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Study of Kidney Repair Mechanisms of Corn Silk (*Zea mays* L. Hair)-Binahong (*Anredera cordifolia* (Ten.) Steenis) Leaves Combination in Rat Model of Kidney Failure

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Abstract: Earlier studies from our laboratory have indicated renal function improving action of corn silk and binahong in gentamicin-piroxicam induced kidney failure. This study was aimed to determine the effects of combination of cornsilk and binahong extracts on kidney failure model in rat and the effects of the extract combination on oxidative stress. Rats were divided into the positive control group, the group treated with 75 mg kg⁻¹ of corn silk, the group treated with 100 mg kg⁻¹ b.wt. of binahong, two groups treated with graded doses of combination of corn silk and binahong and the negative control group. Serum creatinine, urea, organ-to-body weight ratio of the kidney (kidney index) and renal histology were assessed to determine renal function. Meanwhile, the activities of lipid peroxidation, catalase and Superoxide Dismutase (SOD) were measured to analyse oxidative stress level. Administration of combination of the extracts at half dose resulted in marked depletion of serum creatinine and urea which was comparable to the results in corn silk-and binahong-treated groups. In addition, the extract combination was shown to reduce kidney index compared to that of the positive control group. The combination was further revealed to reduce renal damage histologically. Administration of the extract combination was demonstrated to attenuate kidney oxidative stress as shown by the reduction in lipid peroxidation and the increased activity of antioxidant enzymes, such as catalase and SOD. Taken together, results of this study suggest that corn silk in combination with binahong possesses renal function improving activity which is slightly better compared to the activity of each extract alone. The results further indicate that reduction of oxidative stress by each extract as well as their combination might be beneficial to the repair of renal damage.

Key words: Corn silk, binahong leaves, ethanol extract, kidney function improvement, oxidative stress

INTRODUCTION

Kidney is one of vital organ in the body. The kidney plays roles as eliminator, maintainer, regulator and producer. As eliminator, kidney has function to remove metabolic waste products, such as urea, creatinine and ammonia. As the maintainer and regulator, kidney should keep a balance of extracellular fluid volume, concentration of inorganic electrolyte in the extracellular fluid, extracellular fluid osmolarity, acid-base balance and blood pressure. In addition, kidney plays a role in producing vitamin D and hormones (Scanlon and Sanders, 2003; Kelly, 2004; National Kidney Federation, 2003).

Kidney failure is a condition where the kidney is damaged so that it can no longer perform the function of excretion properly. These conditions can result in the accumulation of metabolic waste products that cause toxicity in the body. In addition, kidney failure is often followed by a variety of other physiological disorders,

such as cardiovascular disease, anemia, osteodystrophy, acidosis, etc. (Thye, 1998; Zdanowicz, 2003; CDC, 2010). It is known that the incidence of kidney failure in the United States has doubled during the last two decades. Each year, there is an increase of about 7-8% in the world's population. Approximately 10% of the U.S. population or about 20 million people are suffering from kidney failure (National Kidney Federation, 2003; The American Society of Nephrology, 2012). The incident increases in the diabetic and hypertension population (CDC, 2010).

Kidney disease is generally irreversible and likely to lead to ESRD (End Stage Renal Diseases) conditions. Until now there are no specific therapies to treat the disease. Treatments used are mainly symptomatic and only for replacing kidney function, such as dialysis or kidney transplantation. Consequently, they should be done routinely, however they are costly and provide responses that vary for each person. Therefore,

alternative therapies are needed to inhibit the progression of this disease. Alternative therapy should be safe and not expensive as these disease managements require long duration.

The previous research has shown that the single use either of “binahong” leaves extracts or corn silk extracts could help improve kidney function that has previously been tampered by using nephrotoxic agents (Sukandar *et al.*, 2011; Fadliah, 2008). Therefore, this study tested for activity of the extract combination against kidney function improvement. The purpose of this study was to determine whether the activities provided by the combination of the two extracts are synergistic, additive, or antagonistic. The power of the combination compared to the activity of a single power-sole.

MATERIALS AND METHODS

Animals: Male Wistar rats aged 8-12 weeks, weighing 175-225 g, were kept under usual management conditions in conventional animal house of School of Pharmacy, Bandung Institute of Technology. Rats were fed with standard laboratory diet and water *ad libitum*.

Plant material: Corn silk (*Zea mays* L.) dan binahong leaves (*Anredera cordifolia* (Ten.) Steenis) were purchased from Manoko farm in Lembang, Bandung and identified by experts in School of Biological Science and Technology, Bandung Institute of Technology, Indonesia.

Preparation of extract: Grinded powder of corn silk and binahong leaves each was extracted with ethanol using reflux method and filtered through Whatman filter paper. The total extract was evaporated using rotary vacuum evaporator (Buchi R-124) to obtain viscous extract that referred as ethanol extract.

Experimental procedure: This study was conducted according to Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research. NRC, 1996 Washington, DC: National Academy Press.

