Eco. Env. & Cons. 27 (Special Issue of the 5 IBOC on Sustainability of Natural Resources, December, 2021) : pp. (S6-S12)
Copyright@ EM International
ISSN 0971–765X

In vitro Test of Deazaelliptisina 1 Compound on Phagocytotic activity of Macrophage Cell

Awik Puji Dyah Nurhayati[1], Mardi Santoso[2], Edwin Setiawan[1], Ayuri Cintyasari[1] and Syamsudin Abdillah[3], Fitri Lianingsih[1], Adik Mayang Indiani[1] and Shabrina Syifa Ghaissani[1]

[1]Department of Biology, Faculty of Sciences, Institut Teknologi Sepuluh November, Surabaya, Indonesia
[2]Chemistry Department, Faculty of Sciences, Institut Teknologi Sepuluh November, Surabaya, Indonesia
[3]Department of Pharmacology, Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia

(Received 17 September, 2021; Accepted 2 October, 2021)

ABSTRACT

Cancer is a growth of abnormal cells that invades tissues and possess a metastasis phase. Chemotherapy treatment is often used but it causes side effects, namely immunosuppressive nevertheless. Therefore, natural ingredients that function as immunomodulator is needed for improving immune system. Deazaellipticine 1 compound were synthesized from *Ochrosia elliptica* is a natural ingredient that possess an anticancer potent and being expected as an immunostimulant. This study was conducted to investigate *in vitro* test of Deazaellipticine 1 compound on phagocytosis activity of mice macrophage cells (*Mus musculus*). The concentration of Deazaellipticine 1 used is 6.25; 12.5; 25; 50; 100 µg/ ml and as a positive control, an anticancer drug Doxorubicin was used. Phagocytic activity was expressed in a capacity and an index. The results showed was phagocytosis capacity by 64.4% and phagocytic index 2.29 were increasing at a concentration of 100 µg / ml, while Doxorubicin yield only phagocytic capacity of 17.7% and a phagocytic index of 0.06 at a concentration of 25 ìg / ml. Deazaellipticine 1 compound is able to increase the phagocytic activity of macrophage cells in mice (*M. musculus*).

Key words*: Deazaellipticine 1, Doxorubicin, Phagocytic, Cancer, Macrophage*

Introduction

Cancer is a disease, in which cells experience an abnormal growth and invade nearby tissues, in addition to metastasize other or more distant tissues (Syafaah *et al.*, 2013). The spread of cells occurs either with direct growth in the surrounding tissue (invasion) or with migration of cells to distant places (metastasis) (Martini *et al.*, 2013). In 2012 14.1 million cases of cancer were recorded and expected to continue to increase until 2030 to 23.6 million each year (WHO, 2014). Uncontrolled growth of cancer cells stimulate response of immune guard (immune surveillance) to damage cancer cells. The immune system fight on appearance of cancer cells, whereas macrophage cells destroy tumour cell growth and prevent metastatic cells (Gozales *et al.*, 2018).

Macrophages have a very important role, namely as phagocytes in immune system innate and as antigen presenting cells (APC), in which initiate on adaptiveness of immune response (Herawati *et al.*, 2015). The presence of immune response and cell proliferation can further activate mechanism of

apoptosis or necrosis, so that cell death occurs to keep cells in the normal tissue (Chabane *et al.,* 2013). When cells experience cell death by necrosis, cells will experience inflammation while apoptotic death will occur when apoptotic bodies phagocytized by macrophage cells (Kumra *et al.,* 2013*)*. Macrophages have a large role in immune system to phagocytose bacterial cells, viruses and secrete inflammatory molecules, in addition to repair tissue (Fujiwara *et al.,* 2014).

