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The Effect of Garlic Extract on the Average Number of Lecocytes in Mice Infected with *T. Evansi*

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Abstract

Trypanosomiasis is one of the most widespread diseases in the world. This disease is an infectious disease that often occurs in various types of livestock, especially horses, buffalo, and cattle. This disease is caused by infection with the blood parasite Trypanosoma evansi (T. evansi). The aim of this study was to determine the effect of giving garlic extract on the average number of white blood cells of mice infected with T. evansi. The study was conducted on thirty male mice of the DDY strain with a body weight of 25-30 g per head which were divided into six treatment groups. Each treatment used five mice as replicates, namely: the untreated group, as a normal control (K0); the group infected with T. evansi intraperitoneally at a dose of 103/0.3 mL; divided into several groups, namely those who were not given any treatment (K1, negative control), the group that was given trypanocidal (K2, positive control), the group that was given garlic extract at a dose of 50 mg/kgBW (K3); the group given garlic extract at a dose of 100 mg/kgBW (K4) and the group given garlic extract at a dose of 200 mg/kgBW (K5). In this study, it was shown that at the beginning of T. evansi infection it caused an increase in the average number of leukocytes in the group of mice that did not receive treatment (K1) and those given trypanocidal (K2), while the group of mice that received treatment with garlic extract showed a decrease in the number the mean leukocyte count (K3 and K4) or a non-significant increase in the mean leukocyte count (P<0.05) at K5.

***Keywords**: Trypanosomiasis, T. evansi, garlic extract, leukocytes*

Introduction

Trypanosomiasis is one of the most widespread diseases in livestock. Farm animals such as horses, buffalo and cattle are very sensitive to trypanosomiasis. This disease is a disease caused by infection with *Trypanosoma evansi* (*T. evansi*). Indonesia is an endemic area of Trypanosoma, so the incidence of Trypanosomiasis is still often found in various regions. Trypanosomiasis is also known as surra. Surra is a disease that needs attention because it can

cause high mortality in infected livestock. The Surra outbreak on Sumba Island, East Nusa Tenggara Province resulted in the death of 1760 livestock, consisting of 1159 horses, 600 buffaloes and 1 cow. The data on cases and deaths of livestock are accumulated data from mid-2010 to 2012 (Mardiatmi et al., 2012). Other areas in Indonesia where trypanosomiasis is still found include East Nusa Tenggara, Kalimantan, Sulawesi, Banten, Lampung, and Aceh.

Currently the treatment of Surra disease is still using commercial synthetic drugs. The treatment of Surra disease with commercial synthetic drugs is quite expensive, besides it has been found that some substances are no longer effective in killing *T. evansi* (Subekti DT. et. al, 2015). In addition, it is suspected that some trypanosoma isolates have developed resistance to some trypanocidals. Therefore, for effective treatment of Surra's disease, it must be based on a trypanocidal sensitivity test (Melaku and Birasa 2013). The active ingredients commonly used as trypanocidal today are suramin, melarsomine dihydrochloride, diminazene diaceturate, quinapyramine and isomethamidium chloride (Steverding 2010; Melaku and Birasa 2013). Some researchers have found that there are trypanocidals that are no longer suitable for certain species of *T. evansi*. According to Subekti (2015) isolates of *T. evansi* from different regions have different sensitivity to trypanocidal. The problem with the current use of synthetic trypanosidal is that apart from being quite expensive, there are also several reports that prove that some types of synthetic trypanosidal are no longer being used effectively. Alternative substitutes using plant-based drugs (herbs) can be of particular concern. Currently, some herbal extracts do not give good results as anti-*T.evansi*, as reported by Abdelrahman, S.H. (2011). However, Nzelibe et al. (2013) have tested the trypanocidal activity of extracts of *Azadirachta indica* seeds and leaves of Tridax procumbens (TP) have shown success as trypanocidal.

The plant that can be an alternative treatment for trypanosomiasis is garlic because this garlic has been widely studied and the results are known to contain active compounds, including flavonoids and allicin. Allicin not only has an antibacterial effect, but also has anti-parasitic and antiviral effects (Londhe, 2011). Therefore, it is necessary to do research on the effectiveness of this garlic as an alternative to trypanocidal.

Materials and Methods

Trypanosoma evansi isolate

The *T. evansi* isolate used in this study came from Banjarbaru, South Kalimantan Province. This isolate came from the Trypanosomiasis case in Buntok, South Kalimantan in 2019 and was propagated in mice before being used in the study and stored in the form of a stabilizer and stored in liquid nitrogen. Before being used in this study the isolates of *T. evansi* were revived.

