Kidney protective effect of *acalypha indica* linn. root extract in high-fructose and high-cholesterol diet-fed rats

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**Abstract.** Diet high in fructose and cholesterol may lead to the development of diabetic nephropathy (DN). One of the first drugs of choice in DN treatment is captopril. Prolonged use of this drug may lead to some adverse effects and the treatment can be optimized through using other therapy options. *Acalypha indica* Linn. (AI) may be an alternative herbal therapy for DN. The objective of this research is to investigate the renoprotective effect of AI on DN. For seven weeks, thirty-two *Sprague-Dawley* rats were divided into groups receiving normal diet and high-fructose and high-cholesterol diet (HFCD). Then, the HFCD-fed rats were divided into four groups receiving different treatment: negative control, AI root extract (250 mg/kgBW), captopril (2.5 mg/kgBW), and combination of captopril and AI. Normal diet group was divided into AI and no treatment. After four weeks of treatment, the rats were terminated and serum urea and creatinine levels were measured. In the normal group, AI therapy decreased serum urea and creatinine levels. In the HFCD groups, AI and captopril monotherapy groups had increased serum urea levels, but lower compared to negative control. Meanwhile, serum creatinine levels decreased in both groups. However, these findings are not statistically significant. We found that combination therapy group had the highest increase in serum urea level, which was significantly different with captopril group (p=0.01). Serum creatinine level was also increased in this group. Our present study showed that AI tend to reduce serum urea and creatinine levels in normal diet group and inhibit the increase of serum urea and creatinine levels in rats fed with HFCD diet. Antagonistic interaction between captopril and AI might be present.

1. Introduction
Diabetic nephropathy (DN) is a complication of diabetes mellitus (DM) that is found in about 40% of DM cases [1]. It is the main etiology of chronic kidney disease (CKD), around 90% of which occurred in patients with type 2 DM (T2DM) [2,3]. CKD is initiated by inadequate control of blood glucose and its progress is accelerated with hypertension and hyperglycemia. Two main markers of the disease are albuminuria and lowering of glomerular filtration rate (GFR). Both are associated with increased risk of cardiovascular disease, end-stage renal disease (ESRD), and death [1].

Renal damage occurring in T2DM may result from consumption of diet high in fructose and cholesterol. High fructose diet has been shown to cause characteristics of metabolic syndrome, oxidative stress, and kidney disease in rats [4]. Sánchez-Lozada et al (2007) reported that high fructose diet, in both liquid (10% fructose) and solid (60% fructose) form, for eight weeks in *Sprague-Dawley*
rats induced metabolic syndrome and renal damage, including kidney hypertropho, glomerular hypertension, arteriolopathy, and cortical vasoconstriction [5]. Another study conducted by Palanisamy et al (2008) found that high fructose diet (60 g/100 g) given to Wistar rats for 60 days led to a significant increase in serum and urine concentrations of urea, creatinine, and uric acid [6].

High cholesterol diet has also been shown to result in kidney damage. Guijarro et al (1995) conducted an experiment in which rats were fed with 4% cholesterol to 8-week-old rats for four weeks. No significant increase in serum creatinine level was found, though it was higher than the control group. However, histologic examination of the kidney showed glomerular hypertrophy and foam cells, mesangial matrix expansion, and extracellular lipid deposition [7].

To delay further progression of renal damage in DN, treatment is necessary. It consists of adequate control of hyperglycemia, hypertension, and dyslipidemia [8,9]. The first drug of choice in treating DN is renin-angiotensin-aldosterone system (RAAS) blockers, such as angiotensin-converting enzyme inhibitor (ACE-I). In addition to having hypotensive effect, RAAS blockers have also shown renoprotective effects, including minimizing glomerular damage and other effects such as antioxidant, antithrombotic, and anti-inflammatory [9,10]. However, RAAS blockade mainly affects only one of the various pathophysiologic pathways that result in DN. As a multifactorial and complex condition, interventions from various pathways in the treatment of DN may be necessary. Interventions of other pathophysiologic pathways may lead to a more effective treatment [10,11].

