ORIGINAL ARTICLE

Ethanol Extract of Yacon Leaves (*Smallanthus sonchifolius*) Attenuates Fibroblast and Myofibroblast Expansion in Association With Downregulation of TGF β1 and Snail mRNA Expression in 5/6-Subtotal Nephrectomy Model in Mice

Ermawati[1], Nur Arfian[1], Setyo Purwono[2], Muhammad Mansyur Romi[1], Dwi Cahyani Ratna Sari[1]

[1] Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia
[2] Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia

ABSTRACT

Introduction: Chronic kidney disease (CKD) is characterized by fibroblast activation, myofibroblast formation, and up-regulation of transforming growth factor-β1 (TGF-β1) that may activate Snail in fibroblast to myofibroblast transition. Ethanol extract of Yacon leaves is known to have a renoprotective effect on diabetic nephropathy but its effect in the CKD model is unknown. This experimental study aimed to elucidate the effect of ethanol extract from Yacon leaves in attenuating renal failure in a CKD mice model. **Methods:** Male Swiss-Webster mice (3 months, 30–40 grams, n=25) underwent 5/6 subtotal nephrectomy (SN) to induce CKD. The mice were divided into five groups: SN, SN mice with oral treatment of Yacon leaves ethanol extract with doses 0.735 µg/kg (SN+YK1), 1.47 µg/kg (SN+YK2), and 2.94 µg/kg (SN+YK3), and a Sham operation (SO) group with aquadest 0.1% supplementation. Mice were euthanized on day 14 after the operation and kidneys were harvested. Paraffin sections were used for histological analysis. Immunostaining was done for quantifying fibroblasts and myofibroblasts. We performed RT-PCR to measure TGF-β1 and Snail mRNA expressions. **Results:** The SN group had significantly higher fibroblast number, myofibroblast fraction area, TGF-β1 and Snail mRNA expressions compared to the SO. The fibroblasts number ($p<0.001$) and myofibroblast fraction areas ($p<0.001$) were significantly lower in Yacon treated-groups compared to the SN group. RT-PCR analysis showed lower mRNA expressions of TGF-β1 and Snail, but no significant differences were found among the various Yacon treated-groups. **Conclusion:** Ethanol extracts of Yacon leaves improved kidney damage in male mice with 5/6 subtotal nephrectomy model.

Keywords: Ethanol extract of Yacon leaves, Chronic Kidney Disease, fibrosis, TGF-β1, Snail.

Corresponding Author:
Nur Arfian, PhD
Email: nur_arfian@ugm.ac.id
Tel: +62 274 6492521 / +62 8112640306

INTRODUCTION

Chronic kidney disease (CKD) is a significant threat to world health. It is estimated that more than 10% of the adult global population has CKD (1,2). CKD in the United States (US) is still a major health problem that affects many middle-aged and elderly people (3). Based on data from 2010, the prevalence of CKD in adults was 10.4% of men and 11.8% of women (4). Worldwide, the number of cases of CKD is growing rapidly, especially in developing countries such as Indonesia.

The progression of CKD results in loss of renal function leading to end-stage renal disease (ESRD) as well as death (5). The main characteristic of CKD that is considered to be a common endpoint is renal fibrosis. This characteristic begins with the formation of myofibroblasts, inflammation, and damage to the renal epithelial architecture (6,7). Many cells play a role in renal fibrosis including fibroblasts, tubule epithelial cells, endothelial cells, vascular smooth muscle cells, mesangial cells, and podocytes (8,9). Fibroblasts differentiate to myofibroblasts during kidney fibrosis (10). Myofibroblasts can be identified by the presence of α-SMA expression. In normal kidneys, the myofibroblast with the α-SMA marker are absent in interstitial areas (10) (10). Furthermore, the expression of myofibroblast represents early markers of type I and type III collagen (11).

Transforming growth factor-β1 (TGF-β1) is a cytokine that acts as the main mediator of renal fibrosis. The interaction of TGF-β1 and its molecules called Smads

contributes to renal fibrosis. The components of Smads, which are involved in the development of renal fibrosis, are the proteins: Smad2/Smad3 (11). In addition, TGF-β1 plays an important role in epithelial-mesenchymal (EMT) transition. The most common biochemical change associated with EMT is a decline in the E-cadherin expression that signifies changes in epithelial cells to mesenchymal. This change can cause epithelial cells to lose their cells and extracellular matrix. The decrease of E-cadherin is governed by Snail (12,13).

