

---

**THE SUBCHRONIC EFFECT OF WATER EXTRACT LEAVES OF SOURSOP  
(*Annona Muricata L.*) BAX EXPRESSION OF NEURON RAT HIPPOCAMPUS  
GYRUS DENTATUS (*Rattus Norvegicus*)**

**Desnita Monica<sup>1</sup>, Ety Sari Handayani<sup>2</sup>**

Faculty Of Medicine Islamic University of Indonesia<sup>1</sup>

Department of Anatomy, Faculty Of Medicine, Islamic University of Indonesia<sup>2</sup>

Email: Desnitamonica@gmail.com<sup>1</sup>, 097110415@uii.ac<sup>2</sup>

---

**KEYWORDS**

Gyrus Dentatus  
Hippocampus,  
leaves of  
Annona  
muricata L.  
Acetogenin,  
Expression Bax.

**ABSTRACT**

Soursop (*Annona muricata*) is a plant that is rich in benefits and widely used in the community as one of the traditional medicines. *Annona muricata* itself contains a compound known as acetogenin toxic if consumed continuously, In addition to the benefits so many of *Annona muricata* can give side effects to the body one of which is the part of the brain is called the gyrus dentatus. Anonaine, compound acetogenin could induce Bax expression in cancer cells resulting in apoptosis. This study wanted to find out if *Annona muricata* can induce Bax expression of neuron hippocampus gyrus dentatus female rats (*Rattus norvegicus*). The purpose of this study was to subchronic effect of aqueous extract of soursop leaves (*Annona muricata L.*) Bax expression of neuron rat hippocampus gyrus dentatus (*Rattus norvegicus*). This study was a simple experimental study design using a post-test control group design to see Bax expression of neuron rat hippocampus gyrus dentatus (*Rattus norvegicus*) in subchronic administration of aqueous extract of soursop leaves (*Annona muricata L.*). The subjects were adult female rats (*Rattus norvegicus*) that met the inclusion criteria. The samples were 10 rats. Subjects were divided into 2 groups and each group consisted of 5 rats. The first group was the control group with the administration of water and the second group was the treatment group with the administration of aqueous extract of soursop leaves (*Annona muricata L.*) at a dose of 1000 mg/kg/day for 30 days. The stain of preparation used Immunohistochemistry (IHC) to see Bax expression of neuron rat hippocampus gyrus dentatus (*Rattus norvegicus*). The difference in Bax expression between the control and treatment group with One Sample and Independent Sample T-Test using SPSS for Windows 16.0. The results showed no difference between control and treatment groups regarding Bax expression of neuron gyrus dentatus hippocampus (p-value = 0.101). There was no difference in Bax expression neuron rat hippocampus gyrus dentatus (*Rattus norvegicus*) in subchronic administration of aqueous extract of soursop leaves (*Annona muricata L.*).

---

**INTRODUCTION**

The Indonesian people have long known and used medicinal plants as an effort to overcome health problems. Indonesia is known as a repository of medicinal plants so it gets

the nickname *live laboratory*. There are about 40,000 species of plants in the world and 30,000 of them are found in Indonesia, of which 940 species are medicinal plants (this number is 90% of the number of medicinal plants found in the Asian region). Knowledge of medicinal plants is based on experience and skills that have been passed down from one generation to the next (Harmanto & Ahkam, 2007).

One of the plants used in herbal medicine is the soursop plant. It is estimated that since 1940 soursop plants have been used as herbal medicine. Brazilian people are the first people to use soursop plants to be used as medicine both leaves, seeds, fruits, stems, and roots. Based on National Cancer Institute research (Tanton et al., 2011), *acetogenin* in soursop plants works as a selective toxic in tumor cells, lung cell carcinoma, breast cancer, prostate adenocarcinoma, pancreatic carcinoma, colonic adenocarcinoma, liver cancer, lymphoma, and drug-resistant breast adenocarcinoma (Alvarez Colom et al., 2009).

