

**NEPHROPROTECTIVE OF ETHANOL EXTRACT OF  
*Annona muricata* L. BARK ON PARACETAMOL-INDUCED  
NEPHROTOXICITY IN RATS**

<sup>1</sup>Widya Sari, <sup>2</sup>Masykur\*, <sup>3</sup>Farah Adhilla, <sup>4</sup>Rojatul Maisarah, <sup>5</sup>Fauziah  
<sup>12345</sup>Universitas Syiah Kuala, Banda Aceh, Indonesia

\*Email: masykur@unsyiah.ac.id

DOI: 10.22373/biotik.v11i1.15635

**ABSTRACT**

Kidneys are organs that have the potential for damage caused by toxic substances. The use of paracetamol in high doses or long-term therapeutic doses can cause adverse effects in the form of damage to the kidneys. Plants that have the potential to become medicinal raw materials against kidney damage are *Annona muricata* L. The purpose of this study was to determine the potential protection of ethanolic extract of soursop bark (EESB) against histopathological rat kidney induced by toxic doses of paracetamol. This study used an experimental method using a completely randomized design (CRD). The normal control group (K0) and negative control (K-) were given distilled water, while in the treatment group the doses of EESB were 150, 300 and 600 mg/kgBW dissolved in 1 ml of distilled water for 14 days. On the 7th day, all groups of rats were given paracetamol at a dose of 1350 mg/kgBW except for the K0 group. Each group was 5 repetitions, so that in this study used 25 rats (*Rattus norvegicus* L.). Samples of kidney organs were taken on the 14th day to observe the histopathological structure of the tubular cells undergoing necrosis. The results showed that at a dose of 300 mg/kgBW EESB had the potential for protection against kidney histopathology by suppressing the occurrence of cell death (necrosis).

**Keywords:** kidney, necrosis, paracetamol, soursop bark.

**INTRODUCTION**

Paracetamol (acetaminophen) is one of the antipyretic analgesic drugs used by the community as a fever and pain-reducing drug [1]. Paracetamol works by inhibiting prostaglandins that are weak in tissues (Asmara and Nugroho, 2017) [21]. In several countries, including Pakistan,

paracetamol is included in the class of analgesic drugs most widely used. Its use reaches 42.8% compared to other analgesic drugs [2].

Paracetamol can be tolerated by the body if used in the right dosage. However, if it exceeds the recommended dose consumed or the use of therapeutic doses in the long term can cause toxic effects in the form of damage to the kidneys [3][4][5]. The reactive metabolite formed from the metabolism of paracetamol is N-acetyl-p-benzoquinone imine (NAPQI) which can become a free radical that is both hepatotoxic and nephrotoxic [7].

The kidney is an organ that has the potential for damage caused by toxic substances. Blood circulation that enters the kidneys is 25-30% and will be cleaned by the kidneys. High blood flow, an increase in products excreted by the kidneys, and reabsorption of water in the tubules are the main factors affecting the sensitivity of the kidneys to toxic substances that enter the body [8]. The main target of toxic substances in the kidney is the proximal tubule of the kidney [9]. Structural changes in the kidney can be observed by looking at

the cell injury that occurs, namely cell necrosis [8].

The potential of a plant as a medicinal raw material in healing damage caused by a toxic substance can be seen from the antioxidant activity of the plant. One of the plants that have potential as medicinal raw materials is *Annona muricata* L. [10]. mentions that the phytochemical content in the ethanol extract of soursop bark is alkaloids, saponins, tannins, flavonoids, and phenolics. The antioxidant effects of a plant can prevent an imbalance in the production of free radicals that can trigger oxidative stress, to prevent damage to the kidneys [11].

Soursop bark is hepatoprotection by observing changes in the liver that have been induced by paracetamol [12]. Therefore, in this study, the ethanol extract of soursop stem bark was tested for damage to kidney tissue using paracetamol as an inducer.

## **RESEARCH METHOD**

This research was conducted in August-December 2022. Preparation of ethanol extract of soursop bark (*Annona muricata* L.) (EESB) was carried out at

the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA) USK. Provision and provision of treatment to experimental animals is carried out at the Experimental Animal Laboratory, Faculty of Animals (FKH) USK. The microscopic depiction of the kidney structure was carried out at the Structure and Development Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA) USK.