Snail has three isoforms: Snail1, Snail2, and Snail3. Snail1 is an E-cadherin receptor that acts as a cell adhesion molecule and has an important role in the formation and maintenance of the complex tissue integrity stimulated through Smad2/Smad3. As a result, Snail will increase the proliferation of fibroblasts into myofibroblasts, fibronectin, and accumulation of the extracellular matrix (14). Some research has shown that kidney damage in diabetic nephropathy that is mediated by TGF-β/Smads signals can be prevented using Yacon leaves extract (15).

Yacon (*Smallanthus sonchifolius*) is a plant that originates from the Andean mountains and is known as the insulin leaf by the herbal community. This plant consists of roots, stems, leaves, and yellow flowers (16). The Yacon leaves have active compound ingredients such as sesquiterpene lactones (STLs), flavonoids, and phenolic acids. STLs are known for their highly toxic properties. Flavonoids act as a protective repair agent of the kidneys and have the activity of inhibition towards the angiotensin-converting enzyme (ACE) (16). The effects of Yacon on renal failure by the angiotensin system require further study. In addition to other phenolic acids, Yacon leaves are also known to contain compounds such as protocatechuic acid, gentisic acid, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, p-Coumaric acid, ferulic acid, sinapic acid, and quercetin (17). Chlorogenic acids are known for their anti-oxidant and anti-inflammatory properties (18).

The applications of ethanol extracts of Yacon leaves for the treatment of diabetes mellitus and prevention of cardiovascular disease have been widely studied but experimental research is still required to further study the effects of these extracts on chronic renal failure with the subtotal nephrectomy 5/6 model (19). Based on these facts, this research aimed to investigate the effect of Yacon leaves ethanol extracts in preventing the number of fibroblasts, myofibroblast area fraction, TGF-β1 and Snail expressions in chronic renal failure with 5/6 subtotal nephrectomy model.

MATERIALS AND METHODS

Animal Subjects and Subtotal Nephrectomy (SN) model
This study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada based on the certificate of ethical eligibility with number KE/FK/262/EC/2018. Male Swiss-Webster mice (n = 25, 3-months old of age, 30-40 grams body weights) were obtained from the Integrated Research and Testing Laboratory (LPPT) unit 4, Universitas Gadjah Mada, Indonesia. Mice were housed in cages owned by the Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, with a light-dark cycle of 12:12 hours. Mice were fed with standard chow and had access to water ad libitum. The 5/6 subtotal nephrectomy (SN) procedure was performed to induce chronic renal failure. Briefly, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.1 mg/kg). The right flank from the posterior abdominal wall was opened. Then, the kidney uninephrectomy procedure was performed through ligating the vessels followed by kidney excision. At the following day, the superior and inferior poles of contralateral kidney were removed. The Sham Operation (SO) Procedure was used for control group with an open procedure without the 5/6 subtotal nephrectomy.

The mice were divided into five groups: SO (Sham-SN + aquadest 0.1%), SN (SN + aquadest 0.1%), and three groups treated with different oral Yacon leaves ethanol extract administration doses: 0.735 μg/kg (SN+YK1), 1.47 μg/kg (SN+YK2), and 2.94 μg/kg (SN+YK3). The ethanol extract of Yacon leaves was administered via oral gavage for 14 days. Then, on the following day, mice were euthanized.

Yacon Leaves Ethanol Extract
Yacon leaves ethanol extract were obtained from Wonosobo, Central Java, Indonesia. Later identified in the Plant Systematic Section, Faculty of Biology, Universitas Gadjah Mada. Yacon leaves ethanol extract were macerated in 70% ethanol at Pharmacology and Therapy Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Briefly, we used 0.5 kg of dry leaves soaked in 3 L of ethanol, then the leaves were separated, and dried to get the water extract of dry leaves. The process was repeated for this study. Then, we also did Thin Layer Chromatography to evaluate the flavonoid content in the extract. We used the previously established dose of Yacon extract based on the previous study in diabetic conditions (15). Then, we did dose assessment with the conversion factor from rat to mouse to determine the dose. We performed tests with three different doses to assess the possibility of dose-dependent differences in the effects found in this study.

Kidney harvesting
For euthanasia, mice were anesthetized with injection of sodium pentobarbital (0.1 mg/kg), then the abdomen and thorax were opened. Organs were perfused with Phosphate Buffer Saline (PBS) from the left ventricle. Left kidney tissue was then harvested, and the anterior part

was kept in RNA later for RNA extraction. The posterior part was fixated in Normal Buffer Formalin (NBF) for 24 hours, and used for the paraffin embedded tissue process.