In soursop leaves there are flavonoids, alkaloids, tannins, and several other chemical ingredients including *Annonaceous acetogenin*. *Annonaceous acetogenin* is a compound found in the *Annonaceae* family that has cytotoxic potential (Chao & Korsmeyer, 1998). Cytotoxic compounds are compounds that can be toxic to inhibit and stop the growth of cancer cells. Biological activity and mechanism *Annonaceous acetogenin* is very potent and has a wide range, among which it functions as an antitumor *in vivo*, cytotoxic, pesticide, antibacterial, antiparasitic, and immunosuppressive effect. *Acetogenin* can reduce ATP production by decreasing the production of NADH: *ubiquinone oxidoreductase (Complex I)* in the mitochondrial electron transport system and NADH *oxidase* enzyme in the plasma membrane of cancer cells (Chang & Yang, 2000), due to the inhibition of these substances so that cells lack ATP and cause inhibition of cancer cell growth so that cancer growth can be suppressed (Kim & Diamond, 2002).

Although some books reveal that treatment using soursop leaves is relatively safe compared to chemotherapy, some research facts show that the use of *acetogenin compounds* can affect normal cells. This is evidenced by the research of (Champy et al., 2004), where pure *acetogenin* compounds were found in the brain tissue of healthy rats and the presence of damage to dopaminergic neurons in the brain of rats causing symptoms similar to Parkinson's in healthy mice (Champy et al., 2004).

*Acetogenin compounds* can induce apoptosis of cancer cells and healthy cells. (Nascimento et al., 2012), mentioned an increase in apoptosis index in rat liver cells given ethanol extract of srikaya leaves with HE staining. In cancer cells, there is an increase in apoptosis involving impaired expression of Bax / bcl2 disrupting mitochondrial membrane action potentials. This causes the release of Cytochrome C compounds that will activate caspase and PARP (Chang & Yang, 2000). Furthermore, DNA fragmentation occurs which induces apoptosis resulting in cell death. *Acetogenin* compounds have been shown to increase Bax expression thereby inducing cell apoptosis (Li et al., 2013). In addition to the apoptosis mechanism, soursop leaf extract can result in necrosis of healthy kidney tubular and glomerular cells through the mechanism of caspase 9 (Dayeef, 2013).

Another mechanism *acetogenin* can stop the cancer cell cycle in the G1 phase and inhibit the progress of the cell cycle towards the S phase by inducing the expression of p53, p21, Bax, and Bad in cancer *cell lines* (Hadi, 2011). Based on the description above

*acetogenin compounds* can cause damage to some healthy cells through the mechanism of apoptosis which is thought to involve Bax, so it is necessary to research the effect of subchronic administration of soursop leaf water extract (*Annona muricata*) on Bax expression in neurons of the dentate gyrus hippocampus of rats (*Rattus norvegicus*) (Asyiyah, 2005).

## METHOD RESEARCH

This study is a simple experimental study using a *post-test control group design* to see the effect of subchronic administration of soursop leaf water extract (*Annona muricata L.*) against Bax expression in neurons of the mouse hippocampus dentate gyrus (*Rattus norvegicus*) (Yuwono, 2022).

### Data Analysis and Collection

The difference in the number of cells that secrete Bax between the control and treatment groups. Observations of Bax expression were carried out in all fields of view of each research sample using an Olympus CX21 microscope connected to an optical camera viewer with image raster software. Observation using 1000x exposure. The difference in the mean amount of Bax expression between groups I and II was tested with the *t-test*. The results of the study are presented in the form of a table (Yoo et al., 2012).

## RESULTS AND DISCUSSION

### Description of the Research Subject

The test animal used for this study was a female mouse (*Rattus norvegicus*) developed by the Pharmaceutical Laboratory of Universitas Islam Indonesia (Dwigunasari, 2014). Rats are anesthetized first before decapitation so as not to hurt rats. The animals were placed in cages at room temperature and kept under irradiation for 12 hours (during the day) and without irradiation for 12 hours (at night). Food and drinks of these animals are provided, namely water and pellets. The number of samples used was 10 mice (Wicaksono, 2011). The subjects were divided into 2 groups and each group consisted of 5 mice. The first group was a control group, given sondase aquades and the second group was a treatment group, given sondase water extract of soursop leaves (*Annona muricata L.*) at a dose of 1000 mg/kg/day given for 30 days (Paxinos & Watson, 2005). The following is a table that describes the characteristics of research subjects based on body weight and Hb values (Table 1).

