

THE PROTECTIVE EFFECT OF SARANG SEMUT (*Myrmecodia tuberosa*) TUBERS INFUSION ON GENTAMICIN-PIROXICAM INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

Kidney is very important organ for blood filtration. Impaired renal function often results in chronic kidney disease. Sarang semut (*Myrmecodia tuberosa*) is traditionally used to cure various diseases. This research was conducted to determine the effect of sarang semut infusion in nephrotoxicity rats models by gentamicin-piroxicam. Thirty male Wistar rats were divided into 6 groups. Group I as normal control (CMC Na was treated orally for 7 days), group II was treated sarang semut infusion dose of 1g/kgBW orally for 7 days, group III as nephrotoxic control was injected by gentamicin 100mg/kgBW intraperitoneally and piroxicam 3.6mg/kgBW orally daily for 7 days and group IV until VI were rats simultaneously treated with sarang semut infusion at the dose of 1, 2, and 4g/kgBW, respectively and induced by gentamicin-piroxicam daily for 7 days. Parameters of kidney function such as serum creatinine and blood urea nitrogen (BUN) levels were measured on day 0 and 7 using spectrophotometer. The rats were then sacrificed, kidneys tissue were removed for examination of renal index and kidney histology. These results showed that sarang semut tubers infusion at the dose of 1, 2, and 4g/kgBW reduced BUN and creatinine levels on induced-nephrotoxic rats. Creatinine level was reduced significantly to 1.76 ± 0.13 ; 0.86 ± 0.15 ; 0.60 ± 0.07 mg/dL, respectively. The creatinine level of nephrotoxic control was 5.16 ± 0.27 mg/dL. BU level decreased significantly to 55.66 ± 6.22 ; 18.49 ± 2.61 ; 16.81 ± 1.24 mg/dL, respectively. Meanwhile the BUN level of nephrotoxic control was 276.65 ± 50.52 mg/dL. Rat histological profile of the three doses treatment showed improvement in renal cell after administration of sarang semut infusion for 7 days.

Key words: *Myrmecodia tuberosa*, blood urea nitrogen, creatinine, gentamicin-piroxicam

INTRODUCTION

Kidney is very important organ particularly for filtering blood to discard unnecessary substances. The kidney showed many essential functions in the body such as remove waste products of metabolisms, maintain fluid and electrolyte balances and also the homeostasis system (Kelly, 2004). Impaired kidney function often result in Chronic Kidney Disease (Dowling, 2005).

Chronic renal failure is a serious problem in the world, in both developed and developing countries, which currently increased substantially. From a survey conducted by

PERNEFRI (Association Nephrology Indonesia) in 2009, the prevalence of chronic renal failure in Indonesia approximately 12.5% which means that there are 18 million adults in Indonesia suffered from chronic renal failure (PERNEFRI, 2009).

Gentamicin is an aminoglycoside antibiotic widely used in treating severe Gram negative bacterial infection. The clinical use is limited due to nephrotoxicity effect. There are some evidence indicate that free radical are important mediators of gentamicin toxicity. Gentamicin may induce the formation mechanism of reactive oxygen species (free radicals)

(Sukandar *et al.*, 2011). More severe renal damage can be induced by a combination of NSAIDs (Non Steroid Antiinflammation Drugs) and nephrotoxic drugs (Hosaka *et al.*, 2004).

Renal replacement therapy such as dialysis and kidney transplantation, as well as erythropoietin therapy can inhibit the increased of the disease severity, but requires an expensive cost. Therefore, it is needed to explore agents capable to inhibit the progression of chronic renal failure, which is safe and inexpensive, because kidney disease is a chronic disease that requires longterm treatment (Yokozawa *et al.*, 2002)

Sarang semut contains several chemical compounds such as flavonoids, tannins, and polyphenols having antioxidant activity. Moreover, since the extract of sarang semut capable to inhibit the activity of xanthine oxidase and uric acid, this might improve renal function (Simanjuntak *et al.*, 2010). Abdel-Raheem (2009) proved that the flavonoid quercetin has a protective effect in gentamicin-induced nephrotoxic rats. The flavonoid compounds identified in sarang semut, were kaempferol, luteoline, rutin, quercetin and apigenin (Engida, *et al.*, 2013). Therefore, it was necessary to do research on the protective effects of sarang semut infusion in gentamicin-piroxicam - induced kidney damaged rats.

