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Original Research Article

Combination of Honey and *Nigella sativa* Oil Reduce Oxidative Stress and Inflammation in Unilateral Ureteral Obstruction in Rats

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ABSTRACT

Renal fibrosis plays a significant role in the development of chronic kidney disease (CKD). Therefore, treatment of renal fibrosis is essential in reducing the incidence of CKD-related problems. Due to their function as anti-inflammatory and antioxidant agents, honey and *Nigella sativa* may have therapeutic promise in renal fibrosis. This study aim to investigate the effects of the combination of honey and *Nigella sativa oil* as anti-inflammatory and antioxidant agents in a rat model of unilateral ureteral obstruction (UUO)-induced kidney injury. In this study, thirty male Wistar rats were divided randomly into five groups. One sham group, one UUO group, and three UUO groups that received varied doses of the combination of honey and *Nigella sativa oil* (HoNi). HoNi was administered to the rats orally, once daily for 21 days starting from the 14th day post-UUO induction. After treatment, blood samples were collected, and the levels of antioxidant enzymes; Superoxide Dismutase (SOD), Glutathione (GSH), and Catalase (CAT) were measured. The inflammatory marker; C-reactive protein (CRP) activity, and haemoglobin (Hb) levels were also measured. The results showed that the administration of the combination of honey and *Nigella sativa* for 21 days in UUO rat model significantly reduced CRP, while increasing the activities of SOD, GSH, and CAT, and also enhanced haemoglobin levels in a dose-dependent manner.These findings suggest that the renoprotective effect of the combination of honey and *Nigella sativa* oil maybe associated with its anti-inflammatory and antioxidant properties.
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Keywords: *Unilateral Ureteral Obstruction, Honey, Nigella sativa, Anti-inflammatory, Antioxidant.*

Introduction

Chronic Kidney Disease (CKD) affects more than 10% of the global population and is related primarily to diabetes, hypertension, obesity, and advanced age.[1–3]Existing treatments for CKD are limited and insufficiently effective, prompting a quest for alternative therapies.[4] CKD progression is characterized by renal fibrosis involving inflammation, oxidative stress, pro-fibrotic mediators, myofibroblast activation, and excessive extracellular matrix (ECM) accumulation. These result in irreversible phenotypic changes, loss of parenchymal cells, reduced renal microvasculature, regenerative capacity, and metabolic alterations.[4–6]CKD also results in anaemia due to reduced endogenous erythropoietin (EPO) production, iron deficiency, and inflammation with elevated hepcidin levels.[7]Previous studies suggest natural substances like honey and *Nigella sativa* have potential benefits as anti-inflammatory, antioxidant, and antifibrotic agents in preventing CKD progression.[8–10]

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Honey, rich in antioxidants like quercetin, hesperetin, and chrysin, can inhibit cellular transcription factors, protecting against free radical damage.[8,11,12]*Nigella sativa* contains bioactive compounds such as thymoquinone, known for its anti-inflammatory and antioxidant properties.[11,13,14] Its effectiveness in healing renal injury in rats and fascilitating kidney stone removal in clinical studies has been demonstrated.[10,11]
The level of protection honey and *Nigella sativa* provides in UUO-induced renal fibrosis is unknown. This study investigates the anti-inflammatory, antioxidant, and anti-anaemic effects of honey and *Nigella sativa* on UUO-induced rats to better understand their potential as a dynamic pair.

Materials and Methods

Sample collection
Commercially available honey and *Nigella sativa* oil were obtained from a local market in Indonesia.

Experimental animals
Thirty (30) male Wistar rats were used for the study. The rats were acclimatized to the laboratory conditions for seven days. The study was approved by the Faculty of Medicine Ethics Committee, Sultan Agung Islamic University, with approval number 454/XI/2022/Bioethics Commission.

Animal grouping and UUO induction

The rats were randomly divided into five groups of six animals per group (n = 6). Group 1 served as the control group, and underwent a sham surgical procedure. Group 2 was the UUO group, and underwent unilateral ureteral ligation. Groups 3 – 5 represented the intervention group i.e. UUO rats that received a combination of Honey and *Nigella sativa*oil. Groups 3 – 5 were administered the combination of Honey and *Nigella sativa* oil(HoNi) as follows:

Group 3 (HoNi 1):received combination of honey (0.675 mL/200 g/day) and *Nigella sativa*oil(0.1 mL/200 g BW).

