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Efficacy of Ethanol Extract of Anredora cordifolia (Ten) Steenis Leaves on Improving Kidney Failure in Rats

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Abstract: The kidneys are important organs in the process of filtering blood. Damage of the renal function often leads to Chronic Kidney Disease (CKD). Anredora cordifolia leaves, traditionally, are used to treat various diseases, including kidney failure. Therefore, this study aimed to determine the effect of three different doses Anredora cordifolia leaves extract against renal function improvement in renal failure conditions. The research includes quality analysis and testing of the effects of Anredora cordifolia leaves extract to kidney failure. Rat model of renal failure were developed using gentamicin (100 mg kg⁻¹ b.wt.) intraperitoneally and piroxicam (3.6 mg kg⁻¹ b.wt.) orally for 8 days. Anredora cordifolia extract (50, 100 and 150 mg kg⁻¹ b.wt.) was given from day 8 for four weeks. Evaluation of renal function included serum creatinine levels, serum urea levels, organ index and kidney’s histology. After four week of therapy, Anredora cordifolia extract at doses of 50, 100 and 150 mg kg⁻¹ b.wt. decreased creatinine levels (0.02±0.17, 0.07±0.13 and 0.05±0.12 mg dl⁻¹) which were significantly different to the positive control group. Urea level decrease also occurred significantly in the test dose of 150 mg kg⁻¹ b.wt. of 20.35±2.89 mg dl⁻¹. Anredora cordifolia extract dose of 150 mg kg⁻¹ b.wt. significantly influenced on renal index. Histological results in the three test group also showed improvement in renal cells after administration of the extract. Extracts on all three types of test dose provided improvements to the kidney function.

Key words: Anredora cordifolia, kidney failure, creatinine, urea, histology of kidney

INTRODUCTION

The kidneys are organs specialized to filter the blood. Through the process, the kidneys perform many essential functions for body systems homeostasis, such as removal of metabolic waste products as well as maintenance of fluid and electrolyte balance (Kelly, 2004). Impairment of kidneys function is often referred to as Chronic Kidney Disease (CKD) or chronic renal failure (DiPiro et al., 2008).

CKD is a serious problem in the world, including in developed countries like the United States. The prevalence of renal failure increased from year to year. Patients who needed dialysis or transplantation in the United States in 2010 were 651,000 compared to 340,000 patients in 1999 (National Kidney Foundation, 2002). In developing countries, the incidence is estimated at 40-60 cases in one million populations every year.

Basically, there is no specific therapy for CKD. Renal replacement therapy, such as dialysis and transplantation, as well as drug therapy, such as erythropoietin, may give success in inhibiting the disease’s severity. However, they require a relatively high cost. Moreover, the treatments are only granted for patients in terminal renal failure which show that the damage is relatively severe. Consequently, in the process of therapy, it is required to utilize agents that are capable to inhibit the development of chronic renal failure. Such agents need to be very safe and inexpensive because chronic diseases need ongoing treatment (Yokozawa et al., 2002). In this regard, researchers in all over the world still investigate anti-renal failure activity of many plants/herbal extracts to find out new active principle with no side effect.

Anredora cordifolia is a medicinal plant that originated from China which is known as the original name Dhen San Chi or Madeira vine in South America. In Indonesia, this plant is known as binahong. Binahong is used traditionally to treat various diseases, including skin disease, hypertension, inflammation and gout. Traditional medicine in Colombia and Taiwan use water extract of Anredora leaves as anti-diabetic drug and analgesics. Thus, it can be concluded that this plant has been known to have remarkable healing properties. Anredora leaves are reported to contain saponin, flavonoid, quinon, steroid, monoterpenoid and sesquiterpenoid, while the rhizomes are known to contain flavonoid, poliphenol, tannin and steroid. A study has managed to isolate the

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A triterpenoid saponin from *Anredera* leaves which is known as bousigoside A1 (Lemmens and Buryapraphatsara, 2003).