**Table 1**  
**Characteristics of research subjects based on body weight and Hb levels**

| Group   | Subject | Weight Loss<br>(grams) | Up to<br>hemoglobin<br>(g/dl) |
|---------|---------|------------------------|-------------------------------|
| Control | K1      | 244                    | 14,7                          |
|         | K2      | 203                    | 15,0                          |
|         | K3      | 230                    | 14,1                          |
|         | K4      | 205                    | 13,4                          |
|         | K5      | 215                    | 14,1                          |

|           |    |     |      |
|-----------|----|-----|------|
| Treatment | P1 | 223 | 13,8 |
|           | P2 | 200 | 13,6 |
|           | P3 | 204 | 13,5 |
|           | P4 | 190 | 12,8 |
|           | P5 | 188 | 12,8 |

Based on the results of the rhino weight examination presented in the table above, the criteria for sample inclusion in this study were rats weighing 175 - 300 grams. Based on the results of the Hb examination showed that the test results were within normal limits or the rats were in a healthy state. According to (Mitraka & Rawnsley, 1977), the normal value of Hb in white rats (*Rattus norvegicus*) is 10-6.8 g / dl. During the study, all the mice (*Rattus norvegicus*) were in good health and none died (Trubus, 2012).

#### Description of the relative weight of the organ

The relative weight of organs listed in Table 2 can be calculated by the formula:

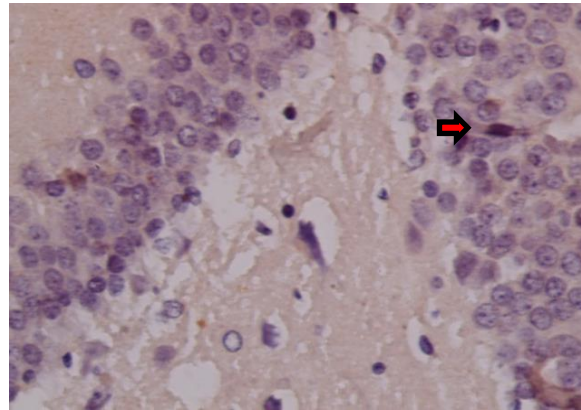
$$\frac{\text{organ weight (grams)}}{\text{rat body weight (grams)}} \times 100\%$$

**Table 2**  
**Berat organ relative**

| Group     | Subject | Brain Weight (grams) | Weight Mouse after Treatment (gram) | Relative Brain Weight (%) |
|-----------|---------|----------------------|-------------------------------------|---------------------------|
| Control   | K1      | 1,671                | 217                                 | 0,77                      |
|           | K2      | 1,706                | 256                                 | 0,66                      |
|           | K3      | 1,827                | 263                                 | 0,69                      |
|           | K4      | 1,874                | 240                                 | 0,78                      |
|           | K5      | 1,937                | 262                                 | 0,74                      |
| Treatment | P1      | 1,811                | 264                                 | 0,68                      |
|           | P2      | 1,913                | 235                                 | 0,81                      |
|           | P3      | 1,073                | 259                                 | 0,41                      |
|           | P4      | 1,919                | 224                                 | 0,86                      |
|           | P5      | 1,791                | 248                                 | 0,72                      |

### Description of Bax Expressions

Based on the results of observations of Bax's expression, the brown color that appears indicates a positive reaction (+). The results of observing the number of Bax expressions are viewed with an objective magnification of 1000x. Here's the expression of Bax mice from the control group and experimental group.



**Figure 1**

**Expression of Bax gyrus dentatus hippocampus at 1000x Objective enlargement**

**Table 3**

**Data Ekspresi Bax**

| Ekspresi Bax | Minimum | Maximum | Mean   | Std. Deviation |
|--------------|---------|---------|--------|----------------|
| control      | 0,37    | 3,57    | 2,3552 | 1,18987        |
| Experiment   | 1,44    | 6,50    | 4,4259 | 2,20121        |

The table above shows the minimum, maximum, mean, and standard deviation values of the Bax expression values from the control group and the experimental group. In the control, a minimum value of 0.37 and a maximum value of 3.57 were obtained with a mean of 2.3552 and a standard deviation of 1.18987. While the experimental group has a minimum value of 1.44 and a minimum value of 6.50 with a mean of 4.4259 and a standard deviation of 2.20121.