### **Tools and Materials**

The tools that will be used in this research are a container, mortar and pestle, analytical scale, sample bottle, staining jar, embedding mold, embedding cassette, oven, rotary evaporator RE100-S, microscope (Zeiss primo star®), sleetec water /heat, computer, microtome, surgical tray, surgical instrument, gastric probe, mouse cage 50 cm x 40 cm x 30 cm, fume hood, and stationery.

The materials used in this study were 500 g soursop (*A. muricata* L.) bark, a syringe, paracetamol, 25 Wistar rats (*Rattus norvegicus*), distilled water, 96% ethanol, 500 mg paracetamol tablets, alcohol 70%, 80% alcohol, 90%

alcohol, absolute alcohol, Bouin's solution, xylol, paraffin, hematoxylin dye, eosin dye, aluminum foil, Whatman filter paper, embedding cassette, slide glass, microtome knife, physiological NaCl and rat feed

### **Research Process**

The initial process of making soursop bark samples is collected and cut into smaller pieces. The bark is then dried. The process was carried out at room temperature without direct sunlight for 3 days.

The preparation of ethanol extract of soursop bark was carried out using the maceration method. Soursop bark was soaked in a container containing 1 L of 96% ethanol solution for 3x24 hours. The next process was carried out using Whatmann filter paper to obtain the filtrate. The filtrate was concentrated using a rotary evaporator to obtain a thick extract. The extract was made in the form of a suspension in the form of a liquid extract dissolved with distilled water to facilitate administration to experimental animals.

This study used 25 males Wistar rats aged 3 months with a body weight

of about 200-250 g and acclimatized for 7 days. Rats are reared by giving them pellets and drinks ad libitum (to taste).

The treatment in each group was administered orally for 14 days using a gavage needle. The determination of the dose of paracetamol and the determination of the dose of EESB in this study followed the dose given in the study [12]. Groups K0 and K- were given distilled water, while group P1 was given EESB 150 mg/kgBW, P2 was given EESB 300 mg/kgBW and P3 was given EESB 600 mg/kgBW. Each feeding was given as much as 1 ml. On the 7th day, all groups of rats were given paracetamol at a dose of 1350 mg/kgBW in 1 ml of distilled water in one feeding except the normal control group.

Kidney organ sampling in rats was carried out on the 14th day. Observation of kidney structure is done by performing histotechnical steps. Staining of renal histological samples was performed using hematoxylin and eosin (HE) staining. Kidney histology was observed with the aid of a light

microscope at a magnification of 10 x 40. Kidney histology was observed with 3 times the microscope field of view in each incision. Observations were made by looking at the cortex of the kidney on tubular cells undergoing necrosis.

Data on the structure of kidney cells undergoing necrosis in this study were analyzed using One Way ANOVA (Analysis of variance). If there is a difference in the effect of treatment, then the analysis is continued with the multiple distance test (Duncan) at the 5% level.

## **RESULTS AND DISCUSSION**

This study used the bark of soursop (*Annona muricata* L.) which was processed using the maceration method. The type of solvent used in this study was 96% ethanol. Ethanol is used as a solvent because it is non-toxic, neutral, absorbs well, and can mix with water in all ratios and ethanol is a universal solvent that can attract polar, semi-polar, and non-polar compounds [13].

