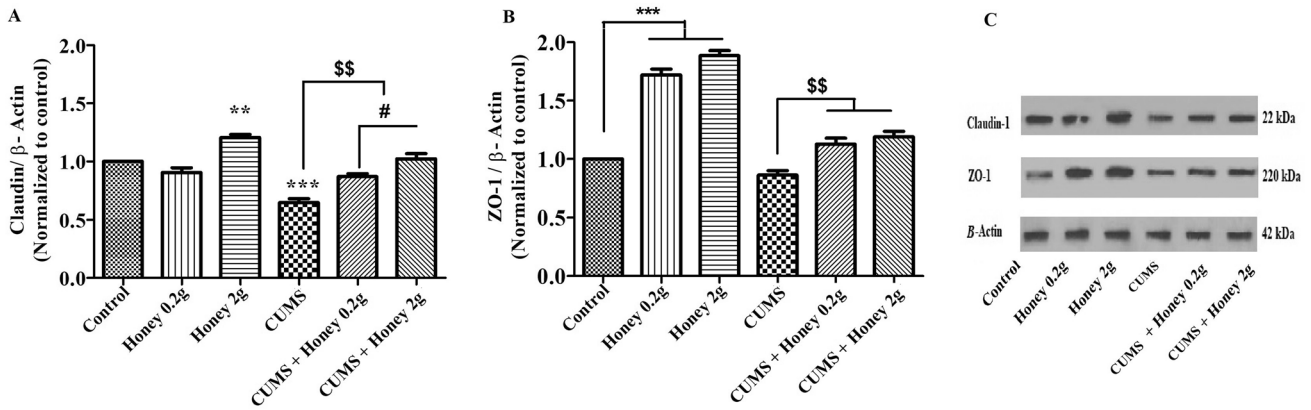

Fig. 2 The protective effects of honey on intestinal barrier function in CUMS. Expression levels of TJ proteins of claudin-1 and ZO1 in ileal tissue following CUMS induction and honey treatment. **a** Stress significantly decreased the expression levels of claudin-1 compared to control, and honey (high and low doses) significantly elevated the expression levels in stressed rats. **b** Stress had no significant effect on ZO-1 levels compared to control, but honey treatment improved the expression levels of ZO-1 in stressed rats. **c** Representative western blot assay. Data are presented as mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Control, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ vs. CUMS

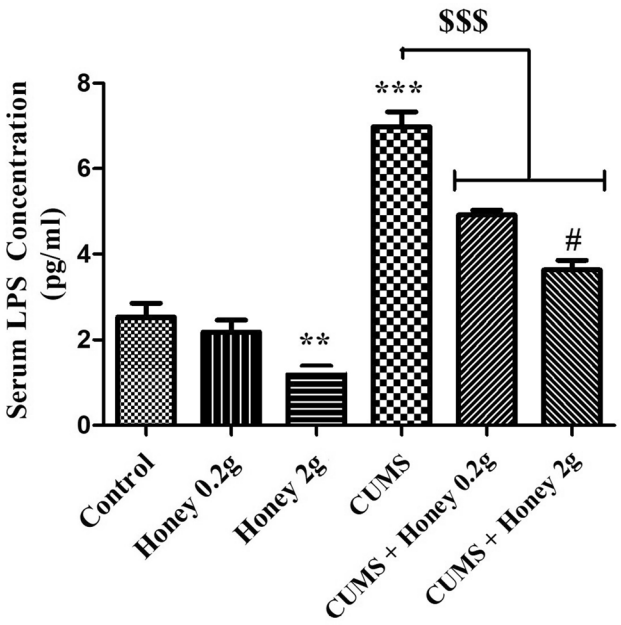

Fig. 3 Effects of CUMS and honey treatment on serum level of LPS. CUMS significantly increased serum LPS compared to control. Honey treatment (low and high dose) markedly decreased LPS compared to stress group. Data are presented as mean ± SEM, **p < 0.01, ***p < 0.001 vs. Control; $^{\$}$p < 0.05, $^{\$\$\$}$p < 0.001 vs. CUMS

suppressed this stress-induced effect in a dose dependent manner where the concentration of serum endotoxin was only slightly elevated in high dose of honey compared to control, suggesting protective effects of honey on intestinal barrier function.

