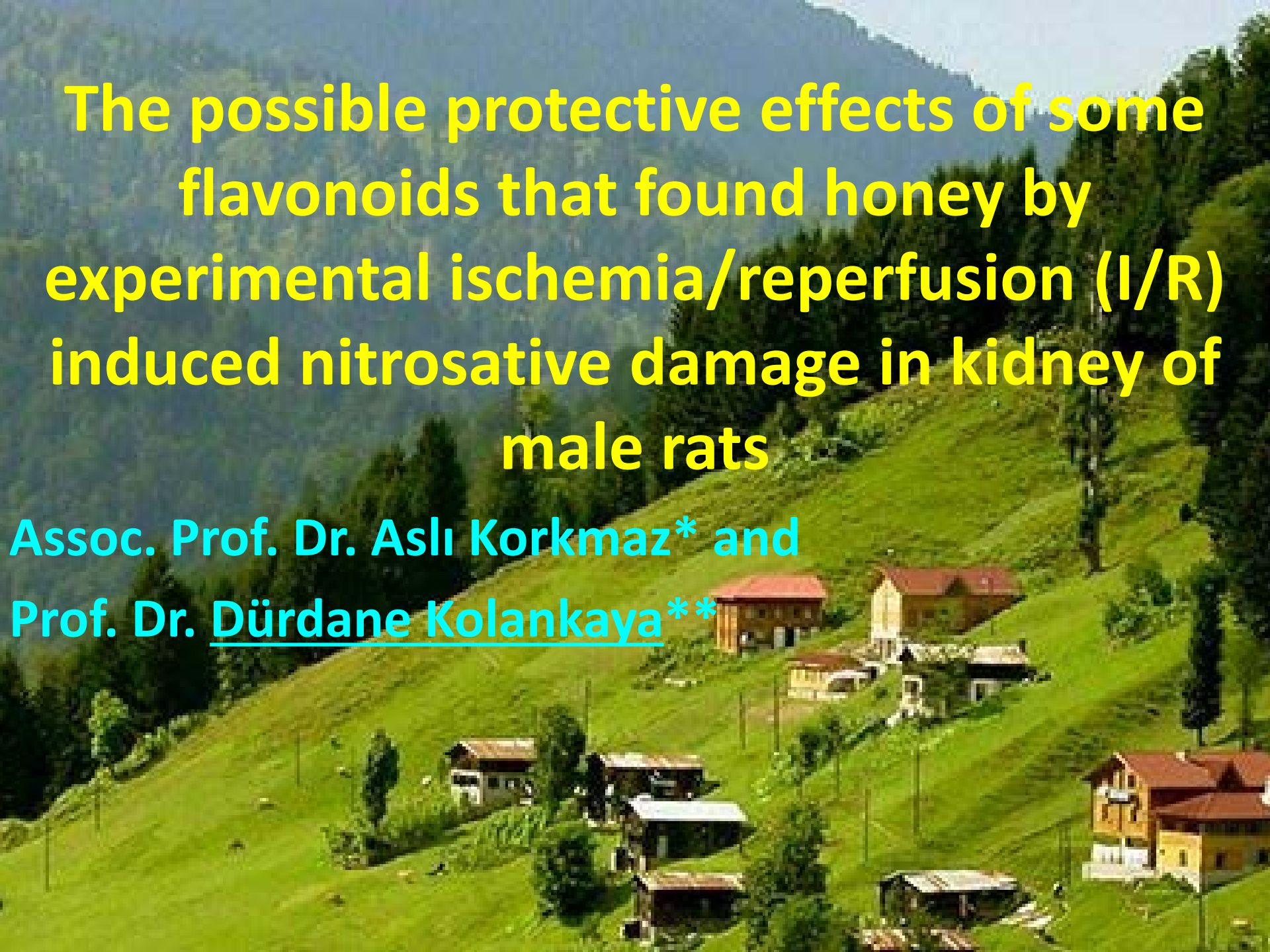


The possible protective effects of some flavonoids that found honey by experimental ischemia/reperfusion (I/R) induced nitrosative damage in kidney of male rats