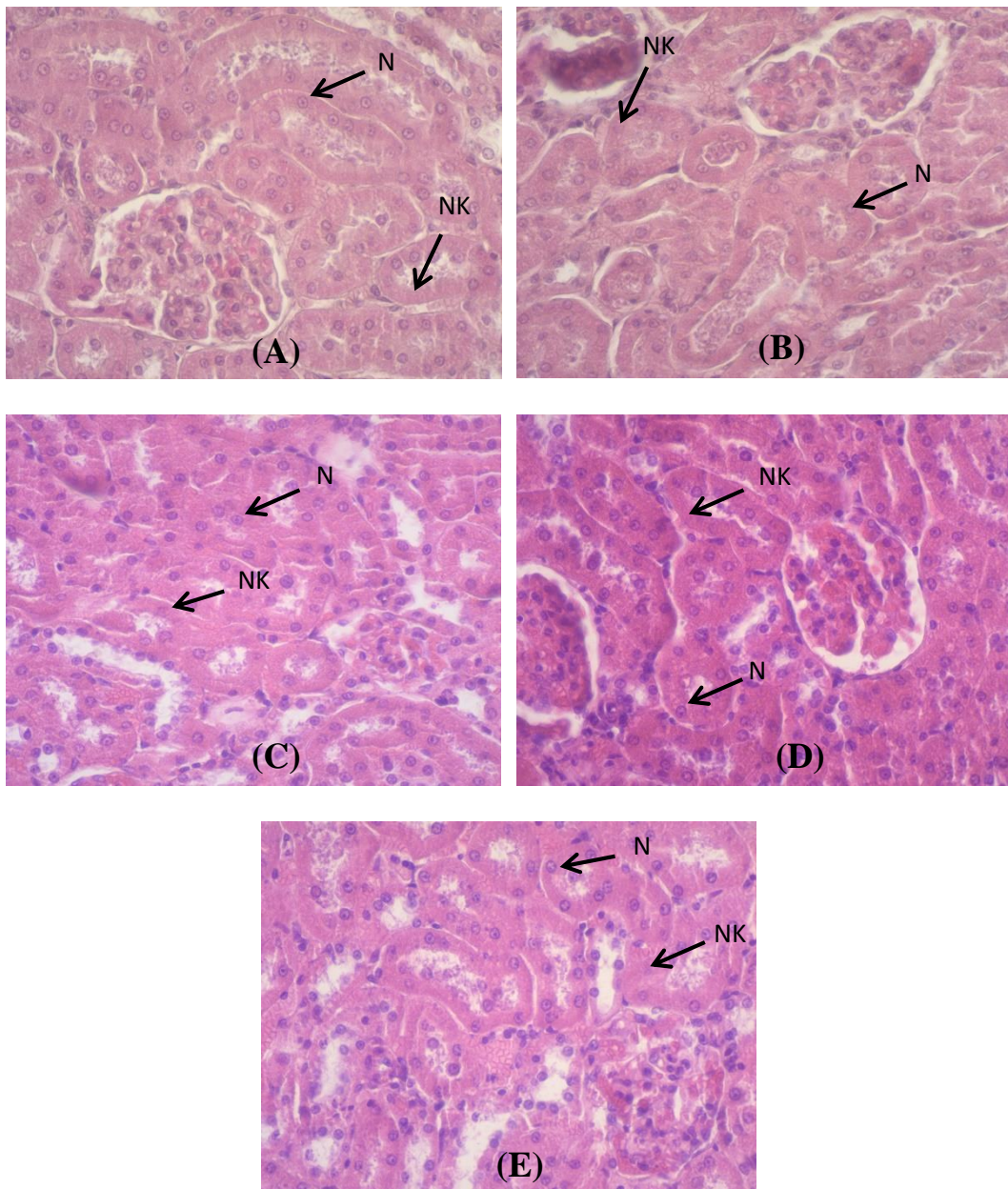


Figure 1. The histological structure of the rat kidney on various treatments of soursop bark ethanol extract (EESB) at 10x40 magnification. Information: (A) normal control (K0), (B) negative control (K-), (C) treatment 1 EESB 150 mg/kgBW, (D) treatment 2 EESB 300 mg/kgBW, (E) treatment 3 600 mg /kgBB. N = Normal cell, N = Necrosis cell

The results of the observations histopathological changes of the rat that have been made, it is found that the kidney which is characterized by the administration of toxic doses of necrosis in the tubular cells of the renal paracetamol can affect the cortex. The results of previous studies

also stated that the administration of toxic doses of paracetamol could affect histopathological changes in the kidneys of white rats which could be seen from the occurrence of necrosis in tubular cells [14].