Chemotherapy is a treatment process using drugs that aim to destroy or to retard the growth of cancer cells. However, side effects of chemotherapy arise because, chemotherapy drugs do not only destroy cancer cells but also attack healthy cells (Liu *et al.,* 2015). The use of Doxorubicin as a chemotherapy agent is able to decrease immune system (Bowles *et al.,* 2012). Doxorubicin is also able to initiate a death of cell by necrosis and furthermore damage normal cells (Tacar *et al.,* 2013). For this reason, natural materials are needed, in which increase cell activity of macrophages without damaging cells. Phagocytosis activity of macrophage cells can be identified by three parameters, namely phagocytic index, phagocytic capacity and phagocytosis efficiency (Hartini *et al.,* 2013). Latex beads is one type of antigen model which is a biomaterial used in medical diagnoses. Polymer microspheres are often used to study the influence of materials on immune function and cell response (Akilbekova *et al.*, 2014).

Elliptisina (5, 11-Dimethyl-6H-pirido [4, 3-b] carbazola) is a natural compound that is isolated from *Ochrosia elliptica* (Apocynaceae) that shows anticancer bioactivity. Elliptisine and deazaellipticine derivatives (6, 11-dimethyl-5H-benzo [b] carbazola) in the form of (2-bromo-6- (5-bromo-3a, 7a-dihidro-1H-indol-3-yl) -5H-benzol [b] carbazola) as one compound (S1) has high efficiency for many types of cancer (Miller *et al.*, 2014). Deazaellipticine 1 that has a similar structure with ellipticine is also known to have an ability to inhibit cancer cell activity (Miller *et al.,* 2019) Deazaellipticine 1 is anticancer against HepG2 Cell Line liver cancer cells with an IC_{50} value of 1.932 µM. While Elliptisina compounds are also able to increase phagocytic activity (Calabro *et al.,* 2015). Therefore, phagocytic activity of Deazaelliptisna 1 was carried out in this study on macrophage cells of *Mus musculus* mice in vitro with latex beads as an antigen model.

Materials and Methodology

The study was conducted in September 2017 - March 2018 in the Laboratory of Parasitology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Natural Material Chemistry Laboratory and Synthesis of the Department of Chemistry and Animal Zoology and Engineering Laboratory, Department of Biology, ITS Faculty of Science Sepuluh Nopember Institute of Technology, Surabaya.

Preparation of Stock Solution

The stock solution was made with a concentration of 1000 µg/ml. Deazaellipticine 1 compounds (2-bromo-6- compounds (5-bromo-3a, 7a-dihidro-1H-indol-3-yl) -5H-benzol [b] carbazola) and Doxorubicin as many as 1000 dissolved in 1000 µl DMSO (Dymethyl sulfoxide).

Dilution

Stock solutions were diluted with RPMI media into 5 series concentrations (6.25; 12.5; 25; 50; 100 µg / ml).

Isolation and Culture of Macrophage

Mice were killed using chloroform. Mice were placed in a supine position, the skin of the abdomen was cleaned with 70% alcohol. Ten cold RPMI (Roswell Park Memorial Institute) was injected into the peritoneum cavity while rolling slowly for 3 minutes. Macrophage cell isolation was carried out by aspirating with a syringe, then the liquid was centrifuged at 1200 rpm, for 10 minutes. The supernatant was removed and the pellet was suspended with complete RPMI media (RPMI plus PBS 10%, amphotericin B 0.5% and streptomycin penicillin 1%). Cell count was calculated by haemocytometer, then suspended with complete RPMI until cell density of 2.5 x 10v / ml was obtained. A total of 200 µl of cell suspension was placed in a well which had been covered with a slips cover so that each well contained 5x105 cells and added 800 mL of RPMI media. Macrophage cells were cultured in a 5% CO2 incubator, 37 ^{o}C for 24 hours (Hartini *et al.*, 2013).

Phagocytosis Activity Test with Latex Beads

The well containing macrophage cell culture was washed twice with RPMI and added a solution of Deazaellipticine 1 compound then left for 4 hours. Two hundred µl of latex suspension was added to

each well. Incubation is carried out for 60 minutes. The well was washed with PBS 300 µl twice. Fixation was carried out with 100% methanol and dried air at room temperature, then stained with 10% Giemsa. After being washed and dried, coverslips are taken from the well, placed above the glass object and seen under a light microscope with a magnification of 400 times. The number of phagocytic beads latex and macrophages calculated which actively phagocytosis every 300 macrophage cells. The phagocytic index and phagocytosis capacity are calculated as follows:

Phagocytosis Capacity

(Macrophage of Phagocytized latex beads ÷ Total of Macrophage counted (300))× 100

(Shanumugam *et al.,* 2015)
Phagocytosis index
Total of latex beads phagocytized macrophage ÷ Total of macrophage active
(Shanumugam *et al.,* 2015)

Phagocytosis Efficiency

Phagocytosis index ÷ Phagocytosis capacity
(Shanumugam *et al.,* 2015)

Data Analysis

Phagocytosis activity was calculated with 3 parameters, namely phagocytosis index (IF), percent phagocytosis (PF), and phagocytosis (EF) efficiency (Hartini *et.al* 2013). Statistical analysis used one way ANOVA and Tukey test.

Results and Discussion

Phagocytosis test is an immunomodulatory attempt of a compound efficiency (Hartini *et.al* 2013). In order to investigate ability of phagocytosis from macrophages, administration of latex as an antigen material is applied (Akrom and Fatimah, 2015)

Morphology Analysis

Macrophage cells that have not been active showed a different morphology with cells that have been actively done a phagocytosis (Figure 1). Sitoplasmic pseudopodia (Figure 1A) have not seen yet when macrophage cells innactivated. Nevertheless, when it started active, size of macrophage cells became larger (Figure 1B) in addition to starting point of pseudopodia formation (Figure 1C). Pseudopodia is a form of morphological substitution that occur due to the activation of macrophages by pathogenic microorganisms or other antigens (Nakada *et.al*, 2104). Characteristics of normal peritonial macrophage cells are indicated by eccentric, round or kidney-shaped nuclei with one or two nuclei and multiple cytoplasm (Wang *et.al,* 2013). The administration of Diazaleptizin 1 caused macrophage cells to be activated while treatment with Doxorubicin causes damage to macrophage cells in the form of lysed cell membranes (Figure 1D) (Shin *et al.,* 2015).

Phagocytosis Capacity of Macrophage Cells

The value of phagocytic capacity from macrophages was increasing along with concentration compound of Deazaellipticine 1 (Table 1).

Deazaellipticine compound at a concentration of 100 µg / ml induced a phagocytic capacity of 64.6% and 22.4% at a concentration of 6.25 /100 µg. Phagocytosis capacity of macrophage cells increased along increasing of Deazaellipticine treatment 1. This high phagocytosis capacity was indicators that Deazaellipticine 1 has a potential as immunostimulant. Doxorubicin at a concentration of 25 µg / ml triggered the phagocytosis capacity of 17.7%, while at a concentration of 100 µg / ml pos-

Table 1. Phagocytosis capacity test of Deazaellipticine 1 compound against macrophage cell *M. musculus*

Concentration	Phagocytosis capacity value (%) of Deazaellipticine	Phagocytosis capacity value (%) of Doxorubicin
0 µg/ml	8,3 ± 0,335 a	8,3 ± 0,335 ab
6,25 µg/ml	22,4 ± 0,470 ab	9,111 ± 2,792 ab
12,5 µg/ml	26 ± 7,37 bc	15,642 ± 2,132 b
25 µg/ml	33,3 ± 0,942 bc	17,712 ± 3,440 b
50 µg/ml	43,1 ± 1,414 c	3,525 ± 0,905 a
100 µg/ml	64,6 ± 1,650 d	0,940 ± 0,648 a

Notes : Different letter notations on the same line show significantly different effects at the level of á = 0.05