MATERIAL AND METHODS

Sarang semut tuber (UD. Fira Papua), gentamicin injection (PT. Indofarma), piroxicam capsul (PT. Sampharindo Perdana), reagent kit urea FS* (DiaSys Diagnostic System GmbH cat. 131019910021), Creatinine FS* (DiaSys Diagnostic System GmbH cat. 117119910021), distilled water, CMC Na, and formalin 10%.

Animal testing

Healthy Wistar male rats (from Faculty of Pharmacy Gadjah Mada University), aged 2-3 months, weight 150-250g.

Experimental procedure

Making infusion of sarang semut

Sarang semut infusion was made with concentrations of 8; 16 and 32%w/v. Dried sarang semut each weighed 8; 16 and 32g, were put in different infusion pans. Water as much as 2 times the weight of dried sarang semut was

added to tend the dried material. After addition of 100mL water the mixture was then heated for 15min at 90°C on waterbath, while stirred occasionally. The mixture was filtered, while still hot, with flannel. Additional hot water was poured through the dregs to obtained desire volume. The infusion was administered to the rats with volume of 2.5mL/200gBW (equal with the dose of 1; 2; and 4g/kgBW, respectively)

Induction nephrotoxic in rats

Induction of nephrotoxic in rats were carried out using gentamicin 100mg/kgBW (i.p) and piroxicam 3.6mg/kgBW (p.o) (Sukandar, *et al.*, 2011). Eventually, 100mg/kgBW gentamicin was injected intraperitoneally (i.p) daily during 7days whereas piroxicam 3.6mg/kg BW was given peroral (p.o) daily for 7days. Rats was considered to be nephrotoxic if blood urea nitrogen (BUN) and creatinine levels increased significantly compared to that of the control group.

Nephroprotective test

Thirty rats were divided into six treatment groups. Each group consisted of five rats. Group I as normal control (CMC Na was administered orally for 7days); group II was treated with sarang semut infusion at the dose of 1g/kgBW orally for 7days; group III as nephrotoxic control was injected with gentamicin 100mg/kgBW intraperitoneally and piroxicam 3.6mg/kgBW orally daily for 7days; group IV until VI, were rats simultaneously treated with sarang semut infusion at doses of 1, 2, and 4g/kgBW, respectively, and induced by gentamicin-piroxicam daily for 7days. Blood was taken from the lateral tail vein on day 0 and 7 for measuring serum creatinine and BUN levels. Later in the day 7, rats were sacrificed and the kidney were taken for determination of renal index and histological evaluation.

Determination of renal index

Renal index is calculated by comparing the kidney weight to body weight of rats.

Determination of BUN level

BUN level was determined using urea FS* (DiaSys) kit reagent. BUN level was measured with UV spectrophotometer at the wavelength (λ) of 340nm.

BUN levels (mg/dL) = concentration of urea (mg/dL) x conversion factor (0.467).

Determination of serum creatinine level

Serum creatinine level was determined using creatinine FS* (DiaSys) kit reagent. It was measured with spectrophotometer at the wavelength (λ) of 546nm.

Statistical analysis

Data of BUN and creatinine levels were analyzed by one way ANOVA test followed by post-hoc LSD (Least Significant Difference) with 95% confidence level using the SPSS program.

RESULTS AND DISCUSSION

Effect of sarang semut infusion on renal function improvement in gentamicin-piroxicam induced nephrotoxicity rats

Sarang semut tubers infusion have effect on the improvement of renal function in gentamicin-induced renal piroxicam using parameters of serum creatinine, Blood Urea Nitrogen (BUN) blood level, renal index and histology of kidney. The higher levels of creatinine and urea in the blood indicating reduction of kidney function.

