Published in final edited form as:

Clin Exp Pharmacol Physiol. 2015 August ; 42(8): 843–848. doi:10.1111/1440-1681.12433.

Honey feeding protects kidney against cisplatin nephrotoxicity through suppression of inflammation

Rania Hamad^{1,2}, Calpurnia Jayakumar¹, Punithavathi Ranganathan¹, Riyaz Mohamed¹, Mahmoud Mohamed Ismail El-Hamamy², Amina A. Dessouki², Abdelazim Ibrahim², and Ganesan Ramesh¹

¹Department of Medicine and Vascular Biology Center, Georgia Regents University, Augusta, GA 30912.

²Department of Pathology, Suez Canal University, Egypt

Abstract

Cisplatin is a highly effective chemotherapeutic drug used to treat a wide variety of solid tumors. However, its use was limited due its dose limiting toxicity to the kidney. Currently, there are no therapies available to treat or prevent cisplatin nephrotoxicity. Honey is a naturally occurring complex liquid and widely used in traditional Ayurvedic medicine to treat many illnesses. However, its effect on cisplatin nephrotoxicity is unknown. To determine the role of honey in cisplatin nephrotoxicity, animals were pretreated orally for a week and then cisplatin was administered. Honey feeding was continued for another three days. Our results show that cisplatin-induced kidney dysfunction as determined by increased serum creatinine. Animals which received honey feeding had less kidney dysfunction. Improved kidney function was associated with better preservation of kidney morphology in honey treated group as compared to cisplatin treated group. Interestingly, honey feeding significantly reduced cisplatin-induced tubular epithelial cell death, immune infiltration into the kidney as well as cytokine and chemokine expression and excretion as compared to cisplatin treated animals. Western blot analysis shows that cisplatin-induced increase in phosphorylation of NFkB was completely suppressed with honey feeding. In conclusion, honey feeding protects the kidney against cisplatin nephrotoxicity through suppression of inflammation and NFkB activation.

Introduction

Drug induced kidney injury is a major cause of kidney failure in clinical settings [1-4]. Chemotherapeutic drug such as cisplatin is widely used to treat many solid tumors such as head and neck and lung cancers[5]. However, its use was limited by its damaging effects on normal tissues which include ototoxicity, bone marrow suppression, nephrotoxicity and intestinal injury[5-7]. Although some of these tissues are able to recover from injury due their high regenerative and proliferative capacity, extensive kidney damage is irreversible

Correspondence Address: Ganesan Ramesh, Ph.D., Department of Medicine and Vascular Biology Center, CB-3702, Georgia Regents University, 1459 Laney-Walker Blvd, Augusta, GA 30912, TEL. NO. (706) 721-9728, FAX. NO. (706) 721-9799, gramesh@gru.edu.

Disclosure: All the authors declared no competing interests.

and may lead to the development of end stage renal disease. For the past several years research was aimed at understanding the underlying pathogenic mechanisms of cisplatin nephrotoxicity which provided us a better understanding of some of the pathways that may mediate tissue injury during cisplatin treatment. Two such major pathways include inflammation [8-10] and oxidative stress [11;12]. Suppression of inflammatory cytokine production or activity reduced cisplatin induced kidney injury [10;13;14]. Moreover, neutralization of oxidative stress or deletion of gene that may increase production of oxygen radicals was able to reduce cisplatin induced nephrotoxicity[12;15]. Although compounds targeting these pathways appear as potent drugs to treat cisplatin nephrotoxicity, their efficacy in humans were never been tested. Moreover, concerns about their unintended activity in reducing anti-tumor effect of cisplatin need to be confirmed further. Use of natural compounds that is known to have less side effects but effective in reducing cisplatin nephrotoxicity is highly desirable.