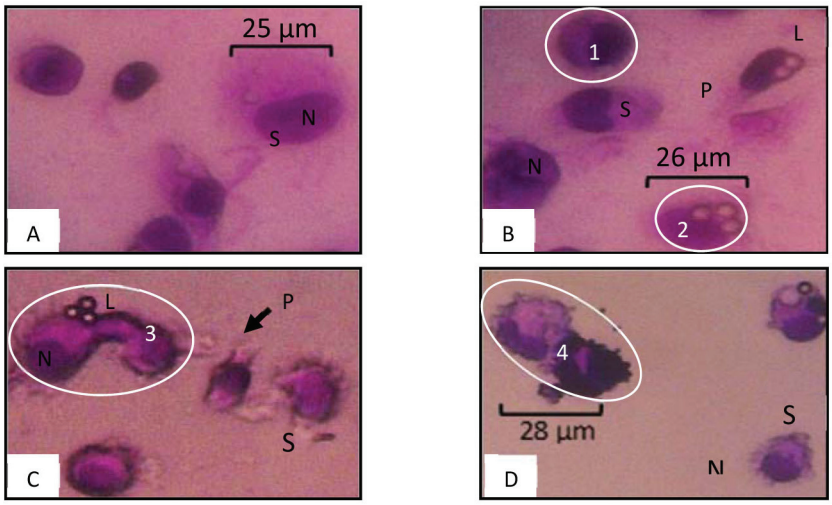

Fig. 1. Morphology of macrophage cells in mice (M. musculus) before the treatment of latex beads and Deazaellipticine (A); after treatment of latex beads and Deazaellipticine (B); Macrophages that phagocytose latex beads (C); Macrophages with Doxorubicin (D) N = Nucleus treatment; S = Cytoplasm; P = Pseudopodia; Inactivated macrophage cells (1); Macrophage cells that phagocyte latex beads (2); Pseudopodia in macrophage cells (3); Macrophage cells that experience necrosis (4); 400x magnification.

sess 0.94%. Increased concentration of Doxorubicin decreased the capacity of phagocytosis. One Way ANOVA statistical analysis at the level of confidence (pˆ0.05) showed that Deazaellipticine and Doxorubicin had an effect on phagocytic capacity.

Increased phagocytic capacity was influenced by the number of phagocytic receptors available. The more phagocytic receptors present in macrophage cells, the higher the capacity (Garcia, 2013). Phagocytic receptors will be activated when there are stimuli from outside the cell such as antigens in the form of pathogens, lipopolysaccharides other proteins and particles (Gordon, 2016). In this study, Deazaellipticine 1 and doxorubicin were used to activate macrophage cell receptors. Deazaellipticine is a modification of the ellipticine compound which is an indole alkaloid type . The addition of the bromo group, one of the VIIA (halogen) groups is thought to be able to activate AhR (Aryl hydrocarbon receptors) because the activation of the AhR receptor can be affected by the presence of indole structures (Hubbard *et.al* 2015). AhR (Aryl hydrocarbon receptor) can activate the STAT1 and NFêB receptors. STAT1 functions to promote transcription of iNOS, IL-12 and activation of macrophages (Tugal *et.al* 2013). More receptors that are activated, the ability of macrophage cells to phagocytosis of antigens is high. Latex beads are polystyrene microparticles consisting of water, polymers (polysaccharides / proteins), surfactants and inorganic salts (Sigma-Aldrich) which will be recognized by TNF-a phagocytic receptors and express cytokines (Akilbekova *et.al* 2014).

Phagocytosis begins with an increase in cell membrane tension, opsonized latex beads and the formation of pseudopodia (Tugal *et al.*, 2013). The transmembrane signal will cause actin polymerization on the cytoplasmic surface so that phagosome formation occurs and phagocytosis subsequently occurs (Arum *et.al* 2014). The more activated receptors the ability of macrophage cells to recognize antigens also increases. The addition of Deazaellipticine 1 compound causes more active number of macrophages, so the value of phagocytic capacity increases with increasing compound concentration. The treatment of doxorubicin causes a decrease in phagocytic capacity of macrophages because doxorubicin is im-

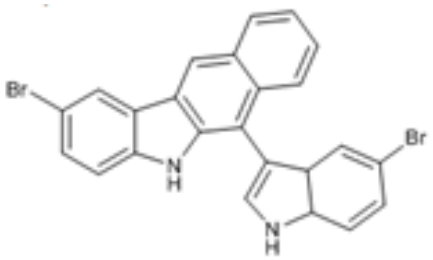

Fig. 2. Chemistry structure of Deazaellipticine 1

Table 2. Test the phagocytosis index of Deazaellipticine 1 compound against *M. musculus* macrophage cells.

