Effect of Ethanol Extract *Sonchus arvensis* Linn Leaf on BUN, SCr, ALT, AST in Healthy Male Albino mice (*Rattus norvegicus*).

N Harun¹, M Indriastuti¹, A Fatimah¹, D Ega¹, Y E Safitri ¹, M Rosmayanti¹

¹Diploma of Pharmacy Muhammadiyah Health School of Ciamis, West Java, Indonesia.

harunnurhidayati@gmail.com; marlina.tirtahadidjaya@gmail.com

**Abstract.** Sonchus leaves are traditional plants whose effects have been widely studied including immunostimulants and diuretics. Both of these effects have benefits for maintaining health and treating diseases. The purpose of this study was to determine the effect of ethanol extract sonchus on levels of BUN, SCr, ALT, AST and acute toxic effects on healthy male albino mice after being extracted 1 time a day for 14 days. Each group of test animals was given a dose of 100 mg/KgBW (n=9) for the BUN, SCr, ALT, AST test and every 10 healthy male albino mice (n=40) with a dose of 700, 1400, 2800, 5600 (mg/KgBW) for acute toxicity test. Blood sampling performed on day 0 and day 14 (BUN, SCr, ALT, AST) and toxicity tests were observed for 14 days. The data obtained were statistically tested with Shapiro Wilk normality test, or Wilcoxon T test and one way Annova or Friedman test and calculated LD50. Statistics show that there were no significant differences (p>0.005) in the administration of extracts between day 0 and day 14 of BUN, SCr, and AST levels but for ALT had significant difference (p<0.005). The results of this study concluded that the ethanol extract of sonchus leaves did not affect the levels of BUN, SCr, AST but neither for ALT on healthy male albino mice.

1. Introduction

*Sonchus arvensis* leaves have the potential as immunomodulators in male mice with an optimal dose of 100 mg / kgBB capable of providing an increase in the number of leukocytes (Sukmayadi et al, 2014). Besides being an immunomodulator, tempuyung can be efficacious as a diuretic (Rustam et al, 2014). In Lumbanraja's research, tempuyung contains organic compounds such as flavonoids (kaemferol, luteolin-7-O-glucoside, and apigenin-7-O-glucoside), coumarin (skepoletin), taraksasteol, inositol, and phenolic acids (synamat, kumarat, vanilat).

*Sonchus arvensis* leaves have properties to facilitate the urinary tract (diuretic), stone urine, gout, hypertension, appendicitis and hemorrhoids (Sitanggang & Dewani, 2002). Therefore, it can be concluded that tempuyung leaves have an important influence on the kidneys, where the kidneys are the body's vital organs that function to maintain body stability, regulate body fluid balance, electrolytes, and acid bases by means of blood filtration, selective reabsorption of water, electrolytes, non-electrolytes, and as an organ of excretion (Price and Wilson, 2002). Disorders of the kidneys can cause inhibition of the excretion process. One of the most important kidney function parameters is the Glomerular Filtration
Rate (GFR), which measures the performance of the kidneys. Clinically it can be measured by looking at serum creatinine (SCr) and blood urea nitrogen (BUN) levels (Pradnyani, 2015). Serum creatinine (SCr) is a glomerular filtration rate index that is more accurate than urea because its production speed is mainly in the function of muscle mass which has little change. Increased creatinine in the blood is an indication of damage to kidney function (Sumarny, 2006).

Other organs that have vital functions are the liver. The liver is a blood vessel, a blood purifier that has been infected, as a metabolic tool (fat, carbohydrates, vitamins, proteins), neutralizing toxic substances that enter the body through the bloodstream and help maintain body temperature (Wijayakusuma, 2008). Abnormalities that occur in the liver can be described by changes in Aminotransferase enzymes including Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). ALT has a greater indicator of liver damage than AST, because in hepatocytes ALT is three times more than AST (Sacher & McPherson, 2004). According to its location, the ALT enzyme is found in liver, heart, muscle and kidney cells. The highest levels of this enzyme in the cytoplasm of liver cells. While AST is found in liver cells, heart, skeletal muscles, brain, pancreas, spleen and lungs (Hall P & Johnny C, 2012). An increase in AST levels one to three times the normal limit can occur in drug poisoning, an increase in AST up to twenty-fold occurs due to chronic necrosis of liver cells caused by drugs and toxins (Setyaningsih, 2015).

Benefit of *Sonchus arvensis* leaves as a traditional herbal medicine not only be used in the treatment of diseases but also in maintaining health. Safety of the kidneys and liver is an important parameter whether traditional medicines are safe to use (BPPOM, 2014).