Group 4 (HoNi 2):received combination of honey (1.3 mL/200 g/day) and *Nigella sativa* oil(0.2 mL/200 g BW).

Group 5 (HoNi3): received combination of honey(2.025 mL/200 g/day) and *Nigella sativa*oil(0.4 mL/200 g BW).

UUO was induced by ligating the left ureter with two ligations. Each rat was anesthetized with ether, and a laparotomy was performed in the left quadrant of the abdomen. The left ureter was exposed and ligated with 4-0 silk sutures (in the UUO, UUO+HoNi 1,UUO+HoNi2, and UUO+HoNi3 groups). Laparotomy was also performed in the control group without ureteral ligation. The combination of honey and *Nigella sativa oil* was administered orally after 14 days post-ligation for 21 days.

Sample preparation

After 21 days of treatment, rats were anaesthesized by placing them in an ether chamber for 30-60 seconds. Blood samples (up to 3 cc) were collected from the ophthalmic vein into a red-capped vacutainer, and any remaining blood on the eye corner was swabbed. Subsequently, a non-heparinized microhematocrit was collected through the ophthalmic vein.

Measurement of antioxidant and anti-inflammatory activities

The collected whole blood sample was placed in the serum separator tube, and allowed to stand for 2 hours at room temperature or overnight at 2-8°C. This period allows natural blood coagulation, which slows down as the temperature drops. Following the coagulation period, the sample was centrifugedat 1000×g for 20 minto separate the components. The supernatant was collected for further analysis. Superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) levels were measured for assessing antioxidant activity, while the anti-Inflammatory activity was assessed by measuring the level of C-reactive protein (CRP) using an ELISA Fine-Test kit.

Measurement of anti-anaemic activity

Anti-anemia activity was assessed by measuring blood hemoglobin (Hb) levels using a Hematology analyzer.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences in mean values of parametric data were statistically analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. Statistical significance was considered when $p < 0.05$.

Results and Discussion

Chronic kidney disease often results from obstructive nephropathy, with the unilateral ureteral obstruction (UUO) model commonly used by researchers for its study via ureteral ligation. On day 14th after UUO induction, there was substantial tubulointerstitial damage in the kidney, including isolated tubular dilatation, flattened epithelium, atrophy, interstitial space enlargement with fibrosis, and inflammation.[6,15,16]C-reactive protein is a primary inflammatory marker in UUO rat model, driving early inflammation and renal fibrosis through FcgRI and TGF-b/Smad2/3 and NF-kB/p65 pathways.[17]In UUO rats, oxidative stress stimulates the Renin-Angiotensin System (RAS), activating the NF-κB cytokine transcription factor. NF-κB triggers the transcription of target genes, leading to inflammation and fibrosis. RAS induces fibrosis through the TGF-β/Smad and Wnt/β-catenin pathways, involving proteins like Snail1, Twist, Matrix Metalloproteinase 7 (MMP7), Plasminogen Activator Inhibitor 1 (PAI-1), and Fibroblast Specific Protein. Snail1, as a major regulator of Epithelial-to-Mesenchymal Transition (EMT), drives tubular epithelial cells to acquire a mesenchymal cell phenotype, promoting migration, apoptosis resistance, and increased extracellular matrix production, ultimately resulting in fibrosis.[18,19]

Antioxidant and anti-inflammatory activities of the combination of honey and Nigella sativa oil

In this study, a notable and statistically significant reduction in antioxidant levels (SOD, CAT, and GSH), along with hemoglobin levels, was observed 14 days after unilateral ureteral ligation (UUO) in comparison to the control (Sham Operation) group. Conversely, the inflammatory marker high-sensitivity C-reactive protein (hs-CRP) exhibited an increase. SOD, CAT, GSH, and Hb levels increased significantly in the groups that were treated with honey and *Nigella sativa oil* combination on day 36 after UUO induction. Conversely, the levels of Hs-CRP declined (Table 1).

