Effect of Ethanol Extract of Puguntano Leaf (Curanga Fel-Terrae (Lour) Merr.) on the Expression of TGF-B1, E-SELECTIN, Glomerulosclerosis, Interstitial Fibrosis, and Renal Function in Wistar Strained Rats Which was Given Doxorubicin

Alwi Thamrin Nasution[1], Dharma Lindarto[2], Rosita Juwita Sembiring[3], Muara Panusunan Lubis[4], Delyuzar[5], Bambang Purwanto[6], Syafruddin Ilyas[7], Juliandi Harahap[8], Santi Syafril[2]

[1]Department of Internal Medicine, Division of Nephrology and Hypertension, Universitas Sumatera Utara, Medan, Indonesia, 20155
[2]Department of Internal Medicine, Division of Endocrine Metabolic and Diabetes, Universitas Sumatera Utara, Medan, Indonesia, 20155
[3]Department of Pathology Clinic, Universitas Sumatera Utara, Medan, Indonesia
[4]Department of Obstetrics and Gynaecology, Universitas Sumatera Utara, Medan, Indonesia
[5]Department of Pathology Anatomy, Universitas Sumatera Utara, Medan, Indonesia
[6]Department of Internal Medicine, Division of Nephrology and Hypertension, Universitas Negeri Surakarta, Surakarta, Indonesia
[7]Biomedical Science Department, Universitas Sumatera Utara
[8]Universitas Sumatera Utara, Medan, Indonesia
Email: alwi_thamrin@yahoo.com

Abstract

The increasing use of Doxorubicin (DXR) in cancer treatment has highlighted its nephrotoxic effects. Puguntano leaf ethanol extract, known for its anti-inflammatory properties, may offer nephroprotective benefits. This study investigates whether combining DXR with Puguntano leaf ethanol extract can mitigate DXR's nephrotoxicity. The study used an experimental posttest control group design, conducted in various USU Medical Faculty laboratories. Thirty male Wistar mice were divided into three groups: control (K1), DXR-treated (K2), and DXR combined with Puguntano leaf ethanol extract (K3). The DXR was administered intraperitoneally weekly for three weeks, while the Puguntano extract was given orally at 200 mg/kg daily for three weeks. In the fourth week, all mice were sacrificed using ketamine. TGF-β1 and E-selectin expressions were assessed via immunohistochemistry. Glomerulosclerosis and interstitial fibrosis were examined using Masson's Trichome. Blood tests for urea and creatinine were conducted using immunoturbidimetry, and proteinuria was assessed with urine dipstick tests. Data were analyzed using ANOVA. Results indicated that DXR significantly increased TGF-β1 (253.5%±65.1), E-selectin (218.0±70.4), glomerulosclerosis, interstitial

fibrosis, proteinuria, urea, and creatinine levels (p = 0.001). However, the combination of DXR and Puguntano extract significantly reduced TGF-β1 (180.0±51.0), E-selectin (172.5±57.98), and the other aforementioned markers (p = 0.001). In conclusion, combining DXR with Puguntano leaf ethanol extract effectively reduces the nephrotoxic effects of DXR, suggesting a potential therapeutic approach to mitigate DXR-induced kidney damage.

Keywords: doxorubicin; ethanol extract of puguntano leaves; glomerulosclerosis.

Chronic kidney disease (CKD) is a global public health problem with increasing prevalence and incidence, has a poor prognosis and costs a lot in its treatment. The prevalence of CKD increases with the increase in the elderly population and the incidence of diabetes mellitus and hypertension. Globally, the prevalence of CKD stage 1-5 is 13.4% (Susanto, 2018). Based on data from the Indonesian Ministry of Health, the prevalence of chronic kidney disease (permil) in the population aged > 15 years increased from 2.0 in 2013 to 3.8 in 2018. (Riskesdas, 2018).