Honey is a complex natural liquid reported to contain hundreds of substances [16]. It's a highly saturated solution consisting of fructose (38%) and glucose (31%) as the major constituents, whereas the rest of the solution's composition is constituted by minor constituents such as phenolic acids, flavonoids, ascorbic acid, certain antioxidant enzymes such as glucose oxidase and catalase, carotenoid-like substances, organic acids and Maillard reaction products [17]. These minor constituents are reported to be mainly responsible for the antioxidant properties of honey[18]. To date, many epidemiological studies have demonstrated the effectiveness of phenolics and flavonoids when used alone or in combination as antitumor [19] or anti-inflammatory agents, in the treatment of cataracts [18;20] or in reducing the risk of cardiovascular diseases [18]. However, no study has been conducted to investigate whether crude honey is protective against cisplatin induced nephrotoxicity. Here we report for the first time that oral administration of crude honey protects kidney against cisplatin induced nephrotoxicity by suppressing oxidative stress and inflammation.

Results

Honey feeding suppresses cisplatin induced acute kidney injury

To determine the protective effects of honey, animals were pretreated for 1 week before cisplatin administration (Figure 1A). Kidney function was evaluated by quantifying serum creatinine. Saline treated animals did not show any increase in serum creatinine whereas cisplatin treated animals showed a time dependent increase in serum creatinine (Figure 1B) suggesting severe kidney injury. Honey feeding largely suppressed serum creatinine rise seen with cisplatin administration suggesting improved kidney function. Interestingly, honey alone fed mice did not show any alteration in serum creatinine level (Figure 1B).

Improved kidney function with honey feeding was associated with better preservation of kidney morphology (Figure 2C) as compared to cisplatin+vehicle treated mice kidney (Figure 2B). Both saline (Figure 2A) and honey (Figure 2D) alone treated mice kidney showed normal morphology. Injury of the kidney was quantified and shown in Figure 2E. Cisplatin treatment caused high level of tissue injury which is largely reduced with honey feeding.

To determine the effect of fenugreek on cisplatin induced apoptosis of tubular epithelium, kidney section was stained with TUNEL kit as described in Methods. Cisplatin induced extensive apoptosis in tubular epithelium (Figure 3B) as compared to saline treated animal kidney (Figure 3A) which was largely suppressed with honey feeding (Figure 3C). Honey alone did not have an effect on kidney epithelium survival (Figure 3D). Quantification of apoptosis is shown in Figure 3E.

Honey feeding suppressed cisplatin induced inflammation in kidney

Inflammation is a hallmark of cisplatin induced kidney injury [5;10]. To determine whether honey feeding has any effect on cisplatin induced inflammation in the kidney, neutrophil infiltration and cytokine excretion was quantified. Administration of cisplatin significantly increased neutrophil infiltration into the kidney (Figure 4B & E) as compared to saline (Figure 4A) treated animal kidney. Honey feeding significantly reduced cisplatin induced neutrophil infiltration (Figure 4C & E). Honey alone did not alter neutrophil infiltration into the kidney (Figure 4D & E). Reduced neutrophil infiltration in the kidney with honey feeding was associated with suppression of cytokine (TNF α , IL-1 β , IL-6) (Figure 5 A-C), chemokine (MCP-1) (Figure 5F) and adhesion molecule (ICAM-1) (Figure 5E) expression in honey treated kidney as compared to cisplatin+vehicle treated kidney. However, the expression of anti-inflammatory gene called activated microglia/macrophage WAP domain protein (AMWAP) is increased (Figure 5D) whereas oxidative stress marker heme oxygenase-1 (Figure 5G) is downregulated with honey treatment. Consistent with suppression of kidney cytokine mRNA expression, urinary cytokine (IL-6) (Figure 6A) and chemokine (MCP-1) (Figure 6B) excretion was reduced with honey feeding as compared to cisplatin +vehicle treated animals.

Honey suppresses cisplatin induced NFkB activation in the kidney

Data in Figure 5&6 had shown that honey feeding suppressed cisplatin induced cytokine mRNA expression and excretion. To determine whether this suppression was associated with decrease in phosphorylation and inhibition of NFkB activation, Western blot analysis for p65-NFkB phosphorylation was carried out. As shown in Figure 7, cisplatin treatment significantly enhanced p65-NFkB phosphorylation. Honey feeding suppressed cisplatin induced increase in p65-NFkB phosphorylation suggesting honey may suppress inflammation through inhibition of NFkB activation.