Gentamicin and piroxicam can be used to induce renal impairment in rats in our previous study. A lot of studies have proven that gentamicin can alter biochemical indicators, such as serum creatinine, serum uric acid, serum urea and also cause histopathological changes. Reactive Oxygen Species (ROS) may have an involvement in the conformation of kidney failure caused by gentamicin (Lakshmi and Sudhakar, 2010; Walker and Shah, 1988; Ali et al., 2001; Erdem et al., 2000). More severe renal injury can be induced by a combination of NSAID with another nephrotoxic drug (Hosaka et al., 2004). Another method to induce renal impairment was done by another researcher using amikacin (Abdelaziz and Kandeel, 2011) and nephrectomized method (Sheikh et al., 2010).

Based on those explanations, this study was an attempt to test the ethanol extract of the leaves to improve renal function.

**MATERIALS AND METHODS**

This study was conducted over 9 months from January 2010 to October 2010 in the laboratory of Pharmacology and Toxicology, School of Pharmacy, Institute of Technology Bandung (ITB), Indonesia.

**Animals:** Male wistar rats aged 8-12 weeks, weighing 150-300 g, were kept under usual management conditions in conventional animal house of School of Pharmacy, Institute of Technology Bandung. Rats were fed with standard laboratory diet and water *ad libitum*.

**Plant material:** Leaves of *Anredera cordifolia* were purchased from Manoko farm in Lembang, Bandung and identified by experts in School of Biological Science and Technology, Bandung Institute of Technology.

**Preparation of leaves extract:** Grinded powder of *Anredera cordifolia* leaves was extracted with ethanol with reflux method and filtered through Whatman filter paper. The total extract was evaporated using rotary vacuum evaporator (Buchi R-124) to obtain a dark green viscous extract that referred as ethanol leaves extract.

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

Experimental rats were divided into 5 groups, 5 rats each. All groups were treated with 100 mg/kg/day of gentamicin intraperitoneally and 3.6 mg/kg/day of piroxicam orally for 7 consecutive days to induce renal failure (Hosaka et al., 2004). Group 1 continued with administration of piroxicam orally until the 4th week of therapy. This group served as a control. Group 2 continued with simultaneously giving 50 mg/kg/day leaves extract and piroxicam orally until the 4th week of therapy. Group 3 and 4 received the same treatment with group 2 but accepted different doses of leaves extract. Group 3 treated with 100 mg/kg/day of leaves extract orally, while group 4 received 150 mg/kg/day of leaves extract. Group 5 were injected with normal saline and CMC-Na solution as placebo for 7 consecutive days and 4 weeks of therapy. CMC-Na was used as a vehicle for piroxicam and ethanol leaves extract. This group served as a normal. Creatinine and urea 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 paraffin for histopathological observation by light microscopy.

**Determination of serum creatinine level:** Serum creatinine level was determined using Human reagent kits according to the kinetic method of Jaffé. Absorbance was measured at 546 nm via photometer (Techno 168).

**Determination of serum urea level:** Serum urea level was measured with spectrophotometer using urease enzyme kit. 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 renal index:** Renal 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 μm-thick paraffin sections were stained with Haematoxylin and Eosin for light microscope examination.
Statistical analysis: The data was analyzed using 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 leaves extract on renal function improvement in gentamicin-piroxicam induced renal failure: Serum creatinine and urea levels, organ index and kidney’s histology were parameters to evaluate consequence of leaves extract treatment.

Effect on creatinine and urea serum levels after 7 consecutive days administration of gentamicin-piroxicam: Each group which was given gentamicin and piroxicam had serum creatinine levels 2-3 times higher than the upper limit (0.41±0.07 mgDL⁻¹) of creatinine levels of normal group (Table 1). The drug combination also led to a significant increase in serum urea levels in every group which was given gentamicin and piroxicam (Table 2) compared to the normal group (the p-scores are 0.001, 0.025, 0.004 and 0.002, respectively for control, test of 50 mg kg⁻¹ b.wt., test of 100 mg kg⁻¹ b.wt. and test of 150 mg kg⁻¹ b.wt.). In the present study, gentamicin-piroxicam-induced renal impairment was evidenced by an increase in serum urea and creatinine after 7 consecutive days administration of both drugs. These changes persisted up to the fourth week of therapy in the control group, while the test groups experienced level degradation which could be seen from the Table 1 and 2.