Assoc. Prof. Dr. Aslı Korkmaz* and

Prof. Dr. Dürdane Kolankaya**

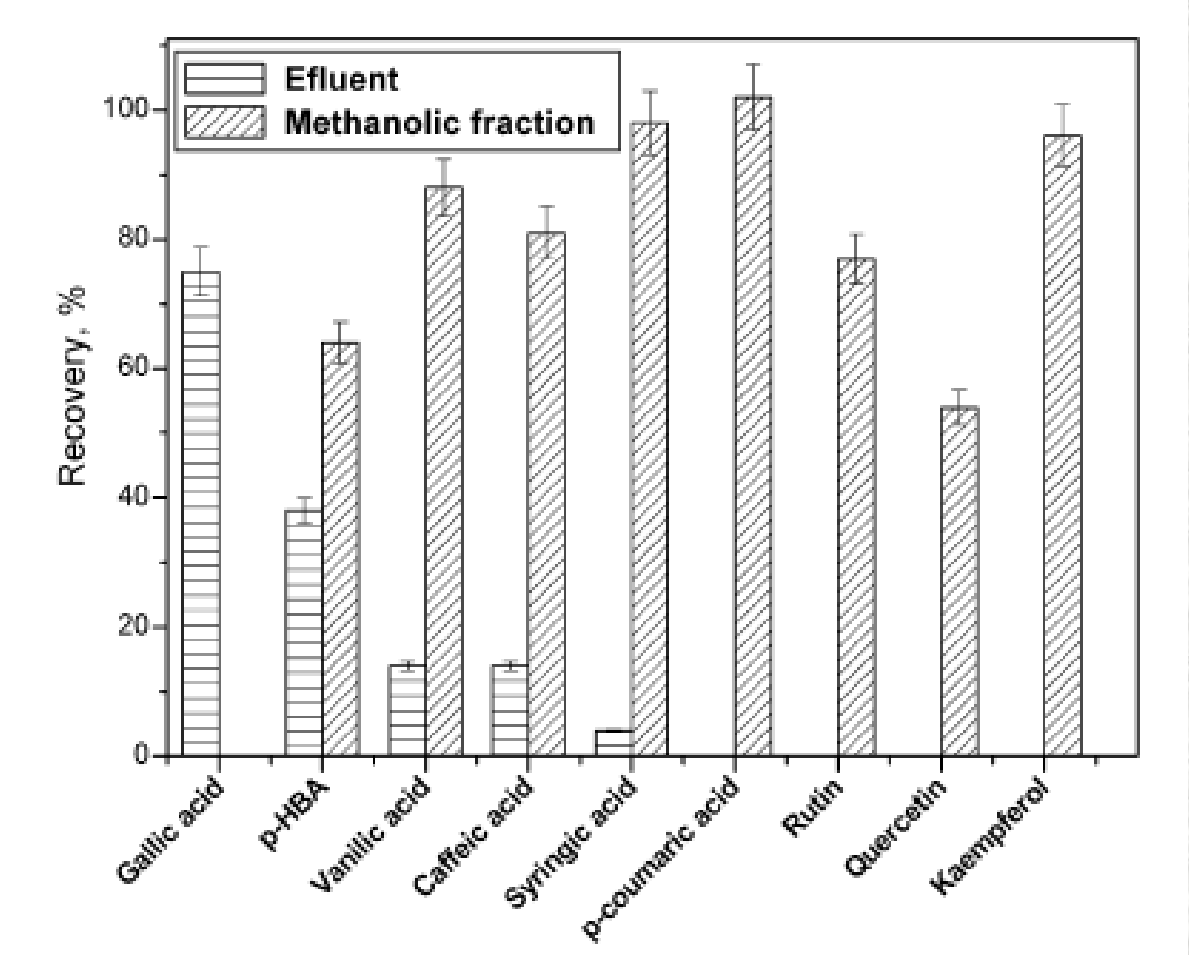
Flavonoids

- Flavonoids occur naturally in plants and cannot be synthesized by humans. In vitro studies have shown that flavonoids, possess anti-inflammatory, anti-allergic, antioxidant and anti-cancerogenic 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.

Flovonoids and Honey

- Many food and honey contain various flavonoids such as quercitrin, quercetin, kaempherol, luteolin, hesperitin, rutin, isorhamnetin, naringin, apigenin and others.
- 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

Phenolic acids and some flavonols in honey



A. Michalkiewicz et al. / J. Chromatogr. A 1187 (2008) 18–24

Flavonoid contents of tarhana, honey and *Urtica* sp.

	Quercetin (mg/100 g)	Luteolin (mg/100 g)	Apigenin (mg/100 g)	Kaempferol (mg/100 g)
Tarhana	5.92 ± 0.12 [a]	–	–	–
Honey	– [b]	–	29.3 ± 4	2.42 ± 0.1
Urtica sp.	0.87 ± 0.02	–	143 ± 55	–

[a] Mean ± standard deviation.
[b] Not detected.

Flavonoids content of black tea, linden flower, sage, rosehip, violet carrot juice and grape molasses

	Quercetin (μg/l)	Luteolin (μg/l)	Apigenin (μg/l)	Kaempferol (μg/l)
Black tea	34.8 ± 9 [a]	– [b]	–	110 ± 9
Linden flower	21.7 ± 8	–	–	113 ± 2
Sage	27.2 ± 0.8	11 ± 0.7	–	–
Rosehip	16.7 ± 0.2	–	–	–
Violet carrot juice	83.7 ± 0.5	–	–	–
Grape molasses	1692 ± 28	–	–	–

[a] Mean ± standard deviation.
[b] Not detected.

