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Research Article

Indonesian Propolis Extractattenuates Unilateral Ureteral Obstruction Induced Renal Damage by Reducing Oxidative Stress and Blood Pressure

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ABSTRACT

Ureteral obstruction may result in permanent kidney damage. Research suggests that the Indonesian Propolis Extract (IPE) play a strong role on free oxygen radicals removal and prevents oxidative stress. This study aims to investigate the efficacy of IPE on malondialdehyde (MDA) reduction and systolic blood pressure (SBP) level down-regulationafter unilateral ureteral obstruction (UUO).A total of 32 rats were divided into four groups. Group 1 as control, Group 2 were rats with UUO, Group 3 were rats with UUO that were given IPE (oral 50 mg kg^{-1}body weight) and Group 4 were rats with UUO that were given IPE (oral 100 $mgkg^{-1}$ body weight). SBP level were measured once every week within duration of experiment and at day30 blood sample were taken for Malondialdehyde analysis. Statistical analysis was performed by one-way analysis of variance. There were statistically significant increase in MDA and blood pressure in Group 2, while there were significant decrease for MDA and blood pressure in Group 3 and 4 ($p< 0.001$). In this experiment we suggest that IPE prevents kidney damage by decreasing oxidative stress (MDA) and SBP.

Keywords: Indonesian propolis, blood pressure, malondialdehyde, ureter obstruction.

INTRODUCTION

Chronic kidney disease (CKD) is a global public health problem which has substantial impact on morbidity, mortality, and health resource utilization. The progression of CKD is defined as a decrease in glomerular filtration rate regardless of primary disease. CKD is related to a variety of metabolic abnormalities including acidosis, hypertension, anemia, and mineral bone disease[1]. Accumulated findings reveal that chronic hypoxic stress is involved in a range of pathogenic conditions, including the progression of CKD. Chronic kidney hypoxia is induced by a number of pathogenic conditions, including renal ischemia, reduced peritubular capillary, and tubulointerstitial fibrosis[2].

Studies have revealed that renal ischemia and hypoxia are caused by microvessel loss during CKD. A unilateral ureteral obstruction (UUO) model of renal interstitial fibrosis (RIF) was established in mice[3]. Obstructive nephropathy is an important cause of end stage renal disease in children and adults. It results in a progressive and permanent loss in renal function that is characterized by interstitial inflammation and tubulointerstitial fibrosis. The acute phase of obstructed kidney in UUO is characterized by dramatic changes in glomerular filtration rate, renal blood flow, and interstitial edema. On the other hand, the chronic phase of the obstructed kidney is characterized by development of hydronephrosis, renal atrophy, interstitial fibrosis, and renal dysfunction[4].

Reactive oxygen species (ROS) are a recently recognized mechanism in the pathogenesis of UUO in experimental studies. Increased lipid peroxidation (LPO) has been reported in renal cortexes of UUO animals. It has been shown that oxidative stress in UUO contributes to the development of tubulointerstitial lesions and renal fibrosis. Various factors with complex cellular and molecular interactions have also been proposed as possible causes that lead to tubulointerstitial lesions and renal fibrosis[3,4].

Ethanolic extract of propolis has been used for centuries to confer health benefits in a number of inflammatory diseases. Due to the presence of compounds such as flavonoids, phenolic acids, and their esters, propolis exhibits anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant, and antiproliferative properties. ROS such as superoxide anions and hydroxyl radicals are scavenged by antioxidants present in propolis. In addition, the extreme reactivity of ROS toward lipids and proteins contributes to their rapid damaging capacity[5]. It has also been shown that propolis can suppress nuclear

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Table 1: Average weight difference before and after study (gram).

Group	mean±SD
control	33,63±1,92
UO	-17,50±1,77
UO + IPE 50 mg.kgBW^{-1}	5,13±1,64
UO + IPE 100 mg.kgBW^{-1}	14±2,13

Values are expressed as mean±SD for eight rats in each group.
SD: Standard deviation, UO: Ureteral obstruction, IPE: Indonesian Propolis Extract.

factor-κB (NF-κB) activation through a novel mechanism in vascular endothelial cells[6].
Recently, a great attention is being paid to lipid peroxidation, which actually is oxidative damage of lipids and increased creation of lipid peroxides, whose final product is malondialdehyde (MDA). Nowadays, MDA is used in many expert researches as oxidative stress marker, i.e. for assessment of lipid peroxidation. Oxidative stress marker, which bonds to malondialdehyde fast and strongly is Thiobarbituric Acid Reactive Substance (TBARS)[7].
As a result of these informations, in this study we investigated the possible inhibitory effects of ethanolic extract of Indonesian propolis against the UUO induced oxidative stress (MDA) and SBP in rat models.