Table 1: Effect of HoNi on antioxidant enzymes, inflammatory markers, and haemoglobin levels in rats

Groups	Time (Days)	SOD (%)	CAT (U/mg)	GSH (U/mg)	Hs-CRP (ng/mL)	Hb (g/dL)
Control (Sham operation)	D14	81.42 ±3.54*	5.93 ± 0.18*	7.59 ± 0.16*	3.05 ± 0.09*	12.99 ± 0.49*
	D36	76.50 ± 4.48$^{\#}$	5.99 ± 0.19$^{\#}$	7.55 ± 0.14$^{\#}$	3.21 ± 0.07$^{\#}$	13.11 ± 0.48$^{\#}$
UUO	D14	24.10 ± 3.32	1.80 ± 0.04	1.55 ± 0.06	17.95 ± 0.63	9.37 ± 0.22
	D36	25.31 ± 3.49	1.89 ± 0.04	1.62 ± 0.06	19.81 ± 0.33	9.18 ± 0.22
UUO+HoNi 1	D14	26.78 ± 3.54	1.66 ± 0.06	1.63 ± 0.04	18.05 ± 0.22	8.99 ± 0.20
	D36	42.62 ± 4.27$^{\#!}$	3.98 ± 0.11$^{\#!}$	4.98 ± 0.04$^{\#!}$	5.91 ± 0.16$^{\#!}$	10.34 ±0.13$^{\#!}$
UUO+HoNi 2	D14	23.22 ± 2.41	1.79 ± 0.06	1.64 ± 0.06	18.01 ± 0.09	9.08 ± 0.18
	D36	54.10 ± 5.29$^{\#!}$	5.07 ± 0.15$^{\#!}$	5.83 ± 0.11$^{\#!}$	4.99± 0.22$^{\#!}$	11.13 ± 0.12$^{\#!}$
UUO+HoNi 3	D14	25.96 ± 4.07	1.79 ± 0.06	1.62 ± 0.06	18.04± 0.07	8.92 ± 0.13
	D36	64.75 ± 3.06$^{\#!}$	5.75 ± 0.11$^{\#!}$	6.87 ± 0.11$^{\#!}$	4.01± 0.19$^{\#!}$	12.01 ± 0.17$^{\#!}$

Values are Mean ± SD (Standard deviation), n = 6.

UUO: Unilateral ureteral obstruction,HoNi: Combination of Honey and *Nigella sativa oil*, SOD: Superoxide Dismutase, GSH: Glutathione, CAT: Catalase, CRP: C-reactive protein, Hb: Haemoglobin.

*p<0.001 compared to UUO D14 group; #p<0.001 compared to UUO D36 group; !p< 0.05 compared to D14 of each group.

This study revealed that the UUO + HoNi groups exhibited significantly higher antioxidant levels than the UUO group. Increased doses of HoNi correlated with elevated antioxidant levels, particularly in SOD, CAT, and GSH levels. Conversely, the inflammation parameter, represented by hs-CRP levels, decreased in the UUO+HoNi groups at all the doses tested (Figure 1).

Honey contains primarily phenolic antioxidants, such as quercetin, hesperetin, chrysin, and Maillard reaction products known as melanoidins. Flavonoids and phenolic acids in honey are essential in its antioxidant and medicinal effects. The content of quercetin in honey has a preventive effect against nephrotoxicity through the suppression of inflammation, inhibition of NF-κB activation, suppression of cell membrane oxidation, inhibition of intracellular reactive oxygen species (ROS) production, and restoration of intracellular GSH.[9]

In healthy individuals, honey boosts the levels and activity of antioxidants such as beta-carotene, vitamin C, glutathione reductase, and uric acid. Although the exact antioxidant mechanism is not fully elucidated, potential actions include sequestering free radicals, hydrogen donation, metallic ion chelation, and flavonoids acting as substrates for hydroxyl and superoxide radicals.9 The primary components responsible for the pharmacological effects and therapeutic benefits of *Nigella sativa* include thymoquinone (TQ), thymohydroquinone, thymol, carvacrol, nigellidine, nigellimine, and α-hederin. Thymoquinone, an active quinone and the primary constituent of *Nigella sativa*, exhibits antioxidant properties and acts as a scavenger for free radicals and superoxide anions. It also demonstrates antibacterial effects and anti-inflammatory properties by inhibiting the cyclooxygenase and 5-lipoxygenase pathways. Interestingly, the fixed oil derived from N. sativa is more potent in antioxidant and anti-eicosanoid properties than thymoquinone alone.21,22 The study results support previous findings, as evidenced by the significantly higher values of SOD, GSH, and CAT. Similarly, C-reactive protein (CRP) level was statistically higher in the UUO + HoNi groups compared to the UUO group.