S. Karakaya, S.N. EL / Food Chemistry 66 (1999) 289–292

- Natural honey has been used in traditional medicine of different cultures throughout the world.
- Various studies on antioxidant properties of honey have been done.
- One of them shows that 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 been found anti-cataract effects.
- The present study is a short review on the antioxidant properties of quercitrin, kaempherol, rutin, luteolin, isorhamnetin and naringin that are contain some of honey and its role against experimental kidney **Ischemia/reperfusion (I/R)** injury.

The Pathophysiology of Ischemic damage

- 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 (Acute Renal Failure) and, in the case of transplantation, delayed graft function or graft rejection.
- Recent experimental research has helped elucidate the pathophysiologic basis behind ischemic ARF, and therapies that can treat or even prevent ischemic ARF may become a reality in the near future.
- 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.

The Pathophysiology of Ischemic damage

- 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. These is problem for the after graft transplantation.

The Pathophysiology of Ischemic damage

- 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 over produced, the administration of exogenous antioxidants such as FLAVONOIDS should be given as a potential scavenger.

The Aim of the study

- This study was designed to investigate the effect of each of flavonoid seperatly; quercitrin, kaempherol, rutin, luteolin, isorhamnetin and naringin in ischemia/reperfusion (I/R) induced nitrosative stress (NRS)in kidney of male rats.
- In this way preventing oxidative stress that produce ischemia/reperfusion (I/R) induced nirtosative stress during operation.

Method

- In this purpose, it has been created ten different experimental groups were seen below:
- 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

Method

- 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.

Method

- At the end of the reperfusion period, kidney samples were taken for histopatological and immunohistochemical 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

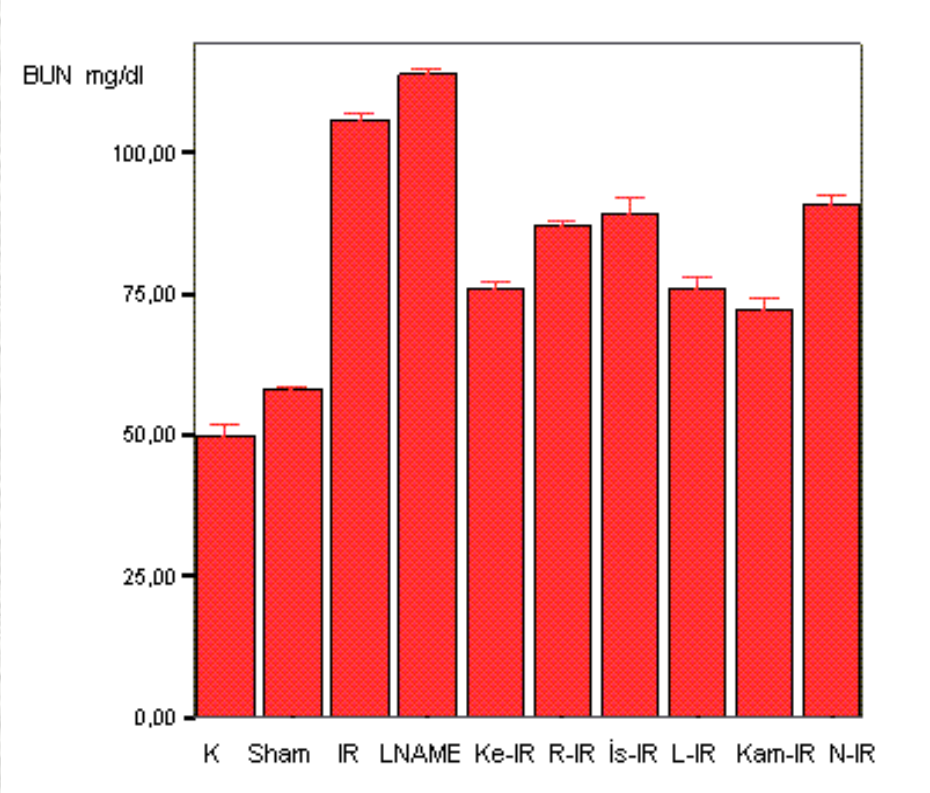
- Serum BUN and creatinine levels were elevated in the I/R group as compared to the control group.
- I/R caused an increase in plasma cGMP level, which was accompanied with an increase in plasma nitrite/nitrate level.
- I/R caused a significant increase in MDA levels which were accompanied by a significant 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.