### Assumption Test

The assumption test is carried out before data analysis is carried out, which consists of the distribution normality test and the variance homogeneity test. The following are the results of the spread normality test and homogeneity test.

### Uji Normality

The data on this normality test are obtained from experimental class data (treatment) and control class data. The normality test was performed using the computer-assisted program *SPSS for Windows 19.00 Shapiro Wilk*. The normality test results for each of the study variables are presented below.

**Table 4**  
**Normality test results with Shapiro Wilk test**

| Ekspresi Bax | Itself | Information |
|--------------|--------|-------------|
| Control      | 0,424  | Normal      |
| Experiment   | 0,218  | Normal      |

The results of the research variable normality test can be known that the experimental and control class data have a significance value greater than 0.05 at ( $p > 0.05$ ), so it can be concluded that the experimental class and control class data are normally distributed.

#### Homogeneity Test

The summary of the results of the homogeneity test of data variants is presented in the following table.

**Table 5**  
**Homogeneity test results with Levene test**

|              | Lavene Statistic | Itself | Information |
|--------------|------------------|--------|-------------|
| Ekspresi Bax | 3,695            | 0,091  | Homogeneous |

From the data above, it is explained that for data in the experimental group and control group, it can be known that the significance value is greater than 5% ( $p > 0.05$ ) and  $F$  count  $< F$  table, which means that the two groups are homogeneous.

#### Test the hypothesis

Based on the normality test and homogeneity test, it can be seen that the Bax expression data in the control and treatment groups are normally distributed and have the same variant, so a parametric test is carried out, namely the *Independent Sample T-test* (Taylor, 2005).

**Table 6**  
**Hasil Independent Sample T-Test**

| Measuremen<br>t results | Group     | mean    | Mean<br>difference | t-count | Sig. 2<br>tail |
|-------------------------|-----------|---------|--------------------|---------|----------------|
| Ekspresi Bax            | Ekperimen | 2,20121 | 1,01134            | 1,850   | 0,101          |
|                         | control   | 1.18987 |                    |         |                |

The results of the analysis of the effect of subchronic administration of soursop leaf water extract (*Annona muricata*) on Bax expression in rat hippocampus dentate gyrus neurons (*Rattus norvegicus*) can be seen in the table above. Based on the results above, show that there is no difference in the results of experimental and control class Bax expression evidenced by a significance value greater than the significance level ( $0.101 > 0.05$ ) that there is no subchronic effect of soursop leaf water extract (*Annona muricata*) on Bax expression in rat hippocampus dentate gyrus neurons (*Rattus norvegicus*).

#### Discussion

Based on data analysis using the *Independent Sample t-test*, it can be concluded that there is no effect of subchronic administration of soursop leaf water extract (*Annona muricata L.*) against Bax expression in neurons of the mouse hippocampus dentate gyrus (*Rattus*

*norvegicus*). Bax is a member of the Bcl-2 gene. Apoptosis regulators Bax promotes apoptosis by binding to and opposition to the Bcl-2 protein (Adams & Cory, 1998). Bax regulatory apoptosis is also known as Bcl-2 protein like the protein that in humans is encoded by the Bax gene (Duvernoy et al., 2005).

Based on the results of research by (Rahman, 2014), who researched the degeneration of rat Gyrus Dentatus Hippocampus neurons (*Rattus norvegicus*) on the administration of soursop leaf water extract (*Annona muricata L.*). There is an effect of giving water extract from soursop leaves (*Annona muricata L.*) on the number of pyramidal gyrus dentatus cells of the mouse hippocampus (*Rattus norvegicus*) and there were differences in the number of neurons of the rat Hippocampus Dentatus Gyrus (*Rattus norvegicus*) between groups exposed to soursop leaf water extract (*Annona muricata L.*) with a control group (Dahana & Warisno, 2013).

Based on research conducted by Putri Rizki D (2014), who researched the effect of subchronic administration of soursop leaf water extract (*Annona muricata L.*) against the number of cell pyramidal CA1 hippocampus rats (*Rattus Norvegicus*). There was vacuolization and differences in the number of CA1 pyramidal cells in the mouse hippocampus (*Rattus Norvegicus*) in the group exposed to soursop leaf water extract (*Annona muricata L.*).