2. **Methods**

This research was conducted in the Pharmacology laboratory of Pharmacy Muhammadiyah Health School of Ciamis. *Sonchus arvensis* leaves were obtained in the Ciamis area and determined at the Faculty of Biology, Galuh University. Extraction of tempuyung leaves using 70% ethanol with meseration method, with suspending agent, which is 1% CMC to be easily homogeneously mixed. Wistar male rats used in this study had inclusion criteria including: healthy, 8-12 weeks of age with 200-250 grams of body weight and not yet treated by any drug. Animal testing were acclimatized for 7 days, not fed before they were given treatment for 14-18 hours but drinking water was given (BPOM, 2014). Animal testing was acclimatized for 7 days, not before they were given treatment for 14-18 hours but drinking water was given(BPOM, 2014).

The method used in this study was pre and post treatment using 9 white male rats for each pair of kidney parameters (BUN & SCr), liver (ALT & AST). Before being given tempuyung extract wistar male rats were first checked for levels of BUN, SCr, ALT and AST. Blood samples were taken at Plexus Retroorbitalis in the eye and accommodated on vacutainer tube. After that, the ethanol extract of tempuyung leaves was given once a day at a dose of 100 mg / kgBB, for 14 days, then the four parameters were examined on the 14th day.

2.1. **Examination of BUN**

BUN level measurement uses the Barthiffot modiff method by using an automed chemical analyzer photometric system with a wavelength of 578 nm. The absorbance data recorded was calculated as BUN levels in units of mg / dl.

2.2. **Examination of SCr**
Measurement of creatinine levels using the Jaffe method is a complex reaction of creatinine picrate. The absorbance is read using a photometric automated chemical analyzer with a wavelength of 492 nm. Measuring the level of creatinine serum using units of mg / dl.

2.3. Examination of ALT and AST
Check using a reagent kit (reagent I and reagent II) based on the photometric system method (Hidayat A., 2013). The wavelength used in the photometer is 365 nm. The units of ALT and AST use UI / L

3. Result and Discussion
The preliminary results of Sonchus arvensis leaves ethanol extract with the Wilstatter method showed positive results containing flavonoids by producing yellow. The yellow color produced is due to a reaction that occurs between the flavonoids induced with red, yellow or orange Mg and HCl metals. HCl functions to hydrolyze flavonoids into their aglycones by hydrolyzing O-Glycosyl, glycosyl will be replaced by H + from acid. This reduction with Mg and HCl will produce complex yellow or orange compounds on flavononol, flavonol, flanon, xanton (Latifah, 2015).

3.1. Blood Ureum Nitrogen and Serum Creatinin
On examination of BUN levels in 9 healthy wistar male rats there was a decrease in mean levels on day 14 compared to day 0 before being given ethanol extract from Sonchus arvensis leaves. However, different levels of SCr did not decrease. Based on the statistical results show that there were no significant differences in the two measurements on day 0 and 14 both of the two parameters (BUN & SCr). The results of observations can be seen in table 1.

Table 1. Comparison of levels of BUN and SCr on day 0 and day 14 at doses of 100 mg / kgBW (n = 9 heads)

| Parameter | Concentration (mg/dl) | P Value |
|-----------|-----------------------|---------|
|           | Day 0                 | Day 14  |         |
| BUN       | 31.44 ± 7,161         | 25,00 ± 6,928 | 0,767 |
| SCr       | 0,80 ± 0,6745         | 0,80 ± 0,6745 | 0,432 |

Sonchus arvensis leaves has the effect of being nephroprotective able to protect the kidneys from injury (Imelda et al, 2018) and also is diuresis and natriuresis (imelda and Andani, 2014). Between diuretics and BUN and SCr have a close relationship where urea is freely filtered at the glomerulus and then undergoes considerable tubular reabsorption while creatinine is secreted in tubular. Diuretics are able to block tubular absorption by producing diuresis so that levels decrease in BUN (Lindenfeld and Schrier, 2011). The flavonoid content of Tempuyung is a promoter of diuretic and antioxidant effects, preventing or thinning kidney injury (Vargas et al, 2018). In this study the levels of BUN and SCr before being given the intervention of tempuyung ethanol extract indicated that the kidneys of the rat were functioning healthily. Giving for 14 days does not have a significant effect on healthy kidneys.

3.2. ALT and AST
The results of ALT examination on the 14th day after being given Sonchus arvensis ethanol extract showed an increase in ALT levels in wistar male rats but were still in the normal range (table 2). The results of statistical tests showed that there were significant differences in the value of ALT enzyme activity (p <0.05) between day 0 and day 14. Different from the results of statistical tests of AST levels
between days 0 and 14 did not show a difference in meaning even though the average value produced on day 14 experienced a decrease in AST levels.