About 1 in 10 global populations experience CKD at some stage. According to the results of the Global Burden of Disease in 2010 , CKD was the 27th leading cause of death in the world in 1990 and increased to 18th in 2010. IRR (Indonesian Renal Registry) data in 2018 recorded 66,433 new hemodialysis patients, in 2007 there were 4,977 people. There was an increase of about 13%. Chronic kidney disease is initially asymptomatic and signs but can progress into end-stage chronic kidney disease, increasing cardiovascular risk and death. Kidney disease can be prevented or treated with effective therapy when known earlier (KDOQI, 2018). In 2018, the proportion of etiology or basic disease of stage 5 dialysis CKD patients is hypertension ranks first.

as much as 36% and Diabetic nephropathy or known as diabetic kidney dieasese (DKD) as the second order (28%) Primary Glumerulopathy (GNC) 10%, Chronic Pyelonephritis (PNC) 3%, Obstruction Nephropathy 3%, Uric Acid Nephropathy 1%, Polycystic Kidney 1%, others 5% and the most age is aged 45-54 years (30.56%). (IRR, 2017)

Various diseases can be the cause of kidney fibrosis which can end up being a late-stage kidney disease, including: diabetes, hypertension, glomerulonephritis, infectious, renal vasculitis, ureteral obstruction, genetic changes, autoimmune diseases and drugs. Renal fibrosis is a consequence of kidney injury and is associated with renal dysfunction that can end in kidney damage. Renal fibrosis is mainly associated with glomerulosclerosis and interstitial fibrosis, characterized by tubule atrophy, tubule dilatation, increased fibrogenesis and deposition of collagen as well as the extracellular matrix. (Nogueira et al., 2017)

Recent studies have shown that renal fibrosis is associated with reactive oxygen species (ROS) and free radicals. ROS continues to be produced physiologically, and plays an important role in the expression of cell functions such as impulse transmission. However, if overproduced from any cause, ROS plays a cytotoxic role as a mediator of adverse events such as inflammation, necrosis, and apoptosis. ROS is an important cause of kidney disease both acute and chronic.

Progressive deterioration of renal function in CKD, will induce several biological and clinical dysfunctions including changes in cellular energy metabolism, changes in Nitrogen input or output, protein malnutrition, insulin resistance and increased synthesis of inflammatory mediators or oxidative stress. Free radicals such as superoxide and hydroxyl radicals easily interact with molecular components in the nephron. (Kao et al., 2010), which produces an inflammatory response. Neutrophils and other recruited phagocyte cells will damage the nephron, producing superoxide through the

nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in the membrane, where electrons are transferred from NADPH in the cell across the membrane combining to oxygen molecules, thus forming superoxide. Superoxide and other radicals continue to increase kidney injury or act as messanger molecules generating a local inflammatory response in the kidneys on an ongoing basis. (Tucker et al., 2015).

Several inflammatory cytokines also play a role in the progressivity of kidney disease, including TNF-α, IL-6, IL-10, E-Selectin, TGF-β, and Caspase-3 In previous studies it was also found that hydrogen sulfide (H2S) administration can improve kidney disease in rats by inhibiting apoptosis and inflammation through the ROS / MAPK and NF-κB signaling pathways (Wu et al., 2016)

E-selectin works by binding PMN to the kidney injury site so as to trigger the release of IL-I and TNF-α at the site of cell damage. Such release will stimulate the expression of e-selectin from the endothelium of blood vessels. The occurrence of endothelial dysfunction in blood vessels, there will also be endothelial dysfunction of the glomerular capillaries which will reduce negativity so that microproteinuria occurs (Purwanto, 2012). According to previous studies the involvement of TGF-β 1 on the formation of renal scar tissue is also through increased synthesis of the extracellular matrix. (Agarwal R, 2002; Park HC, 2003; Praga M, 2006). At first glomerular inflammation occurs followed by injury to tubule epithelial cells and recruitment of inflammatory cells. Next comes the formation of sclerotic tissue and fibrosis, finally terminal renal failure occurs. (Gerritsma JS,1996).