Honey potentiates CUMS-induced NF-kB in rats

NF-κB plays a central role in regulating the immunity response, cell proliferation and apoptosis. In the present study, we examined the impact of honey treatment on the expression of NF-κB protein in ileum tissue following CUMS. As shown in Fig. 4, CUMS significantly increased the expression of NF-κB (~3 fold) as compared to control ($p < 0.001$). The same trend was observed in CUMS + honey (0.2 and 2g/kg) rats, but no significant differences were found in protein expression of NF-κB among CUMS and CUMS + honey groups (Fig. 4). Our results also indicated that honey treatment increases NF-κB expression in unstressed rats ($p < 0.05$).

Honey improves CUMS-induced liver injury via an increase in total antioxidant capacity and inhibition of MDA, NO, TNFα and Nrf2 expression

Inflammation and oxidative stress have been suggested to be the most important pathogenic factors in liver diseases. During liver injuries, oxidative species cause damage of important biomolecules and production of pro/inflammatory factors, and antioxidant and anti-inflammatory therapy has been deliberated to be beneficial in mitigating this damage. The results are as follows:

Honey inhibits CUMS-induced Nrf2 production in rat liver

Exposure to CUMS elicited a significant increase in liver Nrf2 levels compared to control animals ($p < 0.001$; Fig. 5a and e). Honey treatment in CUMS rats induced a

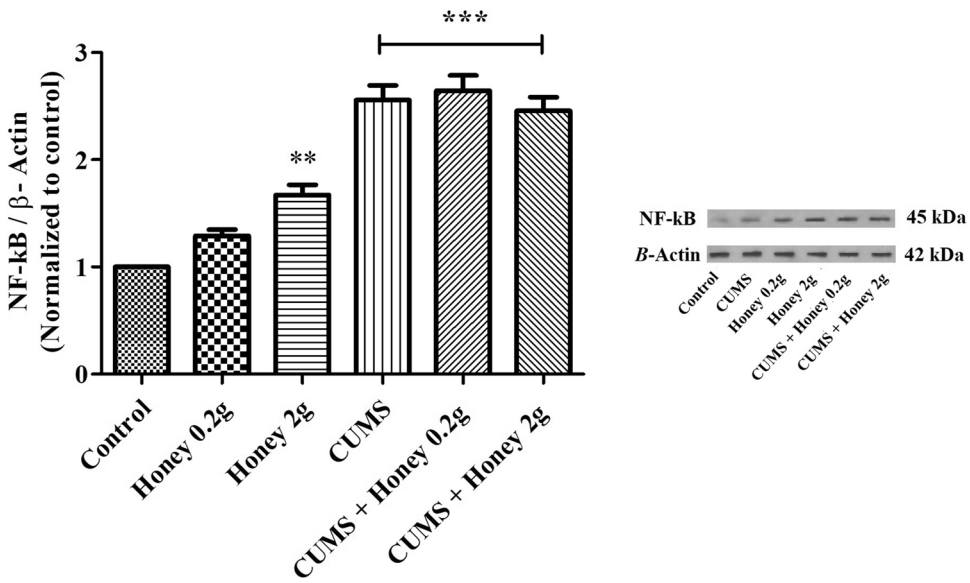

Fig. 4 Expression levels of NF-kB in ileal tissue following CUMS induction and honey treatment. Ileal NF-kB level was significantly increased following CUMS compared to control and honey treatment (low and high dose) failed to decreased it. Data are presented as mean ± SEM, **p < 0.01, ***p < 0.001 vs. Control

