• pISSN: 2085-1545
• eISSN: 2339-093X

Sains Medika Journal of Medicine and Health

http://jurnal.unissula.ac.id/index.php/sainsmedika

RESEARCH ARTICLE

Acute Toxicity Test of Soursop Leaves *(Annona muricata)* on Liver and Kidney of Switzerland Mice

Astika Widy Utomo[1], Neni Susilaningsih[2], Desy Armalina[2]

[1] Pharmacology and Therapeutic Department of Medical Faculty of Diponegoro University, Semarang

[2] Histology Department, of Medical Faculty of Diponegoro University, Semarang

Correspondence: Astika Widy Utomo, email: astikautomo@gmail.com

ABSTRAK

Pendahuluan: Ekstrak daun tanaman sirsak *(Annona muricata)* sudah dipakai luas di masyarakat sebagai obat tradisional untuk pengobatan kanker. Namun hingga saat ini belum ada penelitian untuk mengetahui standar keamanan ekstrak.

Tujuan: Untuk mengetahui efek toksisitas akut per oral ektrak daun sirsak *(Annona muricata)* pada organ hati dan ginjal mencit Swiss.

Metode : Sebanyak 24 ekor mencit, dibagi menjadi 4 kelompok. Kelompok I sebagai kontrol, sedang kelompok II-IV diberikan ekstrak daun sirsak dosis tunggal melalui sonde. Mencit diamati hingga hari ke 7 untuk menentukan LD50 dan di akhir pengamatan mencit dikorbankan untuk diambil organ hati dan ginjal untuk pembuatan sediaan histopatologi dengan pewarnaan Hematoksilin-Eosin. Sediaan hati dan ginjal dibaca dan dianalisis menggunakan mikroskop cahaya untuk dinilai index histopatologinya. Hasil penelitian dianalisis dengan menggunakan uji ANOVA dan dianggap beda bermakna pada level p<0,05.

Hasil : Tidak ada mencit yang mati maupun menunjukkan spektrum gejala toksik setelah pemberian dosis tunggal ekstrak daun sirsak. Dari pemeriksaan mikroskopis tidak didapatkan kerusakan jaringan di ginjal maupun hati pada kelompok perlakuan dibandingkan dengan kelompok kontrol.

Kesimpulan : Nilai LD50 ekstrak daun sirsak adalah lebih besar dari 2000 mg/kgBB. Hasil ini menunjukkan bahwa ekstrak daun sirsak merupakan bahan yang praktis tidak toksik *(practicaly non toxic)*, serta tidak merusak jaringan ginjal dan hati.

Kata kunci : Daun Sirsak, Hati, Ginjal, Uji toksisitas akut, LD50.

ABSTRACT

Introduction: *The soursoup leaves extract (Annona muricata) has widely been used as traditional medicine for cancer. No studies have been conduct to investigate the safety of the extract.*

Objectives: *The purpose of the study was to investigate the acute oral toxicity test of soursoup leaves extract (Annona muricata) on Swiss mice's liver and kidney.*

Methods: *Twenty four mice were divided into 4 groups. Group I was control group, while group II-IV was given soursoup leaves extract as single dose orally via sonde. The mice were obsereved until day 7 to determine the LD50 and at the end were terminated to collect the liver and kidney. The organs later were made into histopathology slides. The slides read with light microscope. The data analyzed with ANOVA and was considered significant at p<0.05.*

Results: *All mice were alive during the 7 days observation and no mice showing the toxic spectrum after the dosing. Microscopically, no damage on the liver and kidney organ among the groups.*

Conclusion: *The LD50 of soursoup leaves extract is more than 2000 mg/kgBW. This result indicate that the extract is practically non toxic and do not damage the liver and kidney.*

Keywords: *Soursoup leaves, liver, kidney, acute toxicity test, LD50*

INTRODUCTION

Soursop *(Annona muricata = A.muricata)* is one of the fruit crops that commonly grow in Indonesia. Soursop have many benefits for human, such as rich in nutrition, and also one of the traditional medicine ingredients that has multiple properties (Mardiana dan Ratnasari, 2011).

The main chemical compounds (phytochemical) in the soursop leaves is the alkaloid known as *acetogenin*. Contains more than 60 types of alkaloid compounds, including at least 13 alkaloid compounds that have the killing power against cancer cells, even when the cancer cells have developed resistance to modern chemotherapy drugs such as Adriamicyn, Vinblastin dan Vincristin (Gonzales et al., 2008). Research about the killing power of *acetogenin* against cancer cells was conducted at Purdue University, West Lafayete, Indiana, USA, in 1997, reported that the *acetogenin* compounds are potent inhibitors towards enzymatic reactions in the cell membrane by blocking ATP *(Adenosine Triphosphate)* (Anonim, 2005). Soursop are commonly used in the society. However, there is no certain dosage

• pISSN: 2085-1545
• eISSN: 2339-093X
http://jurnal.unissula.ac.id/index.php/sainsmedika

or measurement for its use. Therefore, it is necessary to conduct toxicity test to measure the safety of the usage of soursop leaves.

