The possible protective effects of some flavonoids that found honey by experimental ischemia/reperfusion (I/R) induced nitrosative damage in kidney of male rats

Assoc. Prof. Dr. Aslı Korkmaz* and Prof. Dr. Dürdane Kolankaya**

*Ministry of Agriculture and Rural Affairs, National Food Reference Laboratory, Fatih Sultan Mehmet Avenue, No: 70, Yenimahalle, 0643 Ankara/TURKEY.

**H.Ü.Science Faculty Departmen of Biology, Beytepe Campus, 06800 Ankara/TURKEY

Abstract

Honey contain various flavonoids such as quercitrin, kaempherol, luteolin, and naringin. They were used as a marker for particular type of honey. Some flavonoids are a strong antioxidant and help to prevent oxidative damage which effect lipids, proteins and DNA of our cells. The flavonoids kaempferol and quercetin that found in honey seems to act synergistically in reducing cell proliferation of cancer cells, meaning that the combined treatments with quercetin and kaempferol are more effective than the additive effects of each flavonoid.

This study was designed to investigate the effect of quercitrin, kaempherol, rutin, luteolin, isorhamnetin and naringin in ischemia/reperfusion (I/R) induced nitrosative stress in kidney of male rats. In this purpose, it has been created ten different experimental groups as control, sham, IR, L-NAME(20mg/kg)+IR,quercitrin (3 mg/kg)+IR, kaempherol(7mg/kg), rutin(1g/kg)+IR luteolin(0,7mg/kg)+IR, isorhamnetin(4 mg/kg)+IR and naringin(350 mg/kg)+IR was administered intraperitoneally 1h prior to ischemia.

At the end of the reperfusion period, kidney samples were taken for histopatological examinations and immunohistocemical detection of 3-NT, determination of renal malondialdehyde (MDA) and glutathione(GSH) levels, manganase superoxide dismutase (MnSOD) activities. Also, serum creatinine and blood urea nitrogen (BUN) level; plasma cyclic guanosine monophospate (cGMP) and plasma nitrite/nitrate levels were measured in blood sample of rats.

I/R caused a significant increase in MDA levels which were accompanied by a significant decrease in GSH level and MnSOD activities of kidney tissues. Also, I/R caused hemorrhage, infiltration of mononuclear cells, dead cells deposit in tubule lumen of the rat kidney, strong positive immunostaining for 3-NT and drastic lose of renal function. Furthermore, there were greate increse of serum BUN and creatinine levels and plasma cGMP and nitrite/ nitrate levels in IR and L-NAME+IR groups. On the other hand, pretreatment of rats with flavonoids significantly attenuated renal dysfunction, reduced elevated MDA levels, plasma cGMP levels plasma nitrit/nitrat levels and restored the depleted activity of MnSOD and GSH levels. These beneficial changes in the biochemical parameters were also associated with parallel changes in histopathological appearance. Also it has been detected slight posive staining of 3-NT levels in kindey section which belonging to flavonoid groups.

In conlusion the flavonoids are graded according to their effectiveness in preventing NO formation and renal I/R injury, it has been determined that most efficient ones are quercitrin and kaempherol

which found in many honey, the middle efficent ones are rutin and luteolin and the least efficient ones are isorhamnetin and naringin.

Keywords: Rat, kidney, ischemia/reperfusion, nitrosative stres, kaempherol, rutin, quercitrin, luteolin, isorhamnetin, naringin

Introduction

Recent experimental research has helped elucidate the pathophysiologic basis behind ischemic ARF (Acute Renal Failure), and therapies that can treat or even prevent ischemic ARF may become a reality in the near future. Ischemia/reperfusion (I/R) of an organ or tissue is cellular injury triggering a complex cascade of biochemical events that affect the structure and function of almost every organelle and subcellular system of affected cells. Many scientists report that renal I/R injury is a common cause of renal cell death, ARF and, in the case of transplantation, delayed graft function or graft rejection. Many mediators are involved in the pathophysiology of I/R injury, including reactive oxygen species (ROS), reactive nitrogen species (RNS), purine metabolites, neutrophil accumulation, vasoactive substance (endothelin, angiotensin II) and subsequent release of lytic enzymes.