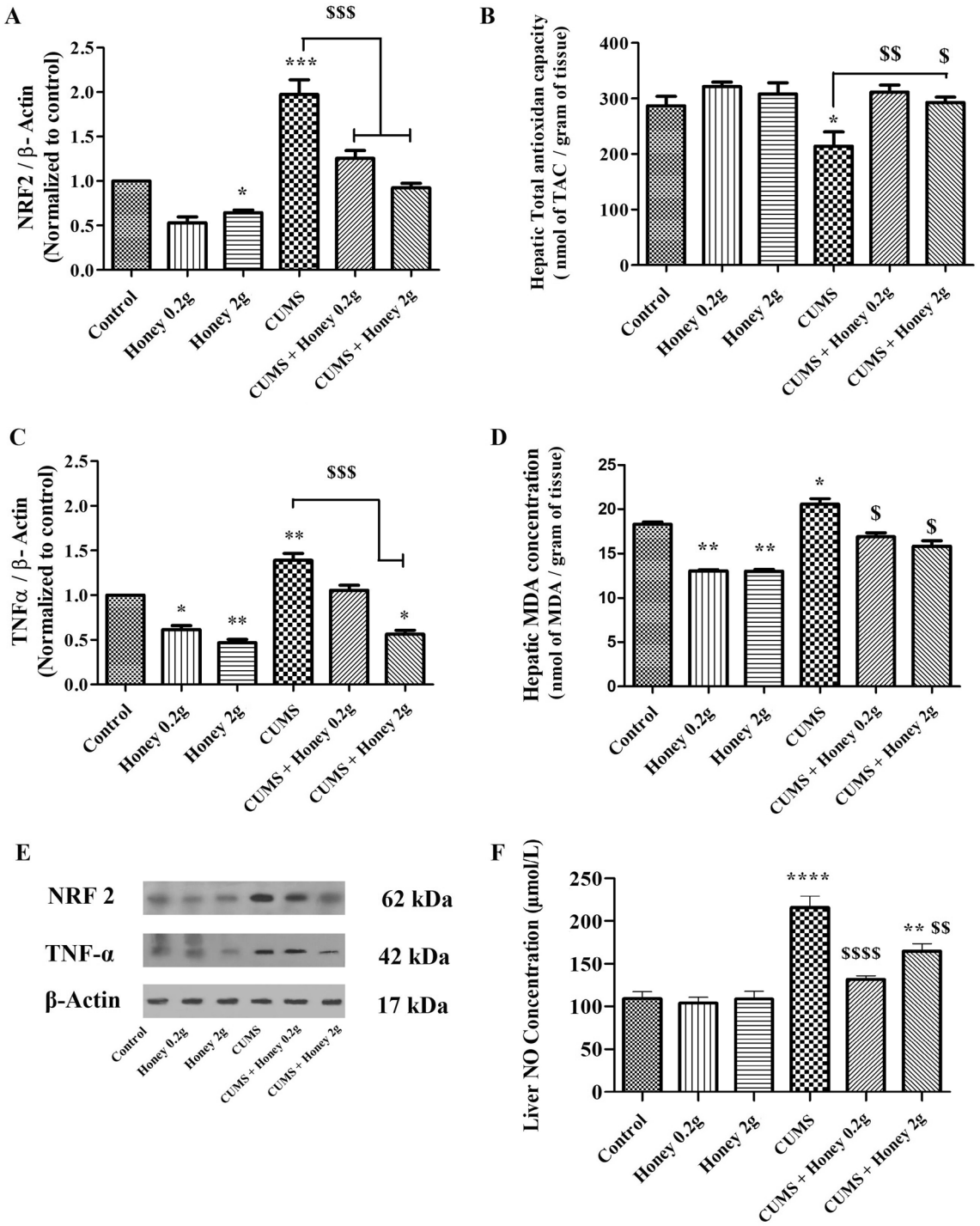

Fig. 5 Protective effects of honey on hepatic antioxidant capacities (Nrf2 and TAC; **a** and **b**), inflammatory properties (TNFα; c) and oxidative markers (MDA and NO; **d** and **f**) following CUMS induced-hepatic injury. **e** Representative Western blot assay. Data are presented as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001 vs. Control; $^{\$}$p < 0.05, $^{\$\$}$p < 0.01, $^{\$\$\$}$p < 0.001 vs. CUMS

substantial inhibition ($p < 0.001$) in the Nrf2 production in a dose dependent manner, as by high dose of honey treatment, the Nrf2 expression reached the level of the control rats.