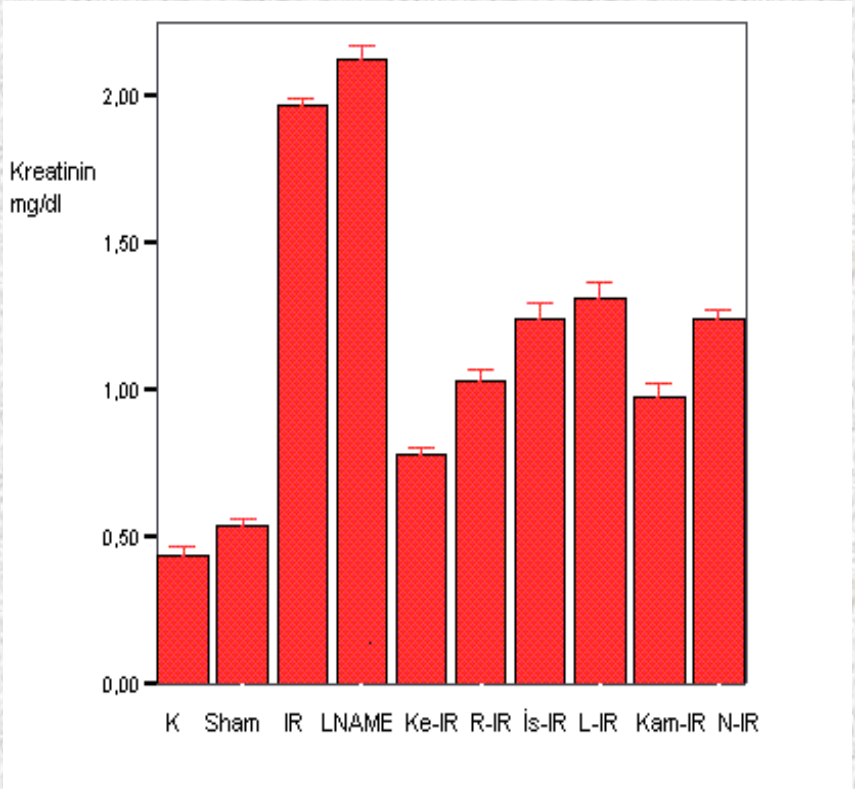
Results

Pretreatment of the flavonoids

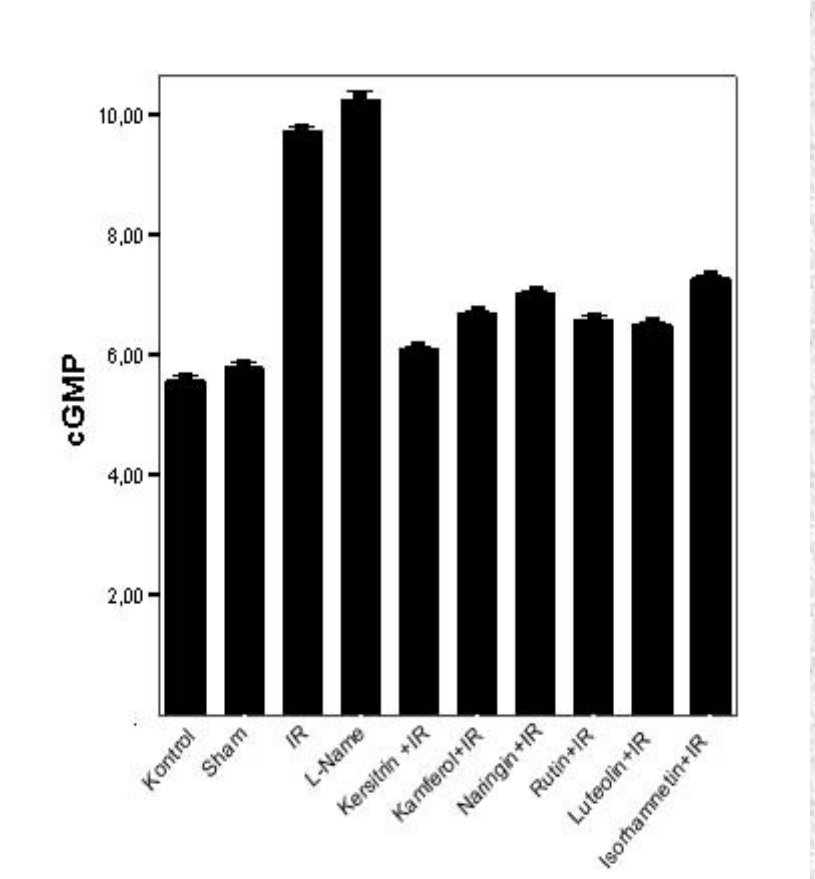
- Prereatments of rats with the flavonoids (quercitrin, kaempherol, rutin, luteolin, isrohamnetin or naringin) produced a significant reduction in the serum levels of creatinine, BUN.
- Pretreatment of rats with flavonoids attenuated both renal dysfunction and elevation in plasma cGMP levels and restored 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.