Experimental rats were divided into 6 groups, 5 rats each (it is used 8 rats each group in the 0th week; 3 rats for each group were dissected after 7 days administration of gentamicin and piroxicam). All groups, except the sixth group, were treated with 100 mg kg⁻¹ per day of gentamicin intraperitoneally and 3.6 mg kg⁻¹ per day of piroxicam orally for 7 consecutive days to induce kidney failure (Hosaka *et al.*, 2004). Group 1 continued with

administration of piroxicam orally until the 4th week of therapy. This group served as a positive control group. Group 2 continued with simultaneously receiving 75 mg kg⁻¹ b.wt. per day corn silk extract and piroxicam orally until the 4th week of therapy. This group served as a corn silk single test group. Group 3 was administrated with 100 mg kg⁻¹ b.wt. per day binahong leaves extract and piroxicam orally until the 4th week of therapy. This group served as binahong single test group. Group 4 was delivered with 37.5 mg kg⁻¹ b.wt. day⁻¹ corn silk extract, 50 mg kg⁻¹ b.wt. day⁻¹ binahong leaves extract and piroxicam up to the 4th week of therapy. This group named as corn silk-binahong half dose combination test group, whereas group 5 which named, corn silk-binahong one dose combination test group was given 75 mg kg⁻¹ b.wt. day⁻¹ corn silk extract, 100 mg kg⁻¹ b.wt. day⁻¹ “binahong” leaves extract and piroxicam. Group 6 were injected with normal saline and tragacanth solution as placebo for 7 consecutive days and 4 weeks of therapy. Tragacanth was used as a vehicle for piroxicam and ethanol extract. This group served as a negative control group.

Creatinine levels were determined in serum samples every week. Twenty four hours after the 4th week of therapy, the rats in all groups were sacrificed and both of the kidneys were quickly removed. The kidneys were weighed and fixed with 10% buffered formalin solution to be embedded in parafine for histopathological observation by light microscopy. The remaining kidneys immediately and thoroughly washed with ice-cold physiological saline. The tissue was homogenized in cold buffer phosphate (pH 7.4) in a homogenizer for 10 min (the concentration was 20%). The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant was used to assay MDA (malondialdehyde), catalase and SOD (superoxide dismutase) activity.

Determination of serum creatinine level: Serum creatinine level was determined using Human[®] reagent kits according to the kinetic method of Jaffe (Lustgarten and Wenk, 1972). Absorbance was measured at 546 nm via spectrophotometer.

Determination of serum urea level: Serum urea level was measured with spectrophotometer using urease enzyme kit (Wilcox *et al.*, 1966). Principle of this method is hydrolyzing of urea in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia ions react with hypochlorite and are catalyzed by nitroferricyanide to give dark blue/green

dye. The color of the dye was measured at 578 nm. The color intensity is proportional to the concentration of urea in the sample.

Determination of lipidperoxidation by measuring thiobarbituric acid reactive substances from kidney supernatant: In 1 mL of the reaction muddle, 0.58 mL phosphate buffer (0.1 M, pH 7.4), 0.2 mL of kidney supernatant (20% w/v), 0.2 mL ascorbic acid (100 mM) and 0.02 mL ferric chloride (100 mM) was incubated at 37°C in a shaking water bath for 1 h. The reaction was clogged by the addition of 1 mL trichloroacetic acid (TCA) (10% w/v), subsequently 1 mL Thiobarbituric Acid (TBA) (0.67% w/v) was added and all the tubes were kept in a boiling water bath for 20 min. The tubes were shifted to ice-bath and centrifuged at 3000 rpm for 10 min. The amount of TBARS formed in each of samples was assessed by measuring the absorbance of the supernatant at 535 nm allied with reagent blank without tissue homogenate (Wright *et al.*, 1981).

Determination of catalase activity from kidney supernatant: Catalase activity was assayed by Clairborne (1985). The assay mixture consisted of 1.95 mL phosphate buffer (0.05 M, pH 7), 1 mL H₂O₂ (0.019 M) and 0.05 mL of kidney supernatant (20% w/v). Changes in absorbance were recorded at 280 nm for 2 min with 60 sec interval using a spectrophotometer.

Determination of SOD activity from kidney supernatant: SOD activity in homogenate was estimated using reagent kit obtained from Sigma-Aldrich Labware.

Determination of kidney index: Organ-to-body weight ratio of the kidney (kidney index) was calculated by comparing the kidneys weight to the rat's body weight.

Histological evaluation: Twenty four hours after the 4th week of therapy, the rats in all groups were sacrificed and both of the kidneys were quickly removed. The kidneys of each animal were fixed in buffered formalin. Kidneys were processed and embedded in paraffin wax. Three micro mitter thick paraffin sections were stained with Haematoxylin and Eosin for light microscope examination.

Statistical analysis: The data was analyzed using t-test and one-way ANOVA followed by post-hoc LSD test using SPSS packages (version 15.0). Values of $p < 0.05$ were taken as significant.

RESULTS

Effect of the extract on renal function improvement in gentamicin-piroxicam induced renal failure: Four parameters of kidney function were determined including biochemical markers (serum creatinine and urea levels), oxidative stress levels, organ index and kidney's histology.