Necrosis is irreversible which is the event of cells that have experienced death [15]. Necrotic events can be characterized by the occurrence of visible cell fragments, cells with no visible nucleus or no visible cells accompanied by an inflammatory reaction, chromatin clumping into coarse strands, loss of the nucleus, shrinking or condensed nuclei or nuclei (pyknosis), pyknotic nuclei breaking into many basophilic particles. into small pieces (karyolysis), the nucleus

undergoes lysis (karyolysis), or damage to the membrane followed by the mitochondria, so that the cell is unable to eliminate water and triglycerides because the tubules are the site of the reabsorption process [16][17].

Based on the Anova test, the Sig value was obtained. of  $0.000 < 0.05$  which indicates that the dose of ethanol extract of soursop bark affects the number of necrotic cells. Based on the results of the Anova test obtained, the data can be continued with the further test (Duncan) for further data analysis.

Based on the Duncan test results from the table of homogeneous subsets of necrosis, if depicted in a bar chart, the following picture is obtained.

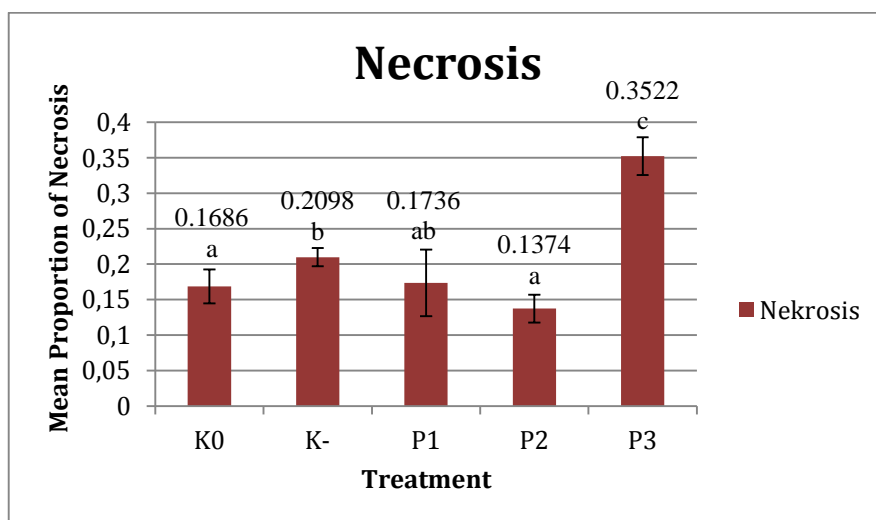


Figure 1. Diagram of the mean histopathological results of the kidneys

Based on the diagram, the average proportion of cells in the normal control group (K0) had necrotic cells. The results of the ANOVA test mean the proportion of K0 necrosis cells is  $0.168 \pm 0.023$ . This can be due to the influence of physiological factors from mice or the occurrence of apoptosis which is a programmed cell death process caused by physiological and pathological factors [18]. Other factors that can influence are due to microbiological agents, immune mechanisms from mice, or human error [19].

Based on Figure 1 in the form of the mean of cells undergoing necrosis, shows that the negative control group (K-) experienced a significant increase ( $P < 0.05$ ) when compared to K0. The results of the ANOVA test mean the proportion of K0 necrosis cells is  $0.209 \pm 0.012$ . This is in line with previous research, high doses of paracetamol can have a toxic effect in the form of damage to the kidneys [3][4][5]. Kidney damage caused by paracetamol is due to the presence of reactive metabolites formed from paracetamol metabolism, namely *N-acetyl-p-benzoquinone imine* (NAPQI),

which is a free radical that can be hepatotoxic or nephrotoxic [7].

The part of the kidney that is often the main target of poisoning cases is the proximal tubule [9]. The higher the dose of paracetamol that enters the body, the amount of NAPQI formed in the body that should be detoxified by glutathione has accumulated. The increase in the number of NAPQI results in a decrease in glutathione which in the next process will be able to cause toxicity to cells because it is released into the blood circulation [20].

The average proportion of necrosis in rat kidney tubular cells given a dose of 150 mg/kgBW of EESB (P1) obtained significantly different results ( $p < 0.05$ ) when compared to K-. The results of the ANOVA test mean the proportion of K0 necrosis cells is  $0.173 \pm 0.046$ . So that EESB with a dose of 150 mg/kgBW has been able to provide potential protection against the kidneys but is not yet maximal. This can also be due to the effect of a dose that is too low so that the optimal antioxidant contained in the extract has not been able to withstand damage to cells.