Herbal medicine offers an alternative as additional treatment. Acalypha indica Linn. (AI), a plant which has been shown to have various healing properties, including antioxidative, anti-diabetic, hypolipidemic, and anti-hyperuricemia, is a potential candidate [12-14]. Several studies have also shown renoprotective effects of the plant, both in normal and streptozotocin (STZ)-induced diabetic rats [15,16]. Therefore, AI may be a possible additional treatment in the management of DN.

In this study, we investigate the effect of AI root extract in kidney damage in Sprague-Dawley rats, with serum urea and creatinine levels as markers of kidney damage. Both substances are metabolism end-products normally excreted by the kidney. Injury to the kidney may result in impaired excretion of the substances, leading to its build-up inside the body [17].

We aim to evaluate the kidney protective effects of AI in rats induced with HFCD. Induction using HFCD is expected to be more representative of kidney damage in T2DM. The change in serum urea and creatinine levels of rats receiving captopril, AI and both captopril and AI were compared. In addition, we also evaluate the effect of AI in rats receiving normal diet. This study may offer further explanation regarding renoprotective effects of AI and how it affects kidney damage in Sprague-Dawley rats induced with HFCD.

2. Methods

2.1. AI root extract
AI roots were dried and mashed until it turned to powder. The root was macerated using 70% ethanol solution. After maceration, the extract then was evaporated with a rotary vacuum evaporator.

2.2. Diet induction
For seven weeks, nine Sprague-Dawley rats received normal diet and twenty-three others were fed with HFCD. HFCD diet consisted of fructose solution (55%) and cholesterol (10%) derived from quail egg yolk, in addition to normal diet.

2.3. Animal grouping
Animals used in this study were 12-week-old male Sprague-Dawley rats, with weights ranging from 240 to 400 grams. For the next four weeks, the animals were divided into six groups with different treatments. Rats who received normal diet were divided into two groups (no treatment and given AI root extract). The HFCD groups were divided into four groups: negative control, AI extract 250 mg/kgBW, captopril 2.5 mg/kgBW, and combination therapy of AI and captopril. The rats continued to receive the same diet throughout the treatment.
2.4. Data collection
Blood samples were obtained at week seven and eleven, before and after the treatment was given. The blood samples were withdrawn from retro-orbital venous plexus. Serum urea and creatinine concentrations were determined using spectrophotometry method.

2.5. Statistical analysis
The data was analysed using GraphPad Prism 5.0, to compare the change in serum urea and creatinine concentrations between the groups. Normality of the data was determined using Saphiro-Wilk test. To compare data between the groups, one-way ANOVA test was used. Results were considered statistically significant if \( p \)-value < 0.05 and Tukey post-hoc analysis was done thereafter.

3. Results

3.1. Serum urea levels
Serum urea levels were found to be increased in all groups who received HFCD, while the rats fed with normal diet had reduced levels. The decrease in serum urea level found in normal diet group treated with AI was greater than the group with no treatment. However, the difference was not statistically significant. No significant association was found between the increase in serum urea levels in HFCD-only group as negative control with other HFCD-fed groups treated with AI, captopril, and combination therapy. The highest increase occurred in combination therapy group, followed by HFCD-only and AI groups, respectively, and the lowest was found in captopril group. The increase of serum urea levels in combination therapy group was significantly different with captopril monotherapy group (\( p=0.01 \)). Data were presented in mean ± SD, shown in table 1 and 2. Comparison between serum urea level changes was illustrated in figure 1.

| Group       | Serum urea level change (mg/dl) |
|-------------|---------------------------------|
| No treatment| 0.308 ± 4.73 (↓)                |
| AI          | 2.177 ± 3.2 (↓)                 |

3.2. Serum creatinine levels
In almost all groups serum creatinine levels were decreased, except for combination therapy group. However, no statistically significant association was found between the change of serum creatinine levels in all groups.

| Group        | Serum urea level change (mg/dl) |
|--------------|---------------------------------|
| No treatment | 12.064 ± 3.51 (↑)               |
| AI           | 10.847 ± 4.78 (↑)               |
| Captopril    | 1.257 ± 15.23 (↑)               |
| Captopril + AI| 24.912 ± 11.98 (↑)             |
Figure 1. Comparison of the change in serum urea levels between groups. Both normal diet groups had a decrease in serum urea levels, while all HFCD groups had increased levels.

