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Research Article

ANTIHYPERTENSIVE AND SUPEROXIDE DISMUTASE EFFECT OF INDONESIAN PROPOLIS EXTRACT IN KIDNEY DAMAGE

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A B S T R A C T

Loss of redox homeostasis and formation of excessive free radicals play an important role in the pathogenesis of kidney disease and hypertension. Redox control of kidney function is a dynamic process with reversible pro– and anti-free radical processes. The imbalance of redox homeostasis within the kidney is integral in hypertension and the progression of kidney disease. Oxidative stress is associated with kidney and vascular defects leading to hypertension and atherosclerosis, being superoxide dismutase (SOD) one of the main intracellular antioxidant defence mechanisms. Ureteral obstruction may result inpermanent 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 onsystolic blood pressure (SBP) levelreduction and increased levels of SOD after 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 day 30 blood sample were taken for SOD analysis. Statistical analyses was performed by one-way analysis of variance. There were statistically significant increased SBP and decreased SOD in Group 2, while there were significant decrease for SBP and increase for SOD in Group 3 and 4 ($p< 0.001$). In this experiment we suggest that IPE prevents kidney damage by decreasing SBP and increasing SOD.

INTRODUCTION

Oxidative stress and impaired endogenous antioxidant defense are associated with hypertension and chronic kidney disease (CKD)[1]. Hypertension is the second most common cause of CKD and represents the primary risk factor of its progression[2]. Further, increased production of reactive oxygen species (ROS) and other free radicals are also involved in unilateral ureteral obstruction (UUO)[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[3,4]. 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].

Although the therapeutic focus has shifted to regenerative cell-based agents, the lack of a comprehensive understanding of the pathogenesis of renal scar formation following injury remains a major challenge to the development of effective therapeutic strategies. Known factors in the pathophysiology of renal obstructive parenchymal injury include renal blood flow impairment, intrapelvic pressure elevation, and vasoactive and inflammatory mediators. Recently, it has been suggested that ROS, which are formed during ureteral obstruction, may play a role in this process[5]. Endogenous defense system involved in neutralization of ROS includes

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antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px)[2].

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 capacity6. It has also been shown that propolis can suppress nuclear factor-κB (NF-κB) activation through a novel mechanism in vascular endothelial cells[7].

This experimental study was designed to produce ischemia-reperfusion injury in rat kidney by performing UUO and investigated the effects of ethanolic extract of Indonesian propolis on the levels of antioxidant enzymes injury parameter and systolic blood pressure (SBP).

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 Norvegicus Rats* (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 silk8. 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 method before the UUO and once every week after 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 and blood samples were collected through cardiac puncture for serum levels of SOD measurement.

SOD 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 SOD 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

Systolic blood pressure at baseline between groups showed no difference.The first week after UUO seen a significant increase in SBP. The effect of IPE on the decrease in SBP was seen in the second week. The effect of IPE on each groups on SBP per week is shown in Figure 1.

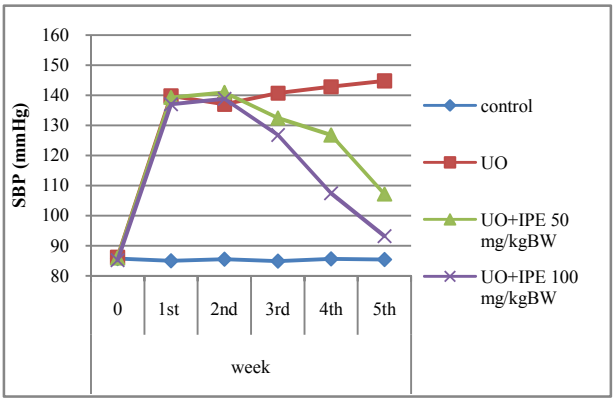

Figure1 Kinetic ofSBPper week on each groups

The mean of 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) in 1st week. Administration of 50 mg.kgBW-1IPE 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).

There was statistically significant difference of SBP between groups (Table 1).

Table 1 Effect of IPE on the levels of SBP in each rat group.