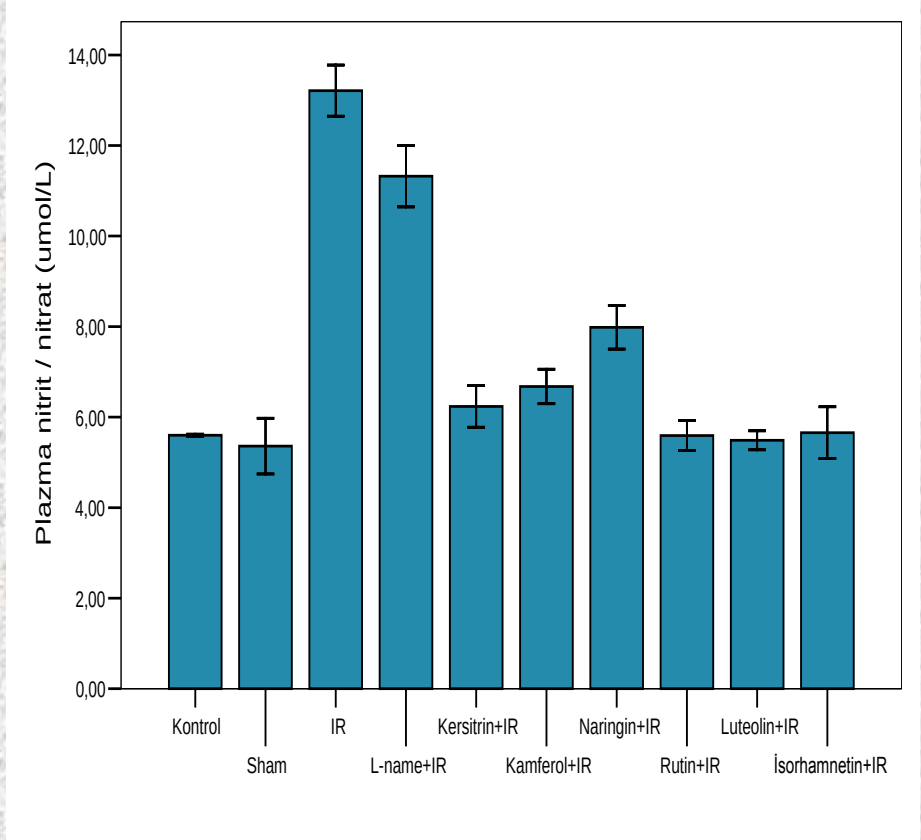
Effects of flavonoids on Blood Urea Nitrogen (BUN) and, Serum Creatinine, Rats Exposed to Renal I/R





Effects of Flavonoids on plasma cGMP levels and plasma nitrite/nitrate levels, in Rats Exposed to Renal I/R.