Table 3. Serum creatinine level change in normal diet groups. Reductions in serum creatinine levels were found in both groups. AI group had a lower decrease compared to the one receiving no treatment, although not statistically significant.

| Group         | Serum creatinine level change (mg/dl) |
|---------------|---------------------------------------|
| No treatment  | 0.055 ± 0.0784 (↓)                     |
| AI            | 0.0123 ± 0.082 (↓)                     |

Table 4. Serum creatinine level change in HFCD groups. No significant association was found between the groups. An increase in serum creatinine level occurred in combination therapy group. The other groups had reduced serum creatinine levels, with no treatment group having the greatest decrease.

| Group            | Serum creatinine level change (mg/dl) |
|------------------|---------------------------------------|
| No treatment     | 0.084 ± 0.0775 (↓)                     |
| AI               | 0.029 ± 0.16 (↓)                       |
| Captopril        | 0.05 ± 0.203 (↓)                       |
| Captopril + AI   | 0.125 ± 0.17 (↓)                       |

4. Discussion
This study aimed to evaluate the effects of captopril, AI root extract, and its combination towards renal damage in HFCD-induced Sprague-Dawley rats. We also evaluated the effect of AI towards kidney function in Sprague-Dawley rats which only induced with normal diet. In this study, renal damage was assessed using two biochemistry parameters, serum urea and creatinine levels.
4.1. HFCD and renal damage
According to a study by Han Z (2010) et al, normal level of serum urea in Sprague-Dawley rats was 19,474 ± 2,996 mg/dl [18]. In our study, HFCD only-fed rats had higher serum urea level compared to normal diet group. Therefore, both HFCD-only and normal diet group had increased level of serum urea. Meanwhile, a slight decrease in serum creatinine level was found in the HFCD and normal diet groups. However, these findings were not statistically significant. Studies have shown that diet high in fructose and cholesterol led to renal damage [5,6,19,20]. Pathological changes in glomerulus were seen in rats fed with 25% fructose for two weeks [19]. A 60-day study has also reported significant increase in the level of serum urea and creatinine concentrations in Wistar rats receiving 60 g/100 g high-fructose diet, accompanied with glomerular changes [6]. High cholesterol diet given to rats for four weeks had been shown to cause abnormalities in the glomerulus, yet did not increase creatinine level [9]. In these previous studies, diet high in fructose and cholesterol content led to early kidney damage, especially found in histological examinations. However, based on findings from other studies, even though serum urea and creatinine levels had not yet elevated significantly, morphological abnormalities should have occurred.

![Figure 2](image-url)  
**Figure 2.** Comparison of the change in serum creatinine levels. Combination therapy group was the only one with an increase in serum creatinine levels. The highest decrease was found in HFCD-only group.

4.2. Captopril as monotherapy
Captopril is an ACE-I, thus works by inhibiting the conversion of angiotensin I to angiotensin II. Allah et al (2015) conducted a study evaluating the effect of captopril (60 mg/kgBW) given for six weeks in STZ-induced diabetic rats [20]. This study reported an improvement of serum urea and creatinine in group treated with captopril. Another study by Akbar et al (2012) showed a similar result, which was a decrease in serum creatinine in STZ-induced diabetic rats treated with captopril (17.5 mg/kgBW) [21]. The results found in this study were in line with the previous ones, in which HFCD group treated captopril (2.5 mg/kgBW) had lower level of serum urea compared to all HFCD groups, while serum creatinine level was decreased.