The anti-inflammatory property of honey results from down regulating inflammatory transcription factors (NF-kB and MAPK) and suppressing pro-inflammatory cytokine production. In addition, it stimulates the production of inflammatory mediators such as prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2).23

The therapeutic potential of N. sativa in addressing UUO-induced kidney damage is comparable to the effects of captopril and losartan. Likewise, thymoquinone (TQ) results in significant improvements in oxidative damage, apoptosis, and TNF-α expression. Unilateral ureteral obstruction (UUO), whether from physical blockage or congenital issues, induces tubular cell apoptosis due to stretching, ischemia, and oxidative stress. This heightened apoptosis leads to cell infiltration, interstitial cell proliferation, and fibrosis. In chronic kidney disease (CKD), the progressive depletion of tubular cells through apoptosis contributes to tubular atrophy and renal fibrosis.[21]Thymoquinone can also reduce pro-inflammatory factors such as nitric oxide (NO), nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2) by inhibiting the AP-1/NF-κB-related-IRAK pathway.20

The findings from this study agree with previous theories and research, confirming the anti-inflammatory properties of honey and *Nigella sativa*.

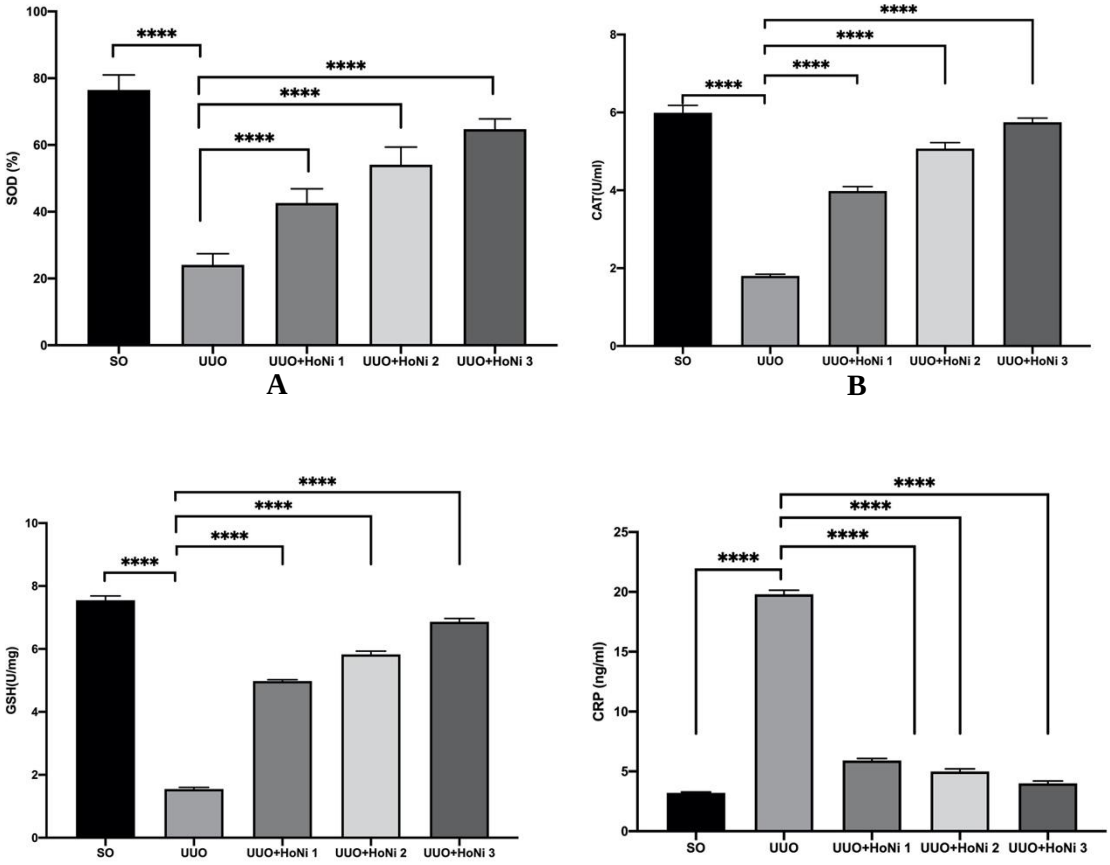

Figure 1: Comparison of antioxidant and anti-inflammatory activity of HoNi21 days after administration. A: SOD (%); B: CAT (U/mL); C: GSH (U/mg); D: CRP (ng/mL).****p<0.001 significantly different compared to the UUO group.
UUO: Unilateral ureteral obstruction; HoNi: Combination of Honey and Nigella sativa oil.

Anti-anaemia activity of the combination of honey and Nigella sativa oil

The Hb levels in the UUO + HoNi groups after 21 days of administration of HoNi increased significantly compared to the UUO group (Figure 2).