Table 2. Comparison of ALT and AST levels on day 0 and day 14 at doses of 100 mg / kgBW (n = 9 heads)

| Parameter | Concentration   | P Value |
|-----------|-----------------|---------|
|           | Day 0           | Day 14  |         |
| ALT       | 54.44±11.555    | 73.89±14.692 | 0.039   |
| AST       | 151.67±44.153   | 136.22±39.255 | 0.412   |

ALT and AST are two markers of disturbances in the liver, both caloric and carbohydrate intake can affect the levels of both enzymes (love et al, 2004). This research has various limitations because it only has one test control so that further research is needed and accurate using other methods. The results of acute toxicity tests according to Dhianawaty 2016, from the preparations of *Sonchus arvensis* leaves are known that dekoko or *Sonchus arvensis* leaves boiled water is relatively safe against the liver and kidneys. (Diah Dhianawaty Djunaedi, 2016). 2017 Journal of Adolescent Research, *Sonchus arvensis* leaves have a very high Flavonoid content even compared to spinach and papaya leaves. As is known, Flavonoids are active compounds that have acidic properties, where acid that is too high can reduce the optimization of metabolic system performance functions in the body that are related to the role of liver performance.

*Sonchus arvensis* leaves have a high antioxidant content and efficacious for relieving fever, heartburn and flu, with a note that the amount given must be limited, because the side effects of *Sonchus arvensis* leaves that are consumed in excess can directly damage the productivity system of liver and bile tissue. Prof. Dr. Geoff McColl, PHd M.Sc (2018). Queensland University.

4. Conclusion
Influence on the kidneys and liver is an important parameter whether a traditional medicine is safe to use. The results of this study on ethanol extract of *Sonchus arvensis* leaves did not have a significant effect on the levels of BUN and SCr, which indicates that the ethanol extract of tempuyung leaves is safe for kidney organs. While the ALT level has a significant effect, this requires further research to determine the effect of *Sonchus arvensis* leaves on the liver.

5. References
[1] Love et al (2004) The influence of diet upon liver function tests and serum lipids in healthy male volunteers resident in a Phase I unit
[2] Dewi, A. K. (2013). Efek Ekstrak Kulit Buah Rambutan Terhadap Peroksida Lipid Hepar Tikus Obesitas. Universitas Negeri Malang, 3.
[3] Djunaedi, (2016). Kajian Etnofarmakologi Tumbuhan untuk Mengatasi Batu Kandung Kemih. Fakultas Kedokteran Universitas Padjadjaran
[4] Latifah. (2015). Identifikasi Golongan Senyawa Flavonoid dan Uji Aktivitas Antioksidan Pada Ekstrak Rimpang Kencur Kaempferiae Galanga L. Dengan Metode DPPH. Universitas Islam Negeri Maulana Malik Ibrahim Malang, 18.
[5] Wijayakusuma, H. (2008). Tumpas Hepatitis Dengan Ramuan Herbal. Jakarta: Pustaka Bunda.
[6] Sacher, R., & McPherson, R. (2004). Tinjaua Klinis Hasil Pemeriksaan Laboratoium Edisi II. Jakarta: Penerbit Buku Kedokteran EGC
Hall P, & Johnny C. (2012). What Is The Real Function of The Liver "function test". Ulster Med J, 81:30-36.

Hidayat, A. (2013). Pengauh Vitamin E Terhadap Kadar SGPT dan SGOT Serum Darah Tikus Putih (Rattus norvegicus) Jantan Galur Wistar yang Terpapar Timbal Per-Oral. Skripsi, 20.

Rosida, A. (2016). Pemeriksaan Laboratorium Penyakit Hati. Berkala Kedokteran, Vol.12, No.1 123-131

Sukmayadi, Asep E., Sri A. Sumiwi, Melisa I. Barliana, dan Anisa D. Aryanti. Aktivitas Immunomodulator Ekstrak Etanol Daun 31 Tempuyung (Sonchus arvensis Linn.). Sumedang, Jawa Barat: Universitas Padjajaran, 2014

Khan, R. (2012). Evaluation of Flavonoid and Divers Antioxydant Activities of Sonchus arvensis. Chem Cent J.

Dri, H. (2014). Normal Clinical Chemistry Values. Universitas Animal Care.

Pearce, Evelyn C. Anatomi dan Fisiologi Untuk Paramedis. Jakarta: Gramedia, 2006.

Sitanggang, & Dewani. (2002). 33 Ramuan Penakluk Asam Urat. Jakarta: Agromedia Pustaka.

Pradnyani, P. R. (2015). Pengaruh Stres Fisik Terhadap Serum Kreatinin Tikus Wistar Jantan (Rattus norvegicus). 13.

Felix Vargas et al (2018) Flavonoids in Kidney Health and Disease.

JoAnn Lindenfeld, MD,† Robert W. Schrier, MD Blood Urea Nitrogen A Marker for Adverse Effects of Loop Diuretics

Erlina Rustam imelda FB dan Andriani (2006) Perbandingan efek diuretik dan Natriuresis Tempuyung

Imelda, Achadiyani, Nanan Sekarwana, (2018) Protective Effect Of Ethanolic Extract Sonchus arvensis L. in Gentamicin-Induced Acute Tubular Necrosis on Wistar Rats.