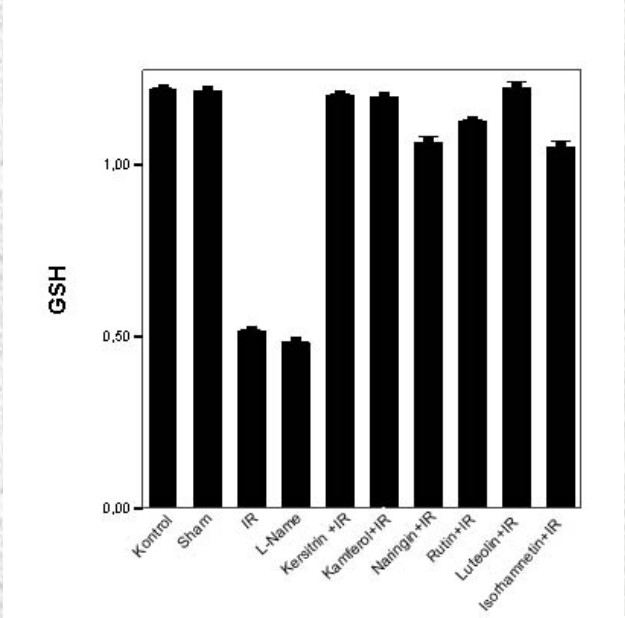


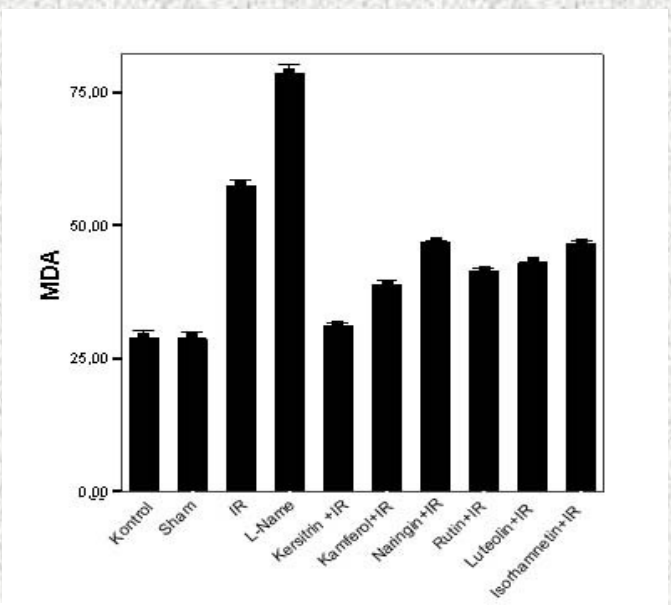


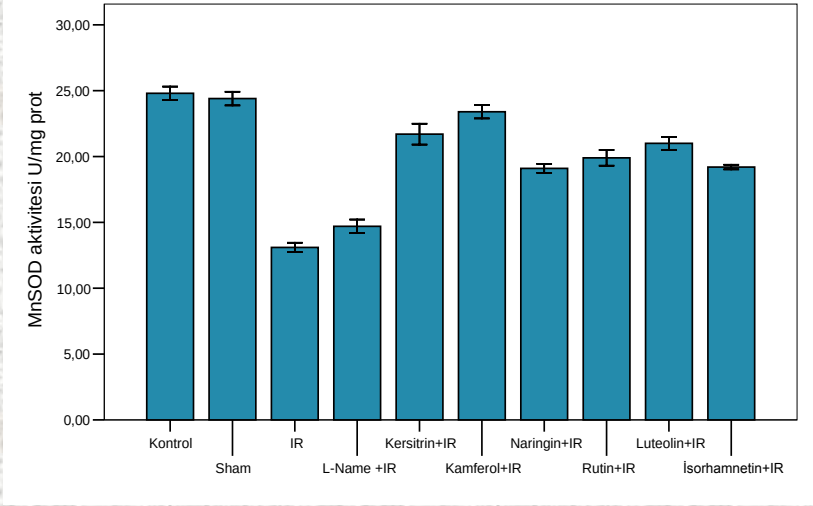
Effects of flavonoids

- 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.

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

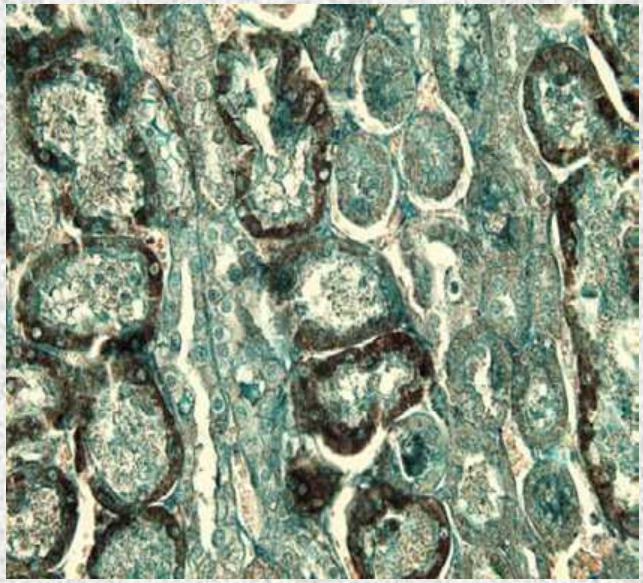


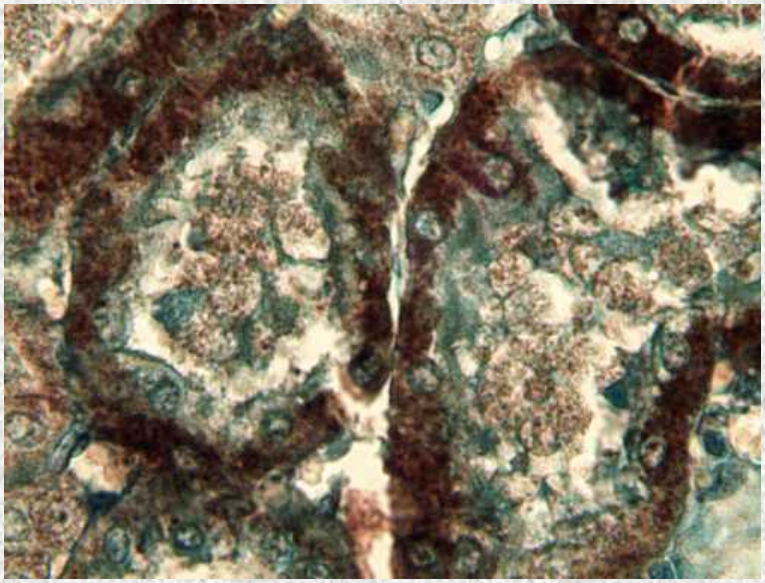




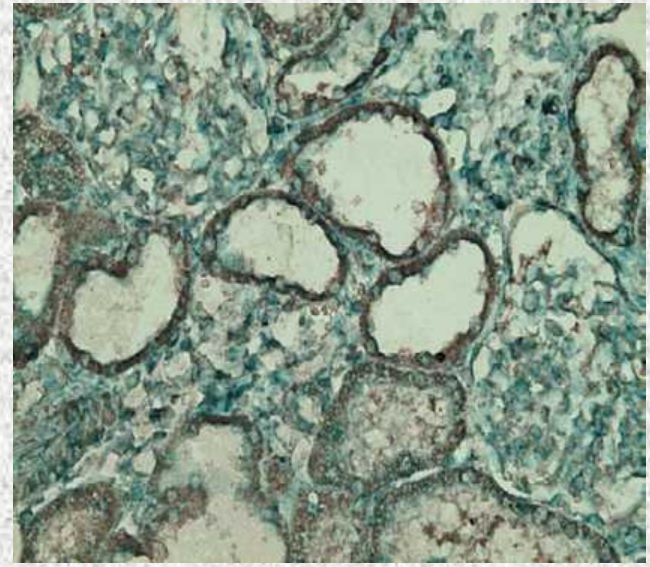
During I/R iduduced 3-nitrotyrosine formation and preventing with flavonoids(Quercitin, kaenferol, rutin, luteolin, Isorhamnetin and naringin.