The normal value of creatinine in blood is less than 1.2mg/dL. On day 0 (base line), all groups showed the serum creatinine level in normal range, it mean that the serum creatinine on day 7 increase due to the treatment. Group III as nephrotoxic control which was only induced by gentamicin and piroxicam (GP) showed an increase serum creatinine level approximately 11 times compared to that of normal values on day 0 (0.46 ± 0.09)mg/dL become (5.16 ± 0.27)mg/dL on day 7 (Figure 1).

Serum creatinine on day 7 in control CMC Na group and sarang semut infusion groups, with the dose of 1g/kgBW; 2g/kgBW; and 4g/kgBW, showed significance different to nephrotoxic control which was only induced by gentamicin-piroxicam (GP) ($p < 0.05$). This result showed that the treatment of sarang semut infusion for 7days could decrease the creatinine level, but only sarang semut infusion at dose of 2 and 4g/kgBW that showed no significant differences ($p > 0,05$) compared to that of normal control group. This mean that sarang semut infusion at doses of 2 and

4g/kgBW could reduce serum creatinine back to normal level. On the other hand based on Pearson test correlation indicated that among the treatment of sarang semut infusion at doses of 1, 2 and 4g/kgBW, there are significant differences ($p < 0.05$) with Pearson correlation 0.930 (strength of the correlation is very strong). This gave information there are a dose dependent trend of sarang semut infusion in lowering serum creatinine.

The normal value of blood urea nitrogen (BUN) is 8-25mg/dL. On day 0 (base line), all groups showed the BUN in normal range, indicating that the increased of BUN on day 7 due to the treatment. Group III, as nephrotoxic control, demonstrated an increase of BUN level approximately 13times, from (21.16 ± 2.00) on day 0 to (276.65 ± 50.52) mg/dL on day 7 (Figure 2). Blood Urea Nitrogen (BUN) in control CMC Na group and treatment groups of sarang semut infusion at doses of 1; 2; and 4g/kgBW showed significance different with nephrotoxic control ($p < 0.05$). This result indicated that sarang semut infusion could decrease BUN level. However, only sarang semut infusion at the doses of 2 and 4g/kgBW that showed no significant difference ($p > 0,05$) compared to that of normal control group. The result indicated that sarang semut infusion at doses of 2 and 4g/kgBW could reduce BUN to normal level. Meanwhile, between the treatment infusion 2g/kgBW and 4g/kgBW showed no significant difference ($p > 0.05$), meaning that the effect of lowering BUN at dose of 2 and 4g/kgBW were equivalent. Meanwhile according to the Pearson test correlation demonstrated that among the treatment of sarang semut infusion at doses of 1, 2 and 4g/kgBW, there are significant difference ($p < 0.05$) with Pearson correlation 0.868 (strength of the correlation is very strong). This indicated that there were a dose dependent trend of sarang semut infusion in decreasing BUN.

The administration sarang semut infusion alone to rats for 7 days did not exhibit any significant change in BUN and serum creatinine parameters (Figure 1 and 2).

Sarang semut infusion was also capable to reduce renal index. The nephrotoxic control showed the highest renal index among the