Immunostaining

Paraffin sections with 4 µm thickness was used for the following analyses. Immunohistochemical (IHC) staining was done for PDGFRβ antibody (1:200; Abcam, ab32570) to detect fibroblasts and done for α-SMA (1:400; Sigma, A2547) antibody to detect myofibroblasts. Briefly, after deparaffinized, paraffin sections were heated in citrate buffer (10 nM Sodium Citrate, 0.05% Tween20) pH 6, then incubated in 3% H2O2 in PBS. Slides were incubated with the primary antibodies for overnight. At the following days, the slides were incubated with the secondary antibodies for 1 hour (Finetest, IHC008), and probed with diaminobenzidine (DAB) (Finetest, IHC008). Quantification of fibroblasts and myofibroblasts were done using ImageJ software with 15 fields in each sample with 400X magnification.

Reverse Transcriptase PCR Examination

RNA was extracted from kidney tissue using Genezol (GENEzol™, Cat No. GZR100). cDNA was synthesized using ReverTra-Ace (TOYOBO Co; Ltd, TRT-101x10). Reverse transcription-polymerase chain reaction (RT-PCR) was done to examine the expression of the following genes: TGF-β1 (forward: 5′ –TTC CGC TGC TAC TGC AAG TCA - 3′, reverse 5′ – GGG TAG CGA TCG AGT GTC A – 3′), Snail (forward: 5′ – CTG CTT CGA GCC ATA GAA CTA AAG - 3′, reverse: 5′ – GAG GGG AAC TAT TGC ATA GTC TGT - 3′) and GAPDH (forward: 5′ – TTG CTG TTG AAG TCG CAG GAG - 3′; reverse: 5′ – TGT GTC CGT CGT GGA TCT GA - 3′) was used as housekeeping gene. The following PCR conditions were used: 35 cycles with temperature initial denaturation 94°C for 2 minutes, denaturation 94°C for 10 seconds, annealing 58°C for 30 seconds and 72°C for 72 minutes for 1 minute ended with the end extension phase with the condition of 72°C for 10 minutes then electrophoresis using 2% of gel red. Expressions of TGF-β1 and Snail were quantified based on densitometry analysis of the band with GAPDH for normalization using ImageJ software.

Statistical Analysis

Data were presented in mean± standard deviation (SD) and statistical analysis was done using SPSS 22 software for Windows (IBM Corp., Chicago). Values of $p<0.05$ were used for measuring significance among the groups when comparing the average number of fibroblasts, area fraction of myofibroblast, and levels of TGF-β1 and Snail. The data had normal distribution and were analyzed by one-way ANOVA test and continued with Post-hoc test.

RESULTS

Yacon treatment attenuated total fibroblast number

Immunostaining revealed positive staining of PDGFRβ in the interstitial areas on day 14 of SN which indicated the fibroblast number. SN induced a significant higher fibroblast number compared with the levels in the SO group. The histologic picture of the number of renal fibroblasts in the SN group showed the presence of brown positive cells in the interstitial areas through semiquantitative examination on day 14 of SN indicating the fibroblast cell number. These findings were associated with lower fibroblast cell number in the SN+YK1, SN+YK2, and SN+YK3 groups compared with the SN group ($p<0.05$) (Fig. 1).

Yacon treatment attenuated myofibroblast area fraction

Immunostaining revealed positive staining of α-SMA in the interstitial area after 14 days of SN which indicated myofibroblast formation. SN induced a significantly higher number of myofibroblast fraction areas compared with the levels in the SO group. These finding were associated with lower myofibroblast fraction areas in the

