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Nephroprotective effect of ethanol extract of *Sonchus arvensis* L. leaves in gentamicin-piroxicam induced rat renal failure

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ABSTRACT: Kidney failure is characterized by a decrease in the glomerular filtration rate, albuminuria, increased serum creatinine and blood urea nitrogen levels, abnormalities in kidney histology, and electrolyte disturbances. *Sonchus arvensis* L. contain high antioxidants such as flavonoids, glycosides, alkaloids, steroids/triterpenoids, tannins, saponins, and quinones, so it has the potential to improve kidney function. Twenty-five male Wistar albino rats were divided into five groups: negative control, positive control (induction of kidney failure), and three test groups which were administered an ethanol extract of *Sonchus arvensis* L. leaves of 50, 100, and 150 mg/kg b.w doses, respectively. Rats kidney failure model were using gentamycin 100 mg/kg b.w. and piroxicam, 3.6 mg/kg b.w. induction. *Sonchus arvensis L.* extracts were then administered for four weeks. The parameters for nephroprotective of *Sonchus arvensis L.* were urea and serum creatinine levels, urine volume, kidney organ histology, TBARS, and catalase activity. There was an improvement in kidney function as all *Sonchus arvensis* L. dosage levels were characterized by a significant decrease in serum urea levels (25-30%) and serum creatinine (16-23%), as well as significantly increased urine volume (83-167%) in the third week of therapy. At the end of treatment, there was a decrease in the TBARS levels (7-17%) and increased catalase activity (41-62%), significantly in all the treatment groups. The best effect was seen when using the 150 mg/kg b.w. the dose of *Sonchus arvensis* L. extract.

KEYWORDS: *Sonchus arvensis* L.; gentamicin; piroxicam; renal failure; nephroprotective.

1. INTRODUCTION

Kidney failure, a condition characterized by a decrease in the glomerular filtration rate, albuminuria, increased serum creatinine levels, blood urea nitrogen levels, abnormalities in kidney histology, electrolyte disturbances, and other abnormalities due to tubular damage, is a primary target of therapeutic drug development due to its high prevalence and mortality rates. Chronic kidney failure lasts at least three months [1]. According to the World Health Organization (WHO, 2018), kidney failure is ranked as the fourteenth most common cause of death worldwide, with as many as 871,000 recorded deaths in 2018 (1.5% of the global total). Furthermore, the WHO predicts that deaths caused by kidney failure will increase to 13 cases per 100,000 population by 2030 [2]. While kidney failure can be caused by glomerulonephritis and nephrotoxic drugs [3], it is predominantly caused by complications resulting from hypertension and diabetes. The strategy for overcoming kidney failure in patients such as these may be to emphasize the treatment of hypertension and diabetes.

Kidney failure can also be caused by drugs such as aminoglycoside antibiotics and nonsteroidal anti-inflammatory drugs. Aminoglycosides are excreted in the urine by glomerular filtration for a half-life of 2-3 hours in patients with normal renal function. The incidence of aminoglycoside nephrotoxicity is 5% -10% [4]. A retrospective cohort study in children who received aminoglycoside therapy for more than five days showed that acute renal failure occurred in 20% of patients [5]. Nonsteroidal anti-inflammatory drugs inhibit prostaglandin synthesis via the arachidonic acid pathway, resulting in vasoconstriction, thereby increasing the risk of kidney failure. Studies in hyperuricemic patients treated with nonsteroidal anti-inflammatory drugs showed that 9.1% of patients had an acute renal failure [6]. Several studies have been conducted on Wistar rats induced by gentamicin 100 mg/kg b.w for seven days showed the occurrence of acute renal failure. The

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combination of gentamicin 100 mg/kg b.w and piroxicam 3.6 mg/kg b.w showed worse renal damage to the kidneys in rats [7,8].