One of the plants that is known to have medicinal properties is Puguntano Leaf (Curanga fel-terrae (Lour.) Merr.) or often called Picria fel-terrae. Puguntano leaf is a plant that grows in Asian regions such as Indonesia, China, India, the Philippines, Malaysia and Myanmar. (USRDS, 2015) Puguntano leaves are traditionally often used by the people of Tiga Lingga Village, Dairi Regency, North Sumatra Province as an anti-diabetic medicine. In addition, this plant is also believed to be efficacious as a pain reliever in the body, increase endurance, and even as an anti-aging to look youthful. Puguntano leaf ethanol extract obtained from percolation and socleation methods contains phytochemicals, namely flavonoids, saponins, tannins, glycosides, and steroids / terpenoids (Harahap et al., 2013; Juwita, 2009). The group of secondary metabolite compounds of puguntano leaf ethanol extract identified is glycosides (Zhou et al., 2005; Huang et al., 1998), flavonoids (Huang et al., 1999), saponins (Fang et al., 2009), and terpenoids (Wang et al., 2006). Flavonoid, tannin, and saponin compounds from various types of plants are known to have antioxidant and anti-inflammatory effects from various previous research results. Research using puguntano leaf ethanol extract showed that the suspension of puguntano leaf ethanol extract dose 100 mg / kg bb provides a decrease in blood sugar levels almost the same as glycenclamide suspension dose of 10 mg / kg bb (Sumantri et al., 2017). Clinical observations by Harfina et al. (2012) using simplisia powder of puguntano leaf ethanol extract dose 2 gr, 3 times a day for 14 days given orally by patients in the form of steeping, obtained the effect of reducing blood sugar levels in DM (Harfina et al. 2012). From preliminary clinical trials, the administration of ethanol extract of puguntano leaves capsules Dhawalsan-1 (Curanga fel-terrae (Lour) Merr.) □ for 12 weeks effectively lowered fasting blood glucose levels, HbA1c, and HOMA-IR and increased adiponectin levels which is statistically meaningful in newly diagnosed DMT2 patients where the study mentioned Adiponectin is known to increase insulin sensitivity through stimulation of glucose use and decreased concentration of free fatty acids. Adiponectin increases insulin sensitivity and has anti-atherogenic as well as anti-inflammatory properties. (Lindarto et al., 2016). Other studies

also stated that there was a decrease in adiponectin levels in patients suffering from kidney disease. Adiponectin can work by lowering NADPH oxidase thereby reducing the formation of ROS and other free radicals thereby lowering the expression of proinflammatory cytokines (Sweis and Sharma, 2014).

Several studies have studied the content of chemical compounds in puguntano leaf ethanol extract and their effects on health. The results of research conducted by Juwita show that the puguntano leaf ethanol extract plant has the potential as an anti-inflammatory. In this study, puguntano leaf ethanol extract can increase PPAR-γ levels (Syafril et al. 2017).

Based on the mechanism, the researchers are trying to take an approach to finding new therapeutic agents that can inhibit progressiveness in chronic kidney disease and renal fibrosis. A promising new medicator is the SGLT 2 and GLP1 antagonists that can be used mainly in patients with diabetes. Another approach is through TGF-β antagonists and the use of antioxidant agents. (Rayego-Mateos et al., 2020).

Doxorubicin (DXR) is one of the most widely used cancer chemotherapy in its own form or combined with other chemotherapy drugs, such as in multiple myeloma cancer, breast, lung, ovarian cancer and others. DXR has side effects against the kidneys, cardiovascular, gastrointestinal, hematopoethic, skin, and others (Storm and Hoesel, 2007; Laghina et al., 2007).

DXR side effects are mediated by reactive oxygen species (ROS) and NFKβ. Doxorubicin will trigger damage from mitochondria and Kβ inhibitors resulting in hyperactivity of NFKβ and ROS. NFKβ and ROS will damage nephron cells. Damaged nephron cells (debris) will activate macrophages through toll-like receptor4 (TLR4) and express cytokines, such as TNF-α1, TGF-β1, IL-1β, IL-6, and IL-8. TGF-β1 in mesangial cells will stimulate the expression of type-IV and type-I collagen, which will produce components of the extracellular matrix and the occurrence of nephron cell fibrosis which eventually occurs damage to glomerulosclerosis nephron cells and interstitial fibrosis. This will cause damage and leakage of renal tubule function so that there is a disturbance in the excretion of metabolic remains such as ureum, creatinine and proteinuria. Proteinuria will be reabsorbed by proximal tubule cells. Proteinuria that lingers for a long time will cause the work of the proximal tubules to increase and is a stressor for the formation of pro-inflammatory cytokines (TNF-α, IL-6, Il-8, IL-1β, TGF-β1). These pro-inflammatory cytokines will damage nephron cells. Proteinuria is an important and sensitive non-invasive sign of kidney damage, proteinuria is also a sign of glomerular endothelial damage (Purwanto, 2010).