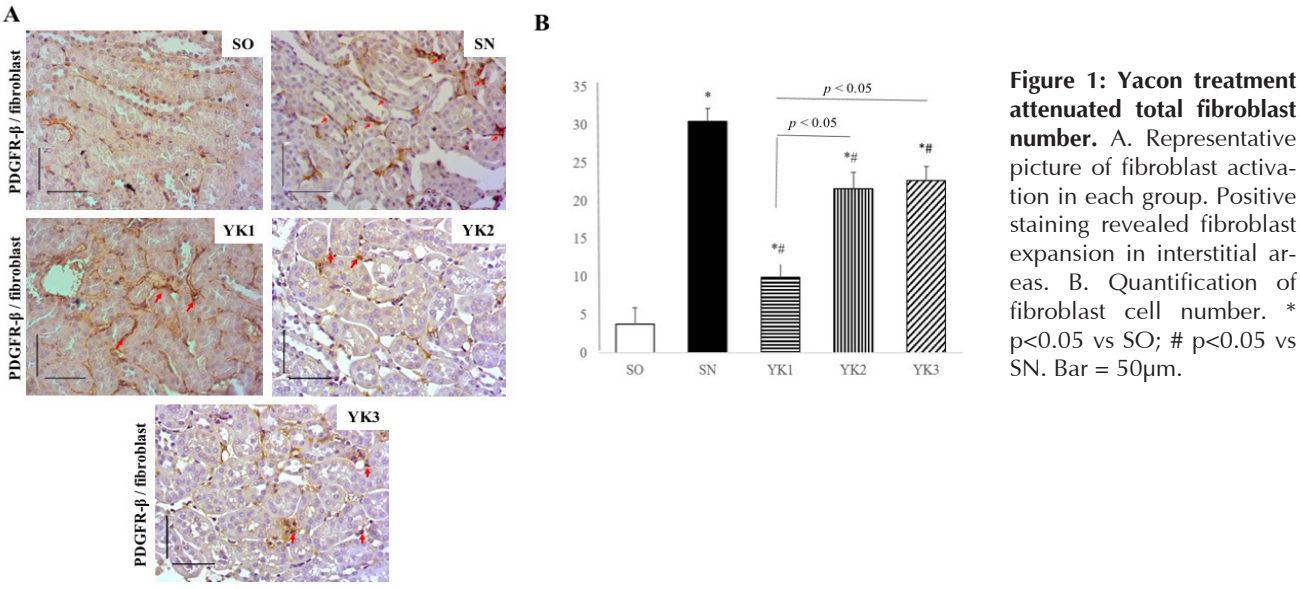

Figure 1: Yacon treatment attenuated total fibroblast number. A. Representative picture of fibroblast activation in each group. Positive staining revealed fibroblast expansion in interstitial areas. B. Quantification of fibroblast cell number. * $p<0.05$ vs SO; # $p<0.05$ vs SN. Bar = 50µm.

SN+YK1, SN+YK2, and SN+YK3 groups compared with the SN group ($p<0.05$) (Fig. 2).

Yacon treatment downregulated TGF-β1 and Snail mRNA expressions

SN groups had significantly higher TGF-β1 expression compared to the SO. This finding showed lower SN induced TGF-β1 secretion. Meanwhile, the Yacon leaves ethanol extract affected the regulation of TGF-β1 mRNA expression compared to the SN group. On the other hand, we found slightly higher expression of Snail in the SN group compared to the SO. Quantification resulted in a reduction in TGF-β1 and Snail expressions in the YK group compared with the SN group (Fig. 3). These finding were associated with lower TGF-β1 and Snail mRNA expressions in the SN+YK1, SN+YK2, and SN+YK3s groups compared with the SN group ($p<0.001$). We did not find any significant difference of TGF-β1 mRNA expressions between the YK1, YK2 and YK3 groups. However, we also did not find any significant difference in Snail mRNA expressions among the various Yacon treated-groups.

DISCUSSION

SN is considered a relevant model for CKD. It is characterized by fibroblast activation, myofibroblast formation, and TGF-β1 up-regulation. SN induces profibrotic growth factors such as TGF-β1, and this profibrotic signaling inhibition attenuates kidney fibrosis. Many cells play a role in renal fibrosis such as fibroblasts, tubule epithelial cells, endothelial cells, vascular smooth muscle cells, mesangial cells, and podocytes (8,9). In this study, Yacon leaves ethanol extract administration reduced interstitial fibrosis. Fibroblasts differentiate to myofibroblasts during kidney fibrosis (10). Myofibroblasts can be identified by α-SMA expression. In normal conditions, myofibroblasts cannot be found in the kidney interstitial space (10,20). Increased α-SMA expression represents the early markers of mesangial damage including fibronectin, type I and III collagen (21). Fibronectin serves as a site for the formation of matrix extension, increased collagen, and other fibronectin molecules thus increasing the expression of TGF-β1 (22). Yacon leaves ethanol extract