However, many drugs used in the treatment of hypertension and diabetes are prone to causing unwanted side effects. Therefore, the use of natural products may be an alternative therapy strategy due to their lower side effects than conventional drugs. One such natural product is *Sonchus arvensis* L, which has the potential to improve kidney function. *Sonchus arvensis* L. has been shown to have a diuretic effect in rats that have induced hyperuricemia [9]. *Sonchus arvensis* L. 100 mg/kg b.w have been shown to decrease serum creatinine levels in rat kidney failure induced by gentamicin [10]. It also has antioxidant activity against *2,2-diphenyl-1-picrylhydrazyl* (DPPH) free radicals, *2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)* ABTS, OH, and superoxide [11]. *Sonchus arvensis* L. is safe for long-term use and pregnant women [12,13]. Besides, it has xanthine oxidase inhibitory activity, vasodilatation effect, anti-dysentery activity, anti-hyperuricemic activities, and antihypertension effect [14-17]. In this study, we have used rat models to investigate the prospective of *Sonchus arvensis* L. as an effective nephroprotective agent.

2. RESULTS

2.1. Rats kidney failure model

Table 1 shows the initial condition of rats with induced kidney failure at week 0 (after induction gentamicin and piroxicam for seven days), including serum creatinine, serum urea, and urine volume. Animals in all groups showed a significant increase in serum creatinine (250%), serum urea levels (213%), and urine volume (102%) when compared to the negative control group ($P < 0.05$) after induced by gentamicin and piroxicam for seven days.

Table 1. Parameters of rat kidney failure induced by gentamycin 100 mg/kg b.w and piroxicam 3.6 mg/kg b.w at week 0 (after induction gentamicin and piroxicam for seven days).

Groups	Week 0		
	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Urine volume (mL)
Negative Control	62.98 ± 3.655	0.624 ± 0.076	8.4 ± 1.517
Positive Control	197.02 ± 49.337*	2.182 ± 0.369*	17 ± 1.517*
Sonchus arvensis L. extract 50 mg/kg b.w	202.28 ± 27.191*	2.202 ± 0.333*	16.4 ± 2.608*
Sonchus arvensis L. extract 100 mg/kg b.w	192.52 ± 54.937*	2.004 ± 0.608*	18 ± 2*
Sonchus arvensis L. extract 150 mg/kg b.w	201.18 ± 31.537*	2.186 ± 0.441*	18.4 ± 3.578*

All values are expressed as Mean ± SD (n = 5); * means $p < 0.05$ compared to negative control (Tukey's test).

2.2. Serum urea, serum creatinine level, and urine volume during therapy

The treatment did not show any effects in any of the animal groups in the two weeks of therapy. Serum urea, creatinine, and urine volume levels of the test groups did not significantly differ to the positive control group. Then in the third and fourth weeks of therapy, the effectiveness of the *Sonchus arvensis* L. treatment started to appear. During this period, all *Sonchus arvensis* L. test groups showed a significant decrease in serum urea levels (25-30%) compared to the positive control group ($P < 0.05$) (Figure 1). At the same time, all of the *Sonchus arvensis* L. groups also showed a significant decrease in serum creatinine levels (16-23%) compared to the positive control group ($P < 0.05$) (Figure 2). In the third week of therapy, the *Sonchus arvensis* L. 100 mg/kg b.w. and 150 mg/kg b.w. groups also showed a significant increase in urine volume (83-167%) compared to the positive control group ($P < 0.05$), while the 50 mg/kg b.w. group did not (Figure 3). However, at the end of therapy, all of the *Sonchus arvensis* L. groups showed significant increases in urine volume (90-210%).

2.3. The kidney index, TBARS, and catalase activity

Figure 4-6 shows that by the end of the treatment period the kidney index, TBARS, and catalase activity values of all the *Sonchus arvensis* L. groups differed significantly compared to the positive control group ($P < 0.05$). Treatment with *Sonchus arvensis* L. resulted in decreased TBARS levels and increased catalase activity.