Discussion

Inflammation and oxidative stress are the major contributors of drug induced nephrotoxicity such as cisplatin induced acute kidney injury. Currently, there are no drugs available to reduce cisplatin induced AKI. Moreover, available anti-inflammatory and antioxidant therapies may reduce cisplatin efficacy against tumors. Hence it is important to develop new methods to treat cisplatin nephrotoxicity. Natural compounds such as honey are rich in antioxidants and also may have anti-inflammatory effects has been used to treat wide variety of illnesses in Ayurvedic medicine in Asia. However, its usefulness in reducing cisplatin nephrotoxicity is unknown. Our studies demonstrate for the first time that oral

administration of crude honey is effective in preventing cisplatin induced kidney injury by reducing inflammation and oxidative stress through inhibition of NFkB activation.

Honey feeding to animals suppressed cisplatin induced inflammation in the kidney which includes leukocyte infiltration, cytokine and chemokine production and cytokine excretion in urine. Honey is known to have phenolic compounds [16;18] that may be responsible for the observed effects on inflammation. NFkB activation by cisplatin is a major mediator of inflammatory response in the kidney in response to cisplatin administration [5;10;14]. Our studies show that cisplatin induced a large increase in phospho-P65 (NFkB) levels in the kidney which was largely reduced with honey feeding suggesting that honey may reduce inflammation through suppression of NFkB activation.

The mechanism of NFkB inhibition is unknown. Oxidative stress is a hallmark of cisplatin induced nephrotoxicity[12]. Oxidants such as superoxide and hydrogen peroxide, hydroxyl radicals are known activators of NFkB [21]. RT-PCR analysis of oxidative stress marker such as HO-1 is highly elevated after cisplatin nephrotoxicity which was significantly downregulated with honey treatment suggesting reduction of oxidative stress with honey. Therefore, it is possible that reduction of oxidative stress may reduce NFkB activation and thereby reduction in inflammation in the kidney.

In summary, our studies demonstrate that oral administration of honey is effective in preventing cisplatin nephrotoxicity through suppression of inflammation. Suppression of inflammation may be related to reduction of oxidative stress. We conclude that honey intake could benefit patients who receive chemotherapy for cancer.

Materials and Methods

Animals and drug administration

Experiments were performed using 8-10 week old male C57BL/6 mice. Mice were maintained on a standard diet and water was freely available. This study was approved by IACUC at the Georgia Regents University. Cisplatin was dissolved in saline at a concentration of 1 mg/ml. Mice were given a single intraperitoneal injection of either saline or cisplatin (30mg/kg body weight). Some of these animals received oral feeding of crude honey (over the counter) (500mg/kg BW per day) in water or water alone. Honey feeding was started seven days before cisplatin administration continued till 72hr after cisplatin administration. Animals were sacrificed at 72 hr after cisplatin administration to collect blood and kidney tissues. Kidney tissues were processed for histology, TUNEL assay, neutrophil and RNA isolation.

Renal function

Renal function was assessed by measurements of serum creatinine (cat no: DZ072B, Diazyme Labs, USA).

Quantitation of mRNA by real-time RT-PCR

Real-time RT-PCR was performed in an Applied Biosystems Inc. 7700 Sequence Detection System (Foster City, California, USA). 1.5 µg total RNA was reverse transcribed in a