Concentration	Value phagocytosis index of Deazaellipticine compound	Value phagocytosis index of Doxorubicin compound
0 µg/ml	1.31 ± 0.306 a	1.31 ± 0.306 b
6.25 µg/ml	1.45 ± 0.240 ab	0.410 ± 0.39 ab
12.5 µg/ml	1.50 ± 0.443 abc	0.916 ± 0.648 ab
25 µg/ml	2.04 ± 0.072abc	0.375 ± 0.104 a
50 µg/ml	2.17 ± 0.376 bc	0.069 ± 0.004 a
100 µg/ml	2.29 ± 0.247c	0.022 ± 0.006 a

munosuppressive and causes cell death (Weir *et al.*, 2013).

Phagocytosis Index of Macrophage Cells

The value of phagocCytosis index from macrophages increased in-line with concentration of of Deazaellipticine 1. The lowest phagocytic index value wass obtained at a concentration of 6.25 µg / ml with a value of 1.31. (Table 2).

One Way ANOVA analysis at the level of confidence ($p>0.05$) showed that Deazaellipticine 1 and Doxorubicin had an effect on phagocytic capacity, then in the Tukey test showed a significant difference in each treatment group, whereas in Doxorubicin there was no significant difference (Table 2). Deazaellipticine 1 was able to increase the index phagocytosis of mice macrophage cells at a concentration of 100 µg / ml, whereas Doxorubicin began to influence at a concentration of 12.5 µg / ml. Deazaellipticine 1 is able to activate cell receptor macrophages to phagocyte latex beads. Fc receptor is one receptor that can recognize antigens in the form of peptides (Zhao *et al.*, 2019).

Deazaeliptisina 1 compound and latex beads as antigens cause IgG antibodies that trigger the activation of Fc receptors so that the number of phagocytic latex beads by macrophage cells will increase. Deazaellipticine 1 is able to act as an immunostimulant by increasing metabolism in macrophage cells, whereas the addition of doxorubicin is not able to trigger phagocytic activity of macrophage cells suspected because doxorubicin causes immunogenic cell death and is immunosuppressive (Weir *et al.*, 2013).

Phagocytosis Ratio of Macrophage Cells

The efficiency of phagocytosis is the ratio between the phagocytic index and percent phagocytosis (phagocytic capacity) (Shanmugam *et al.*, 2015). The results of the phagocytosis test of Deazaellipticine compound on macrophage cells, obtained the index ratio and phagocytosis capacity as follows:

The highest phagocytosis ratio was in the treatment of Diazaleptizin 1 concentration of 6.25 µg / ml (Table 3). This means that the most efficient phagocytosis in phagocytosis of latex beads in the addition of Deazaelipticine 1 compounds concentration of 6.25 µg / ml, while in Doxorubicin at a concentration of 100 µg / ml. The results of the efficiency of phagocytosis can be used as a reference to determine the dose as an immunodulator.

Conclusion

Deazaellipticine 1 compound is able to increase the phagocytic activity of macrophage cells in mice (*M. musculus*). At a concentration of 100 µg / ml it was able to increase the phagocytic capacity by 64.6% and the phagocytic index by 2.29. Doxorubicin at a concentration of 25 µg / ml increased capacity by

Table 3. Phagocytosis test ratio of Deazaellipticine 1 compound to mice macrophage cells (*M. musculus*)

Concentration (µg/ml)	Phagocytosis Capacity (%)		Phagocytosis Index		Index Ratio: Capacity	
	Deazaellipticine 1	Doxorubicin	Deazaellipticine 1	Doxorubicin	Deazaellipticine 1	Doxorubicin
0	8.333	8.3	1.314	0.41	0.157	0.04
6.25	22.444	9.111	1.452	0.91	0.064	0.09
12.5	26	16.642	1.509	0.37	0.058	0.02
25	33.333	17.712	2.046	0.06	0.061	0.003
50	43.111	3.525	2.179	0.02	0.050	0.005
100	64.667	0.94	2.298	0.41	0.035	0.43

17.7% and index 0.06.