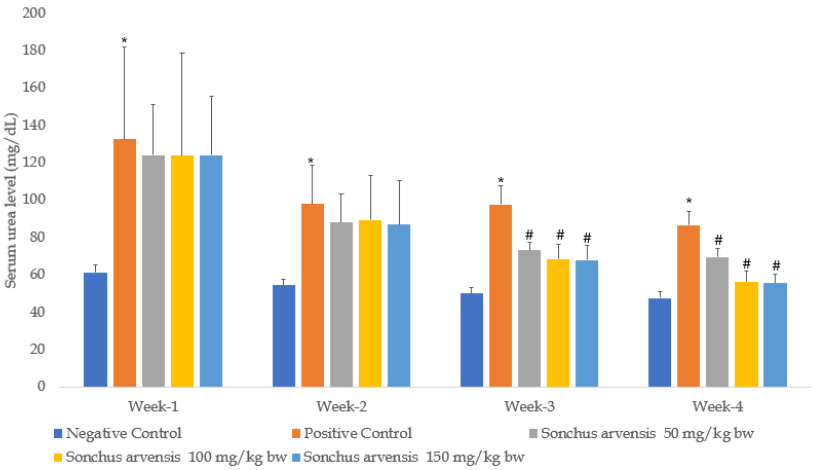

Figure 1. Ethanol extract of *Sonchus arvensis* L. reduced the serum urea level in gentamicin-piroxicam induced rat renal failure. All values are expressed as Mean ± SD (n = 5); * means $p < 0.05$ compared to negative control and # means $p < 0.05$ compared to the positive control (Tukey's test).

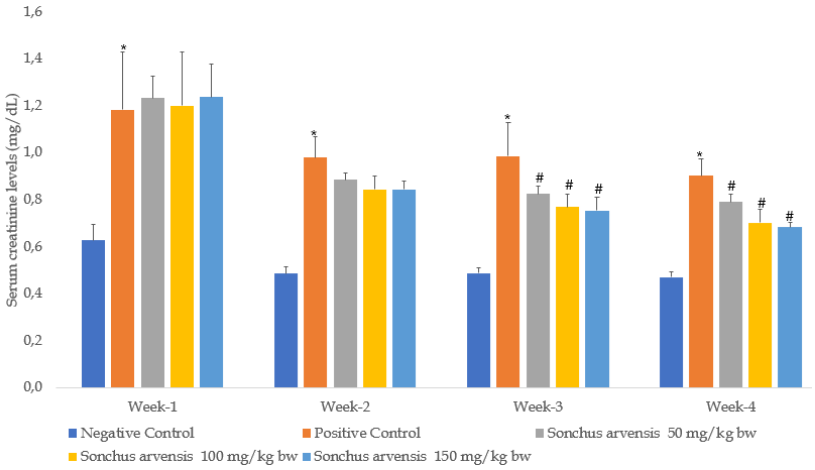

Figure 2. Ethanol extract of *Sonchus arvensis* L. reduced the serum creatinine level in gentamicin-piroxicam induced rat renal failure. All values are expressed as Mean ± SD (n = 5); * means $p < 0.05$ compared to negative control and # means $p < 0.05$ compared to the positive control (Tukey's test).

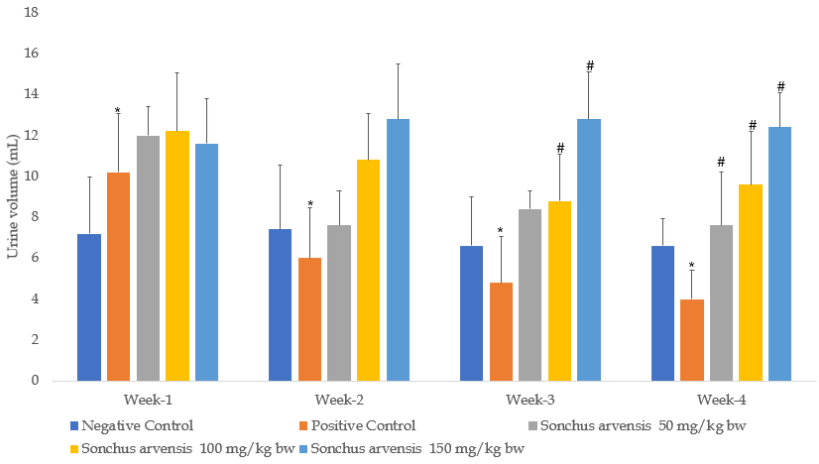

Figure 3. Ethanol extract of *Sonchus arvensis* L. reduced the urine volume in gentamicin-piroxicam induced rat renal failure. All values are expressed as Mean ± SD (n = 5); * means $p < 0.05$ compared to negative control and # means $p < 0.05$ compared to the positive control (Tukey's test).