This study shows that exposure to soursop leaf water extract can affect the decrease in the number of neurons at a dose of 1000 mg / kgbb and this is influenced by several mechanisms by the theoretical framework above, that acetogenin compounds contained in *Annona muricata* can inhibit the ATP process through inhibition of complex 1 mitochondria so that there is a decrease in oxidative phosphorylation and ATP reduction. As a result of the decrease in ATP, neuron cells will degenerate, resulting in cell death which will reduce the number of neurons (Lannuzel et al., 2002).

From previous studies, subchronic administration of soursop leaf extract only causes cell degeneration, not to the point of causing apoptosis of neuron cells dentatus hippocampus. This is possible due to the lack of dose or less prolonged exposure to soursop leaf water extract (*Annona muricata L.*). This is supported by this study, where there was no significant difference in Bax expression in the administration of 1000 mg/kg body weight/day of soursop leaf extract between the treatment group and the control group.

In addition to being influenced by dose and duration of administration, these results may also be influenced by the special abilities of the hippocampus dentate gyrus. Neurogenesis occurs after a neuron degenerates. The presence of neural precursors in the dentate gyrus allows regeneration in the area (Parent, 2003); John & Martin, 2013). The results of this study support the theory that acetogenin compounds can act as antidepressants if used acutely or momentarily (14 days) (Li et al., 2013). This antidepressant property is possible because acetogenin compounds have a structure similar to serotonin so that it can stimulate serotonin receptors (Wiar, 2007). Some of the conditions that trigger neurogenesis are serotonin, antidepressants, and ischemic conditions (Parent, 2003).

Other processes of cell apoptosis may occur; 1) As a result of an intrinsic trigger from within the cell, 2) Triggered by an external "death activator" attached to receptors on the cell surface, and 3) Triggered by oxidizing agents from outside the cell (Hadi, 2011).

## CONCLUSION

The study revealed that an aqueous extract of soursop leaves (*Annona muricata L.*) had a subchronic effect on Bax gene expression in the hippocampal dentate gyrus of rats (*Rattus norvegicus*). This suggests that the use of soursop leaf extract over some time may affect gene activity in the brains of mice, which could have implications for their neuron function.

## REFERENCES

- Adams, J. M., & Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. *Science*, 281(5381), 1322–1326.
- Alvarez Colom, O., Neske, A., Chahboune, N., Zafra-Polo, M. C., & Bardon, A. (2009). Tucupentol, a Novel Mono-tetrahydrofuranic Acetogenin from *Annona montana*, as a Potent Inhibitor of Mitochondrial Complex I. *Chemistry & Biodiversity*, 6(3), 335–340.
- Asyiyah, N. (2005). *Tanaman Obat Indonesia*. Penerbit: IPTEK. Jakarta.
- Champy, P., Höglinger, G. U., Féger, J., Gleye, C., Hocquemiller, R., Laurens, A., Guérineau, V., Laprévotte, O., Medja, F., & Lombès, A. (2004). Annonacin, a lipophilic inhibitor of mitochondrial complex I, induces nigral and striatal neurodegeneration in rats: possible relevance for atypical parkinsonism in Guadeloupe. *Journal of Neurochemistry*, 88(1), 63–69.
- Chang, H. Y., & Yang, X. (2000). Proteases for cell suicide: functions and regulation of caspases. *Microbiology and Molecular Biology Reviews*, 64(4), 821–846.
- Chao, D. T., & Korsmeyer, S. J. (1998). BCL-2 family: regulators of cell death. *Annual Review of Immunology*, 16(1), 395–419.
- Dahana, K., & Warisno, S. (2013). *Daun Sirsak-Langkah Alternatif Menggempur Penyakit*. Gramedia Pustaka Utama.
- Dayeef, A. (2013). *The Influnce Of Annona Muricata Leaves Extract In Damaging Kidney Cell, Increasing Creatinine Serum And Inducing Caspase-9 Activity*. Universitas Brawijaya.
- Duvernoy, H. M., Cattin, F., & Risold, P.-Y. (2005). *The human hippocampus: functional anatomy, vascularization and serial sections with MRI* (Issue 3). Springer.
- Dwigunasari, P. R. (2014). *Pengaruh Pemberian Ekstrak Air Daun Sirsak (Annona Muricata L.) Terhadap Jumlah Sel Piramidal Cal Hippocampus Tikus (Rattus Norvegicus)*. Universitas Islam Indonesia.
- Hadi, R. S. (2011). Mekanisme Apoptosis Pada Regresi Sel Luteal. *Majalah Kesehatan Pharmamedika*, 3(1), 246–254.
- Harmanto, N., & Ahkam, S. M. (2007). *Pilih Jamu Herbal Tanpa Efek Samping*. Penerbit PT. Elex Media Koputindo. Jakarta, 8.
- Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature Reviews Neuroscience*, 3(6), 453–462.
- Lannuzel, A., Michel, P. P., Caparros-Lefebvre, D., Abaul, J., Hocquemiller, R., & Ruberg, M. (2002). Toxicity of Annonaceae for dopaminergic neurons: potential role in atypical parkinsonism in Guadeloupe. *Movement Disorders*, 17(1), 84–90.
- Li, H.-T., Wu, H.-M., Chen, H.-L., Liu, C.-M., & Chen, C.-Y. (2013). The pharmacological activities of (-)-annonaine. *Molecules*, 18(7), 8257–8263.