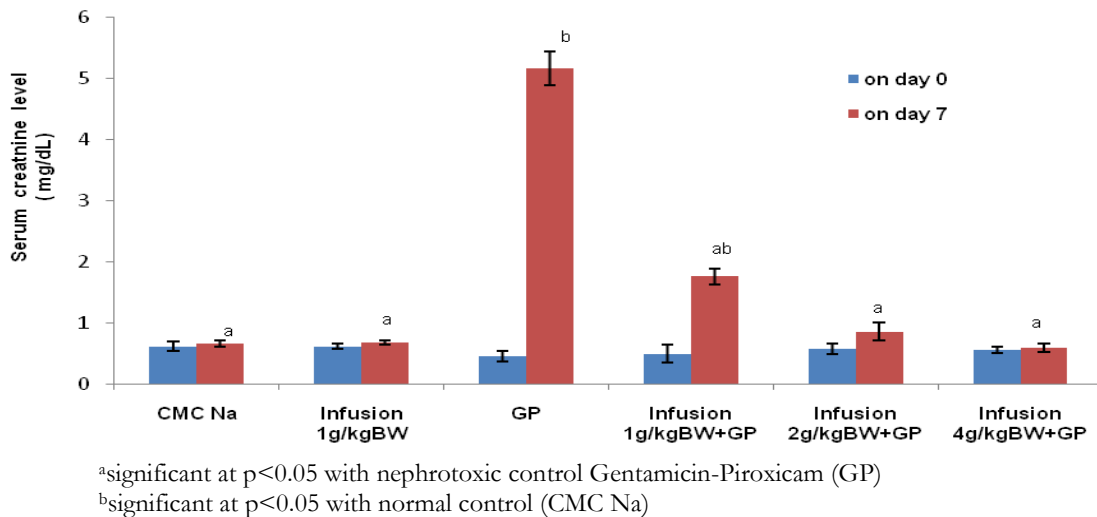


Figure 1. Serum creatinine (mean±SD) on day 0 and after treatment of sarang semut infusion for 7 days in induced by gentamicin-piroxicam rats (GP) (n=5).

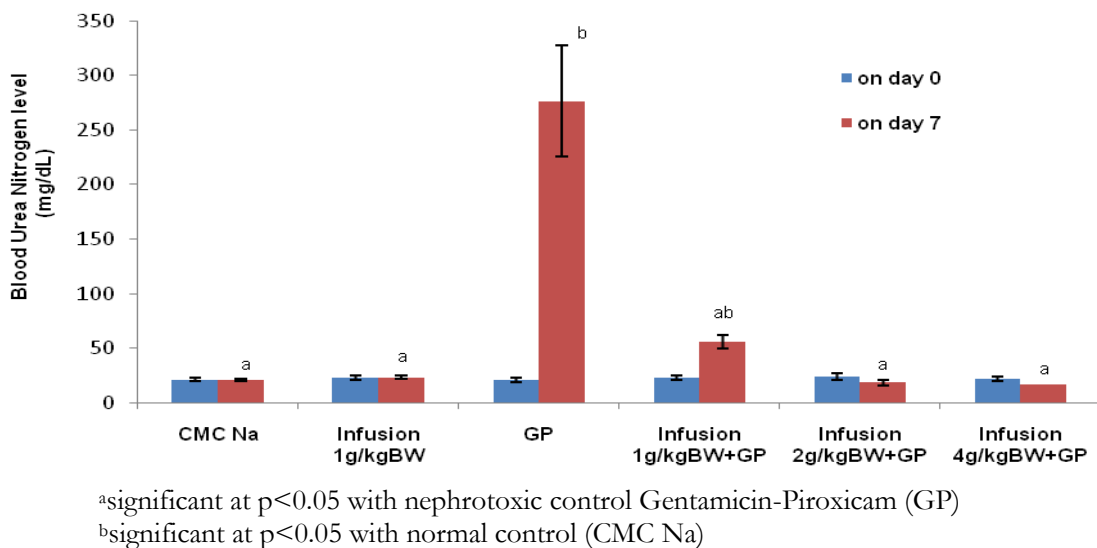


Figure 2. Level of BUN (mean±SD) on day 0 and after treatment of sarang semut infusion for 7 days in induced by gentamicin-piroxicam rats (GP) (n=5).

other groups and significantly different from the treated infusion dose of 1, 2 and 4g/kgBW ($p < 0.05$) (Figure 3).

Histology of kidney

Histological examination revealed that in nephrotoxic group (Figure 4b) (induced by gentamicin-piroxicam only), showing nephrosis characterized by eosinophilic homogeneous mass in the lumen of the tubular part, partly tubular epithelial degeneration and necrosis. On

the other hand in Figure 4c and 4d the damaged of glomeruli and tubuli could return to normal or similar to normal control (Figure 4a).