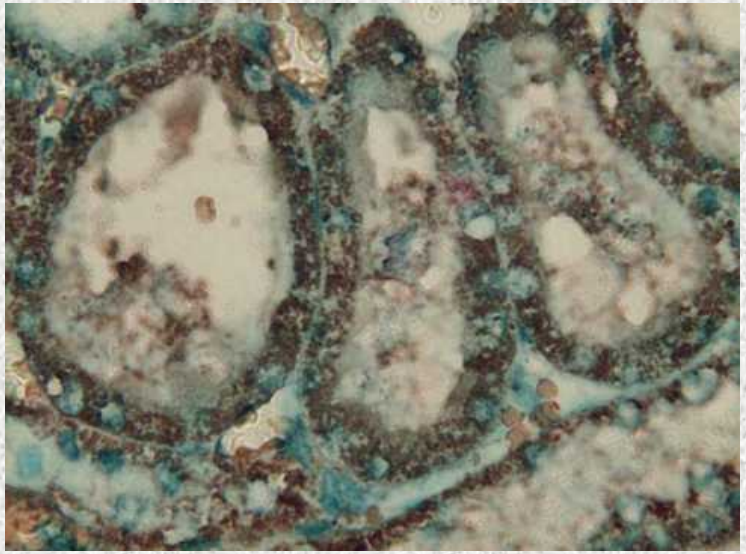
- Immunohistochemical evidence of 3-nitrotyrosine formation in rat kidney following I/R. Pictured are representative photographs.
- Immunohistochemical analysis showing representative expression of iNOS after I/R.
- These flavonoids : the studies shows that most effective are kersitrin and keamferol modarate effective are rutin and luteolin least effectif are isorhamnetin and naringin.



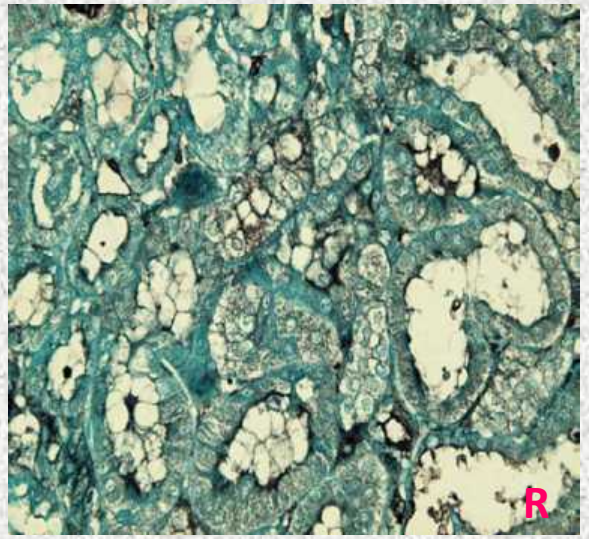


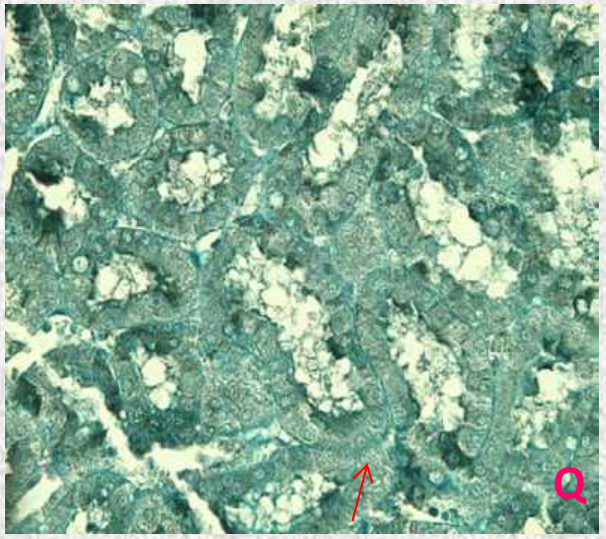
IR grubuna ait böbrek korteks kesitinde hücreler içinde 3-NT birikimi (kahverengi), 40X ve 100X