Effect of leaves extract on serum creatinine levels: Each group, except normal group, experienced reduction in creatinine serum level in the first week of therapy (2.61, 2.41, 3.47 and 3.41 point counted from the 0th week, respectively for control, test of 50 mg kg⁻¹ b.wt., test of 100 mg kg⁻¹ b.wt. and test of 150 mg kg⁻¹ b.wt.; the value was obtained by subtracting creatinine concentration between the first week and the 0th week of therapy). Although the amount of reduction was not significantly different among the groups, creatinine serum level of each group that received extract was lower (respectively 0.55 mgDL⁻¹ (p = 0.001), 0.63 mgDL⁻¹ (p = 0.011) and 0.64 mgDL⁻¹ (p = 0.018) for test of 50 mg kg⁻¹ b.wt., test of 100 mg kg⁻¹ b.wt. and test of 150 mg kg⁻¹ b.wt.) and significantly different to the positive control group (0.80 mgDL⁻¹). Administration of extract inhibited the elevation of creatinine level in the fourth week of therapy so that the serum concentration did not exceed the positive control group. In the fourth week of therapy, serum level of group 2, 3 and 4 were lowered (respectively 0.53 mgDL⁻¹ (p = 0.001), 0.50 mgDL⁻¹ (p = 0.001) and 0.54 mgDL⁻¹ (p = 0.002) for test of 50 mg kg⁻¹ b.wt., test of 100 mg kg⁻¹ b.wt. and test of 150 mg kg⁻¹ b.wt.) and reached statistical significance by the comparison with the group 1 (0.91 mgDL⁻¹). There was no significant difference among the three groups which received extract and the negative control normal group in the fourth week. It was observed that creatinine profile of the normal group remained stable from week to week (0.41, 0.42, 0.42, 0.49, 0.50 mgDL⁻¹), except at the third week (0.49 mgDL⁻¹). The results also showed that serum creatinine level of control group was constantly higher and significantly different to the normal group since the first week up to the fourth week. This observation can be seen in Table 1.

Effect of leaves extract on urea serum levels: Generally, all treated groups, except the negative control normal group, showed decreased urea serum concentration in the first

Table 1: Effect of leaves extract of Anredera cordifolia on serum creatinine levels

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.41±1.91</td>
<td>0.80±0.06*</td>
<td>0.66±0.10*</td>
<td>0.66±0.05*</td>
<td>0.91±0.08*</td>
</tr>
<tr>
<td>The test of 50 mg kg⁻¹</td>
<td>2.96±1.66</td>
<td>0.55±0.06**</td>
<td>0.48±0.16</td>
<td>0.54±0.12**</td>
<td>0.53±0.10**</td>
</tr>
<tr>
<td>The test of 100 mg kg⁻¹</td>
<td>4.10±3.68</td>
<td>0.63±0.10**</td>
<td>0.54±0.04</td>
<td>0.56±0.07</td>
<td>0.50±0.20**</td>
</tr>
<tr>
<td>The test of 150 mg kg⁻¹</td>
<td>4.65±3.90</td>
<td>0.64±0.06**</td>
<td>0.58±0.18</td>
<td>0.59±0.06</td>
<td>0.54±0.15**</td>
</tr>
<tr>
<td>Normal</td>
<td>0.41±0.07</td>
<td>0.42±0.10**</td>
<td>0.42±0.11**</td>
<td>0.49±0.03**</td>
<td>0.50±0.13**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD (n = 4). *Significant difference against the normal group. **Significant difference against control group.