- Mitruka, B. M., & Rawnsley, H. M. (1977). *Clinical biochemical and hematological reference values in normal experimental animals*. Masson Publishing USA Inc.
- Nascimento, G. N. L. do, Valadares, M. C., Nishijo, H., & Aversí-Ferreira, T. A. (2012). Investigation of the toxic potential of crude ethanol extract of *Annona coriacea* (araticum) seeds in acute exposed mice. *Revista Brasileira de Farmacognosia*, 22, 580–586.
- Parent, J. M. (2003). Injury-induced neurogenesis in the adult mammalian brain. *The Neuroscientist*, 9(4), 261–272.
- Paxinos, G., & Watson, C. (2005). *The rat brain in stereotaxic coordinates*. 5th Elsevier Academic Press. San Diego, CA.
- Rahman, A. (2014). *Pengaruh Pemberian Ekstrak Air Daun Sirsak (Annona Muricata) Terhadap Jumlah Neuron Pyramidal Gyrus Dentatus Hippocampus Tikus (Rattus Norvegicus)*. Universitas Islam Indonesia.
- Tanton, C., Weiss, H. A., Le Goff, J., Changalucha, J., Rusizoka, M., Baisley, K., Everett, D., Ross, D. A., Belec, L., & Hayes, R. J. (2011). Correlates of HIV-1 genital shedding in Tanzanian women. *PloS One*, 6(3), e17480.
- Taylor, L. (2005). *The healing power of rainforest herbs: a guide to understanding and using herbal medicinals*. Square One Pub.
- Trubus, R. (2012). Daun Sirsak Vs Kanker. *Depok. PT Trubus Swadaya*.
- Wiat, C. (2007). Plants affecting the central nervous system. *Ethnopharmacology of Medicinal Plants: Asia and the Pacific*, 57–153.
- Wicaksono, A. (2011). Kalahkan Kanker dengan Sirsak. *Citra Media Mandiri, Yogyakarta*.
- Yoo, D. Y., Kim, W., Lee, C. H., Shin, B. N., Nam, S. M., Choi, J. H., Won, M., Yoon, Y. S., & Hwang, I. K. (2012). Melatonin improves D-galactose-induced aging effects on behavior, neurogenesis, and lipid peroxidation in the mouse dentate gyrus via increasing pCREB expression. *Journal of Pineal Research*, 52(1), 21–28.
- Yuwono, M. R. A. (2022). Analisis Perbandingan Metode Eoq, Metode Poq, Dan Metode Min-Max Dalam Pengendalian Persediaan Komponen Pesawat Terbang Boeing 737ng (Studi Kasus: PT Garuda Maintenance Facility Aeroasia Tbk.). *Industrial Engineering Online Journal*, 11(3).

**Copyright holders:**

**Desnita Monica<sup>1</sup>, Ety Sari Handayani<sup>2</sup> (2023)**

**First publication right:**

**JoSS - Journal of Social Science**



**This article is licensed under a Creative Commons Attribution-ShareAlike 4.0  
International**