Honey improves CUMS-induced reduction in liver total antioxidants capacity

The concentrations of total antioxidants were measured

in the homogenates of the animal liver and were shown in Fig. 5b as hepatic TAC capacity. As observed, CUMS exposure elicited a significant reduction in liver TAC levels ($p < 0.05$), and honey treatment at doses of 0.2 and 2 g/kg ($p < 0.01$) significantly elevated TAC levels in the animals.

Honey inhibits CUMS-induced TNF-α production in rat liver

CUMS exposure induced a significant enhancement of liver TNF-α levels compared to control group (Fig. 5c and e), and honey treatment significantly decreased and restored it to control levels ($p < 0.001$). Honey also decreased liver TNF-α level in unstressed rats compared to controls ($p < 0.05$).

Honey inhibits CUMS-induced MDA production in rat liver

The impacts of honey treatment on hepatic MDA levels are shown in Fig. 5d. The hepatic levels of MDA were significantly influenced by honey treatment and CUMS induction. In the liver of stressed rats, there was a significant elevation in MDA levels as compared to those of control ($p < 0.05$) and both doses of honey could restore them to control levels. Honey also significantly decreased MDA levels in unstressed rats compared to controls ($p < 0.01$).

Honey inhibits CUMS-induced NO production in rat liver

The hepatic NO levels of each group are illustrated in Fig. 5f. CUMS exposure induced a marked increase in hepatic NO levels ($p < 0.0001$) compared to control. The stressed rats receiving 0.2 and 2 g/kg of honey revealed a significant decreased level of NO ($p < 0.0001$ and 0.01) compared to CUMS group and dose of 0.2 g/kg could strongly restore it to control level. Honey didn't change NO level in unstressed rats compared to control.

Table 1 Physicochemical properties of honey (Dehdar –Taleghan-Iran)

	Parameters	Values
Physical properties	Conductivity (mS/cm)	0.39 mS/cm
	Acidity	31.9 meq /kg
	pH	4.2
Chemical properties		
	Proline amino acid (mg/100 g)	569.4
	Glucose (g %)	33.92
	Fructose (g %)	36.9
	Sucrose (g %)	< LOD
	F/G	1.09
	Turanose (g %)	1.4
	Maltose (g %)	1.06
	Melezitose (g %)	0.25
	Raffinose	–
Antioxidant properties		
	Total phenolic content (mg / ml)	0.04

Honey protects CUMS-induced liver injury

Histological analysis of the liver tissue in the control rats revealed the normal hepatic cells and veins, and normal arrangement of the parenchyma in the liver with well-defined hepatic lobules (Fig. 6a). After four weeks of CUMS induction, histopathological alterations were clearly observed in liver including degeneration of hepatic cells,