Effect on creatinine serum levels and histopathological cross section after 7 consecutive day administration of gentamicin-piroxicam: The administration of gentamicin and piroxicam for 7 consecutive days to each of tested group could increase creatinine concentration 2-3 fold significantly at the first week (week 1) when compared to the negative control group (Fig. 1). There were no significant differences among the induced groups. The findings on the biochemical parameters were also supported by the histopathological results which were showing changes in kidney structure profile both in the cortex and medulla (Fig. 2, 3). It was clearly found any tubular degeneration, glomerular atrophy, vacuole formation and hematuria in several places in the cross section of groups induced. Enhancement of creatinine concentration followed by more severe damage that can be observed on microscopic section.

Effect of extract on serum creatinine levels: At week 2, as general, each of induced group (include positive control group) experienced reduction of creatinine concentration (it is predicted because of the body homeostasis). However, creatinine concentration of the positive control group was higher and significantly different compared to the test group and the negative control group. In the positive control group, there were no significant differences which were found between the concentrations of creatinine in the treatment's week (week 2, 3 4 and 5) against the baseline value (serum creatinine levels in the week 1). Each of the test group, either single or in combination, experienced reduction creatinine concentration which was significantly different compared to the positive control group and the baseline value of each group. There were no significant differences among the test groups. From these data, it could be seen that the extract in half dose combination could produce effects more or less comparable to the single form of extract. Administration in one dose combination did not provide any significant better activity than single extract and half dose combination. This could be seen clearly in Fig. 1.

Effect of extract on serum urea levels: Similar profiles were also found on the second parameter, serum urea

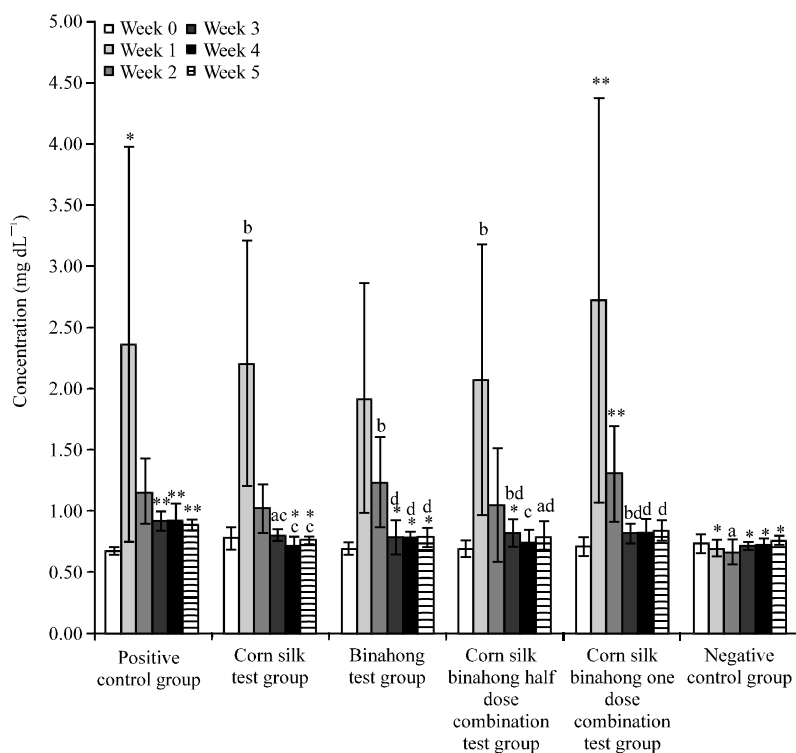


Fig. 1: Effect of extract administration, single and combination, against creatinine concentration each week; *Different significantly against positive control group ($p < 0.05$); **Different significantly against negative control group ($p < 0.05$); a: Different significantly against positive control group ($p < 0.10$); b: Different significantly against negative control group ($p < 0.10$); c: Different significantly compared to the first week's value of itself ($p < 0.05$); d: Different significantly compared to the first week's value of itself ($p < 0.10$)

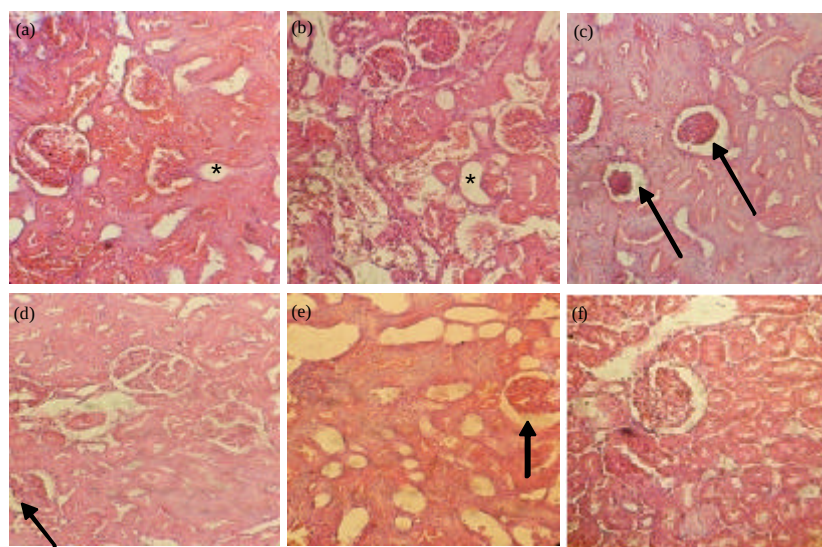


Fig. 2(a-f): Histopathological profile of kidney cortex one week after inductor administration on 100x magnification; (a) Positive control group, (b) Corn silk test group, (c) Binahong test group, (d) Corn silk binahong half dose combination test group, (e) Corn silk binahong one dose combination test group and (f) Negative control group. Arrow sign (→) showed glomerular atrophy, whereas, (*) showed vacuolization