The average proportion of necrosis in the renal tubular cells of rats

given a dose of 300 mg/kgBW of EESB (P2) showed significantly different results ( $p < 0.05$ ) when compared to K-. The results of the ANOVA test mean the proportion of K0 necrosis cells is  $0,137 \pm 0,019$ . So that EESB with a dose of 300 mg/kgBW has been able to provide potential protection for the kidneys that have been exposed to toxic substances.

The EESB dose of 600 mg/kgBW (P3) resulted in significantly

different results ( $p < 0.05$ ) when compared to K-. The results of the ANOVA test mean the proportion of K0 necrosis cells is  $0,352 \pm 0,026$ . The amount of necrosis increased at the EESB dose of 600 mg/kgBW. This can be due to the extract dose being too high where the antioxidant content is too high and also does not always have a good effect on the body. So that the optimal dose is needed to get a good effect as well.

## CONCLUSION

Based on the results of this study, it can be concluded that from the three doses of ethanol extract of soursop bark (*A. muricata* L.) given, it was obtained that the administration of

EESB at a dose of 300 mg/kgBW can provide potential protection against kidney damage by suppressing the level of damage that occurs in cells so that no cell death (necrosis).

## REFERENCE

- [1] Hanifah, R.A., Ningrum, F.H., Kresnadi, E., & Wicaksono, S.A. (2020). The effect of parasetamol and codeine analgesic combination on serum glutamic oxaloacetate transaminase levels in male wistar rats. *Diponegoro Medical Journal*, 9(2), 148-153.
- [2] Oktaviana, E., Hidayati, I.R., & Pristianty, L. (2017). Pengaruh pengetahuan terhadap penggunaan obat parasetamol yang rasional dalam swamedikasi (studi pada ibu rumah tangga di desa sumberpoh kecamatan maron kabupaten probolinggo). *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 4(2), 44-51.
- [3] Sudira, I.W., Merdana, I.M., Winaya, I.B.O., & Parnayasa, I.K. (2019). Perubahan histopatologi ginjal tikus putih diberikan ekstrak sarang semut diinduksi parasetamol dosis



- toksik. *Jurnal Buletin Veteriner Udayana*, 11(2), 136-142.
- [4]Basith, A., Listyaningsih, E., & Handayani S. (2016). Gambaran histologi sel ginjal mencit (*Mus musculus*) yang diinduksi parasetamol ditambahkan ekstrak daun kemangi (*Ocimum sanctum*). *Jurnal Syifa' Medika*, 7, 1-8..
- [5]Rafita, I.D., Kusmiawati,T., Nur'ain. (2015). Pengaruh ekstrak kayu manis (*Korteks sinamum*) terhadap gambaran histopatologi ginjal tikus (*Rattus norvegicus*) yang diinduksi parasetamol. *Jurnal Sains dan Teknologi*, 13(2), 127-134.
- [6]Hidayat, R.P. (2020). N-acetylcysteine sebagai terapi toksisitas acetaminophen. *Jurnal Medika Hutama*, 2, 231-237
- [7]Febilani, E., Berata, I.K., Samsuri, Merdana, I.M., & Sudimartini, L.M. (2017). Pengaruh pemberian propolis terhadap gambaran histopatologi ginjal tikus putih yang diberikan parasetamol dosis tinggi. *Jurnal Buletin Veteriner Udayana*, 9, 9-15.
- [8]Jannah, D.R., & Busijastuti,W. (2022). Gambaran histopatologis toksisitas ginjal tikus jantan (*Rattus norvegicus*) yang diberi sirup umbi yakon (*Smallanthus sonchifolius*). *Jurnal LenteraBio*, 11(2), 238-246.
- [9]Duppa, M.T., Djabir, Y.Y., & Murdifin, M. (2020). Uji aktivitas ekstrak etanol jahe merah (*Zingiber officinale* Rosc var rubrum) dalam memproteksi dan memperbaiki gangguan fungsi hati dan ginjal tikus akibat induksi parasetamol. *Jurnal Majalah Farmasi dan Farmakologi*, 24(2), 33-36.
- [10]Olakunle, S., Onyechi, O., & James, O. (2014). Toxicity, anti-lipid peroxidation, invitro and invivo evaluation of antioxidant activity of *Annona muricata* ethanol stem bark extract. *American Journal of Life Sciences*, 2(5), 271-277.
- [11]Agustiani, T.Y., Betha, O.S., Dhillasari, E.M. (2020). Deskripsi komponen major metabolit sekunder tanaman dengan khasiatnya terhadap organ ginjal dalam buku Al-qanun fi'l tibt II. *Pharmaceutical and Biomedical Sciences Journal*, 2(2), 87-102.
- [12]Maslakhah, F.N. (2018). *Metabolit Profilling* Bagian Akar, Batang, Daun, dan Biji *Helianthus annuus* L. Menggunakan Instrumen UPLC-MS (Tugas Akhir). Universitas Malik Ibrahim Malang, Malang.
- [12]Masykur, Sari, W., Sari, N.Y. (2021). Pengaruh pemberian ekstrak etanol kulit batang