reaction volume of 20 µl using Omniscript RT kit and random primers. The product was diluted to a volume of 150 µl, and 6µl aliquots were used as templates for amplification using the SYBR Green PCR amplification reagent (Qiagen) and gene-specific primers. The primer sets used were: mouse TNFα (forward: GCATGATCCGCGACGTGGAA; reverse: AGATCCATGCCGTTG GCCAG), MCP-1 (forward: ATGCAGGTCCCTGTTCATG; reverse: GCTTGAGGTGGTTGTGGA), ICAM-1 (forward: AGATCACATTCACGGTGCTG; reverse: CTTGAGAGGCAGGAAACAGG), hemeoxygenase-1 (HO-1) (forward: AGCATGCCCCAGGATTTG; reverse: AGCTCAATGTTGAGCAGGA), and AMWAP (forward: CTTGCCCCAAAGAATTTGAA; reverse: GGAGTTTTGCAGACATGACCA). The amount of DNA was normalized to the β-actin signal amplified in a separate reaction (forward primer: AGAGGGAAATCGTGCGTGAC; reverse: CAATAGTGATGACCTGGCCGT).

Serum cytokine measurement

Serum cytokines and chemokines were measured using ELISA array kit from SA biosciences and ELISA kit from eBiosciences.

TACS TdT *in situ* Apoptosis Detection

To identify apoptotic cells, tissue sections were stained using TACS TdT *in situ* Apoptosis Detection kit (R&D Systems, Inc.) according to the manufacturer's instructions. Briefly, tissue sections were deparaffinized, hydrated and washed with PBS. Sections were digested with proteinase K for 15 minutes at 24° C. Slides were then washed and endogenous peroxidase activity was quenched with 3% H₂O₂ in methanol. Slides were washed and incubated with TdT labeling reaction mix at 37° C for 1 hour and then with streptavidin-HRP. Color was developed using TACS blue label substrate solution. Slides were washed, counterstained, and mounted with Permount. Sections were photographed, and labeled cells were counted and quantified.

Histology

Kidney tissue was fixed in buffered 10% formalin for 12 hours and then embedded in paraffin wax. For assessment of injury, 5 µM sections were stained with periodic acid-Schiff (PAS). Acute tubular necrosis was assessed in the outer strip of the outer medulla and cortex using a semiquantitative scale in which the percentage of tubules showing epithelial necrosis, brush-border loss and cast formation was assigned a score: 0 = normal, 1 = <10%, 2 = 11–25%, 3 = 26–45%, 4 = 46–75%, and 5 = >76% [22;23]. Ten fields of 40X magnification were examined and averaged. The slides were scored in a blinded manner and de-identified. To quantify leukocyte infiltration, sections were stained with rat anti-mouse neutrophil antibody (Abcam) (1:200 dilution) followed by goat anti-rat biotin conjugate. Color was developed after incubation with ABC reagent (Vector Lab). Stained sections were photographed and five 40X fields of neutrophils were examined for quantitation of leukocytes.

Western blot analysis

Protein extraction from kidney and Western blot analysis were carried out as described before[14;24]. The membrane was probed with rabbit anti-mouse phospho p65 (NFkB) antibody and rabbit anti-actin antibodies (Cell Signaling Technology, Danvers, MA). Proteins were detected using enhanced chemiluminescence detection reagents (Thermo Scientific Pierce, Rockford, IL USA). Signals were normalized to beta-actin.

Statistical Methods

All assays were performed in duplicate. The data are reported as mean $\pm$ SEM. Statistical significance was assessed by an unpaired, two tailed Student *t*-test for single comparison or ANOVA for multiple comparisons.

Acknowledgements

This work was supported by American Heart Association (AHA) Postdoctoral Fellowship to RM and PR, National Institutes of Health (NIH) R01 DK083379 to GR and predoctoral fellowship from Egypt government to RH.