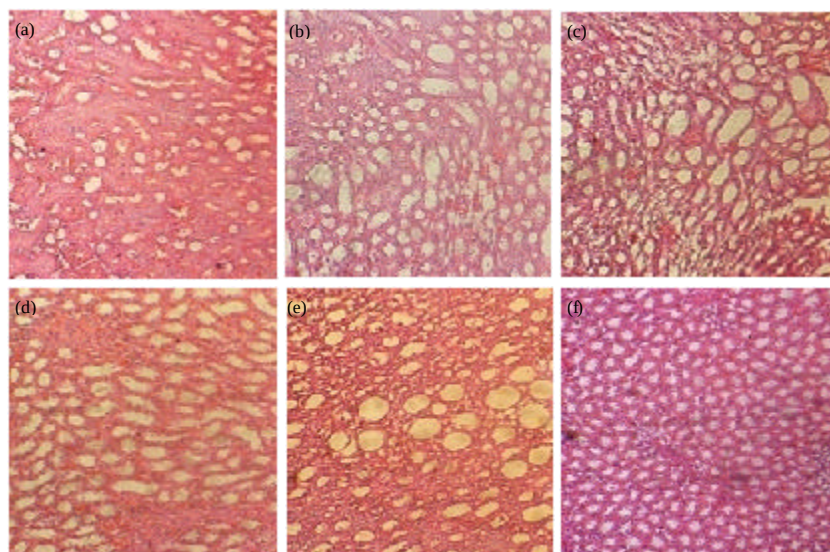


Fig. 3(a-f): Histopathological profile of kidney medulla one week after inductor administration on 100x magnification; (a) Positive control group, (b) Corn silk test group, (c) Binahong test group, (d) Corn silk binahong half dose combination test group, (e) Corn silk binahong one dose combination test group and (f) Negative control group

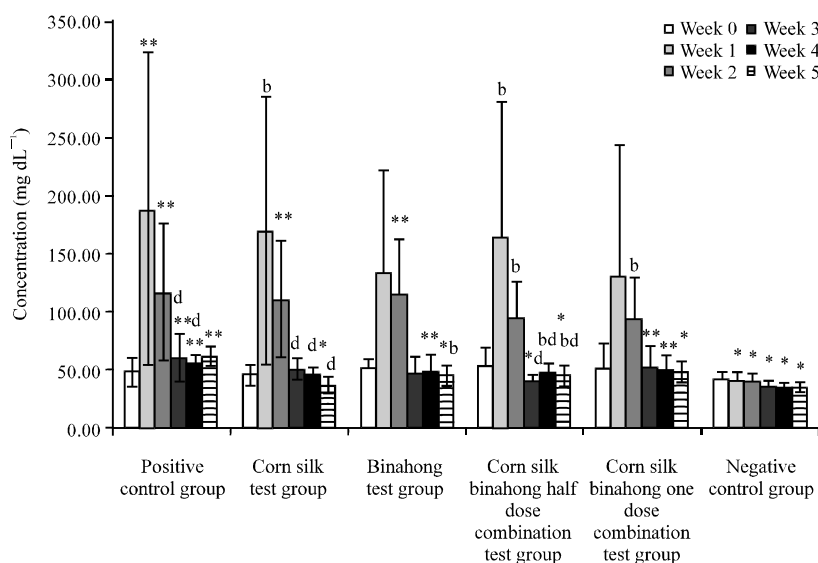


Fig. 4: Effect of extract administration, single and combination, against urea concentration each week; *Different significantly against positive control group ($p < 0.05$); **Different significantly against negative control group ($p < 0.05$); a: Different significantly against positive control group ($p < 0.10$); b: Different significantly against negative control group ($p < 0.10$); c: Different significantly compared to the first week's value of itself ($p < 0.05$); d: Different significantly compared to the first week's value of itself ($p < 0.10$)

level (Fig. 4). Kidney's damage in all induced groups caused by administration of gentamicin and piroxicam was confirmed by increase in serum urea concentration in the week 1. Serum urea levels of positive

control group were decreased in the following weeks, but tend to increase at week 5. The tendency was not found in the test groups which were given extract.

Effect of leaves extract on renal histopathological:

The results of microscopic cross-sectional showed an improvement of kidney structure after extract's administration. Although improvement was also observed

in the positive control group, each of microscopic cross sectional of the test group exhibited better performance and started to approach negative control group, especially in the medulla part (Fig. 5, 6).