The induction of kidney damage using gentamicin is one of the most common methods used at present time. Gentamicin is an aminoglycoside antibiotics which have side effects such as nephrotoxic. Mechanism of nephrotoxicity probably is caused by the formation of Radical Oxygen Species (ROS)

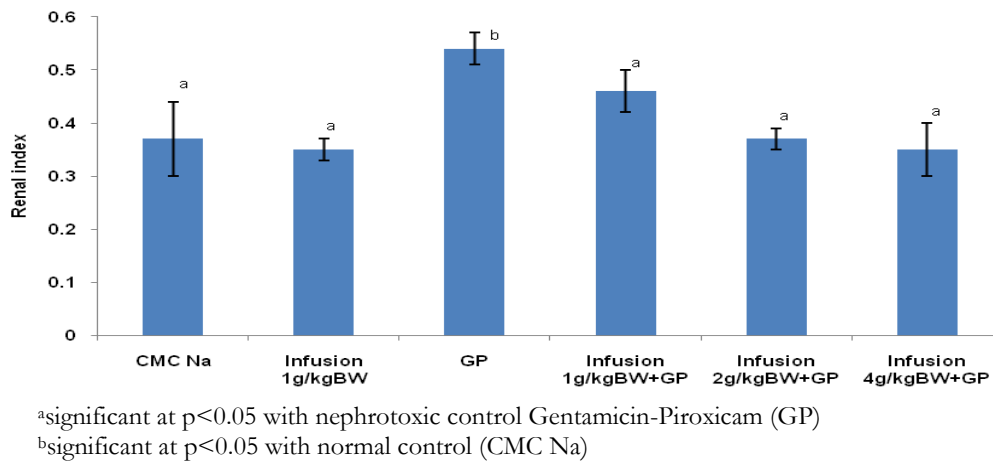


Figure 3. The effect of sarang semut infusion on renal index profile (Mean ±SD) (n=4)

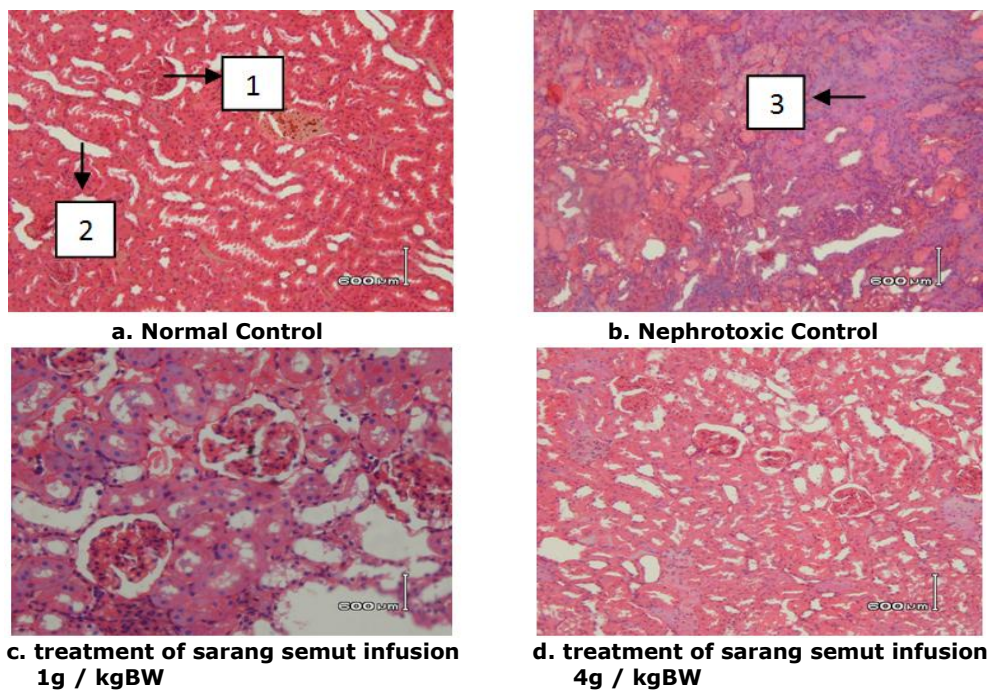


Figure 4. Kidney's histology with hematoxilin and Eosin staining. (1) normal glomeruli, (2) normal tubuli, (3) nephrosis (indicated by eosinophilic homogeneous mass in the lumen of the tubular part, partly tubular epithelial degeneration and necrosis)

and nitrosative which induce nephrotoxic. It is shown that O_2^\bullet , OH and H_2O_2 are involved in the renal damage induced by gentamicin (Petronilho *et al.*, 2009; Poormoosavi *et al.*, 2010).