4.3. AI as an alternative treatment
A study conducted by Hazali et al (2015) evaluated the kidney toxicity of AI n Sprague-Dawley rats, with dosage of 100, 200, and 300 mg/kgBW for 90 days. Decrease in serum urea and creatinine concentrations were found, with the reduction in serum urea level of male Sprague-Dawley rats receiving the highest dose of AI being significantly different from the control group. Creatinine level...
didn’t differ significantly, though lower in the groups treated with AI [16]. Another study by Larasati (2010) reported that AI treatment, given at the dose of 1000 mg/kgBW for two weeks in STZ-induced diabetic rats, resulted in less glomerular damage compared to control [17]. Mechanisms underlying kidney protective effects of AI may come from its flavonoid content, including flavone, isoflavone, and flavanol [23]. Studies have shown that flavonoid may play a role in kidney protection, mainly through its antioxidative effects [24]. In this study, serum urea concentrations were decreased in both normal diet groups, with the AI group having a slightly greater decrease. This might suggest a kidney-protective role of AI as preventive therapy. In HFCD-fed rats, treatment with AI still increased serum urea level, but lower than negative control group. Serum creatinine level was lowered in the HFCD group with AI treatment. These findings suggested that AI tend to reduce serum urea and creatinine level. In addition, it also tend to inhibit the increase of serum urea and creatinine level in rats fed with HFCD.

4.4. Captopril and AI as combination therapy
With the potential renoprotective effects of captopril and AI, we predicted that combination therapy would result in a greater protective effect. However, our results indicated the opposite. Combination therapy group had the greatest increase in serum urea level, even more than negative control. It was significantly different from the group receiving captopril (p=0.01). Creatinine level was raised in this group, while other groups had decreased level. Up to this time, we have not found any study who evaluated the combination therapy of captopril and AI, thus unable to compare our finding directly. However, a finding that may be of support was found study conducted by Sengottuvel T (2012) comparing the effect of captopril with quercetin, a type of flavonoid found in AI. From this study, it was found that combination therapy of captopril and quercetin in Wistar rats had lower cardioprotective effect compared to monotherapies. This was potentially caused by antagonistic effects between the two drugs [25]. Further study evaluating antagonistic effect between captopril and AI in Sprague-Dawley rats may be needed.

5. Conclusion
Our present study showed that AI tend to show kidney protective effect. In addition, contrary to our initial prediction, AI and captopril dual treatment did not result in combined renoprotection, but might be the opposite. Antagonistic interaction might have occurred between AI and captopril. Similar studies using histological examinations as kidney damage parameter, in addition to serum urea and creatinine levels, should be conducted to give more evidence.