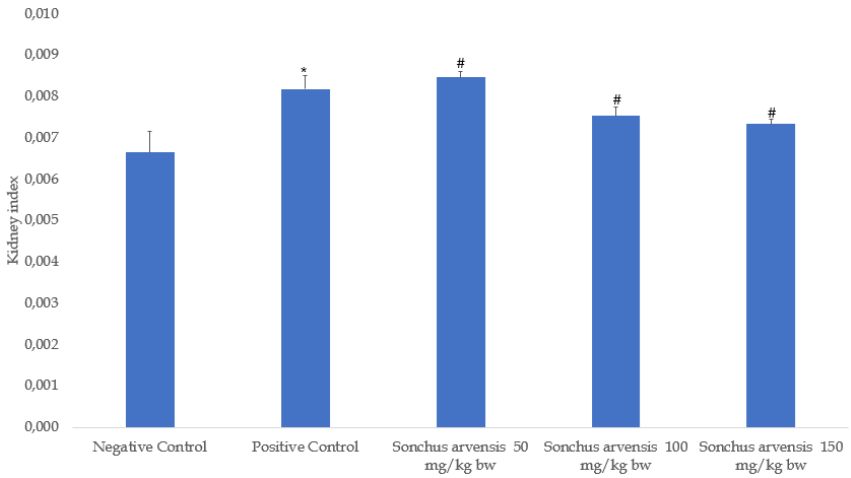

Figure 4. Ethanol extract of *Sonchus arvensis* L reduced the kidney index in gentamicin-piroxicam induced rat renal failure. All values are expressed as Mean ± SD (n = 5); * means p <0.05 compared to negative control and # means p <0.05 compared to the positive control (Tukey's test).

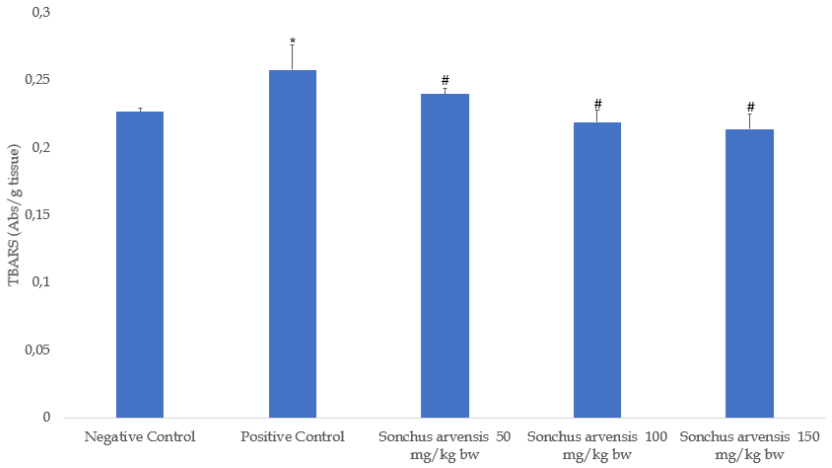

Figure 5. Ethanol extract of *Sonchus arvensis* L reduced the TBARS in gentamicin-piroxicam induced rat renal failure. All values are expressed as Mean ± SD (n = 5); * means p < 0.05 compared to negative control and # means p < 0.05 compared to the positive control (Tukey's test).