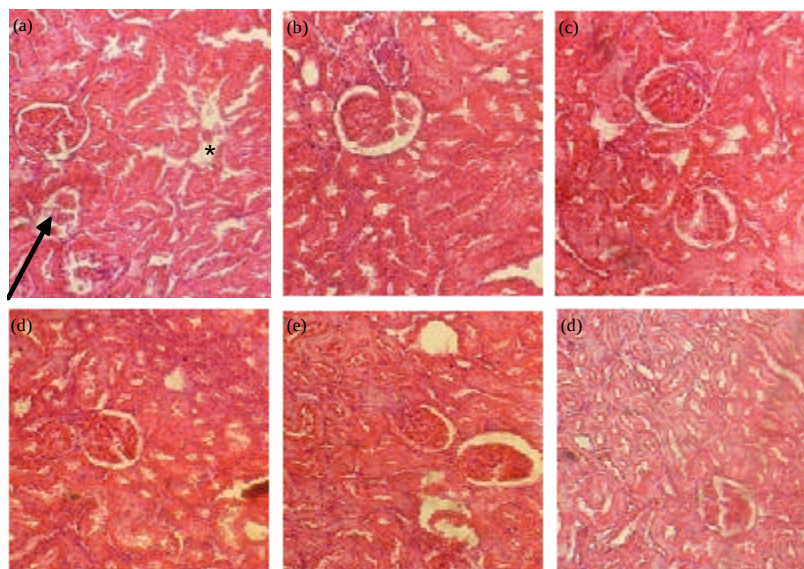


Fig. 5(a-f): Histopathological profile of kidney cortex in week 5 on 100x magnification; (a) Positive control group, (b) Corn silk test group, (c) Binahong test group, (d) Corn silk binahong half dose combination test group, (e) Corn silk binahong one dose combination test group, (f) Negative control group. Arrow sign (→) showed glomerular atrophy, whereas, (*) showed tubular degeneration

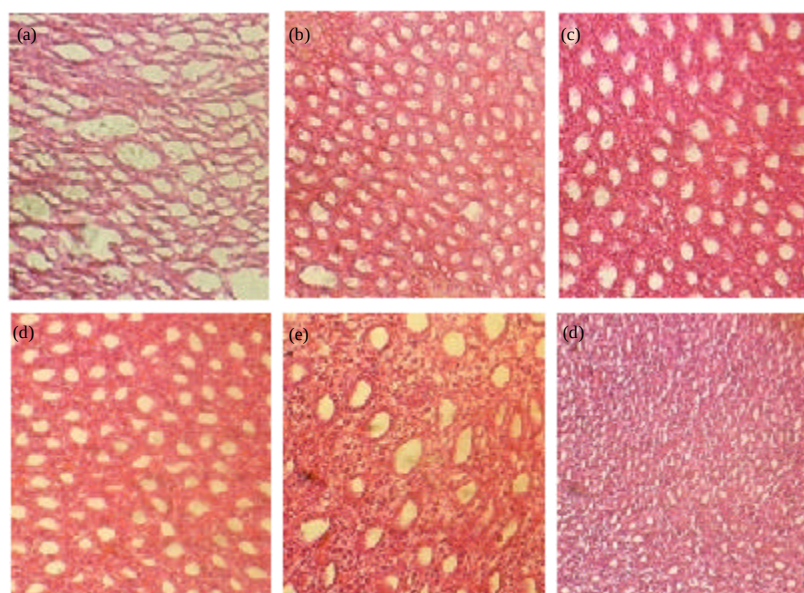


Fig. 6(a-f): Histopathological profile of kidney medulla in week 5 on 100x magnification; (a) Positive control group, (b) Corn silk test group, (c) Binahong test group, (d) Corn silk binahong half dose combination test group, (e) Corn silk binahong one dose combination test group and (f) Negative control group

Effect of extract on TBARS levels: In this study, there was a finding about an increase in the amount of TBARS in group induced by gentamicin and piroxicam. Each test group which was given extract had less amount of TBARS than and significantly different from the positive control group (Fig. 7).

Effect of extract on catalase activity: Positive control group recorded a significantly lower level of catalase compared to the negative control group. The group which was treated with extract recorded significantly elevated levels of catalase indicating restoration of levels of catalase closer to normal, untreated animals (Fig. 8).

Effect of extract on SOD activity: Group induced kidney failure was found to have decrease SOD activity. Treatment with extract produced significant increase in these enzyme levels (Fig. 9).

Effect of extract on organ index: The positive control group had the highest renal index among the other groups and was significantly different compared to the negative

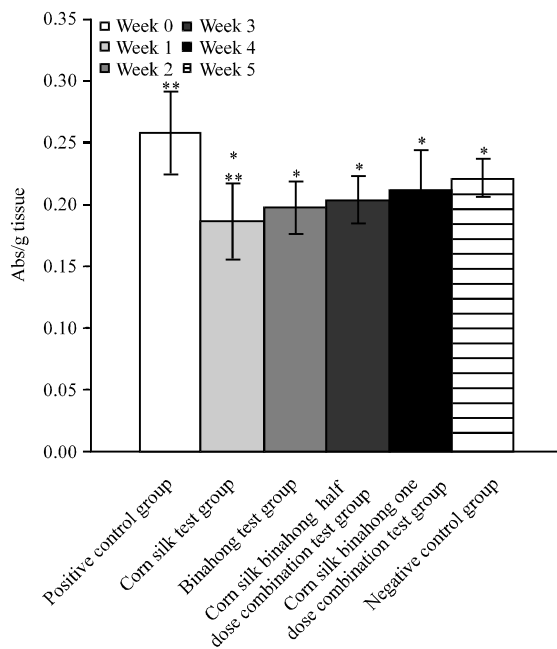


Fig. 7: Effect of extract administration, single and combination, against TBARS level in week 5; *Different significantly against positive control group ($p < 0.05$); **Different significantly against negative control group ($p < 0.05$)