The significant increase in lipid peroxide (TBARS) levels and decrease in glutathione

(GSH) levels, superoxide dismutase (SOD) and catalase (CAT) activities, showed that gentamicin induced nephrotoxicity was mediated through oxidative stress reactions (Abdel-Raheem *et al.*, 2009). Gentamicin causes an increase in the concentration of biochemical renal function such as serum urea and

creatinine along with the total protein excreted in the urine (Abdel-Raheem *et al.*, 2010). These changes lead to nephrotoxic as evidenced by the decrease in glomerular filtration rate (GFR) (Ozbek, *et al.*, 2009).

Other drugs that having toxic effects on the kidney were the class of NSAIDs (Non Steroid Anti-Inflammation Drugs) such as piroxicam. The toxic effects were associated with the inhibition of prostaglandin causing imbalance compensation in the form vasodilatory leading to the damage of glomerular filtration rate (Hosaka *et al.*, 2004)

Previous studies have shown that *Myrmecodia pendan*, which have similar genus to *Myrmecodia tuberosa*, contain flavonoids. The flavonoid compounds identified were kaempferol, luteoline, rutin, quercetin and apigenin (Engida *et al.*, 2013). Flavonoids have benefits for corresponding plants and humans who consume them. Flavonoids contained in the sarang semut infusion are suspected as an antioxidant that can neutralize Reactive Oxygen Species (ROS) which are formed from induction gentamicin. Flavonoids content in the sarang semut infusion persumably was polar flavonoid, rutin. Rutin is a glycoside flavonoid widely distributed in plant kingdom and contains quercetin as its aglycon part and rutinose as glycon part (Sreedevi *et al.*, 2010). The previous study showed that rutin worked as an antioxidant in vivo by scavenging ROS. This property might serves to prevent oxidative renal damage in rat treated with potassium bromate (Khan *et al.*, 2012). Rutin have also shown protective and beneficial effects on gentamycin induced oxidative stress in rats (Akondi *et al.*, 2011). Beside that, in other research showed that simultaneously administration of quercetin and gentamicin protected kidney tissues against nephrotoxic effects of gentamicin, as proved from improvement of histopathological profile and normalization of kidney biochemical parameters such as creatinine and BUN (Abdel-Raheem *et al.*, 2009)

Another study proved that sarang semut showed other biological activity such as an immunomodulator (Hertiani *et al.*, 2010). Sarang semut water extract showed anticancer activity on HeLa cells and MCM-B2 greater

than that of non-polar extract (Soeksmanto *et al.*, 2010), water extract also has antioxidant and cytotoxic activity with IC₅₀ of 30.66mg/mL (Bustanussalam, 2010).

The data of this research showed that the sarang semut infusion at dose of 1, 2 and 4g/kgBW had the nephroprotective effects. They could reduce the level of BUN and serum creatinine compare to the nephrotoxic control group ($p < 0.05$). However, at dose of 1g/kgBW, the decline did not return to normal values. Meanwhile, sarang semut infusion at doses of 2 and 4g/kgBW could prevent impaired kidney function characterized by the return of BUN and creatinine levels decreased to normal levels as before the nephrotoxic induction by gentamicin-piroxicam.

CONCLUSION

Sarang semut tuber infusion at doses of 1, 2, and 4g/kgBW could improve the kidney function of rats based on parameters of BUN, creatinine blood levels, renal index and histopathology of kidney in rats induced-nephrotoxic by gentamicin-piroxicam.

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