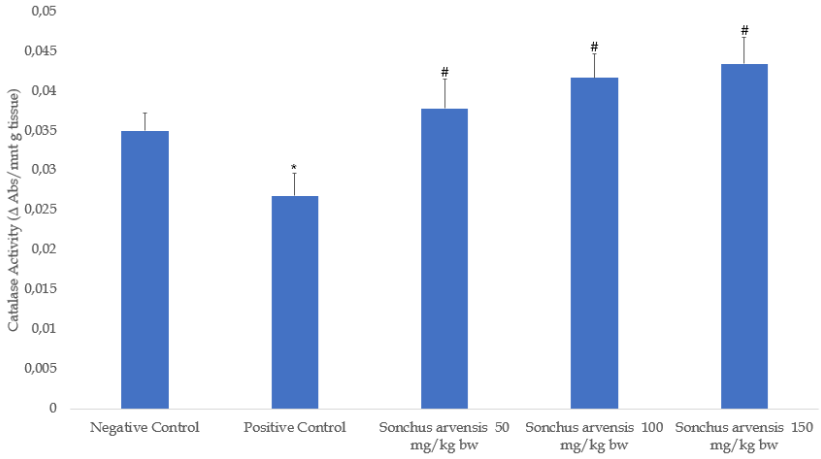

Figure 6. Ethanol extract of *Sonchus arvensis* L increased the catalase activity in gentamicin-piroxicam induced rat renal failure. All values are expressed as Mean ± SD (n = 5); * means p < 0.05 compared to negative control and # means p < 0.05 compared to the positive control (Tukey's test).

2.4. Histology

Histopathological examination illustrated in Figure 7 revealed that kidney in the negative control group showed normal glomerular and tubular histology. On the other hand, the positive control group revealed dilatation of the urinary space between the glomerular epithelium and the bowman capsule. The area within Bowman's capsule surrounding the loops and lobules of the glomerulus is called the urinary space. Furthermore, there were the degeneration of renal tubular epithelial cells (tubular necrosis) in the positive control group. The kidney histology showed there are some improvements in the glomerulus and proximal tubules from the *Sonchus arvensis* L. 100 mg/kg b.w., and 150 mg/kg b.w. groups at the end of the therapy.

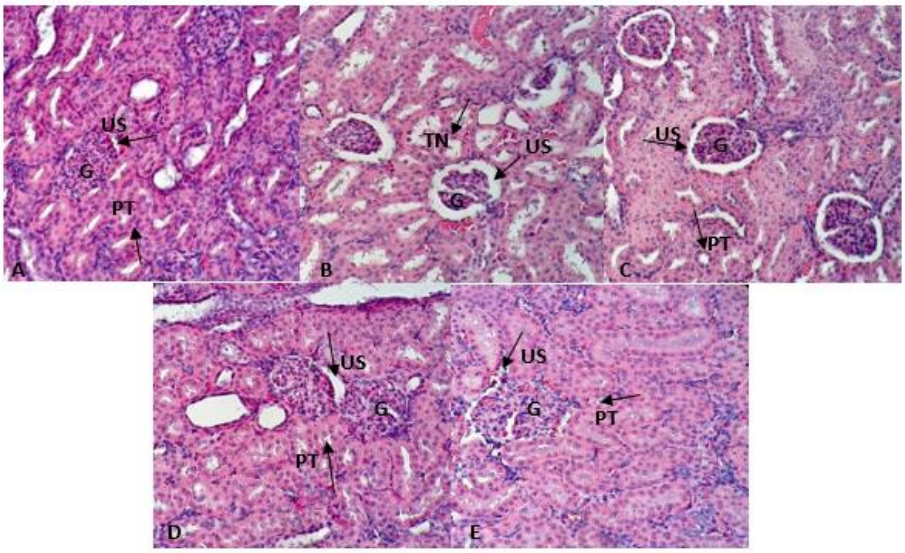

Figure 7. Histopathology profile of kidney cortex on week four after extract treatment, stained with Hematoxylin and Eosin (400x magnification) (A): negative control, urinary space (US) and proximal tubule (PT) are normal. (B): positive control, dilatation of the urinary space (US) and tubular necrosis of proximal tubule (TN). C: SA 50, D: SA 100, E: SA 100. In the treatment group, the kidney profile showing improvement in the glomerulus (G) and proximal tubule (PT).