In previous studies, rat kidney damage occurred at a dose of 0.0019 mg/grBB. By administering a combination of pentoxifilin (PTX) and DXR prevents an increase in TGF-β1 expression that decreases the expression of type-IV and type-I collagen, then inhibits the glomerulosclerosis and interstitial fibrosis processes (Purwanto, 2010). Other studies using alpha lipoic acid (ALA) significantly reduced the effects of DXR-induced renal toxicity (Sayeed E, et al, 2017).

Benzer F, et al. (2018) showed that DXR (40 mg/kg) induces glomerular and tubulointersital damage. Renal damage induced by DXR is manifested by an increase in ureum and serum creatinine. DXR increases TNF-α, COX 2, and iNOS. where COX-2 increases the production of cytokines TNF-α and IL-6 which are inflammatory mediators. (Benzer F et al., 2018).

Other studies have also shown that reducing oxidative stress and inflammation are two commonly used approaches to slow the progressiveness of kidney function deterioration. Oxidative and inflammatory stress have a significant correlation with the estimated glomerular filtration rate so that biological agents that have anti-inflammatory and antioxidative activity can be developed in the management of renal disorders (Sundaran et al., 2014).

This study utilizes traditional medicine as an inhibitor / reduce the nephrotoxic effect of DXR. Traditional medicine is a hereditary heritage based on experience / empirics developed rapidly in society in Indonesia and further developed through scientific substantiation of pre-clinical trials and clinical trials. For this reason, it is necessary to develop traditional medicine in a sustainable manner so that it is utilized optimally to improve public health services. One of the complementary traditional health service methods contained in Government Regulation Number 103 of 2014 concerning traditional health services is traditional health services using potions. The development of traditional health services using potions is currently increasing rapidly, as evidenced by the results of Basic Health Research (Riskesdas, 2010) that the percentage of Indonesians who have consumed herbal medicine is 59.12%. When administering DXR combined with puguntano leaf ethanol extract is expected to cause a decrease in the expression of TGF-β1, E-selectin, glomerulosclerosis, interstitial fibrosis, ureum creatinine, and proteinuria.

In connection with the description above, researchers are interested in conducting research on the effect of puguntano leaf ethanol extract on TGF-β1, E-selectin, glomerulosclerosis, interstitial fibrosis, ureum, creatinine, and proteinuria levels in DXR-induced wistar strain rats.

Method

This study used purely experimental with posttest with control group design. Data were analyzed using the Anova assay, to obtain differences in expression of TGF-β1, E-Selectin, glomerulosclerosis, interstitial fibrosis, ureum, creatinine and proteinuria under normal circumstances, nephrotoxic and nephroprotective at week 4 post-treatment. The data were processed and analyzed using SPSS with a meaningfulness limit of $p < 0.05$.

Result and Disscusion

Result

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) against TGF-β 1 expression in wistar strain mice

Table 1 Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) against TGF-β 1 expression in wistar strain mice

Group	TGF-β1 (%)	P-value
K1	89,7 ± 21,4	
K2	253,5 ± 65,1	0,001*
K3	180,0 ± 51,0	

The data are presented in the form of the Mean ± SD, Kruskal Wallis Test, significant if the value *p<0.05.

The results of the post Hoc test of TGF-β1 expression in wistar strain mice are presented in table 4.2.2. In the results, a significant difference was obtained between TGF-β1 expression in the control group (K1) compared to the group that obtained DXR (K2) (p=0.001). The test results also showed a significant difference between the control group (K1) and the group given DXR and puguntano extract (K3) (p=0.001), and there was a significant difference between the group given DXR alone (K2) compared to the group given DXR plus puguntano leaf extract (K3).

In the mean difference results , it was seen that the toxicity effect of DXR (K2) could increase TGF-β1 expression by 163.8% in wistar strain mice compared to the control group (K1). Meanwhile, the administration of puguntano leaf extract (K3) had a protective effect on reducing TGF-β1 expression by 73.5% in wistar strain rats given DXR (K2).

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) to E-selecttin expression in wistar strain mice

Table 2 Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) to E-selectin expression in wistar strain mice

Group	E-selectin (%)	P-value
K1	103,0 ± 40,22	0,001*

K2	218,00 ± 70,4
K3	172,5 ± 57,98

The data are presented in the form of the Mean ± SD, Kruskal Wallis Test, significant if the value *p<0.05.