Reference List

1. Liangos O, Wald R, O'Bell JW, Price L, Pereira BJ, Jaber BL. Epidemiology and outcomes of acute renal failure in hospitalized patients: a national survey. *Clin J Am Soc Nephrol*. 2006; 1:43–51. [PubMed: 17699189]
2. Musu M, Finco G, Antonucci R, Polati E, Sanna D, Evangelista M, Ribuffo D, Schweiger V, Fanos V. Acute nephrotoxicity of NSAID from the foetus to the adult. *Eur.Rev Med Pharmacol.Sci*. 2011; 15:1461–1472. [PubMed: 22288307]
3. Allgren RL, Marbury TC, Rahman SN, Weisberg LS, Fenves AZ, Lafayette RA, Sweet RM, Genter FC, Kurnik BR, Conger JD, Sayegh MH. Anaritide in acute tubular necrosis. Auriculin Anaritide Acute Renal Failure Study Group. *N Engl J Med*. 1997; 336:828–834. [PubMed: 9062091]
4. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. *N.Engl.J Med*. 1996; 334:1448–1460. [PubMed: 8618585]
5. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of Cisplatin nephrotoxicity. *Toxins.(Basel)*. 2010; 2:2490–2518. [PubMed: 22069563]
6. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov*. 2005; 4:307–320. [PubMed: 15789122]
7. Pabla N, Dong Z. Cisplatin nephrotoxicity: Mechanisms and renoprotective strategies. *Kidney Int*. 2008; 73:994–1007. [PubMed: 18272962]
8. Ramesh G, Reeves WB. TNFR2-mediated apoptosis and necrosis in cisplatin-induced acute renal failure. *Am J Physiol Renal Physiol*. 2003; 285:F610–F618. [PubMed: 12865254]
9. Zhang B, Ramesh G, Uematsu S, Akira S, Reeves WB. TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. *J Am Soc Nephrol*. 2008; 19:923–932. [PubMed: 18256356]
10. Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin.Invest*. 2002; 110:835–842. [PubMed: 12235115]
11. Arany I, Safirstein RL. Cisplatin nephrotoxicity. *Seminars in Nephrology*. 2003; 23:460–464. [PubMed: 13680535]
12. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. *Drug Metab Rev*. 1999; 31:971–997. [PubMed: 10575556]
13. Deng J, Kohda Y, Chiao H, Wang Y, Hu X, Hewitt SM, Miyaji T, McLeroy P, Nibhanupudy B, Li S, Star RA. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int*. 2001; 60:2118–2128. [PubMed: 11737586]
14. Ramesh G, Reeves WB. Salicylate reduces cisplatin nephrotoxicity by inhibition of tumor necrosis factor-alpha. *Kidney Int*. 2004; 65:490–499. [PubMed: 14717919]

15. Ramesh G, Reeves WB. p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. *Am J Physiol Renal Physiol.* 2005; 289:F166–F174. [PubMed: 15701814]
16. White, JW. Composition of honey. In: Crane, E., editor. *Honey: A Comprehensive Survey*. Bee Research Association and Chalfont; St Peter, London, UK: p. 157-206.1-1-1975
17. El Denshary ES, Al Gahazali MA, Mannaa FA, Salem HA, Hassan NS, Abdel-Wahhab MA. Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats. *Exp Toxicol. Pathol.* 2012; 64:753–760.
18. Khalil MI, Sulaiman SA, Boukraa L. Antioxidant properties of honey and its role in preventing health disorder. *The Open Nutraceuticals Journal.* 3:6–16. 15.
19. Facino RM. Honey in tumor surgery. *Arch Surg.* 2004; 139:802.
20. Galal RM, Zaki HF, Seif El-Nasr MM, Agha AM. Potential protective effect of honey against paracetamol-induced hepatotoxicity. *Arch Iran Med.* 2012; 15:674–680. [PubMed: 23102243]
21. Morgan MJ, Liu Zg. Crosstalk of reactive oxygen species and NF-[kappa]B signaling. *Cell Res.* 2011; 21:103–115. [PubMed: 21187859]
22. Wang W, Li C, Summer SN, Falk S, Wang W, Ljubanovic D, Schrier RW. Role of AQP1 in endotoxemia-induced acute kidney injury. *Am J Physiol Renal Physiol.* 2008; 294:F1473–F1480. [PubMed: 18434389]
23. Ramesh G, Zhang B, Uematsu S, Akira S, Reeves WB. Endotoxin and cisplatin synergistically induce renal dysfunction and cytokine production in mice. *Am J Physiol Renal Physiol.* 2007; 293:F325–F332. [PubMed: 17494092]
24. Ranganathan P, Jayakumar C, Navankasattusas S, Li DY, Kim Im, Ramesh G. UNC5B Receptor Deletion Exacerbates Tissue Injury in Response to AKI. *J Am Soc Nephrol.* 2014; 25:239–249. [PubMed: 24115477]

Figure 1.