6. References
[1] Chen J 2014 Diabetic nephropathy: scope of the problem Diabetes and kidney disease (Springer) pp 9–14
[2] Bargmann J and Scorecki K 2015 Chronic kidney disease Harrison’s principles of internal medicine ed D Kasper, S Hauser, J Jameson, A Fauci, D Longo and J Loscalzo (New York: McGraw-Hill Education) pp 1811–3
[3] Jerums G, Ekinici E, Premaratne E, Baker S, Panagiotopoulos and Macisaac R 2015 Diabetic nephropathy International Textbook Of Diabetes Mellitus ed R DeFronzo, E Ferrannini, P Zimmet and G Alberti (West Sussex: John Wiley & Sons Ltd.) pp 895–923
[4] Johnson R J, Nakagawa T, Sanchez-Lozada L G, Shafiu M, Sundaram S, Le M, Ishimoto T, Sautin Y Y and Lanasa M A 2013 Sugar, uric acid, and the etiology of diabetes and obesity Diabetes 62 3307–15
[5] Sánchez-Lozada L G, Tapia E, Jiménez A, Bautista P, Cristóbal M, Nepomuceno T, Soto V, Ávila-Casado C, Nakagawa T and Johnson R J 2007 Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats Am. J. Physiol. Physiol. 292 F423–9
[6] Palanisamy N, Viswanathan P and Anuradha C V 2008 Effect of genistein, a soy isoflavone, on whole body insulin sensitivity and renal damage induced by a high-fructose diet Ren. Fail. 30 645–54
[7] Guijarro C, Kasiske B L, Kim Y, O’Donnell M P, Lee H S and Keane W F 1995 Early glomerular changes in rats with dietary-induced hypercholesterolemia Am. J. kidney Dis. 26 152–61
[8] Evans T and Capell P 2000 Diabetic nephropathy Clin Diabetes 18
[9] Kassi E, Pervanidou P, Kaltzas G and Chrousos G 2011 Metabolic syndrome: definitions and controversies BMC Med. 9 48
[10] Polonsky K and Burant C 2016 Type 2 diabetes mellitus Williams Textbook of Endocrinology ed S Malmed, K Polonsky, P Larsen and H Kronenberg (Philadelphia: Elsevier, Inc.) p 1386
[11] Vivian E and Mannebach C 2013 Therapeutic approaches to slowing the progression of diabetic nephropathy—is less best? Drugs Context 2013
[12] Lozano-Maneiro L and Puente-Garcia A 2015 Renin-Angiotensin-Aldosterone system blockade in diabetic nephropathy. Present evidences. J Clin Med 4 1908–37
[13] Reddy A, Punchchakalaya G, Venkateshwarlu E, Srinivas N, Ramya C and D N 2012 Anti-diabetic and hypo-lipidemic effect of Acalypha indica in streptozotocin-nicotinamide induced type-II diabetic rats Int J Pharm Pharm Sci 4 205–12
[14] Hartanto M 2008 Pengaruh sari fraksi heksana, kloroform, etil asetat, dan sisa air dari rebusan akar tanaman akar kucing (Acalypha indica Linn.) dosis 10.8 g/200 g BB terhadap kadar asam urat dalam darah tikus putih jantan yang diinduksi kalium oksonat (Universitas Indonesia)
[15] Jagatheeswari D, Deepa J, Ali H S J and Ranganathan P 2013 Acalypha indica L-An important medicinal plant: A review of its traditional uses and pharmacological properties Int. J. Res. Bot. 3 19–22
[16] Hazali N, Nazri N N M, Ibrahim M and Masri M 2016 Subchronic toxicity of Malaysian Acalypha Indica: biochemistry and haematology analysis of rat J. Teknol. 78 21–6
[17] Lara’sati C 2010 Pengaruh pemberian ekstrak anting-anting (Acalypha indica L.) terhadap gambaran histologis glomerulus ginjal mencit induksi streptozocin (Universitas Sebelas Maret)
[18] Han Z-Z, Xu H-D, Kim K-H, Ahn T-H, Bae J-S, Lee J-Y, Gil K-H, Lee J-Y, Woo S-J and Yoo H-J 2010 Reference data of the main physiological parameters in control Sprague-Dawley rats from pre-clinical toxicity studies Lab. Anim. Res. 26 153–64
[19] Manitius J, Baines A D and Roszkiewicz A 1995 The effect of high fructose intake on renal morphology and renal function in rats. J. Physiol. Pharmacol. an Off. J. Polish Physiol. Soc. 46 179–83
[20] Ying Y, Xing-kui T, Xiao-cheng L and Ju-fang S 2005 Early renal morphological changes in high-cholesterol diet rats model Wuhan Univ. J. Nat. Sci. 10 1063–8
[21] Abd Allah E S H and Gomaa A M S 2015 Effects of curcumin and captopril on the functions of kidney and nerve in streptozotocin-induced diabetic rats: role of angiotensin converting enzyme 1 Appl. Physiol. Nutr. Metab. 40 1061–7
[22] Akbar D H, Hagras M M, Amin H A and Khoshshid O A 2013 Comparison between the effect of glibenclamide and captopril on experimentally induced diabetic nephropathy in rats J. Renin-Angiotensin-Aldosterone Syst. 14 103–15
[23] Pambudi A, Noriko N, Azhari R and Azura P R 2015 Identifikasi Bioaktif Golongan Flavonoid Tanaman Anting-Anting (Acalypha indica L.) J. Al-AZHAR Indones. SERI SAINS DAN Teknol. 2 178–87
[24] Dahal A and Mulukuri S 2015 Flavonoids in kidney protection World J Pharm Pharm. Sci 4 362–82
[25] Sengottuvel T 2012 Evaluation Of Pharmacodynamic Interaction Of Quercetin With Captopril In Doxorubicin Induced Myocardial Toxicity In Wistar Rats