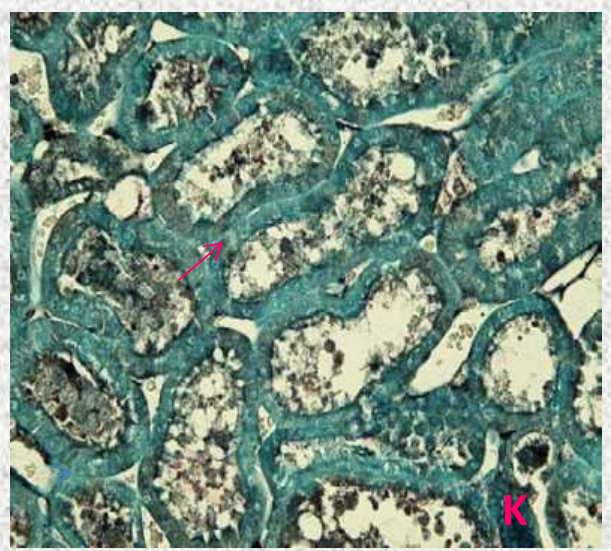


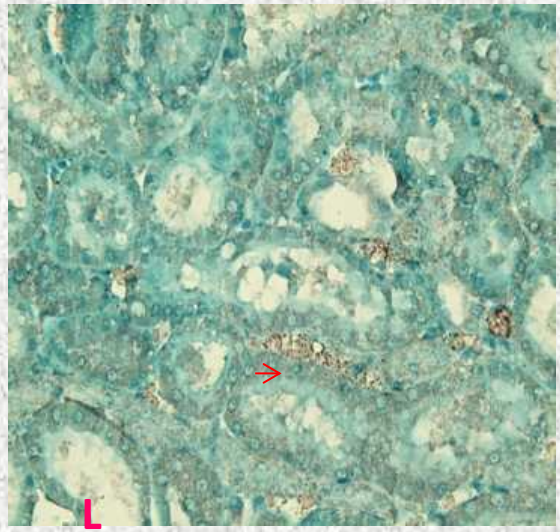


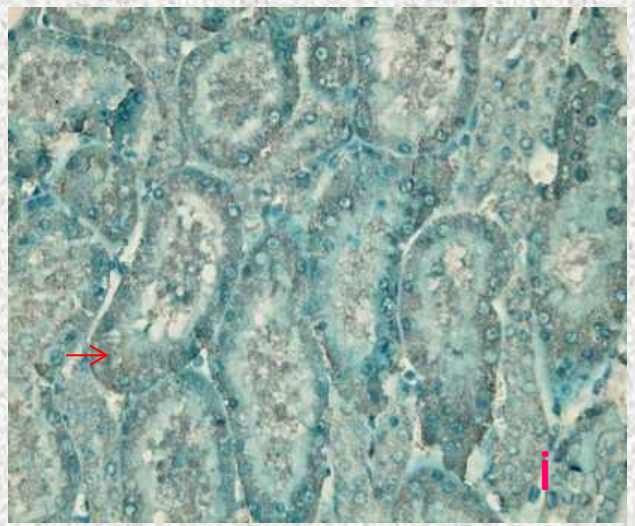
L-Name+IR grubuna ait böbrek korteks kesitinde hücreler içinde 3-NT birikimi (kahverengi), 40X ve 100X

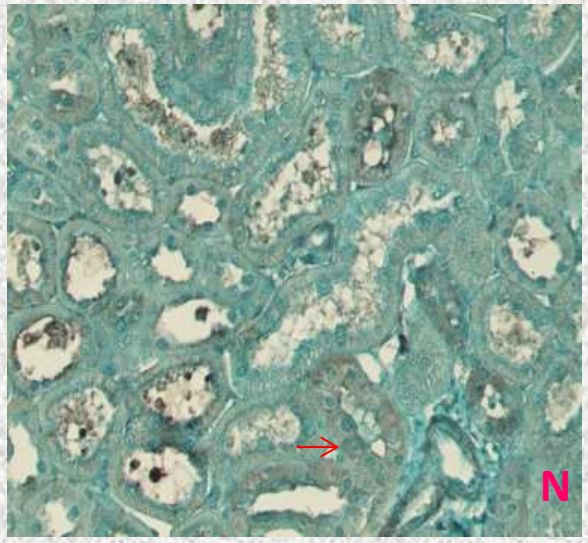












Rutin+IR, Quercitin+IR, Kamferol+IR, Luteolin+IR, İsorhamnetin+IR and naringin+IR grublarına ait böbrek korteks kesitinde hücreler içinde 3-NT birikimi (kahverengi), 40X

In conlusion

- The findings of the current study shows, for the first time, that flavonoids were used to prevent short period I/R injury in the kidney of male rats. It is important to inhibit oxidative stress to prevent renal I/R injury, and we suggest that acute administration of flavonoids might be helpful in clinical practice, particularly in transplantation and renal surgery. Further studies are necessary to improve our understanding of the role of flavonoids on I/R induced ARF.
- 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.

These beneficial changes in the biochemical parameters were also associated with parallel changes in histopathological appearance. These findings suggest that ROS play a causal role in I/R induced renal injury, and that flonanoids exerts renal-protective effects, probably by inhibiting ROS/NRS and antioxidant activities.

So, I want to say these antioxidands chemicals two or three of quercitrin, kaempherol, rutin, luteolin, isorhamnetin and naringin that have honey if consumed enough amonunt, it may be not need anyother antioxidand sourses to protected I/R injury.

Thank you very much