The results of the post Hoc test of E-selectin expression in wistar strain mice are presented in table 4.3.2. In the results, a significant difference was obtained between E-selectin expression in the control group (K1) compared to the group that got DXR (K2) (p=0.008). The test results also showed a significant difference between the control group (K1) and the group given DXR and puguntano extract (K3) (p=0.012), and there was a significant difference between the group given DXR alone (K2) compared to the group given DXR plus puguntano leaf extract (K3).

In the mean difference results , it was seen that the toxicity effect of DXR (K2) could increase E-selectin expression by 115% in wistar strain mice compared to the control group (K1). Meanwhile, the administration of DXR with puguntano leaf extract (K3) had a protective effect of reducing E-selectin expression by 69.5% in wistar strain rats given DXR (K2).

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) Against the process of glomerulosclerosis in wistar strain rats

Table 3 Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) Against the process of glomerulosclerosis in wistar strain rats

Group	Glomerulosklerosis (%)				
	<5	5-25	26-50	50-75	76-100
K1	4	6	0	0	0
K2	0	0	1	7	2
K3	0	2	7	1	0
Total	4	8	8	8	2

The data is presented in the form of a frequency distribution.

In this study, the results of the glomerulosclerosis sclerosis index (table 4.4.2), where the average glomerulosclerosis sclerosis index in group 1 was 65.5 ± 27.6%, the average glomerulosclerosis index in group 2 was 313.9 ± 48.9%, the average glomerulosclerosis sclerosis index in group 3 was 202.2 ± 48.67% . There was a statistically significant difference in glomerulosclerosis index values between groups with p<0.05 values (p=0.001).

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) Against Interstitial Fibrosis Processes in Wistar Strain Mice

Table 4 Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) Against the process of interstitial fibrosis in wistar strain rats.

Group	Interstisial Fibrosis (%)				
	<5	5-25	26-50	>50	75-100
K1	3	7	0	0	0
K2	0	0	2	7	1
K3	0	4	3	3	0
Total	3	11	5	10	1

Data presented in the form of frequency distribution

In this study, the results of the interstitial fibrosis sclerosis index were obtained (table 4.5.2), where the average interstitial fibrosis sclerosis index in group 1 was 79.60 ± 33.50, the average interstitial fibrosis sclerosis index in group 2 was 283.90 ± 35.90 the average interstitial fibrosis sclerosis index in group 3 was 181.10 ± 54.60. There was a statistically significant difference in the value of the interstitial fibrosis sclerosis index between groups with a value of p<0.05 (p=0.001).

Disscusion

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour) Merr.) on the expression of TGF-β1 and E-selecttin

This study is a preliminary study that examines the effect of puguntano leaf extract on reducing TGF-β1 and E-selecttin levels so that it is expected to prevent an increase in the process of glomerulosclerosis and interstitial fibrosis, as

well as ureum, creatinine, and proteinuria levels in CKD rats.

TGF-β is one of the main mediators in the pathogenesis of renal fibrosis that can induce kidney scarring. The Ren et al study mentioned that ROS can be induced by administering DXR and mediating fibrosis through TGF-β-dependent pathways. DXR has a major role in the nephrotoxicity of DXR ($p < 0.001$) (Ren et al., 2016). The study of Hekmat et al found that TGF-β levels in the kidneys and urine were higher in the DXR group than in the control group ($p < 0.001$) (Hekmat et al., 2021). In this study, K3 given puguntano leaf extract treatment at a dose of 200mg/kgbb/day had TGF-β 1 levels lower than K2 (with DXR treatment alone (253.5%), and showed statistically significant results ($p=0.001$).

This research is in line with Purwanto's research. The results of immunohistochemistry studies after 4 weeks of treatment showed that in the normal mice group had TGF-β1 expression in the kidney tissue of 3.25 ± 4.98 immunoreactive cells / 1000 macrophage cells. Administration of DXR to animals

try to significantly increase the mean expression of TGF-β1 to 74.63 ± 12.50 immunoreactive cells / 1000 macrophage cells ($p = 0.001$). Meanwhile, in the administration of the combination of DXR with Pentoxivilin to 15.75 ± 3.41 immunoreactive cells / 1000 macrophage cells ($p = 0.001$) (Purwanto et al., 2010).