References

Akilbekova, D., Philiph, R., Graham, A. and Bratlie, K.M. 2014. Macrophage reprogramming: Influence of latex beads with various functional groups on macrophage phenotype and phagocytic uptake *in vitro*. *Journal Of Biomedical Materials Research A*. 00A:33-35.

Akrom and Fatimah 2015. Ekstrak Heksan Biji Jintan Hitam (*Nigella sativa* L) Meningkatkan Aktivitas Fagositosis Makrofag Tikus Betina Galur SD (Sprague Dawley) Yang Diinduksi DMBA (7,12 Dimetilbenz (α) antrasen) Secara *In vitro*. *Pharmana.* 5(1) : 69-76.

Arum, P., Suromo, L.B. and Niken, P. 2014. Pengaruh pemberian klorofilin berbagai dosis terhadap indeks fagositosis makrofag dan kadar nitric oxide mencit BALB/c yang diinfeksi denga *Salmonella typhimurium*. *Jurnal Gizi Indonesia.* 2(2): 78-82.

Bowles, E.J., Wellman, R. and Feigelson, H.S. 2012. Risk of Heart Failure in Breast Cancer Patients After Anthracycline and Trastuzumab Treatment: A Retrospective Cohort Study. *Journal of National Cancer Institute*. 104 : 1293-305.

Chaabane, W., User, S.D., Gazzah, M.E., Jaksik, R., Sajjadi, E., Rzesowska, J. and Eos. Autophagy, M.J. 2013. Apoptosis, Mitoptosis and Necrosis: Interdependence Between Those Pathways and Effects on Cancer. *Arch. Immunol. Ther.* 61 : 43–58.

Calabro, S.K., Alzhoui, R., Bissinger and Lang, F. 2015. Stimulation of Suicidal Erythrocyte Death by Ellipticine. *Basic & Clinical Pharmacology & Toxicolog.* 116 : 485–492.

Fujiwara, Y., Takeya, M. and Yoshiro, K. 2014. A Novel Strategy for Inducing the Antitumor Effects of Triterpenoid Compounds: Blocking the Protumoral Functions of Tumor-Associated Macrophages via STAT3 Inhibition. *BioMed Research International.* 1: 1-11.

García-García, E. and Rosales, C. 2013. Adding Complexity to Phagocytic Signaling: Phagocytosis-Associated Cell Responses and Phagocytic Efficiency. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience.

Gonzalez, H., Hagerling, C. and Werb, Z. 2018. Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev.* 32(19-20) : 1267-1284. doi:10.1101/gad.314617.118.

Gordon, S. 2016. Phagocytosis: *An Immunobiologic Process.* Cell Press. Volume 44. Pages 463-475.

Herawati, I., Husin, U.A. and Sudigdoadi, S. 2015. Pengaruh Ekstrak Etanol Propolis Terhadap Aktivitas and Kapasitas Fagositosis pada Kultur Makrofag yang Diinfeksi Enteropathogenic *Escherichia coli* (EPEC). *MKB.* 47 (2) : 102-108

Hartini, Y.S., Wahyuono, S., Widyarini, S. and Yuswanto,. 2013. Uji Aktivitas Fagositosis Makrofag Fraksi-fraksi dari Ekstrak Metanol Daun Sirih Merah (Piper crocatum Ruiz & Pav.) Secara *In vitro*. *Jurnal Ilmu Kefarmasian Indonesia*. 11(2): 108-115.Nriagu, J.O. (Ed.). 2011. *Encyclopedia of Environmental Health*. Second Edition. Elsevier, pp. 526-533.

Hubbard, T.D., Murray, I.A., Bisson, W.H., Lahotti, T.S., Gowda, K., Amin, S.G., Patterson, A.D. and Perdew G.H. 2015. *Science Reports.* 5 (12689) : 1-13.

Martin, T.A., Ye, L. and Sanders, A.J. *et al*. Cancer Invasion and Metastasis: Molecular and Cellular Perspective. In: Madame Curie Bioscience Database [Internet]. Austin (TX): *Landes Bioscience.* 2000-2013.