- sirsak (*Annona muricata* L.) dan srikaya (*Annona squamosa* L.) terhadap hati tikus (*Rattus norvegicus* L.) galur wistar. *Prosiding Seminar Nasional Biotik* (hal. 336-345). Banda Aceh : Prosiding Seminar Nasional Biotik.
- [13]Utami, I.K., Nurzafika, & Tandil, J. (2021). Uji efek ekstrak etanol daun jarak merah histopatologi ginjal tikus putih jantan diinduksi streptozotocin. *Farmakologika Jurnal Farmasi*, 18(2), 117-126.
- [14]Deviana, A. (2018). Pengaruh Pemberian Ekstrak Biji Petai (*Parkia speciosa*) Terhadap Gambaran Histopatologi Ginjal Bagian Tubulus Proksimal Pada Tikus Putih (*Rattus norvegicus*) Jantan Galur Wistar yang diinduksi Paracetamol. *Hang Tuah Medical Journal*, 15(2), 233-251.
- [15]Nazarudin, Z., Muhimmah, I., & Fidianingsing, I. (2017). Segmentasi citra untuk menentukan skor kerusakan hati secara histologi. *Seminar Nasional Informatika Medis* (hal. 15-21). Yogyakarta : Magister Teknik Informatika, Universitas Islam Indonesia.
- [16] Lagho, E.E., Kardena, I.M., & Jayawardhita, A.A.G. (2017). Gambaran histopatologi ginjal tikus putih (*Rattus norvegicus*) yang diberi amoxicillin dikombinasikan dengan deksametason dan asam mefenamat pasca operasi. *Jurnal Indonesia Medicus Veterinus*, 6(4), 262-269.
- [17]Dewi, R., & Sutejo, I.R. (2011). Pemberian minyak goreng bekas pakai dalam menimbulkan kerusakan sel-sel hati mencit dan penurunan kadar albumin serum mencit. *Jurnal Moluska Medica*, 4, 61-69.
- [18]Sari, Y. E. K., 2018. Gambaran histologi ginjal tikus wistar yang terpapar msg setelah perlakuan diberikan jus tomat dan diberhentikan perlakuan saja, *The Journal of Muhammadiyah Medical Laboratory Technologist*, 1, 62 – 69.
- [19]Setyawati, A.B., & Yani,. (2020). *Patofisiologi Untuk Masiswa Keperawatan*. CV. Pena Persada, Jawa Tengah.
- [20]Julistiono, H., Saragih, E., & Yulineri, T. (2017). Penguraian parasetamol oleh sel dan protein ekstraseluler khamir *Candida tropicalis* dan *Rhodotorula minuta*. *Jurnal Media Litbangkes*, 27(3), 169-174.
- [21]Amara, D.T., & Nugroho, T.E. (2017). Pengaruh pemberian analgesik kombinasi parasetamol dan tramadol terhadap kadar serum glutamat oksaloasetat transaminase tikus wistar. *Jurnal Kedokteran Diponegoro*, 6(2), 417-426.