Table 2: Effect of leaves extract of Anredera cordifolia on serum urea levels

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.31±107.38*</td>
<td>62.95±19.51*</td>
<td>67.31±8.83*</td>
<td>66.60±18.02*</td>
<td>61.37±6.64*</td>
</tr>
<tr>
<td>The test of 50 mg kg⁻¹</td>
<td>229.85±140.60*</td>
<td>52.05±12.66*</td>
<td>48.67±7.55*</td>
<td>47.66±7.53**</td>
<td>44.52±5.40***</td>
</tr>
<tr>
<td>The test of 100 mg kg⁻¹</td>
<td>303.25±136.90*</td>
<td>60.27±23.00*</td>
<td>54.98±13.10</td>
<td>47.80±5.96***</td>
<td>44.52±5.40***</td>
</tr>
<tr>
<td>The test of 150 mg kg⁻¹</td>
<td>329.82±106.55*</td>
<td>39.87±13.53*</td>
<td>55.32±20.21</td>
<td>58.12±3.72*</td>
<td>37.77±4.23**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD (n = 4). *Significant difference against normal group. **Significant difference against control group.
Fig. 1(a-e): Kidney histology with H and E staining after treated with *Anredera cordifolia* extract; (a) normal, (b) control, (c) 50 mg kg\(^{-1}\) b.wt., (d) 100 mg kg\(^{-1}\) b.wt. and (e) 150 mg kg\(^{-1}\) b.wt.

Table 8: Effect of leaves extract of *Anredera cordifolia* on renal index profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Renal index value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.82±0.01**</td>
</tr>
<tr>
<td>The test group of 50 mg kg(^{-1}) b.wt.</td>
<td>0.79±0.05*</td>
</tr>
<tr>
<td>The test group of 100 mg kg(^{-1}) b.wt.</td>
<td>0.81±0.06*</td>
</tr>
<tr>
<td>The test group of 150 mg kg(^{-1}) b.wt.</td>
<td>0.72±0.03**</td>
</tr>
<tr>
<td>Normal</td>
<td>0.70±0.08**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD (n = 4). *Significant difference against the normal group. **Significant difference against control group.

week (283.36, 177.20, 242.98 and 289.95 point counted from the 0th week, respectively for control, test of 50 mg kg\(^{-1}\) b.wt., test of 100 mg kg\(^{-1}\) b.wt. and test of 150 mg kg\(^{-1}\) b.wt.; the value was obtained by subtracting urea concentration between the first week and the 0th week of therapy). However, the positive control group experienced relatively smaller reduction than the test groups which were given extract and was significantly different, especially compared to group 4 in the fourth week of therapy (p = 0.030). Group 3 had larger reduction than group 2 (9.46 point for group 3 and 3.14 point for group 2, the value was obtained by subtracting urea concentration between the 4th week and the 3rd week of therapy). This indicated that extract at dose of 100 mg kg\(^{-1}\) b.wt. had larger activity than dose of 50 mg kg\(^{-1}\) b.wt. Increased activity in larger doses was also seen in group 4's profile that showed inhibition of increased concentration on second and third week. Table 2 showed that serum urea concentration of each test groups was always lower than the positive control group. Significance of the test group concentration values appeared gradually; started from the second week of therapy for group 2. This observation could be seen in Table 2.

**Effect of leaves extract on organ index:** The positive control group had the highest renal index among the other groups and was significantly different compared to the normal group (p-score 0.007) and the test group of dose 150 mg kg\(^{-1}\) b.wt. (p-score 0.018) (Table 3).

**Effect of leaves extract on renal histopathological:** Histopathological examination revealed the presence of glomerular cells segmentation in the positive control group which treated with gentamicin and piroxicam (Fig. 1b), segmentation was not present in the normal groups (Fig. 1a). Segmentation was reduced after treated with *Anredera cordifolia* extract at doses of 50 mg kg\(^{-1}\) b.wt. and 100 mg kg\(^{-1}\) b.wt. (Fig. 1c, d) and the segmentation was not present at the dose 150 mg kg\(^{-1}\) b.wt. (Fig. 1e).