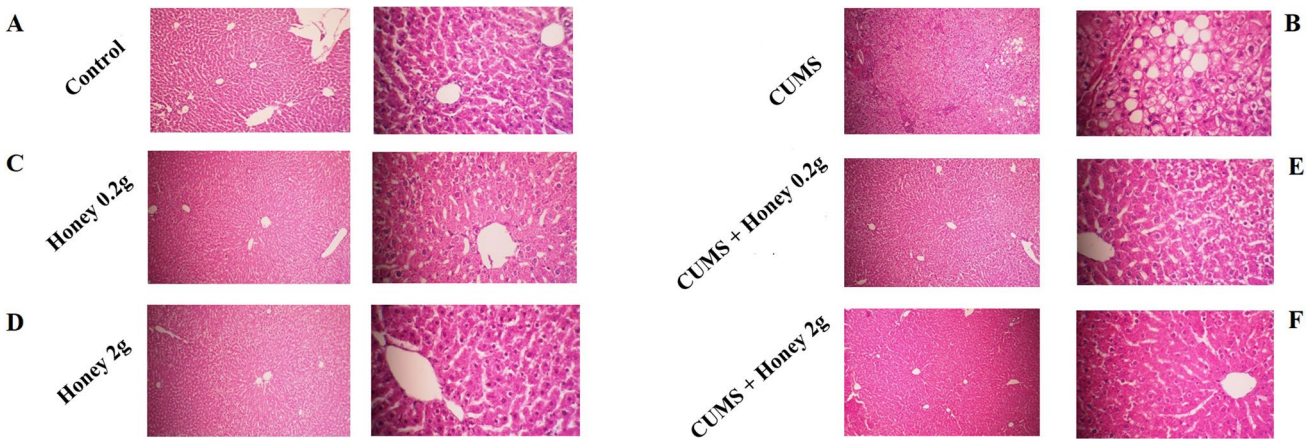

Fig. 6 Histological representation of liver tissue following CUMS induction and honey treatment. Hematoxylin and Eosin staining, X=10 and 40. Chronic unpredictable mild stress = CUMS

intracellular fat and fluid accumulation (ballooning of hepatocytes), and necrotic changes in the hepatic cells with sporadic foci of inflammatory cells (immune cells) in the hepatic lobules (Fig. 6b). Honey at doses of 0.2 and 2 g/kg decreased CUMS-induced tissue injuries in liver (Fig. 6e and f), although in zone 1 of each lobules, a few necrotic changes and degeneration of hepatic cells were observed. In unstressed rats, a better histological feature was observed in the liver structure in honey treatment (0.2 g/kg) compared to control (Fig. 6c &d).

Discussion

A growing body of evidence has highlighted a clear link between chronic stress and enhanced intestinal permeability in the pathogenesis of chronic liver diseases. Under stress states, a perturbed intestinal barrier results in enhanced bacteria translocation from intestinal lumen into blood stream, influencing peripheral tissue particularly liver [9]. Hence, intestinal barrier integrity is necessary for liver homeostasis, and proteins in the TJ barrier have been shown to principally be involved in regulation of this integrity [30, 31]. The transmembrane proteins of TJ, occludin and claudins, interact with the scaffolding proteins of ZO (zonula occludens) and form a defense system against invading pathogens in the lumen [32]. Perturbation of TJ-related barrier integrity has been suggested to be an essential gateway of the bacterial translocation in diseases. Different types of stress can cause the intestinal mucus layer more permeable to bacterial toxins and result in local and peripheral inflammation [4, 7, 28, 33]. Hence, improving intestinal barrier function may relieve the inflammation. In the current study, we found, for first evidence, that honey protects against loss of the TJ proteins of claudine-1 and ZO-1 in CUMS rats, resulting in decreased intestinal hyperpermeability and LPS plasma level. Consistent with our data, Lambert et al., 2003 reported that animals with co-treatment of ethanol and zinc (as an inhibitor of intestinal permeability) did not indicate increased LPS plasma levels and liver injury [14]. In another study, antioxidant therapy with honey prevented intestinal barrier dysfunction and liver injury in bile duct ligated animals model [34]. Furthermore, Gencay et al. (2008) demonstrated an improvement in ileal morphology in presence of obstructive jaundice following honey treatment that was associated to a decrease in bacterial translocation [35]. Although, a recent study indicated that high concentration of honey through inhibition of phosphorylated Akt reduced claudin11 expression in human lung squamous cell carcinoma [36].