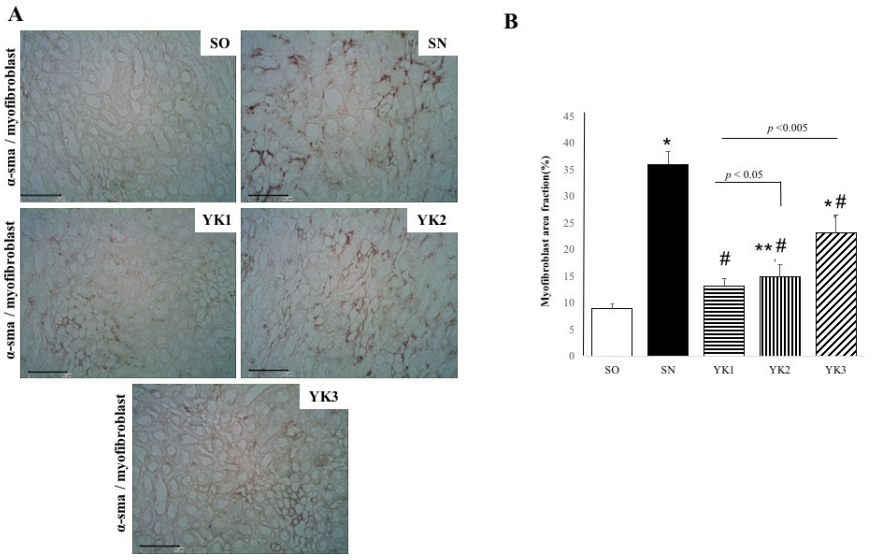

Figure 2: Yacon treatment attenuated myofibroblast area fraction. A. Representative picture of myofibroblast formation in each group. Positive staining revealed myofibroblast expansion in interstitial areas (red arrow). B. Quantification of myofibroblast cell number. * $p<0.05$ vs SO; # $p<0.05$ vs SN. Bar = 50μm.

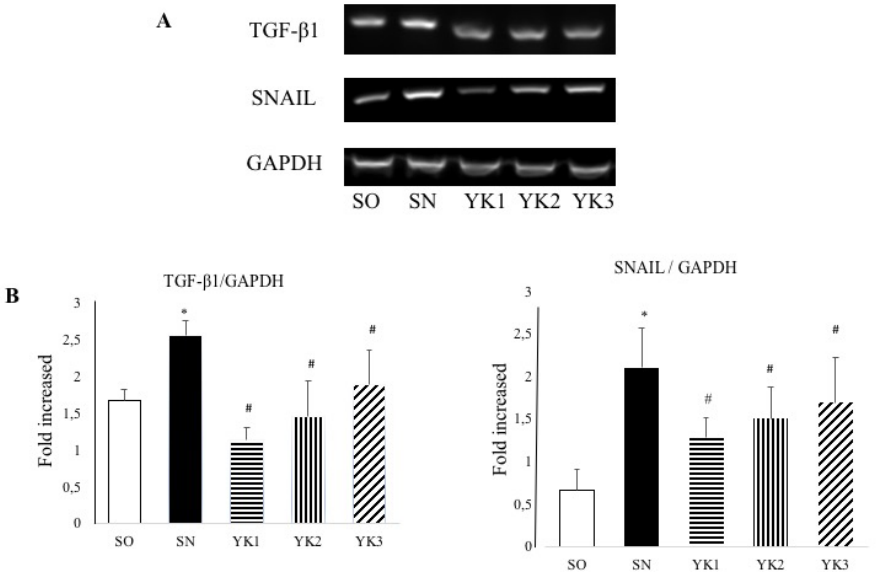

Figure 3: Yacon treatment downregulated TGF-β1 and Snail mRNA expression. A. Representative pictures of TGF-β1 and Snail mRNA expression after SN and Yacon leaves ethanol extract treatment. B. Densitometric analysis of TGF-β1 and Snail mRNA expression after RT-PCR. *$p<0.05$ VS SO, #$p<0.05$ VS SN.

can decrease fibrosis through decreased fibronectin expression (19).

Yacon leaves ethanol extract have active compound ingredients such as sesquiterpene lactones (STLs), flavonoids, and phenolic acids. The beneficial effects of Yacon leaves ethanol extract are from polyphenol compounds which act as free radical inhibitors in lipid peroxidation and cause decreased production of vasoactive mediators that play a role in increasing renal dysfunction (23). The Yacon leaves' content of phenolic acids have also been known to contain various compounds, namely the chlorogenic acid with its known antioxidant and anti-inflammation properties (17,24,25). The common mechanism of free radical inhibition by phenolic antioxidants is through redox reactions that will decrease hydrogen and reactive oxygen levels. Flavonoid acts as a protector for improvement in the kidney (26). In addition, Yacon leaves also contain melampolide compounds. STLs have the activity of forming nitric oxide (NO) (27). Nitric oxide (NO) may play a role in inflammatory and vasodilation reactions (28). However, at excessive levels, NO can play a direct role in various mechanisms of tissue injury such as causing endothelial damage with thrombosis and inactivation of permeability. The nucleotide-binding oligomerization domain (NOD) mechanism starts from cellular necrosis and ends with apoptosis (27).