Effects of honey feeding cisplatin induced nephrotoxicity. A. Schematic representation of protocol used for honey oral administration and cisplatin administration. B. Serum creatinine levels at different time after various treatments. Serum creatinine was quantified as a function of kidney as described in Methods. *, $p < 0.001$ vs. saline treated. #, $p < 0.001$ vs cisplatin treated. $n = 8-10$.

Figure 2.

Histopathological assessment of tissue injury with cisplatin and honey administration. A-D. Kidney injury was assessed in a PAS stained tissue section. 72hr after various treated kidney tissue was processed for PAS staining. A. Saline treated showing normal morphology. B. Cisplatin treated showing extensive tubular necrosis and cast formation. C. Cisplatin+honey treated showing less tubular necrosis. D. Honey treated kidney showing normal morphology. E. Semiquantitative scoring of tubular injury as described in Methods. *, $p < 0.001$ vs. other groups. #, $p < 0.05$ vs. cisplatin treated group. $n = 5$. C.

Figure 3.

Honey feeding reduced cisplatin induced tubular epithelial cell apoptosis in the kidney.

Apoptotic cells in the kidney was quantified by TUNEL assay. A. Saline treated kidney. B. Cisplatin treated kidney. C. Cisplatin and honey treated kidney. D. Honey treated kidney. E. Quantification of TUNEL positive cells in five 40X fields per animals. *, $p < 0.01$ vs. other groups. #, $p < 0.001$ vs. cisplatin treated groups. $n = 5$.

Figure 4.

Honey feeding suppressed cisplatin induced leukocyte infiltration in to the kidney. A-D. Immunohistochemical localization of monocyte and neutrophils infiltration at 72hr after different treatments. A. Saline treated kidney. B. Cisplatin treated kidney. C. Cisplatin +honey treated kidney. D. Honey treated kidney. E. Quantification of infiltrated leukocytes in the kidney. *, $p < 0.001$ vs. other groups. #, $p < 0.001$ vs. cisplatin treated groups. $n = 5$.

Figure 5.

Honey feeding suppressed cisplatin induced increase in proinflammatory cytokine expression. 72hr after cisplatin administration cytokine and adhesion molecule levels in the kidney was quantified by RT-PCR. TNF α (A), IL-6 (C), ICAM-1 (E), MCP-1 (F) and HO-1 (G) levels in were significantly increased with cisplatin treatment which was significantly reduced with honey treatment. However, cisplatin induced IL-1 β increase (B) was not reduced with honey treatment. Interestingly, anti-inflammatory molecule AMWAP upregulated with honey treatment (D). *, $p < 0.01$ vs. other groups. #, $p < 0.05$ vs. cisplatin treated. n=4-6.

Figure 6.

Honey feeding suppressed cisplatin induced increase in proinflammatory cytokine excretion in urine. 72hr after cisplatin administration cytokine levels in urine was quantified by ELISA. IL-6 (A), MCP-1 (B) levels in urine expressed as pg/mg of creatinine. *, $p < 0.001$ vs. other groups. #, $p < 0.05$ vs. cisplatin treated. $n = 4$.

Figure 7.

Honey feeding suppressed cisplatin induced NFκB phosphorylation in the Kidney. Western blot analysis for NFκB (A) was carried out as described in Methods. Protein was normalized to β-actin expression. Blot was scanned and signal was quantified by densitometry and quantitative data expressed as a fold over saline treated (B). *, $p < 0.05$ vs. other groups. $n = 3-4$.