Kumar, S. and Pandey, A. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. The *Scientific World Journal.* 1 : 16.

Liu, B., Ezeogu, L., Zellmer, L., Yu, B., Xu, N. and Joshua, Liao D. 2015. Protecting the normal in order to better kill the cancer. *Cancer Med*. 4(9) :1 394-1403. doi:10.1002/cam4.488

Masters, T.A., Pontens, B., Viasnoff, V., Li, Y. and Nils, G.C. 2013. Plasma membrane tension orchestrates membrane trafficking, cytoskeletal remodeling, and biochemical signaling during phagocytosis. *PNAS.* 110 (29) : 11875–11880.

Mustafiah, S.E., Dina, F. and Iwang, Y. 2011. Indeks Daya Fagosit Makrofag Peritoneum setelah Pemberian Propolis pada Mencit (*Mus musculus*). Universitas Islam Sultan Agung (Unissula) *Semarang*. 3 (2) : 121-128

Nakada, K., Fujisawa, K., Horiuchi, H. and Furusawa, S. 2014. Studies on morphology and cytochemistry in blood cells of ayu *Plecoglossus altivelis* altivelis. *J Vet Med Sci*. 76(5) : 693-704. doi:10.1292/jvms.13-0584.

Saliva, L.F.R.A., Montoiaa, Amorima, R.C.N., Meloa, M.R., Henriquea, M.C., Nunomuraa, S.M., Costad, M.R.F., Andrade, V.F., Costaa, N.D.S., Dantase, G., Lavradof, J., Moreiraf, R., Paulof, A., Pintog, A.C., Tadeia, W.P., Zacardih, R.S., Eberlinh, M.N. and Pohlita, A.M. 2012. Comparative *in vitro* and in vivo antimalarial activity of the indole alkaloids ellipticine, olivacine, cryptolepine and a synthetic cryptolepine analog. *Phytomedicine.* 20: 71– 76.

Shanmugam, L., Ravinder, S.S., Johnson, P., Padmavathi R., Rajagopalan, B. and Kindo, A.J. 2015. Assessment of phagocytic activity of neutrophils in chronic obstructive pulmonary disease. *Lung India*. 32(5) : 437-440. doi:10.4103/0970-2113.164159.

Shin, H.J., Kwon, H.K., Lee, J.H., Gui, X., Check, A., Kim and Sangdun, J.H. 2015. Doxorubicin-induced necrosis i mediated by poly-(ADP-ribose polymerase 1 (PARP1) but i independent of p53. *Science Report.* 5 (15798) : 1-17.

Syafaah, A.N., Maulani, H. and Tri, S. 2013. Angka Kejadian Karsinoma Urothelial di Bagian Patologi.

Anatomi RSUP dr. Mohammad Hoesin Palembang Periode Tahun 2009-2013. *MKS.* 47 (1) : 10-16.

Tacar, O. and Dass, C. 2013. Doxorubicin-induced death in tumour cells and cardiomyocytes: is autophagy the key to improving future clinical outcomes? *Royal Pharmaceutical Society, Journal of Pharmacy and Pharmacology.* 65 : 1577–1589.

Tugal, D., Liao, X. and Mukesh, K.J. 2013. Transcriptional Control of Macrophage Polarization. *Arterioscler Thromb Vasc Bhubard Aliol.* 33 : 1135-1144.

Wang, C., Yu, X., Cao, Q., Wang, Y., Zheng, G., Tan, T.K., Zhao, H., Zhao, Y., Wang, Y. and Harris, D Ch. 2013. Characterization of murine macrophages from bone marrow, spleen and peritoneum. *BMC Immunology.* 14 : 6.

Weir, G.M., Robbert, S. and Marc, M. 2013. Immune Modulation by Chemotheraphy or Immunotheraphy Enhance cancer Vaccines. *Cancers.* 3114-3142.

World Health Organization. 2014. Cancer Country Profile Indonesia.

Zhao, J., Nussinov, R. and Ma, B. 2019. Antigen binding allosterically promotes Fc receptor recognition. *MAbs*. 11(1) : 58-74. doi:10.1080/19420862.2018.1522178.