Other studies have also mentioned that Yacon leaves ethanol extract can decrease the proliferation of fibroblast cells and myofibroblasts, while decreasing the apoptotic level in the group that obtained the ethanol extract of Yacon leaves (29). These results are similar to other in vitro studies, where the Yacon leaves ethanol extract could inhibit the production of α-SMA through inhibition of myofibroblast-fibroblast activity and inhibit myofibroblast proliferation by inhibiting TGF-β1 expression by reducing the receptor expression of the reseptoma (15,20). Mechanisms of chronic renal failure in the 5/6 subtotal nephrectomy model will lead to inflammation that will make the macrophages present in the interstitial space of the kidney. Macrophages then produce cytokines used in tubular apoptosis, activation and proliferation of fibroblasts. One of the pro-fibrotic cytokines in action is TGF-βl. TGF-β1 binds to the TGF-β1 receptors in the membrane, then phosphorylates the Smad2 and Smad3 proteins. Smad2/3 phosphorylation may activate gene transcription, which results in fibroblasts activation and proliferation into myofibroblasts to form an extracellular matrix (30,31). Yacon leaves ethanol extract can inhibit signal transduction of TGF-β/Smad by inhibiting recruitment of pSmad3 to the promoter area at the TGF-β gene target in the diabetic nephropathy model (15).

As a result of continued induction of the CKD the disease progression causes increased inflammatory responses and triggers fibrosis progression (12). The central factor which contributes to fibrosis progression with fibroblast recruitment, proliferation and activation is Transforming Growth Factor-β (TGF-β), then lead to formation of myofibroblast and production of extracellular matrix (32). Myofibroblast formation occurs through TGF-β1 inducing mesenchymal transition from many cells, such as endothelial cells and epithelial cells (33). The most common biochemical changes associated with EMT are a decrease in expression E-cadherin which signifies changes in epithelial cells to mesenchymal. This change can cause epithelial cells to lose their cells and extracellular matrix (12,13). Some genes also contribute to this phenotype transformation, such as Snail which is increased by TGF-β1. Epithelial cells transition to mesenchymal cells (EMT) is promoted by snail (12,13,34–36) (12,13, 18). Other condition also demonstrated role of snail in mesenchymal transition, such as obstructive nephropathy (37), and tumor progression (36). The Snail is an E-cadherin receptor that acts as a cell adhesion molecule and plays an important role in the formation and maintenance of the complexity of complex tissues stimulated by Smad2/Smad3. As a result, Snail will increase the proliferation of fibroblasts into myofibroblasts, fibronectin, and accumulation of extracellular matrix. The cytokine that plays a central role is TGF-β1 which is involved in the activation and proliferation of fibroblasts. TGF-β1 will enhance the EMT process through the Smad2/Smad3 receptors with the change into mesenchymal cells marked by a decrease in the expression of E-cadherin (21,38). In a model of kidney fibrosis, Snail expression upregulation occurs in seven day which represented tubular injury and early event of fibrogenesis process in this model (39).

This finding is similar to other in vitro studies, in which the Yacon leaves ethanol extract can decrease fibrosis by the inhibitory mechanism of TGF-β1 expression by reducing the expression of its receptor so that it can inhibit the activity of Snail which is the E-cadherin receptor gene. When the E-cadherin receptor is inhibited, the E-cadherin transcription will return to normal (40). This inhibitory mechanism occurs via a specific Snail transcription factor that inhibits E-cadherin so that the EMT mechanism is also inhibited (41). Based on results of our study, it revealed attenuation of fibrosis associated with downregulation of TGF-B and snail, thus inducing reduction of fibroblast activation and myofibroblast formation.

This study also demonstrated the lower dose of Yacon extract has more protective effect, although our reference dose was based on the highest dose from previous study (15). The difference of subject selection and model may have some effect in the dose responses, since mice were used instead of rats in this study. The animal model was also different. Diabetes Mellitus (DM) with hyperglycemia was used in the previous study, which is a model with slow progression to induce kidney injury, while we used the 5/6 subtotal nephrectomy which

demonstrated rapid disease progression to induce kidney injury. Not elucidating the difference in the effect of the Yacon doses may become another of the limitations of this study, while cytotoxic analysis of the Yacon extracts needs to be done by the future research for continuation of this study.