In contrast to the findings of Szalay et al, where TGF-β1 mRNA levels in the renal cortex did not differ markedly between the control groups compared to DXR-injected Black Hooded (BH) mice, but increased significantly in the DXR-injected group of Charles Dawley (CD) mice. In the study, CD rats were more sensitive to progressive proteinuria as early as two weeks after administration of 5mg/kg of DXR. Single administration of DXR causes tubulointerstitial inflammation and fibrosis shown by PAS, fibronectin and collagen synthesis, as well as the discovery of pro-fibrotic transformation growth factor (TGF-β1) (Szalay et al., 2015).

The amount of TGF-β1 in the tubulo-interstitial region correlates with the degree of interstitial inflammation and atrophy of the tubules. TGF-β1 levels in the urine in cases of glomerulonephritis with proteinuria were significantly higher compared to levels in IgA nephropathy patients without proteinuria or compared to healthy people (Goumenos DS, 2002; Murakami K,1997).

As for the E-selectin level of this study, in the K3 group (172.5 %) was lower than the K2 group (218%) but higher than the K1 group (103%) ($p=0.001$). E-selectin is the main cell adhesion molecule expressed by endothelium cells. Higher circulating E-selectin concentrations are associated with the development of atherosclerotic plaques and endothelial dysfunction of the glomerular capillaries which will reduce negativity so that microproteinuria occurs (Wong et al., 2021). E-Selectin cell-surface is a mediator of leukocyte adhesions to the endothelium that plays a role in the extravasation of leukocytes at the site of inflammation, so this E-selectin factor plays a key role in the local inflammatory response (Juan F., 2011). The administration of flavonoids is considered to be able to inhibit the production of these adesi molecules so as to protect endothelial cell damage due to the excretion of iNOS and COX-2 (Crespo et al., 2008). Some flavonoids such as flavones, flavonols, flavonons, isoflavones, and chalcones have been studied to inhibit E-selectin by down-regulating transcription levels of these molecules (Takano-Ishikawa, Goto and Yamaki, 2003; Choi et al., 2004).

Another research that supports the results of this research was delivered by Al-Waili N, et al (2017) from guava leaf extract inhibiting the production of LPS-induced NO and PGE2, TNF-α, IL-1β, IL-10, iNOS, COX-2, and transcription activity of NFkB, LPS-induced E-selectin in diabetic rats. The flavonoid fraction of guava leaf extract suppresses the expression of inflammatory mediators involving inhibition of NFkB activation through suppression of LPS-

induced IκB-α degradation. Proanthocyanindin (one form of flavonoid oligomer) from grape seeds also showed significant improvement in kidney markers by lowering levels of caspase-3, iNOS, NFkB, TNF-α and bax, also increasing levels of Bcl-2 protein expression (Al-Waili N, et al., 2017).

TNF-α can trigger endothelium, so the endothelium will express e-selectin, e-selectin will bind to PMN leukocyte cells, PMN will express lysosim. Lysosim is proteolytic so it can cause cell necrosis. E-selectin works by binding PMN to the site of injury so as to trigger the release of IL-I and TNF-α at the site of cell damage. Such release will stimulate the expression of e-selectin from the endothelium of blood vessels. Leukocytes in the blood express proper ligands then bind to e-selectin, causing the leukocytes to move along the walls of blood vessels. The occurrence of endothelial dysfunction in blood vessels, there will also be endothelial dysfunction of the glomerular capillaries which will reduce negativity so that microproteinuria occurs (Purwanto, 2012).

Studies by Patilaya et al and Syafril et al mentioned phytochemical extraction of Puguntano leaf extract containing flavonoid group compounds, glycosides, saponins, tannins, steroids, and terpenoids (Patilaya and Husori, 2017; Syafril et al., 2019). Olomanira et al added flavonols to Pugutano leaves allegedly working by increasing the antioxidant activity of gluthation s-transferase (GSH), increasing GSH synthesis, and directly trapping the ROS formed so that it can inhibit COX and reduce kidney damage. Antioxidant activity is influenced by the content of active compounds in extracts such as flavonoids. Flavonoids will donate hydrogen or electrons to free radicals to stabilize radical compounds, so the higher the flavonoid content in the extract, the higher the antioxidant activity will also be higher (Olomanira B., 2019).