MATERIAL AND METHODS

Indonesian propolis extract was dissolved in distilled water and administered via nasogastric gavage. The average of 0.2 mL diluted IPE contains 50 mg kgBW^{-1} and 100 mg kgBW^{-1} per day.
Male *Rattus Norvegicusrats* (200-300 g) were housed in clean plastic cages in a temperature and humidity-controlled facility with a constant 12 h light/dark cycle with free access to food and water. The use of animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council.
After one week acclimatization, UUO were performed. Briefly after induction of general anesthesia by intramuscular injection of ketamine (0.5 mg.kg^{-1} i.m), the abdominal cavity was exposed via midline incision and the left ureter was ligated at 2 points with 3-0 silk[8]. After a quarantine period of 7 days, 32 rats were randomly divided into four groups, each consisting of eight animals as follows: Rats in Group 1 as control; rats in Group 2 underwent unilateral ureteral ligation and received no treatment; rats in Group 3 were subjected to unilateral ureteral ligation and received IPE (50 mgkgBW^{-1}) for 30 days. Rats in Group 4 were subjected to unilateral ureteral ligation and received IPE (100 mg kgBW^{-1}) for 30 days.
Systolic Blood Pressure (SBP) was measured using tail cuff methodbefore the UUO and once every weekafter the procedure.
Twenty-four hours after the administration of last dose of IPE, on the 30th day, rats were anesthetized by intra-peritoneal injection of ketamine andblood samples were collected through cardiac puncture for serum levels of MDA measurement.
MDA referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm using a spectrofluorometer, as described previously.
Results of all groups were shown as mean values ± standard deviation. Statistical analyses of MDA and blood pressure levels were analyzed by the one-way analysis of variance. The significance between two groups was determined by the Dunnett's multiple comparison tests, and $P < 0.05$ was accepted as statistically significant value.

RESULT

There was difference significantly for body weight between groups (Table 1).
Effect of IPE on the body weight in each rat group is presented in figure 1. Body weight (BW) in control group

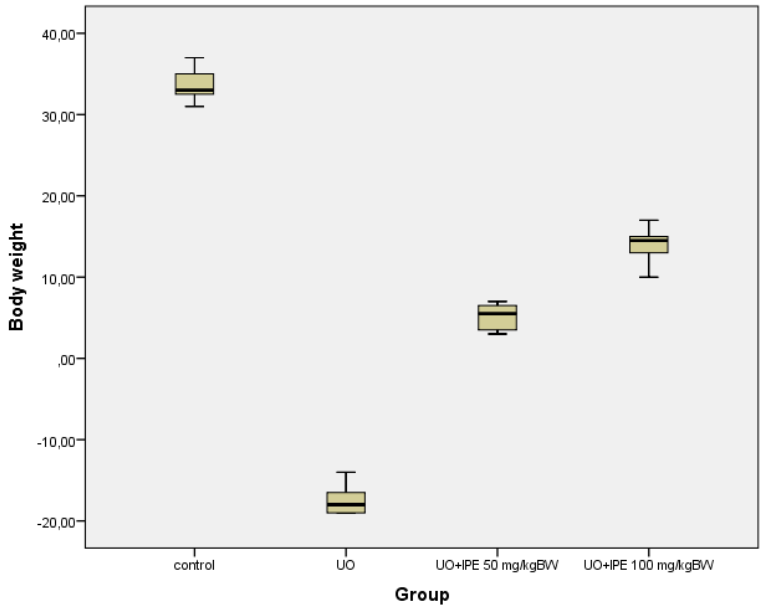

Figure 1: Effect of IPE on the body weight in each rat group.

Table 2: Effect of IPE on the levels of MDA and SBP in each rat group.