Although, honey does not have a direct anti-anaemia effect, it is known to have vitamins such as vitamin C that can help the absorption of iron. A previous study concludes that honey supplementation with iron increases hemoglobin levels more effectively in pregnant women compared to other groups treated only with iron supplement.24Nigella sativa is known to reduce oxidative stress markers and restore normal kidney functions.25 One vital function of the kidneys is the production of erythropoietin (EPO), which stimulates the bone marrow to generate red blood cells. Consequently, anaemia is a distinct feature of chronic kidney disease (CKD). The mechanisms leading to anaemia in CKD are intricate and encompass reduced endogenous erythropoietin (EPO) production, iron deficiency, and inflammation with elevated hepcidin levels.7 Although the data regarding the effect of Nigella sativa on EPO is still limited, a study done by El-Shanshory *et al*. (2019) shows that Nigella sativa was proven to increase blood haemoglobin level while at the same time decreased iron-induced oxidative stress and hemolytic anaemia.26 Another study also shows that 12-week Nigella sativa supplementation in CKD patients increases hemoglobin level significantly compared to the control group.[27]

The results of this study reinforce the theory about the anti-anaemic properties of honey and Nigella sativa. The UUO + HoNi groups showed a significantly higher hemoglobin level than the UUO group. The higher the honey and Nigella sativa dose, the higher the antioxidant, anti-inflammatory, and anti-anaemia properties.

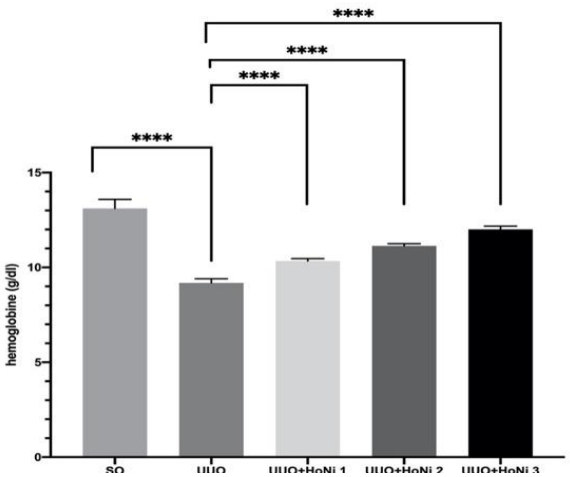

Figure 2: Anti-anaemic activity of HoNi 21 days after administration. ****p < 0.001 compared to UUO group. UUO: Unilateral ureteral obstruction; HoNi: Combination of Honey and *Nigella sativa oil*.

Conclusion

This study explored the potential of combining honey and Nigella sativa oil in preventing kidney fibrosis in UUO-induced rats. The outcomes demonstrated notable improvements, including heightened antioxidant levels, reduced inflammation, and enhanced haemoglobin levels in the HoNi groups compared to the UUO group. These findings encourage further exploration and investigation in human studies to confirm the effectiveness of this combination as a potential therapeutic approach for chronic kidney disease and its associated complications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be born by them.

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