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour) Merr.) on glomerulosclerosis process and interstitial fibrosis process in wistar strain rats

In this study, the average glomerulosclerosis index in K3 given Puguntano extract was 202.2 ± 48.67%, which is lower than K2 who received DXR alone, namely 313.9 ± 48.90%. A total of 4 mice in this study had interstitial fibrosis <5%, and 8 mice each in the process of interstitial fibrosis 5-25%, 26-60%, and >50%. Significant results were obtained on the difference in the fibrosis percentage index in the group with and without pugutano leaf extract treatment (p=0.001).

This research is in line with the Purwanto study, 2010, which compared the sclerosis index in mice with DXR compared to DXR+Pentoxivilin. The results showed that the sclerosis index of the normal mice group was 35.38 ± 12.83. The administration of DXR can increase the meaningful sclerosis index to 152.63 ± 47.04 (p= 0.001). In the combination of Pentoxivilin with DXR, there was a significant decrease in the sclerosis index to 76.00 ± 31.32 (p=0.002). Meanwhile, the interstitial histology examination showed that normal mice were 14.50 ± 4.38, DXR administration increased fibrosis interstitials meaningfully to 29.38 ± 12.06 (p=0.002). Combined administration of DXR and Pentoxivilin can significantly reduce fibrosis interstitials to 20.50 ± 6.39 compared to DXR administration alone (p=0.002) (Purwanto et al., 2010).

DXR causes renal fibrosis due to the production of ROS by excessive DXR metabolism in the body so it is considered the main cause of oxidative damage to the kidneys. DXR is a major cause in nephrotoxicity (Ren et al., 2016). The study of Guo et al evaluated pathological renal tissue in the DXR group and saw significant widening of the diffuse glomerular mesangial area, capillary lumen stenosis and glomerular focal segmental sclerosis. Fibrosis and inflammation are basic pathological changes of CKD and are also provoking factors for renal fibrosis, especially characterized by the infiltration of immune cells and the secretion of

inflammatory mediators such as IL-1, TGF-β, CTGF, IL-1, MCP-1, and osteopontin, which are also the cause of the accumulation of the extracellular matrix (Guo et al., 2019).

Research by Zhou, et al (2013), found that the transcription factor LIMX1B which plays a role in the process of kidney development at the time of the fetus correlates to the occurrence of nephropathy and affects kidney function. Weakened LIMX1B expression in hypoxic and reoxygenation due to oxidation stress will increase the expression of TGF-β1, the extracellular matrix component, and Co1-III, caspase 3 activation and cell apoptosis that triggers renal fibrosis (Zhou, Xu, & Qin, 2013). These processes include inflammation, proliferation of interstitial fibroblasts and excessive Matrix Eccellular Deposition (ECM). Damaged tubular cells act as antigent presenting cells that express cell adhesion molecules and release inflammatory mediator cells such as cytokines, chemokines, and growth factors, as well as increase ECM production and invade periglomerular and peritubular spaces. The resolution of ECM deposition depends on two pathways namely matrix activation of metalloproteinase and activation of proteolytic enzyme plasmin by plasminogen activators. Excessive production of TGF-β1 can result in too much accumulation of extracellular matrice, scarring (fibrosis) that will eventually lead to interstitial fibrosis. Renal scarring occurs due to disruption of the two collagenolytic pathways, resulting in disruption of the balance of ECM production and breakdown of ECM resulting in irreversible fibrosis (Zhou, Xu, & Qin, 2013).

Terpenoids, which are also found in Pugutano leaf extract, are one of the natural compound ingredients that have long been studied to have various effects on body health, including antioxidant and anti-inflammatory effects. The main mechanism of terpenoids is as an inhibitor of NF-kB signals. Inhibition in NF-kB signals has an impact on the inhibition of pro-inflammatory products such as TNF-α and IL-12, decreases the expression of E-selectin cell adhesion molecules, and prevents the occurrence of leukocyte adhesions mediated by E-selectin (Heras et al., 2009). The nephroprotective activity of saponins in B. sensitivum extract is also seen because it has anticrystallizing and anti-urolyhetic properties (Negi K et al., 2020). Tannins may decrease renal fibrosis in diabetic mouse models. In the kidneys, tannin administration decreases the area of necrosis and inflammatory infiltration, decreases the proliferation of mesangial cells, and restores the tubular structure of the kidneys as normal in the kidneys exposed to the nephrotoxic agent arsenic trioxide (Jin et al., 2020). Pugutano leaf extract containing saponins and tannins is also expected to have a similar effect, where in this study the effect of phytopharmaceutical content was found to be significant.