3. DISCUSSION

Ethanolic extract of *Sonchus arvensis* L. has high antioxidant activity due to its secondary metabolite contents. *In vivo* study show that *Sonchus arvensis* L. has diuretic effects and can reduce serum creatinine levels in rats induced by kidney failure. In line with *in vivo* study, our results show that *Sonchus arvensis* L. is having a nephroprotective impact by increasing endogenous antioxidant activity and reducing free radical levels in test animals. In general, the results exhibit the potential of *Sonchus arvensis* L. to be developed as a nephroprotective drug.

In this study, gentamycin and piroxicam were used to induce rat kidney failure. Gentamycin accumulated in the kidney cortex causes changes in kidney morphology [18]. Studies in humans and animals have shown that gentamycin gets in the proximal tubular epithelial cells of the kidneys [19]. Gentamicin enters cells through an endocytosis mechanism mediated by the megalin/cubilin complex, resulting in a negatively charged phospholipid membrane. Also, gentamicin enters the endosomal compartment through a pinocytosis mechanism. An accumulation of gentamycin in the proximal tubular epithelial cells results in changes in the function of several cell organelles [20], which causes cell death [18]. Piroxicam is a non-steroidal anti-inflammatory drug that has nephrotoxic side effects, reducing blood flow to the kidneys and causing a decrease in prostaglandin. Prostaglandins function in the regulation of blood vasodilation in the glomerulus. Non-steroidal anti-inflammatory drugs will interfere with the vasodilatory response of the kidney prostaglandins so that vasoconstrictors are released to maintain blood flow to the kidneys. Continuous release of vasoconstrictors will disrupt the glomerular filtration rate [21].

One of the causes of gentamycin-induced nephrotoxicity is oxidative stress. Hydroxyl radicals that are formed from the release of hydrogen peroxide and superoxide anions from the mitochondria will cause

oxidative stress. Gentamycin increased mitochondrial production of Reactive Oxygen Species (ROS) [22]. The main ROS was superoxide anion and hydroxyl radicals which cause cell damage and cell death by inhibiting the electron transport pathway, suppressing cell respiration, ATP production, and cytochrome c release from the mitochondrial membrane, damaging DNA, lipid peroxidation, destabilizing cell membranes, and resulting in necrosis [18]. This condition leads to kidney failure [23]. Several parameters can be used to predict the level of oxidative stress leading to kidney failures such as lipid peroxidation, antioxidant enzymes, antioxidant activity, peroxidation product of metilguanidin as creatinine, and blood urea nitrogen level [8]. At the same time, ROS can be eliminated by endogenous antioxidants, such as catalase, in the human body. Aldehydes are compounds that result from lipid peroxidation. They react with acid to form pink thiobarbiturates, referred to as Thiobarbituric Acid Reactive Substances (TBARS). TBARS can be used as references to predict the amount of radical production [8].

Furthermore, the results of the study show that *Sonchus arvensis* L. can also reduce TBARS levels. TBARS is used in measuring the level of lipid peroxidation and antioxidant ability in inhibiting the oxidation process [24]. In kidney failure conditions, TBARS levels are increased due to free radical oxidation and increased damage caused by oxidative stress. A study in rat kidney failure induced by gentamicin 100 mg/kg b.w and piroxicam 3.6 mg/kg b.w showed increased TBRAS level [8]. The results also show that *Sonchus arvensis* L. increased catalase activity of the kidney failure-induced animal models. Catalase is an endogenous antioxidant enzyme that neutralizes and accelerates the degradation of free radical compounds to prevent damage to cell macromolecule components [25]. Antioxidant enzymes, such as catalase, can experience decreased activity during kidney failure due to oxidative stress. The treatment with *Sonchus arvensis* L. restored the enzymatic activity of these antioxidant enzymes. *Sonchus arvensis* L. reduced oxidative stress damage because of its potency as an antioxidant; it has antioxidant activity against *2,2-diphenyl-1-picrylhydrazyl* (DPPH) free radicals, *2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid*) ABTS, OH, and superoxide [11, 26]. Phytochemical screening of *Sonchus arvensis* L. has shown that it contains flavonoids, glycosides, alkaloids, steroids/triterpenoids, tannins, saponins, and quinones [27, 28]. It also contains kaempferol, orientin, and quercetin: substances that have high antioxidant activity. It is this high antioxidant activity, present in *Sonchus arvensis* L., that provides it with the ability to increase TBARS levels and improve kidney function.