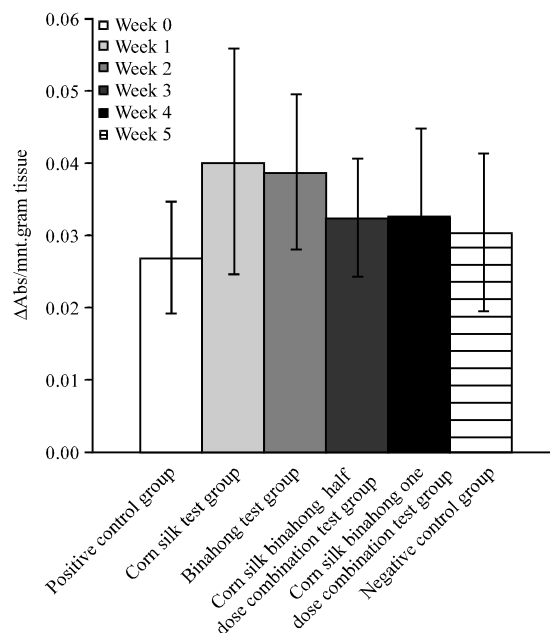


Fig. 8: Effect of extract administration, single and combination, against catalase activity in week 5; a Different significantly against positive control group ($p < 0.10$)

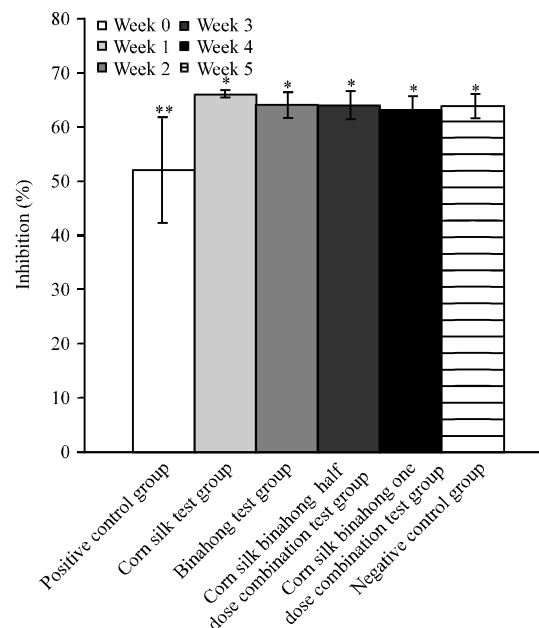


Fig. 9: Effect of extract administration, single and combination, against SOD activity in week 5; *Different significantly against positive control group ($p < 0.05$); **Different significantly against negative control group ($p < 0.05$)

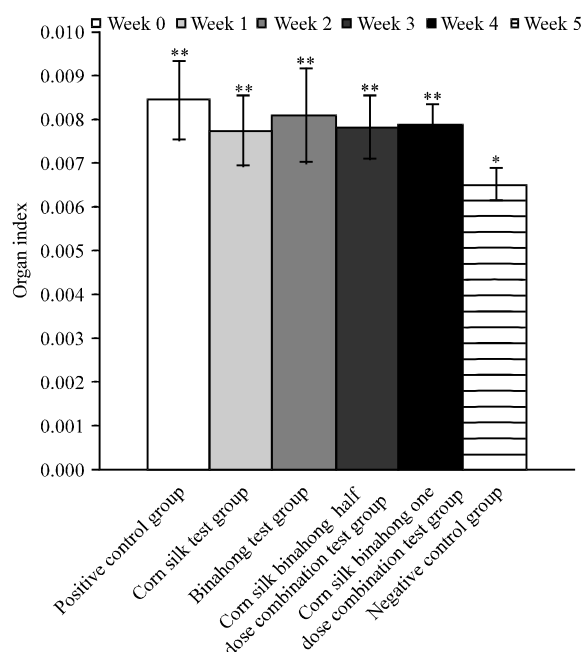


Fig. 10: Effect of extract administration, single and combination, against kidney index in week 5; **Different significantly against negative control group ($p < 0.05$)

control group. The extract group had lower renal index compared to positive control group and there were no differences among the test groups (Fig. 10).