Parameter	Control	UO	UO + IPE 50 mg.kgBW^{-1}	UO + IPE 50 mg.kgBW^{-1}
MDA (nmol/mL)	1.62±0.14	9.09±0.43[a]	4.29±0.27	2.16±0.22
SBP (mmHg)	85.38±1.69	144.75±4.27[a]	107.13±4.09	93.13±3.98

Values are expressed as mean±SD for eight rats in each group.
[a]Significantly different from control, [s]ignificantly different from UO group (p <0.001)
SD: Standard deviation, UO: Ureteral obstruction, IPE: Indonesian Propolis Extract, BW: body weight, SBP: Systolic Blood Pressure

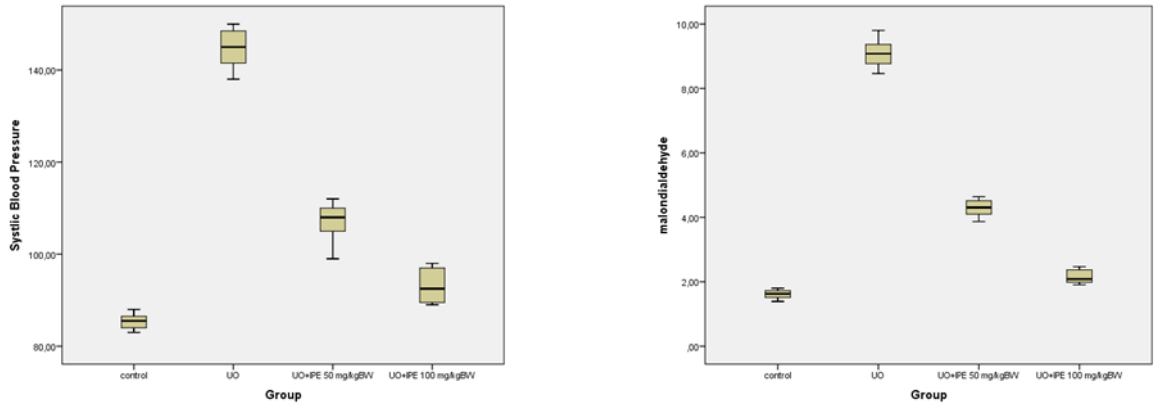

Figure 2: Effect of IPE on the SBP and MDA in each rat group.

increase by 33.63±1.92 gr at the end of the study in contrasttoratswhich received uretheric ligation (rat model CKD) showed significant decrease of BW (-17.50±1.77 gr vs 33.63± 1.92 gr, p <0.001). Indonesian Propolis extract with the dose of 50 mg.kgBW^{-1} in UO rats was able to increase the BW significantly (-17.50±1.77 gr vs 5.13±1.64 gr, p <0.001), as well as for IPE dose of 100 mg.kgBW^{-1} (-17.50±1.77 gr vs 14±2.13 gr, p <0.001). The results of this study also showed that increasing the dose of IPEwill also significantly increased BW (5.13± 1.64 gr vs 14±2.13 gr, p <0.001).

There was statistically significanttdifference ofSBP and MDA levels between groups (Table 2).

The mean of Systolic blood pressure (SBP) in control group was 85.38±1.69 mmHg. Ureter ligation in rats (rat model of CKD) in this study werefound to be able to increase the SBP significantly compared to the control group (144.75±4.27 mmHg vs 85.38±1.69 mmHg, p <0.001). Administration of 50 mg.kgBW^{-1}IPE to UO rats significantly decreased SBP (144.75±4.27 mmHg vs 107.13±4.09 mmHg, p <0.001), as well as for IPE dose of 100 mg.kgBW^{-1} (144.75±4.27 mmHg vs. 93.13±3.98 mmHg, p <0.001). The results of this study also showed an increase in IPE dose significantly decreased SBP almost to the level of the control rat (107.13±4.09 mmHg vs 93.13±3.98 mmHg, p <0.001).

The mean level of oxidative stress (MDA) in control rat was 1.62±0.14 nmol/mL. Rats that underwent ureter ligation (rat model of CKD) in this study, were shown to have significantly higher MDA levels compare to control(9.09±0.43 nmol/mL vs. 1.62±0.14 nmol/mL, p <0.001). IPE dosage of 50 mg/kgBW in rats of CKD model have significantly lower MDA levels (9.09±0.43 nmol/mL vs 4.29±0.27 nmol/mL, p <0.001), as well as for IPE dose 100 mg/kgBW (9.09±0.43 nmol/mL vs. 2.16±0.22 nmol/mL, p <0.001). This results also showed that increase in IPE dose significantly decreased MDA levels (4.29± 0.27 nmol/mL vs 2.16±0.22 nmol/mL, p <0.001). There was positive correlation between blood pressure and MDA values, respectively higher MDA value corelates withhigher blood pressure number (r=0.994, p <0.001).