CONCLUSION

We conclude that the administration of Yacon leaves ethanol extract ameliorates kidney fibrosis through reducing fibroblast number, myofibroblast fraction area, and downregulating the mRNA expressions of TGF-β1 and Snail.

ACKNOWLEDGEMENTS

The authors would like to extend our thanks to Mrs. Wiwit Ananda Wahyu Setyaningsih and Mr. Mulyana for all of their help as the laboratory assistants in the Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Some of the data were used for completing of master program for Ermawati. This research was funded by Dana Masyarakat, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada.

REFERENCES

1. Kaze F, Halle M, Mopa H, et al. Prevalence and risk factors of chronic kidney disease in urban adult Cameroonians according to three common estimators of the glomerular filtration rate: a cross-sectional study. BMC Nephrology. 2015;16(1).
2. Lypez-Novoa JM, Martнnez-Salgado C, Rodrнguez-Peca AB, Lypez-Hernбndez FJ. Common pathophysiological mechanisms of chronic kidney disease: therapeutic perspectives. Pharmacol Ther. 2010;128(1):61-81.
3. Lane BR, Demirjian S, Derweesh IH, Riedinger CB, Fergany AF, Campbell SC. Is all chronic kidney disease created equal? Current Opinion in Urology. 2014;24(2):127-34..
4. Cernaro V, Santoro D, Lacquaniti A, et al. Phosphate binders for the treatment of chronic kidney disease: role of iron oxyhydroxide. Int J Nephrol Renovasc Dis. 2016;9:11-9.
5. Prodjosudjadi W, Suhardjono, Suwitra K, et al. Detection and prevention of chronic kidney disease in Indonesia: initial community screening. Nephrology (Carlton). 2009;14(7):669-74.
6. Mack M, Yanagita M. Origin of myofibroblasts and cellular events triggering fibrosis. Kidney Int. 2015;87(2):297-307.
7. Arfian N, Muflikhah K, Soeyono SK, et al. Vitamin D attenuates kidney fibrosis via reducing fibroblast expansion, inflammation, and epithelial cell apoptosis. Kobe J Med Sci. 2016;62(2):E38-44.
8. Boor P, Sebekovб K, Ostendorf T, Floege J. Treatment targets in renal fibrosis. Nephrol Dial Transplant. 2007;22(12):3391-407.
9. Zeisberg M, Neilson EG. Mechanisms of tubulointerstitial fibrosis. J Am Soc Nephrol. 2010;21(11):1819-34.
10. Strutz F, Zeisberg M. Renal fibroblasts and myofibroblasts in chronic kidney disease. J Am Soc Nephrol. 2006;17(11):2992-8.
11. Lan HY. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. Int J Biol Sci. 2011;7(7):1056-67.
12. Smith BN, Odero-Marah VA. The role of Snail in prostate cancer. Cell Adh Migr. 2012;6(5):433-41.
13. Horiguchi K, Shirakihara T, Nakano A, Imamura T, Miyazono K, Saitoh M. Role of Ras signaling in the induction of Snail by transforming growth factor-beta. J Biol Chem. 2009;284(1):245-253.
14. Grande MT, Sбnchez-Laorden B, Lypez-Blau C, et al. Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. Nat Med. 2015;21(9):989-97.
15. Honorй SM, Cabrera WM, Genta SB, Sбnchez SS. Protective effect of yacon leaves decoction against early nephropathy in experimental diabetic rats. Food and Chemical Toxicology. 2012;50(5):1704–15
16. Habib NC, Honorй SM, Genta SB, Sбnchez SS. Hypolipidemic effect of Smallanthus sonchifolius (Yacon) roots on diabetic rats: biochemical approach. Chem Biol Interact. 2011;194(1):31-9.
17. de Andrade E, Leone R, Ellendersen L, Masson M. Phenolic profile and antioxidant activity of extracts of leaves and flowers of Yacon (Smallanthus sonchifolius). Industrial Crops and Products. 2014;62:499-506.
18. Valentovб K, Ulrichovб J. Smallanthus sonchifolius and Lepidium meyenii: prospective Andean crops for the prevention of chronic diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2003;147(2):119-30.
19. Baroni S, Suzuki-Kemmelmeier F, Caparroz-Assef SM, Cuman RKN, Bersani-Amado CA. Effect of crude extracts of leaves of Smallanthus sonchifolius (Yacon) on glycemia in diabetic rats. Rev. Bras. Cienc. Farm. 2008;44(3):521-30.
20. Tan X, Li Y, Liu Y. Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. J Am Soc Nephrol. 2006;17(12):3382-93.
21. Liu Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. Kidney Int. 2006;69(2):213-7.22. Kadler KE, Hill A, Canty-Laird EG. Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. Curr Opin Cell Biol. 2008;20(5):495-501.
23. Valentovб K, Sersen F, Ulrichovб J. Radical scavenging and anti-lipoperoxidative activities of Smallanthus sonchifolius leaf extracts. J Agric Food