Another renal dysfunction is shown by elevated serum levels of creatinine and blood urea nitrogen. Creatinine is a nonprotein waste product of creatine phosphate, resulting from muscle metabolism. At the same time, urea is the product of protein metabolism breakdown by the liver. Creatinine and urea are cleared from the blood and filtered by the glomerulus of the kidney. The increase of creatinine serum and urea nitrogen can be caused by a weakness in the renal filtration process as a result of declined renal function [29].

Sonchus arvensis L. has also shown to have a nephroprotective effect by reducing serum urea and creatinine levels in animals with induced kidney failure. Decreased serum urea and creatinine levels revealed an improvement in kidney function of the test animals [30-32]. These findings are in line with the report of Imelda et al. showed that *Sonchus arvensis* L. 100 and 200 mg/kg b.w reduced serum urea and creatinine levels after administration of *Sonchus arvensis* L. extract and gentamicin for ten days, simultaneously [10, 33].

One of the main characteristics of kidney failure is a marked decrease in urine volume. Therefore, it is possible to use an increase in urine volume as an indication of improved kidney function. In this experiment, increased urine volume was observed in all of the *Sonchus arvensis* L. groups, confirming that *Sonchus arvensis* L. does, indeed, have a diuretic effect [9].

Finally, histopathological studies reveal renal structural pathological abnormalities in the glomerulus and tubules in rats that were administered gentamycin. In the positive control group, revealed dilatation of the urinary space between the glomerular epithelium and the bowman capsule. However, treatment with *Sonchus arvensis* L. shows that the histology of the kidneys is the same as that of normal rats (negative control group).

Nephrotoxicity induced by gentamycin and piroxicam administration causes high renal oxidative stress; the potent antioxidant action of *Sonchus arvensis* L. might explain the possible nephroprotective mechanism of *Sonchus arvensis* L. in alleviating gentamycin and piroxicam- induced renal structural and functional abnormalities. The nephroprotective effect of *Sonchus arvensis* L. needs to be studied further through its molecular mechanism. One of the limitations of this study is the nephroprotective effect of *Sonchus arvensis* L. is the only test on the rat. More studies should be carried out using kidney cells to certify the nephroprotective mechanism of *Sonchus arvensis* L.

4. CONCLUSION

Sonchus arvensis L. exhibits a nephroprotective effect at doses of 50, 100, and 150 mg/kg b.w. in kidney failure-induced animal models. Increased dosage was linear with increased effects. The potent antioxidant action of *Sonchus avensis* L. might explain the nephroprotective effect.

5. MATERIALS AND METHODS

5.1. Material

Sonchus arvensis L. leaves were freshly obtained from the Herbal Jaya Garden, Tawangmangu, Karanganyar, Central Java and determined in the Herbarium Bandungense, School of Life Sciences and Technology, Institut Teknologi Bandung (No. 652/I1.CO2.2/PL/2018). Creatinine and urea kits were obtained from Rajawali Nusindo, Indonesia. Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were supplied by Tokyo Chemical Industry Co., Ltd. Gentamycin injections, piroxicam capsules, and physiological sodium chloride were provided by PT. Indofarma Tbk. All other reagents and chemical's material used for this research were of analytical grade and were acquired from authorized organizations.

5.2. Extraction of *Sonchus arvensis* L.

Sonchus arvensis L. leaves crude drug was extracted using the reflux process, which involved using ethanol 96% as a solvent. Then the crude drug was evaporated to obtain a thick extract with a rotary evaporator. The crude extract 50 mg, 100 mg, and 150 mg was suspense in each 10 mL 0.5% CMC-Na suspension to get ethanol extract of *Sonchus arvensis* L. in dose 50 mg/kg BW, 100 mg/kg BW and 150 mg/kg BW. The rat was given 1 mL/100 gram of extract.