CUMS-induced hepatotoxicity has been demonstrated to be associated with enhancement of oxidative stress markers (MDA and NO), and inflammatory cytokine of TNF-α. In the present study, honey inhibited MDA, NO and TNF-α production in rats subjected to chronic stress. Therapeutic approach by management of the gut-liver axis by antibiotics, antioxidant and anti-inflammatory therapy has been reported to be effective for preventing of liver injury [37–40]. Nrf2 is an important transcription factor that serves as the first line of cellular defense against damage via regulating numerous detoxifying and antioxidant defense gene expression in the liver, and is activated in response to exogenous and endogenous stressors including reactive oxygen and nitrogen species, LPS, lipid aldehydes and xenobiotics [41–44]. Nevertheless, recent studies have identified a role of Nrf2 in cancer progression and metastasis as well as in resistance to cancer therapy [45]. This suggests that Nrf2 may have harmful consequences under certain conditions. In our study, Nrf2 expression was upregulated in liver in response to CUMS, and honey interestingly restored Nrf2 level to normal level and even downregulated it in unstressed rats. Considering that honey is a natural antioxidant and that in the present study it improved the antioxidant capacity of the liver, hence, reduced Nrf2 levels following honey treatment in stressed rats may prevent Nrf2 from reaching to negative effects on hepatocytes under stress conditions.

In the current study, CUMS resulted in increased levels of intestinal NF-κB in rats and honey treatment did not affect NF-κB expression in the animals. While some studies have indicated a role of NF-κB in ROS production and development of a variety of acute/chronic inflammatory disorders, other studies have suggested that NF-κB signaling may play an essential role in the maintenance of immune homeostasis [46–48]. Therefore, NF-κB may display two faces in chronic inflammation: on the one hand increased and persistent NF-κB activation induces inflammation by the expression of a large number of genes related to inflammatory responses, but on the other hand NF-κB controls the expression of antiapoptotic, proproliferative and antioxidant proteins and thus NF-κB inhibition can perturb immune homeostasis. Since in our study, honey treatment was associated with an increase in intestinal NF-κB expression in both unstressed and stressed rats, it may that honey through sustaining NF-κB activation contribute to maintenance of immune homeostasis and help to improve the intestinal barrier integrity. Consistent to our findings, Raynaud et al. reported an overexpression and activation of the NF-κB transcription factor subunits in response to thyme honey in murine macrophages [49]. Since this effect of honey was shown to be dose-dependent, it is possible that the high dose of honey provide a preferred environment for intestinal bacterial overgrowth and produce an excess amount of secondary bile acids that induces stress response signaling and causes intestine damage [50]. Further research on dose and duration is required to better conclude. Taken to gather, our data revealed that honey could protect intestinal barrier

integrity through promotion of TJ proteins expression as well as activation of NF-κB signaling.

Honey contains many types of compounds such as acids, sugars, enzymes, proteins, vitamins and different phytochemicals. The therapeutic impacts of honey are attributed to features such as high osmolarity, acidity, the presence of hydrogen peroxide inhibitors, flavonoids, phenolic acids, minerals and vitamins, which influence cytokine production and decrease oxidative stress as well as stimulate tissue re-growth [22]. Although a previous research indicated that pure fructose, as an abundant monosaccharide in honey, could increase the gut permeability and promote leaky gut [51], in the present study, honey treatment induced a protective effect against stress-induced gut permeability, suggesting an essential role of other components in the protective effect of honey.

Together, these data suggest that honey protects against CUMS-induced liver damage, via modulation of at least two pathways; intestinal barrier protection via upregulation of TJ protein and NF-kB modulation as well as by increasing hepatic TAC, which in turn may contribute to inhibition of MDA, NO, TNFα and Nrf2 expression.

Acknowledgements This work was supported by the Isfahan University of Medical Sciences (Grant number 198158 to M.G.). This work did involve animals (rats). The experimental procedures were approved by the Ethics Committee of the Isfahan University of Medical Sciences in accordance with ethical guidelines for the Care and Use of Laboratory Animals (IR.MUI.RESEARCH.REC.1398.533).

Compliance with ethical standards

Conflict of interest The authors declare that no conflict interests exist.

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