Effect of puguntano leaf ethanol extract (Curanga fel-terrae (Lour) Merr.) on ureum, creatinine, and proteinuria levels in wistar strain rats

Administration of DXR is known to cause proteinuria, hypoalbuminemia, increased serum levels of BUN and creatinine (two significant indicators of renal function), and decreased creatinine clearance (GFR indicator), all of which are associated with increased oxidative stress and inflammatory factors. Administration of DXR causes an increase in pro-inflammatory cytokines, including the pro-fibrotic proteins TGF-β, NF-κB, renal MDA, and IL-1β as well as IL-6 (Hekmat et al., 2021).

Our study showed an increase in ureum and creatinine levels in the K2 group (DXR treatment) compared to K1 and K3, with statistically significant results ($p<0.05$). In all K2 mice experienced positive proteinurea (+) to (+++) and 9 (90%) K3 mice experienced positive proteinurea (+) to (+++). There was 1 rat K3 (given Puguntano leaf extract) not that had proteinurea. This difference in proteinurea levels was also found to be statistically significant in the anta group ($p<0.05$).

Research by Hekmat et al, in DXR treatment mice showed an increase in serum BUN ($p<0.001$) and serum creatinine ($p<0.001$) while significantly lowering urinary creatinine ($p = 0.033$). In addition, serum albumin levels were significantly lower ($p = 0.002$) compared to the control group, and urine albumin was significantly higher ($p < 0.001$) (Hekmat et al., 2021). Rat treatment with DXR led to a significant increase in BUN and plasma creatinine levels by 2.3 and 4.1 times, respectively, compared to the control group ($p<0.05$) (Ren et al., 2016).

The nephroprotective effect of flavonoids was seen from the ethanol extract of E. scaber leaves which significantly (p value <0.01) lowered serum creatinine levels, total protein, and serum urea but showed a slight increase in electrolyte levels in a dose-dependent manner (Negi K et al., 2020). The study by Sitorus et al. (2017), stated that Puguntano leaf excavities have flavonoid levels of 14.43±0.03 mgQE/g which may have strong potential as antioxidants that have anti-inflammatory, antihepatotoxic, anti-ulcer, anti-allergic, anti-viral, and anti-cancer effects (Sitorus, Hasibuan and Satria, 2017).

The study also agreed, Alasmari et al. also examined blood creatinine, albumin, and BUN levels to determine whether administering DXR caused kidney injury. As expected, the administration of DXR led to a significant increase in BUN and creatinine, in addition to a significant decrease in albumin. With the administration of the antioxidant Geraniol, pre-treatment, it is proven to correct abnormalities observed in creatinine, albumin, and BUN levels, it is thus proven that Geraniol can protect DXR-mediated kidney injury (Alasmari et al, 2022).

Albumin, creatinine and BUN are indicators used to assess kidney function and damage. Normal kidney tissue does not allow albumin to flow out of the bloodstream and out through the urine. However, in the event of kidney damage, this process is disturbed, albumin is excreted in the urine, resulting in a decrease in its concentration in the serum, and creatinine and BUN are not properly filtered, so the serum values of creatinine and BUN increase. In a study conducted by Alasmari et al., the administration of DXR led to an increase in serum creatinine and BUN along with a significant decrease of albumin. The results are in accordance with the research carried out at the moment. This concludes that the administration of antioxidants can prevent kidney damage caused by DXR (Alasmari et al, 2022).

Conclusion

1. Puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) may prevent a significant increase in TGF-β1 expression in DXR-given wistar strain mice.
2. Puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) may prevent a significant increase in E-Selectin expression in wistar strain mice given DXR.
3. Puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) may prevent a significant increase in the glomerulosclerosis process in wistar strain rats given DXR.
4. Puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) may prevent a significant improvement in the process of interstitial fibrosis in wistar strain mice given DXR.
5. Puguntano leaf ethanol extract (Curanga fel-terrae (Lour.) Merr.) can prevent improved kidney function by significantly lowering ureum, creatinine and proteinuria levels in wistar strain mice given DXR.

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