The acute oral toxicity test is one of the important preclinical trials. This test is designed to determine the toxic effect of a substance that will occur within a short period of time after oral administration in a certain dose (Donatus, 2001). The quantitative data obtained from acute toxicity test, which is lethal dose 50 (LD50), are able to qualify the toxicity of a substance whether extremely toxic or practically non toxic. LD50 also able to predict other toxicology tests, such as subchronical and chronical tests. The qualitative data obtained include the clinical manifestations, and mechanisms of toxic effects on various organs such as the liver and kidneys. The liver is the organ where the metabolism and detoxification of various foodstuffs including those containing toxin, while the kidney is a major organ for excretion (Loomis, 1978; Nurlaila, et al., 1992; Hodgson dan Levi, 2000; Donatus, 2001).

METHODS

This research is a *quasi-experimental* design with *Post test only control group design.* The study population was Swiss mice with the following inclusion criteria: male mice, aged 8-10 weeks, weighs between 25-35 grams, and healthy mice without anatomical abnormalities. The exclusion criteria were mice which have an infection or trauma during treatment. Mice were then randomized into three treatment groups and one control group, each consisting of 6 mice. Once adapted for 7 days, mice were then treated orally (sonde). T1 group received 2000 mg/kg body weight soursop leaves extract, group T2 1000 mg/kg body weight, and T3 group received 500 mg/kg body weight. While the control group (C) received distilled water. The aqueous Soursop leaves extract used is obtained from PT. Sido Muncul.

During the first 24 hours, the mice were observed carefully, including whether any mice died, any changes in behavior such as mice became more aggressive or passive. Observations were continued until day 7, if any mice died, surgery were performed immediately to collect the liver and kidneys stored in a solution buffered formalin. On day 8, the kidney and liver of mice were collected to proceed to histopathology slides. Both liver and kidney slides were then evaluated microscopically to see if there any change in a cell as a result of the provision of soursop leaves extract. Results of the study were analyzed using ANOVA test and considered a significant difference at the level of $p<0.05$.