DISCUSSION

The results which were shown by enhancement of creatinine and urea concentration in the first week of treatment, supported previous research that suggested that rat models of kidney failure could be formed rapidly by giving a combination of gentamicin and piroxicam (Sukandar *et al.*, 2011). The doses were given for 7 days for the duration had been reported to induce kidney damage. Enhancement of creatinine levels indicated that the inductor, especially gentamicin, could cause a decrease in glomerular filtration function, whereas, the histological results showed that gentamicin give a major influence on tubule, especially the proximal tubule (Rybak *et al.*, 1987; Dehghani *et al.*, 2011; Ozbek *et al.*, 2009). Tubular damage was probably derived as a result of the apoptosis which is essentially one of the important processes to maintain homeostasis. In that process, both lysosomes and mitochondria sends signals that cause the rupture of membranes and release of lysosomal acid hydrolases (De Souza *et al.*, 2009; Denamur *et al.*, 2008).

Creatinine concentration's profile of the combination test group shows a tendency that extracts are working in additive and time-dependent way. Therefore, improvement of kidney function could be achieved by prolong the application period rather than increasing the dose.

The increasing level of urea was clearly seen in the fifth week of therapy which was confirmed the severe level of kidney damage (Parlakpınar *et al.*, 2005). The increasing indicated that the damage was likely to remain in the positive control group. The elevation tendency was not found in the test groups which were given extracts. This result confirmed the kidney protective activity provided by the extract.

Kidney damage was also supported by the values of organ index which were higher compared to the negative control group. The high values index of the positive control group and the test group might be due to the occurrence of edema and fluid accumulation in the pathological extravascular space, especially within the interstitium, due to tubular necrosis (Meliani, 2006; Zulkarnain, 2009) and might also be caused by the proliferation and apoptosis of mesangial cells simultaneously. These mesangial cells are special cells which are located in around blood vessels in the kidneys. In one study, either *in vivo* or *in vitro* way, it was revealed that gentamicin stimulated mesangial cell contraction and proliferation. Gentamicin was also able to increase the expression or activation of the proapoptosis protein so that, it could cause rupture of the lysosomal membrane and release of acid hydrolases that contributed to apoptosis and necrosis of proximal tubular cells (De Souza *et al.*, 2009; Martinez-Salgado *et al.*, 2004).

Since the high value index of the test group, it could be concluded that the extract didn't work in preventing or repairing the damage of mesangial cells. However, the extract was able to show improvement in biochemical and histological parameters so, it was possible that the extract could maximize the performance of mesangial cells which were still functioning.

Other parameters which are analyzed in this study are related to oxidative stress. Oxidative stress is an abnormal condition in which increased oxidant product could not be opposed by increased production of antioxidants. The absence makes the body's equilibrium are not in a safe zone (Rico *et al.*, 2006; Kandemir *et al.*, 2011).

Various studies have shown the involvement of oxidative stress in many of degenerative diseases, one of which is kidney failure (Polat *et al.*, 2006). Uremia itself causes increased production of free radicals and even hemodialysis procedure could sometimes increase the amount of radicals (Galle, 2001). These things cause

higher risk of morbidity and probability of suffering other diseases of the kidney failure patient compared to common patients without kidney failure (Luciak, 2004). There are several parameters that can be used to predict the level of oxidative stress in patients with kidney failure, such as conjugated diene, antioxidant enzymes, peroxidation products of metilguanidin as creatinine, serum antioxidant activity and lipid peroxidation (Gotoh *et al.*, 1997).

It was found that oxygen radical species, such as superoxide anion, hydrogen peroxide and hydroxyl radicals, took a role in the pathophysiology of gentamicin. Oxygen radical species causes rapid changes in the composition of the membrane lipids or better known as lipid peroxidation. Furthermore, the cells quickly lose their osmotic balance resulting in increased intracellular calcium levels. This leads to edema/swelling of the cells which are an early manifestation of the damage that is still reversible (Kadkhodae *et al.*, 2005; Derakhshanfar *et al.*, 2007). On the other hand, radical species can consume most of the performance of the enzyme, such as Superoxide Dismutase (SOD) and catalase, thereby reducing the activity of these enzymes (Abdel-Raheem *et al.*, 2010).

Lipid peroxidation could be defined as oxidative damage to lipid structures that contain double bonds between carbons. Another definition reveals that lipid peroxidation is a process associated with free radicals, a process that is not controlled and can run continuously causing disruption on the membrane, lipids and other cell components. Lipid peroxidation that occurs continuously can be a major factor in the pathogenesis of complications of kidney failure (Shanmugam *et al.*, 2009). As stated in the previous paragraph, in the human body, the structure of the lipids are commonly found in cell membranes. That makes it more complex is that the membrane becomes first defense for each of the cell, such as mitochondria, plasma, endoplasmic reticulum, lysosomes, peroxisomes and others. Additionally metabolites formed between the oxidation processes can give adverse effects on other places than the place of origin oxidation (Devasagayam *et al.*, 2003).

One result of lipid peroxidation is aldehydes compounds that can react with the acid to form a pink thiobarbiturat which are easily detected using a spectrophotometer. Such compounds are often referred to as acid reactive compounds tiobarbiturat or TBARS (thiobarbituric acid reactive substances). TBARS as one of the products of lipid peroxidation can be used as a reference for predicting how many are the radicals production. In this study, it was found an increase in the amount of TBARS induced by gentamicin and piroxicam.

Each test group has less than the amount of TBARS and significantly different from the positive control group (Fig. 7).