An inflammatory response also leads to vascular congestion that propagates the hypoxic environment and reduces the ability to clear the toxic radicals. Thus the corticomedullary region is the most vulnerable region of the kidney to tubular injury, inflammation and vascular alterations that extend the cellular injury beyond the initial insult and propagate continued hypoperfusion . Renal transplant recipients who experience delayed graft function have increased risks of rejection and long-term graft failure. Ischemic damage is the most common cause of delayed graft function, and although it is known that tissue inflammation accompanies renal ischemia, it is unknown whether renal ischemia affects the production of antibodies by B lymphocytes, which may lead to chronic humoral rejection and allograft failure (Fuquay et al,2013).

ROS and RNS react with biomolecules such as cell membrane lipid as well as proteins, carbohydrates, nucleic acids, and thiols resulting in organic radical formation, lipid peroxidation, enzyme inactivation, glutathione oxidation, and cell destruction. ROS and RNS have an important role in I/R injury, especially through lipid peroxidation.

Cellular defense against oxidative injury is provided by several mechanisms. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), as well as nonenzymatic compounds such as reduced glutathione (GSH), all help to cope with potential damage. The increased production of ROS during I/R injury results in consumption and depletion of endogenous antioxidants. When ROS and RNS is overproduced, the administration of exogenous antioxidants such as FLAVONOIDS should be given as a potential scavenger.

Flavonoids occur naturally in plants and cannot be synthesized by humans. In vitro studies have shown that flavonoids, possess anti-inflammatory, antiallergic, antioxidant and anticancerogenic properties. The health benefits of flavonoids attributed to polyphenols are usually linked to 2 properties: antioxidant activity and inhibition of certain enzymes. Because of these flavonoids protect cells from ROS and RNS. Also, flavonoids reduce the damaging effects of free radicals by stimulating the production of glutathione, which is a strong anti-oxidant.

As a strong antioxidant flavonoids help to prevent oxidative damage which effect lipids, proteins and DNA of our cells. Many food and honey contain various flavonoids such as quercitrin, kaempherol, luteolin, rutin, luteolin, isorhamnetin and naringin.They were used as a marker for particular type of honey. For exp. Black weath honey conain high amonunt of kaempherol and quercitrin. Hesperetin, A marker of the floral origin of citrus honey. Sunflower honeys contained an important relative amount of quercetin. Natural honey has been used in traditional medicine of different cultures throughout the world.

Honey is being used since long time both in medical and domestic needs, but only recently its antioxidant property has come to limelight. With increasing demand for antioxidant supply in the food, honey is becoming popular as a source of antioxidant since it is rich in phenolic acids and flavonoids and other antioxidants including glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, amino acids and proteins.

Figur 1. Phenolic acids and some flavonols in honey

The antioxidants have several preventative effects against different diseases like cancer, cardiovascular diseases, inflammatory disorders, neurological degeneration, wound healing, infectious diseases and aging, which led to search for foods rich in antioxidants. Various studies on antioxidant properties of honey have been done.

The flavonoids kaempferol and quercetin that found in honey seems to act synergistically in reducing cell proliferation of cancer cells, meaning that the combined treatments with quercetin and kaempferol are more effective than the additive effects of each flavonoid. Luteolin that contain Honey has anti-cataract effects

The present study is a short review on the antioxidant properties of quercitrin, kaempherol, rutin, luteolin, isorhamnetin and naringin that is some of contain honey and its role against experimental kidney I/R injury.

Methods and material

In this purpose, it has been created ten different experimental groups were :control, sham, IR (The animals received 0.5 mL of saline 1 hour before ischemia, and then the left renal pedicle was occluded for 45 minutes to induce ischemia followed by 3 hours of reperfusion.), L-NAME+IR, quercitrin+IR, kaempherol+IR , rutin+IR , luteolin+IR, isorhamnetin+IR, naringin+IR.