**DISCUSSION**

Induction of kidney failure by using gentamicin is one of the most common methods used at this time. Gentamicin belongs to the class of aminoglycosides that has nephrotoxic side effects. The mechanisms of nephrotoxicity by gentamicin are not well understood. However, evidence indicates that Radical Oxygen Species (ROS) and nitrosative stress may be involved in gentamicin-induced nephrotoxicity. It has been shown that O\(_2^-\), OH and H\(_2\)O\(_2\) are involved in renal damage induced by gentamicin. The process probably implicates the reaction with iron (Pedraza-Chaverri et al., 2004; Petronilho et al., 2009; Kadkhodaei et al., 2005;
Poormoosavi et al., 2010, Yokozawa et al., 2002). Results from many studies (Silan et al., 2007; Soliman et al., 2007; Abdel-Raheem et al., 2010) have shown that gentamicin produced an elevation in the concentrations of biochemical indicators of kidney function, such as serum urea and creatinine and total protein excretion in urine. The alterations in biochemical changes produce nephrotoxicity as evidenced by the reduction of Glomerular Filtration Rate (GFR) (Ozbek et al., 2009).

Similar to gentamicin, other drugs also show preferential toxicity for the kidney, including the non-selective COX-inhibiting NSAIDs, such as piroxicam. The toxicity effect is closely related to inhibition against prostaglandin so that promote compensatory vasodilatory disequilibrium which leading to a deterioration of GFR. A research finding confirms that the toxic effects of this drug are limited to situations of pre-existing damage or when the drug is administered in combination with other nephrotoxic drugs. Thus, non-selective NSAIDs would potentiate the reduction in urinary concentrating capacity observed with gentamicin alone after a period of treatment (Hosaka et al., 2004).

Those previous studies support this experimental that indicated the presence of elevated serum level of creatinine and urea after gentamicin-piroxicam intensive administration for 7 days. Creatinine and urea levels continued to be maintained high in the control group up to the fourth week of therapy, whereas the extract test group showed suppression of elevated serum level both of creatinine and urea.

Pharmacological activities of Anredera leaves extract are not much known scientifically, but this plant is widely used traditionally in some countries. Some published articles also suggest efficacy Anredera extract as wound healing (Panyaphu et al., 2011; Villegas et al., 1997) and antimicrobial (Tshikalange et al., 2005). This study may add to the scientific data of Anredera cordifolia.

In the previous study, it was found that Anredera shoot contained flavonoids, such as quercetin (Yang et al., 2008). Flavonoids are beneficial to both plants and humans who consume them. For example, flavonoids are known as antioxidants and have an antiproliferative effect, so widely used in the treatment of cancer, cardiovascular disease and inflammation. In another study, quercetin itself can protect renal damage by improving cross-sectional renal histopathology and ameliorating biochemical parameters. This is allegedly due to the antioxidant activity given by the flavonoids (Abdel-Raheem et al., 2009).

The ability of Anredera cordifolia to enhance wound healing and the presence of flavonoids become some possible factors that are likely related to the activity against kidney failure. The renal index and histopathological report supported the biochemical findings. Occurrence of edema and fluid accumulation in the interstitium space, as a result of tubular necrosis, made the renal index of control and test groups higher compared to the normal group. It was predicted that the lumen of the tubules were filled with degenerate and desquamated epithelial and apoptotic cells (De Souza et al., 2009; Martinez-Salgado et al., 2004). Our data showed that leaves extract at dose 150 mg kg⁻¹ b.wt. may be able to prevent or even repair damage that has occurred to cells. The activity at dose 50 dan 100 mg kg⁻¹ b.wt. were lower than that of 150 mg kg⁻¹ b.wt. Extracts on all three types of test dose were able to improve biochemical parameters so that there is a possibility that the extract can repair damage cell and maximize the performance of cells that are still functioning.

CONCLUSION

Anredera leaves extract at doses of 50, 100 and 150 mg kg⁻¹ b.wt. provided improvement to the kidney function of rats based on three parameters, i.e. creatinine and urea serum level, kidney index and histology of kidney. Improvement increases with increasing dose. Therefore, Anredera leaves extract has prospect to be used in treatment of renal failure.

ACKNOWLEDGMENT

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REFERENCES