Parameter	Control	UO	UO + IPE 50 mg.kgBW-1	UO + IPE 50 mg.kgBW-1
SBP (mmHg)	85.38±1.69	144.75±4.27a	107.13±4.09	93.13±3.98

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

The mean level of antioxidant enzyme (SOD) in control rat was 74.56±4.30 U/mL. Rats that underwent ureter ligation (rat model of CKD) in this study, wereshownto have significantly lowerSOD levels compare to control (17.76±5.09U/mLvs. 74.56±4.30 U/mL, p <0.001). IPE dosage of 50 mg/kgBW in rats of CKD model have significantly increasesSOD levels (44.30±4.94U/mL vs 17.76±5.09U/mL, p <0.001), as well as for IPE dose 100 mg/kgBW (62.72±4.57 U/mL vs 17.76±5.09U/mL, p <0.001). These results also indicate that the increase in IPE doses significantly increases SOD levels (62.72±4.57 U/mL vs 44.30±4.94U/mL, p <0.001). There was negative correlation between blood pressure and SOD values,

respectively lowerSOD value corelates withhighersystolic blood pressure(r=–0.960, p <0.001).

There was statistically significantdifference of SOD levels between groups (Table 2).

Table 2 Effect of IPE on the levels of SOD in each rat group.

Parameter	Control	UO	UO + IPE 50 mg.kgBW-1	UO + IPE 50 mg.kgBW-1
SOD(U/mL)	74.56±4.30	17.76±5.09a	44.30±4.94	62.72±4.57

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

Depending on the duration and severity of obstruction UUO may cause detrimental changes on glomerular filtration rate (GFR) and renal blood flow (RBF). Gillenwater *et al*. denoted that 4-28 days after partial unilateral ureteral obstruction (PUUO) GFR was reduced to 20-70% of normal levels. They also reported that in PUUO condition, RBF was decreased to 25% of normal levels. Many studies proved that monocystic infiltration of the interstitial compartments was associated with renal injury. In obstructive nephropathy, leucocyte infiltration (particularly macrophages) occurs in paranchymal space within 24 hours of the obstruction. These macrophages are capable of releasing different products such as proteolytic enzymes, ROS, platelet derivated growth factor, cyclooxygenase, and lipooxygenase products and these products play an active role in establishment of interstitial fibrosis due to PUUO[8].

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 increase SBP and peroxidation of lipids (MDA)[9], on the other hand decrease SOD 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.

Indonesian propolis extract is rich in antioxidants and anti-inflammatories of the polyphenolic class that includes quercetins and caffeic acid phenethyl ester/CAPE, that able to protect kidney cells from free radicals and lipid peroxidation 10. 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.

Reactive oxygen species (ROS; e.g. superoxide, hydrogen peroxide and hydroxyl radical) are intermediary metabolites that are normally produced in the course of oxygen metabolism. ROS can destroy proteins, lipids, nucleic acids, carbohydrates and other molecules, and cause inflammation, apoptosis, fibrosis and cell proliferation. However, under normal conditions, ROS play a critical role as signal molecules and ROS produced by activated leucocytes and macrophages are essential for defense mechanism against invading microorganisms. ROS are generated by mitochondrial cytochrome oxidase, nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, lipooxygenase, cyclooxygenase, hemeoxygenase, cytochrome P-450 enzymes, nitric oxide synthase (NOS), and various other oxidase enzymes. Additionally, ROS may lead to severe injury to the cell membrane by lipid peroxidation reactions and may have an important role in tubulointerstitial inflammation associated with obstructive nephropathy[8]. Membrane lipid peroxidation may generate reactive carbonyl compounds such as MDA, one of the reliable indicators of ROS-induced ischemia–reperfusion tissue damage.Unilateral ureteral obstructionincreased 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[7, 8] and hypertension[11].

Ischemia–reperfusion (I/R) injuries are well known in surgical practice, but I/R injury in obstructive nephropathy has not been extremely studied yet. Recent reports indicate that the release of ureteral obstruction leads to enhanced renal production of ROS. In this study, we observed that recovery of UUO induced elevations in ROS levels which played role in mechanism of renal paranchymal injury. Antioxidant enzyme levels rised to compensate the ROS simultaneously and this was an indirect indicator of increased ROS levels. Elevated SBP and MDA levels9 and lowering SOD levels in blood of UUO group compared to controls (P<0.001) showed that ureteral obstruction produced an ischemic event in renal tissue after UUO.

In this study, the SBP and SOD 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 SBP and SOD level features. On the other hand, the SBP and SODlevels from rats of the UUO+IPE group were nearly normal in SBP and SOD levels. That there was negative correlation between SBP and SOD values, respectively lower SOD value had higher value of blood pressure.

CONCLUSION

The results reported here indicate that IPE exerts a preventative effect on UUO-induced kidney damage in rats by increasing antioxidant enzyme. We therefore propose that IPE 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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