The animals received L-NAME (20 mg/kg in 0.5 mL of saline) intraperitoneally 5 minutes before ischemia, and then the left renal pedicle was occluded for 45 minutes to induce ischemia followed by 3 hours of reperfusion. The animals in different groups received ,quercitrin (3 mg/kg)+, kaempherol (7mg/kg), luteolin (0,7mg/kg), isorhamnetin (4 mg/kg), rutin (1g/kg) and naringin (350 mg/kg)

intraperitoneally 1 h before ischemia, and then the left renal pedicle was occluded for 45 minutes to induce ischemia followed by 3 hours of reperfusion.

At the end of the reperfusion period, kidney samples were taken for histopatological examinations, determination of renal malondialdehyde (MDA) and glutathione(GSH) levels, manganase superoxide dismutase (MnSOD) activities. Also, serum creatinine and blood urea nitrogen (BUN) level; plasma cyclic guanosine monophospate (cGMP) and plasma nitrite/nitrate levels were measured in blood sample of rats.

Results

Effects of I/R

Serum BUN and creatinine levels were elevated in the I/R group as compared to the control group. I/R caused an increase in plasma cGMP level, which was accompanied with an increase in plasma nitrite/nitrate level. I/R caused a significant increase in MDA levels which were accompanied by a significant decrease in GSH level and MnSOD activities of kidney tissues. Also, I/R caused hemorrhage, infiltration of mononuclear cells, dead cells deposit in tubule lumen of the rat kidney. Furthermore, there were greate increse of serum BUN and creatinine levels and plasma cGMP and nitrite/ nitrate levels in IR and L-NAME+IR groups.

Pretreatment of the flavonoids.

Prereatments of rats with the flavonoids (quercitrin, kaempherol, rutin, luteolin, isrohamnetin or naringin) produced a significant reduction in the serum levels of creatinine, BUN. Pretreatment of rats with flavonoids attenuated both renal dysfunction and elevation in plasma cGMP levels and restored the increased plasma nitrite/nitrate level. The rats treated with flavonoids prior to I/R produced a significant reduction of MDA and MnSOD levels . Also, in the flavonoids pretreated groups GSH concentration was found to be preserved. Treatment with flavonoids preserved the normal morphology of the kidney demonstrating normal glomeruli and slight edema of the tubular cells.

Figur2. Effects of flavonoids on Blood Urea Nitrogen (BUN) and, Serum Creatinine, Rats Exposed to Renal I/R

Fügure 3. Effects of Flavonoids on plasma cGMP levels and plasma nitrite/nitrate levels,

in Rats Exposed to Renal I/R.

Figure 4. Effects of Flavonoids on Lipid Peroxidation, Reduced Glutathione (GSH) Level, and MnSOD Activity in Rats Exposed to Renal I/R.

Effects of flavonoids

Pretreatment of rats with flavonoids significantly attenuated renal dysfunction, reduced elevated MDA levels, plasma cGMP levels plasma nitrit/nitrat levels and restored the depleted activity of MnSOD and GSH levels. These beneficial changes in the biochemical parameters were also associated with parallel changes in histopathological appearance.

Figure 5. Immunohistochemical evidence of 3-nitrotyrosine formation in rat kidney following I/R. Pictured are representative photographs. (A) Negative control without primary antibody; (B) Sham; (C) Sham+NP; (D) NP+I-R . (E) NP+L-NIL+ I-R; (F) NP+RU+ I249x281mm (88 x 88DPI).

Figure6. Immunohistochemical analysis showing representative expression of iNOS after I/R. (A) Negative control without primary antibody; (B) Sham; (C) Sham+NP; (D) NP+I-R . (E) NP+L-NIL+ I-R; (F), NP+RU+ IR. 249x281mm (88 x 88 DPI).