This study confirmed the protective role of IPE on renal tissue damage after the induction of UUO in rats. Our results showed that the obstructed kidney cause significant weight loss, increase MDA and SBP levels. The current data demonstrate that UUO structural and functional alterations in the kidney with a concomitant increase in pro-inflammatory cytokines in the blood. IPE, on the other hand, reduced the severity of injury, depressed the concentration of these cytokines and increased the anti-oxidative capacity. It is evident from the increased bodyweight, decreased MDA and SBP.

Indonesian propolis extract is rich in antioxidants and anti-inflammatoriesof the polyphenolic class that includes quercetins and caffeic acid phenethyl ester/CAPE, that able to protect kidney cells from free radicals and lipid peroxidation[9]. The present study demonstrated ameliorative effects of IPE, a phenolic antioxidant and anti-inflammatory, on UUO-induced nephrotoxicity, in line with the consideration that oxygen-free radicals are important mediators of UUO-induced acute renal failure.

Unilateral ureteral obstruction increased renal angiotensin type 1 receptor (AT1R), nuclear factor (NF)-κB, monocyte chemotactic protein 1 (MCP-1), and fibronectin expression. Through binding to its receptor AT1R, angiotensin II activates NF-κB and other downstream mediators, thereby inducing inflammation and fibrosis, which are thought to be directly related to the pathogenesis of UUO[10]and hypertension[11].

The role of the renin–angiotensin system (RAS) in the pathogenesis of hypertension and other cardiovascular diseases is widely acknowledged. However, the traditional view that Ang II is the sole key effector peptide of the RAS has been questioned by the subsequent discovery of angiotensin-converting enzyme 2 (ACE2) and the growing evidence for a physiological role for Angiotensin-(1–7). Angiotensin-converting enzyme 2 (ACE2) is a homologue of the angiotensin-converting enzyme (ACE) that catalyzes Ang II into Ang-(1–7). Angiotensin-(1–7) is a peptide that binds to the G-protein-coupled receptor Mas (MasR) and initiates vasodilator and anti-proliferative responses. Also, Ang-(1–7) antagonizes the cardiovascular actions of Ang II[11,12]. RAS is associated with hypertension in the spontaneously hypertensive rats (SHR). Cardiac ACE2 was suppressed and ACE upregulated in the SHR compared to WKY rats. Moreover, it was implicated in pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β both centrally and in the periphery[11].

When the kidney is exposed to hypoxia (UUO), the expression of some genes changes. The master regulator of the adaptation to hypoxia is hypoxia inducible factor (HIF), a transcription factor. HIF is composed of an α-subunit (HIF-1α,2α,3α) and β-subunit [HIF-1β/AhR nuclear translocator (ARNT)]. Although HIF-1β is constitutively expressed, HIF-α members are degraded in normoxic conditions. HIF-α is hydroxylated by a prolyl hydroxylase domain-containing protein (PHD), and the binding of HIF–α protein to the von Hippel Lindau protein (pVHL) results in ubiquitination and degradation. Under hypoxia, HIF-α escapes this degradation and dimerizes with HIF-1β. The dimer translocates into the nucleus and binds to the hypoxia-response element (HRE) of HIF-target genes. This results in the activation of target genes involved in angiogenesis, erythropoiesis, and glycolysis[2,13].

Malondialdehyde, a stable lipid hydroperoxide, provides an index of the peroxidation of lipids (LPO) in biological tissues. In the present study, we found increased MDA levels in UUO group and as protective effect of IPE lower MDA levels in group determined by UUO+IPE. These findings strongly indicate that IPE is important in protecting the kidney from UUO-induced injury through improvement in oxidant status.

In this study, the MDA and SBP levels showed severe and extensive damage in UUO rats. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by UUO. The kidneys of the control group showed normal MDA and SBP level features. On the other hand, the MDA and SBP levels from rats of the UUO+IPE group were nearly normal in MDA and SBP levels. That there was positive correlation between blood pressure value and MDA values, respectively higher MDA value had higher value of blood pressure.

CONCLUSION

The results reported here indicate that PE exerts a preventative effect on UUO-induced kidney damage in rats by reducing oxidative stress. We therefore propose that PE supplementation therapy can be used for kidney protection in patients with UUO, such as ureteral stones. However, further animal and clinical studies are needed to confirm our suggestion.

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