5.3. *In vivo* experimental design

Twenty-five male Wistar rats, weighing 250–300 g each, were purchased from PT. Biofarma. The animals were maintained under laboratory conditions at 25 ± 5 °C with a 12-h period of light and dark cycle. The rats were divided into five groups (5 in each group): a negative control group that was only administered saline, a positive control group, and three test groups in which each animal in the group was administered *Sonchus arvensis* L. leaves ethanol extract at doses of 50 mg/kg., 100 mg/kg, and 150 mg/kg, respectively. On the first day, gentamycin 100 mg/kg b.w. was administered intraperitoneally for seven days, and piroxicam 3.6 mg/kg b.w was administered orally for 40 days, consecutively to all of the rats except for those in the negative control group, to induce and maintain animal models of kidney failure [34]. The extracts were given 30 minutes after piroxicam to the test animals starting on the 12th day and continuing for four weeks orally as a treatment for kidney failure. All experimental animal protocols have been approved by the Institutional Animal Ethics Committee (IAEC), following the Committee's guidelines for the Objective of Supervision and Control of Animal Experiments (No. 09/KEPHP-ITB/8-2018).

5.4. Sample collection

Blood samples were obtained for measuring creatinine and urea levels. The animal was sedated with a combination of ketamine and xylazine (1 mL/kg b.w) to collect the blood from the heart puncture. The blood was collected at 500 µL and was centrifuged for 20 min at 3000 rpm. Urine was analyzed every week in the period of therapy. Urine was collected by putting the animal in the metabolic cage for 12 hours. The kidney of each animal was processed for histopathological studies with haematoxylin and eosin staining for observed under a light microscope examination. The kidney was homogenized with buffer phosphate (pH 7.4). The homogenates were used to determine thiobarbituric acid reactive substances (TBARS) and catalase activity.

5.5. Biochemical measurements in kidney tissue homogenate

Biomarkers of oxidative stress including TBARS levels (thiobarbituric acid reactive substance assay) and catalase activity were measured by in kidney homogenate as stated by to the method of Ohkawa et al. [35] and Aebi [36]. The absorbance was measured using a spectrophotometer at wavelength 280 nm.

5.6. Biochemical measurements in the serum

Serum urea was determined using urea and creatinine kit from Greiner Diagnostic GmbH, Germany. Urea is hydrolyzed by urease to produce carbon dioxide and ammonia [37]. While, creatinine was determined using Jaffe's kinetic method. The red complex is the result of the reaction between creatinine with alkaline

picrate. The intensity of the colour formed is equivalent to the creatinine concentration in the sample. Absorbance was measured at 546 nm with the time interval using a spectrophotometer [38].

5.7. Histopathological studies

The kidneys were isolated and fixed in 10% formalin and implanted in paraffin. Tissue sections were stained with haematoxylin and eosin and then observed under a light microscope at 400x magnification for histopathological examination.

5.8. Statistical analysis

The results were represented as the mean ± standard deviation (SD). Analysis of the results was conducted using one-way ANOVA in SPSS 20.0. Tukey's post hoc test was used for multiple comparisons. Values of $P<0.05$ were considered significant.

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Author contributions: Concept – N.S., E.Y.S., N.F.K., M.F.; Design – N.S., E.Y.S., N.F.K.; Supervision – E.Y.S., N.F.K.; Data Collection and Processing – N.S., M.F.; Analysis and Interpretation – N.S., E.Y.S., N.F.K.; M.F.; Literature Search – N.S., E.Y.S., N.F.K., M.F.; Writing –N.S.; Critical Reviews – N.S., E.Y.S., N.F.K., M.F.

Conflict of interest statement: The authors declared no conflict of interest.

Ethics committee approval: All experimental animal protocols have been approved by the Institutional Animal Ethics Committee (IAEC), following the Committee's guidelines for the Objective of Supervision and Control of Animal Experiments (No. 09/KEPHP-ITB/8-2018).

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