In conlusion

It is clear that the antioxidants properties of honey are due to the presence of some antioxidant compounds such as Vitamin C, monophenolics, flavonoids, and polyphenolics. Although there is a wide spectrum of antioxidant types, Caffeic acid, Caffeic acid phenyl ester, Chrysin, Galangin,

Quercetin, Acacetin, Kaempferol, Pinocembrin, Pinobanksin and Apigenin predominate in many honeys. These antioxidant compounds have a promising pharmacological agent for preventing cancer, cardiovascular diseases, inflammatory disorders, neurological degeneration, wound healing, infectious diseases and aging as well as it can be used as food preservatives.

The flavonoids are graded according to their effectiveness in preventing NO formation and renal I/R injury, it has been determined that most efficient ones are quercitrin and kaempherol which found in many kind of honey, the middle efficent ones are rutin and luteolin and the least efficient ones are isorhamnetin and naringin. So, I want to say these antioxidands two or three of quercitrin, kaempherol, rutin, luteolin, isorhamnetin and naringin that is honey contain if consumed enough amonunt, it is not need anyother antioxidand sourses to protected I/R injury.

References

Frankel S, Robinson GE, Berenbaum MR. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. J Apic Res 1998; 37: 27-31.

Gheldof N, Engeseth NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agric Food Chem 2002; 50: 3050-5.

Gheldof N, Wang XH, Engeseth NJ. Buckwheat honey increases serum antioxidant capacity in humans. J Agric Food Chem 2003; 51: 1500-5.

Kücük M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C, Candan F. Biological activities and chemical composition of three honeys

of different types from Anatolia. Food Chem 2007; 100: 526-34.

Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, Keen CL. Honey with high levels of antioxidants can provide protection to healthy human subjects. J Agric Food Chem 2003; 51: 1732- 5.

Nagai T, Inoue R, Kanamori N, Suzuki N, Nagashima T. Characterization of honey from different floral sources. Its functional

properties and effects of honey species on storage of meat. Food Chem 2006; 97: 256-62.

Kabasakal L, Sehirli O, Cetinel S, Cikler E, Gedik N, Sener G. Protective effect of aqueous garlic extract against renal ischemia/reperfusion injury in rats. J Med Food. 2005 Fall;8(3):319-26.

Korkmaz A, Kolankaya D. The protective effects of ascorbic acid against renal ischemia-reperfusion injury in male rats. Ren Fail. 2009;31(1):36-43.

Fuquay R , RennerB , Kulik L, McCullough JW, Amura C , Strassheim, Pelanda, Torres and Thurman M Renal Ischemia-Reperfusion Injury Amplifies the Humoral Immune Response. the American Society of Nephrology, Febç 21, 2013.

Munshi R HsuC, HimmelfarbJ. Advances in understanding ischemic acute kidney injury. Munshi et al. BMC Medicine 2011, 9:11

Sutton TA: Alteration of microvascular permeability in acute kidney injury. Microvasc Res 2009, 77:4-7. 7.

Legrand M, Mik EG, Johannes T, Payen D, Ince C: Renal hypoxia and dysoxia after reperfusion of the ischemic kidney. Mol Med 2008, 14:502-516.

Doğan A., Kolankaya D., Protective effect of Anzer honey against ethanol-induced increased vascular permeability in the rat stomach. Experimental and Toxicologic Pathology, 2005, 57: 173-178.

Korkmaz A., Kolankaya D. Kaempherol decreases nitric oxide level in renal ischemia/reperfusion injury. 7th World Congress on Alternatives & Animal Use in the Life Sciences, August 30-September 3, 2009, Rome, Italy. Abstracts 7th World Congress Rome 2009, Volume: 26, S328.

Korkmaz A, Kolankaya D. The protective effects of ascorbic acid against renal ischemia-reperfusion injury in male rats. Renal Failure 2009, 31: 36-43.

Korkmaz A., Kolankaya D. Protective effect of rutin on the ischemia/reperfusion induced damage in rat kidney. Journal of Surgical Research, . 2010,164, 309-315.