Chem. 2005;53(14):5577-82.
24. Russo D, Malafronte N, Frescura D, et al. Antioxidant activities and quali-quantitative analysis of different Smallanthus sonchifolius [(Poepp. and Endl.) H. Robinson] landrace extracts. Nat Prod Res. 2015;29(17):1673-7.
25. Sousa S, Pinto J, Rodrigues C, et al. Antioxidant properties of sterilized yacon (Smallanthus sonchifolius) tuber flour. Food Chem. 2015;188:504-9.
26. Asl MN, Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza radix and its bioactive compounds. Phyther Res. 2008;22(4):709–24.
27. Sugahara S, Ueda Y, Fukuhara K, et al. Antioxidant effects of herbal tea leaves from yacon (Smallanthus sonchifolius) on multiple free radical and reducing power assays, especially on different superoxide anion radical generation systems. J Food Sci. 2015;80(11):C2420-9.
28. Maia LB, Moura JJ. Nitrite reduction by molybdoenzymes: a new class of nitric oxide-forming nitrite reductases. J Biol Inorg Chem. 2015;20(2):403-33.
29. Rocha D, Ribeiro P, Caldas A, da Silva B, da Silva A, de Almeida A et al. Acute consumption of yacon shake did not affect glycemic response in euglycemic, normal weight, healthy adults. Journal of Functional Foods. 2018;44:58-64.
30. Sun YB, Qu X, Caruana G, Li J. The origin of renal fibroblasts/myofibroblasts and the signals that trigger fibrosis. Differentiation. 2016;92(3):102-107.
31. Ito I, Waku T, Aoki M, et al. A nonclassical vitamin D receptor pathway suppresses renal fibrosis. J Clin Invest. 2013;123(11):4579-94.
32. Xu C, Wang WEI, Xu M, Zhang JIE. Asiatic acid ameliorates tubulointerstitial fibrosis in mice with ureteral obstruction. Exp Ther Med. 2013;(13):731–6.
33. Chevalier RL, Forbes MS, Thornhill BA. Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. Kidney Int. 2009;75(11):1145–52.
34. Baroni S, da Rocha BA, Oliveira de Melo J, Comar JF, Caparroz-Assef SM, Bersani-Amado CA. Hydroethanolic extract of Smallanthus sonchifolius leaves improves hyperglycemia of streptozotocin induced neonatal diabetic rats. Asian Pac J Trop Med. 2016;9(5):432–6.
35. Boutet A, Frutos CA De, Maxwell PH, Jose M, Romero J, Nieto MA. Snail activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. EMBO J. 2006;25(23):5603–13.
36. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat rev Mol Cell Biol. 2002;3(March):155:66.
37. Yoshino J, Monkawa T, Tsuji M, Inukai M, Itoh H. Snail1 is involved in the renal epithelial – mesenchymal transition. BBRC. 2007;362:63–8.
38. Lim S, Becker A, Zimmer A, Lu J, Buettner R, Kirfel J. SNAI1-mediated epithelial-mesenchymal transition confers chemoresistance and cellular plasticity by regulating genes involved in cell death and stem cell maintenance. PLoS One. 2013;8(6):1–12.
39. Sato M, Muragaki Y, Saika S, Roberts AB. Targeted disruption of TGF-β1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. J Clin Invest. 2003;112(10):1486–94.
40. Velez E, Castillo N, Mesyn O, Grau A, Bibas Bonet ME, Perdigyn G. Study of the effect exerted by fructo-oligosaccharides from yacon (Smallanthus sonchifolius) root flour in an intestinal infection model with Salmonella Typhimurium. Br J Nutr. 2013;109(11):1971-9.
41. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15(3):178-96.