It appears that kidney failure tends to weaken the defense system against free radicals. This makes the high amount of free radicals that have an impact on the high levels of tissue damage. These facts confirm the microscopic cross section results which are showing higher levels of tubular necrosis in the positive control group than the test group or the negative control group. Much evidence suggested that the excessive amount of free radicals due to kidney failure may worsen the disease and increase the risk of complications. From these results, it appears likely that the extract has antioxidant activity because it can decrease the level of lipid peroxidation in the test group. The decline may be caused by the activity of membrane lipid protection, repair tissue that is provided by the extracts, or free radical damping which reduce directly amount of free radicals found so that there are not radical that can attack the lipid membranes. Furthermore, these results corroborate previous data showing that administration of combination do not give superior activity rather than single extract use (Prasanna and Purnima, 2011).

From Fig. 7, it can be seen that the level of lipid peroxidation of each test group is lower than the negative control group although only the corn silk test group which was significantly different compared to the negative control group. It shows that the extract can improve the resistance of the cell membrane against oxidant so that tissue damage can be prevented or corrected. Higher level of resistance cause lower serum creatinine concentration of the test group compared to its initial value and can mimic serum creatinine of negative control group.

Protection system against oxygen radical species or oxidation products of lipids, proteins and DNA is provided by antioxidant enzymes such as SOD and catalase. In other words, SOD and catalase are the two main examples of radical fighting enzymes *in vivo*. It was found that there was decreased activity of both of enzymes, SOD and catalase, in kidney failure condition. As the result, there was increased number of superoxide anion and hydrogen peroxide to produce hydroxyl radicals. In the end, the hydroxyl radical can initiate lipid peroxidation. This process can increase the level of damage to the kidneys (Palani *et al.*, 2009). SOD can catalyze dismutase of superoxide anions into hydrogen peroxide which are then deactivated by catalase into water (Kim *et al.*, 2012). This indicates that in a state of oxidative stress, there was decreased activity or inactivation of the antioxidant defense system (Olagunju *et al.*, 2009;

Palani *et al.*, 2009). Decreased activity of the enzyme sometimes is a compensation for the increased number of radicals. In other words, an increase in the number of radical forces the immune system to issue more work to eliminate the oxidant. However, it can trigger a series of other, more harmful effects (Geo Vigila and Baskaran, 2011). The extract, either alone or in combination, can increase the activity of SOD and catalase enzyme compared to the positive control group (Fig. 8, 9).

The results of this study confirmed that administration of inductors, gentamicin and piroxicam, could increase lipid peroxidation in the kidney that was characterized by increased TBARS and decreased antioxidant enzyme activity although other damage mechanisms may occur as well. There was a tendency that either corn silk or binahong extracts have antioxidant activity that could protect kidney from nephrotoxicity. Antioxidant agents have been shown to have the ability to prevent or repair damage to the kidneys. The above data indicated that the extract can help improve antioxidant status in rat model of kidney failure.

These data confirm the results of previous studies related to corn silk. Various studies have revealed the antioxidant activity of the corn silk which may be caused by the high content of flavonoid or phenolic compounds (Ebrahimzadeh *et al.*, 2008; Alam, 2011; Bhaigyabati *et al.*, 2011). In connection with its antioxidant extract, corn silk extract are also potentially be used to treat other diseases associated with oxidative stress.

While binahong leaves, not many research publications that reveal about the activities associated with antioxidants. Some studies reveal binahong activity as antibacterial and wound healing (Astuti *et al.*, 2011). However, it is possible that binahong also have antioxidant activity given the flavonoid contained in the extract.

Flavonoids are phenolic compounds found in many plants. Plants used to use flavonoids to protect themselves against oxidative damage by inhibiting or reduce free radicals and reactive oxygen species that arise due to sun exposure. It is caused by the presence of conjugated ring structures and hydroxyl groups. Therefore, it is suggested that high flavonoids have a role in preventing oxygen radical species to cause cytotoxicity and tissue damage in humans (Anila and Vijayalakshmi, 2003).

Suppression activity against oxidative stress may also be generated by the interaction among components contained in the extract. Some studies reveal that isolates containing alkaloid have antioxidant effects and can improve liver function previously damaged by the use of CCl₄ (both corn silk and "binahong" leaves extract

contain alkaloid). In this case, CCl₄ produce damage through free radical production so, there are similarities that can be rationalized on the conditions of this study (Maiza-Benabdesselam *et al.*, 2007; Singh *et al.*, 2010; Parthasarathy *et al.*, 2009; Ravikumar and Gnanadesigan, 2011). It is also predicted that the presence of tannin substances in corn silk may provide protection against damage. Tannin substances have astringent like activities that can cause precipitation of proteins in the cell membrane which form the barrier that prevents the attack by free radicals.

CONCLUSION

Both silk of corn (*Zea mays* L.) and binahong (*Anredera cordifolia* (Ten.) Steenis) leaves extracts could improve kidney function in rat model of kidney failure. Combination of a half dose of each extract showed effects comparable or slightly better than an individual extract showing to have at least additive effect. Reduction of oxidative stress provided by each extracts and their combinations might be correlated with mechanism of repairing kidney failure.

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