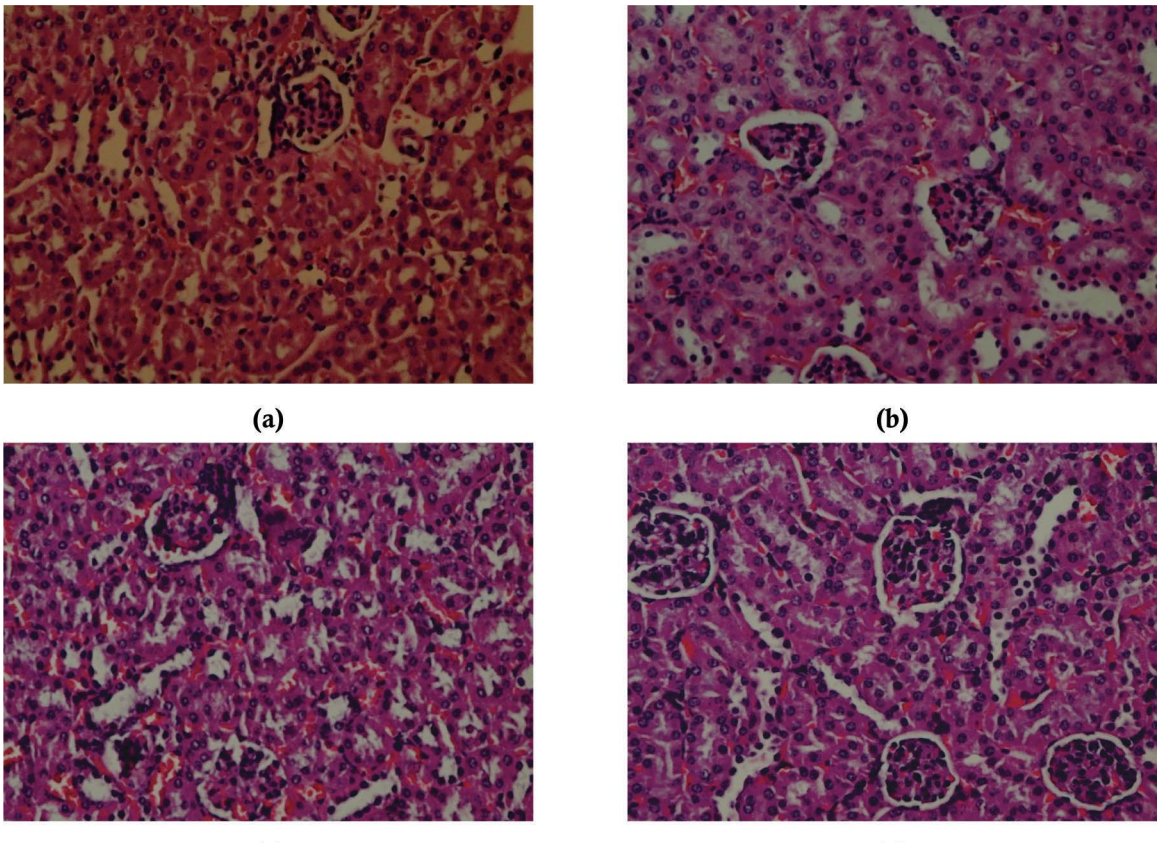

Figure 1. Kidneys's microscopic view of each group (a) Group C (Control), (b) Group T1 (dose 2000 mg/KgBW), (c) Group T2 (dose 1000 mg/kgBW), (d) Group T3 (dose 500 mg/kgBW)

• pISSN: 2085-1545
• eISSN: 2339-093X

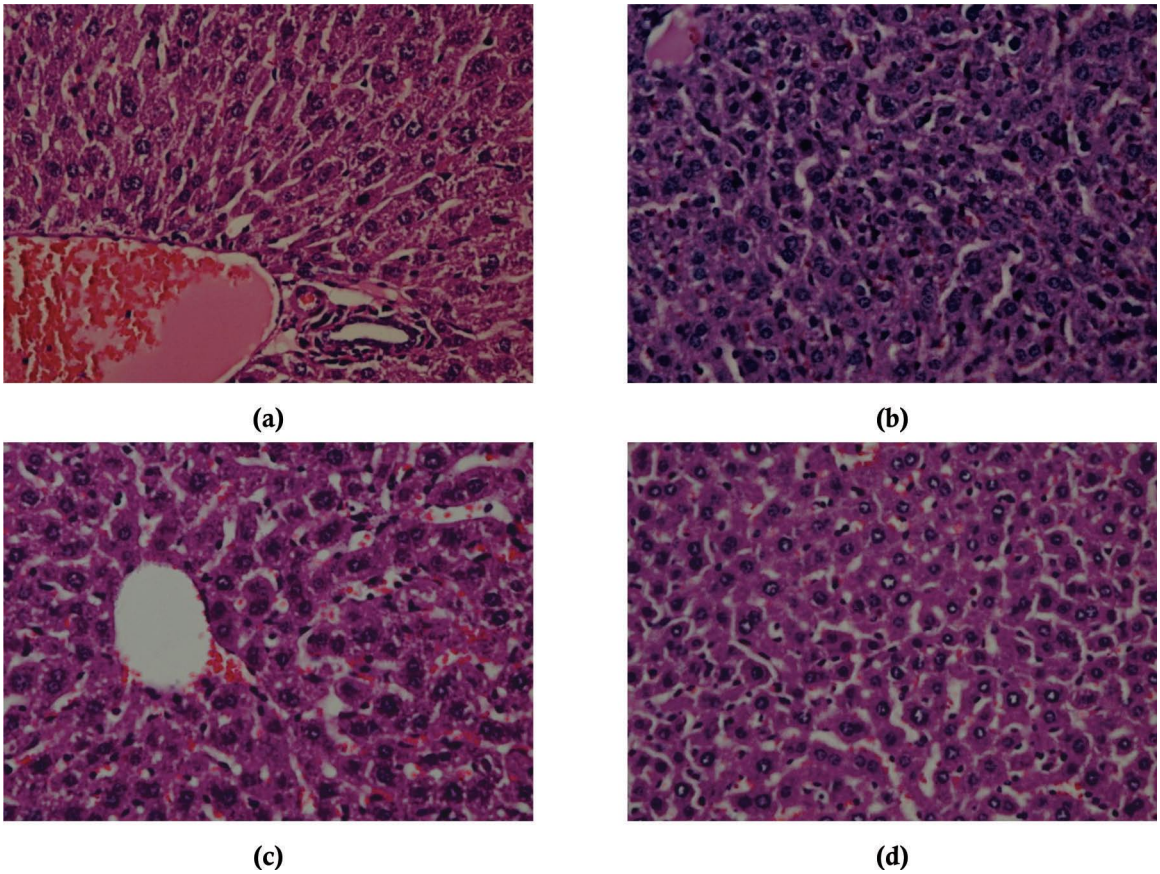

Figure 2. Livers's microscopic view of each group (a) Group C (Control), (b) Group T1 (dose 2000 mg/KgBW), (c) Group T2 (dose 1000 mg/kgBW), (d) Group T3 (dose 500 mg/kgBW))

RESULTS

No mice died during treatment. Behavioral change of mice after getting treatment were also not present. On histopathology examination of the kidney, found no degeneration and necrosis of the glomerolus, tubules, and interstitium, both in the control group and treatment. Similarly, in the histopathology examination of liver, which also has no degeneration and necrosis. Test statistical analysis of histopathology with ANOVA results were not significantly different between control and treatment groups (0.00 ± 0.00; $p<0.05$).

DISCUSSIONS

No mice died during the treatment, change of the behavior of mice after getting treatment was not present as well. The criteria of the OECD *(Organisation for Economic Cooperation and Development)* Guidelines for the Testing of Chemicals was met, because of it. Soursop leaves extract LD50 is greater than 2000 mg/kg body weight. This result shows the soursop leaves extract is an ingredient that is practically non-toxic to the single oral dose administration.

Further examination on histopathological preparation of the mice's livers and kidneys also did not indicated necrosis or degeneration. These results are in contrast to studies conducted by Arthur, et al. in 2011 stating that at high doses, soursop leaves extract causes damage to the kidney tissue (Arthur, et al., 2011). Research conducted by Dayeef et al. (2013) also showed that consumption of soursop leaves extract within 40 days (subchronic) cause kidney damage at the cellular and molecular level (Dayeef et al., 2013). Toxic effects on the kidneys from soursop leaves extract is thought to be the result of a decrease in the amount of caspase 9 and most visible in the cells of the renal tubules. Research by Handy et al., 2015 also gave the result that at high doses, soursop leaves extract caused liver cell necrosis due to the mechanism that inhibits the effect acetogenin mitochrondia complex I in hepatocytes (Hand et al., 2015). The yield difference raises a new question how security soursop leaves extract when used in high doses in the long term. Therefore, further toxicity studies as subchronic and chronic toxicity tests need to be done to get the complete security data of soursop leaves extract.

CONCLUSION

The soursop leaves extract is non toxic ingredient to Switzerland mice on single oral dose administration.

SUGGESTION

Extensive use of soursop leaves extract in the community as herbal medicine should be considered especially for the safety part. Therefore, a comprehensive toxicology test include acute toxicology test followed by subchronical toxicity test, chronic toxicity test and special toxicity tests such as teratogenic test. In addition, determination of other LD50 in other different species with higher levels.

REFERENCES:

Anonim. 2005, Graviola. http://www.graviola.org/cgi/gpp.cgi, cited on 11.05. 2012.

Arthur, F.K.N., Woode, E., Terlabi, E.O., dan Larbie, C, 2011, Evaluation of acute and subchronic toxicity of *Annona Muricata* (Linn.) aqueous extract in animals, *Euro. J. Exp. Bio.,* 1(4):115-124.

Dayeef, A. Y. M., Karyono, S., dan Sujuti, H., 2013, The influence of *Annona muricat*a leaves extract in damaging kidney cell and inducing caspase 9 activity, Int. *J. Pharm. Biol. Sci.,* 8(5): 48-52.

Donatus I. A, 2001, *Toksikologi Dasar,* Laboratorium Farmakologi dan Toksikologi, Fakultas Farmasi UGM, Yogyakarta.

Gonzales, I. A., Agiirre, K. K., dan Roaro, L. M., 2008, Anticarcinogenic and genotoxic effects produced by acetogenins isolated from *Annona muricata, Toxicol Lett,* 180 : 32–246.

Handayani, E. S., Nugraha, Z. S., Fidianingsih, I., dan Pahlevawati, P. R., 2015, Soursoup leaves extract increases neuroglia and hepatocytes degeneration in female rats, *Univ Med,* 34: 17-24.

Hodgson, E., dan Levi, P. E., 2000, *A Textbook of Modern Toxicology. 2nd Edition.* Mc Graw-Hill Co. Philadelphia.

Loomis, T. A., 1978, *Toksikologi Dasar,* Edisi 3, IKIP Semarang Press, Semarang.

Mardiana, L., dan Ratnasari, J. 2011. *Ramuan dan Khasiat Sirsak,* Penebar Swadaya, Jakarta: 17-18.

Nurlaila, Donatus I. A., Sugiyanto, Wahyono, J., Suhardjono, D., 1992, *Petunjuk Praktikum Toksikologi,* Edisi 1, Laboratorium Farmakologi dan Toksikologi, Fakultas Farmasi UGM. Yogyakarta.