Garlic Extract

For the purposes of this study, garlic bulbs were obtained from locations around the city of Makassar. Before being extracted, the good garlic was selected first, then the outer skin was peeled, then extracted using ethanol by the maceration method.

The experimental animals used in this study were *Deutch Democratic Yokohama* (DDY) mice which had a body weight of 25-30 g. Before use, all mice were adapted for one week. During the study all mice were fed and watered ad libitum. After one week of adaptation, all experimental animals were reweighed and randomized according to the treatment group that had been planned and grouped into six treatments, each group consisted of five animals as follows, K0: Normal control, no treatment; K1: Experimental animals were infected with *T. evansi* without treatment (negative control); K2: Experimental animals were infected with *T. evansi* and treated with commercial trypanocidal (positive control); K3: Experimental animals were infected with *T. evansi* and given garlic extract at a dose of 50 mg/kg BW; K4:

Experimental animals were infected with *T. evansi* and given garlic extract at a dose of 100 mg/kg BW; K5: Experimental animals were infected with *T. evansi* and given garlic extract at a dose of 200 mg/kg BW.

Infection of experimental animals, treatment, and observation of the average number of leukocytes

The mice used were male DDY strains and were divided into 6 groups with 5 mice in each group (Table 1). Each mouse in the test group was infected with 10^4 trypanosomes/0.3 ml intraperitoneally (Sones et al. 1998). After infection, all experimental animals were placed according to their group and given food/drink as usual. The treatment was carried out when the infected mice had reached a parasitemia rate of 2+ or equivalent to 10^4-10^6 trypanosomes/mL of blood, on the 3rd day after infection. The treatment in group K2 was using synthetic trypanosidal at a dose of 7 mg/kg.BW (Subekti DT. et al. 2015). Groups K3, K4 and K5 were given garlic extract at a dose of 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW, respectively.

The treatment using garlic extract with several alternative doses was given orally, while for positive control using tryppanosidal which was on the market intraperitoneally. The doses of garlic given were 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW, respectively. Blood collection and observation of the mean white blood cell (WBC) count were carried out at the beginning before infection, and shortly after the last treatment was given (sixth day). Mice blood sampling was done by cutting off the tail of the mice a little.

Results and Discussion

T. evansi when it enters the host's body will penetrate the endothelium of blood vessels and enter the circulatory system. In this blood circulation, the parasite will multiply and increase logarithmically (Noble and Noble, 1982; Jefrey, H.C and Leach, 1983). Trypanosoma (stage trypomastigote) will immediately multiply by binary fission that occurs longitudinally (Desquesnes et al. 2013). Parasites will increase in the blood periodically accompanied by fever. Fever occurs due to massive parasite invasion into the blood or very fast propagation (Ressang, 1984). Flagella and kinetoplasts of trypanosoma divide together (Liu et al. 2005). Trypanosoma will release a toxin called trypanotoxin which will affect the body's metabolism, including causing fever.

Leukocytes or White Blood Cells (WBC) act as the body's defense system and respond to the immune system to provide a strong and fast defense against foreign objects that can cause inflammation and infection in the body. Guyton (1996) stated that leukocytes have two functions, namely destroying infectious agents through the process of phagocytosis or by forming antibodies (immunity) and sensitized lymphocytes. White blood cells are part of the blood that functions in the body's defenses. White blood cells will increase in the blood for a moment when an infection occurs. As well as the current incidence of *T. evansi* infection. Toxins released will cause an increase in the number of white blood cells (Wayan et.al, 1981). Giving garlic will also cause the process of white blood cell formation to increase, in line with the understanding that garlic can function as an immunostimulant (Nwabueze, 2012).

Garlic contains sulfur compounds that act as a starter to increase the immune system (Iciek et. al., 2009). Garlic contains sulfur which can initiate an increase in the immune system (Lau, et al., 1991). An increase in the number of leukocytes is associated with a stimulating effect on immune function and phagocytic capacity (Salman, et al., 1999). Other studies suggest that an increase in the number of leukocytes may result from garlic extract to inhibit neutrophil migration. Administration of garlic extract also causes activation of non-specific defense

mechanisms (granulocytes, monocytes, and lysozyme) and specific defenses (lymphocytes, IgG) (Srivastava and Pathak, 2012). Inhibition of neutrophil migration is likely to increase the number of neutrophils in the circulatory system, thereby increasing the total leukocyte count. Activation of non-specific and specific defenses causes an increase in the total leukocyte count.

Table 1. Division of experimental animal groups

No.	Group	Quantity (head)	Treatment		
			Inoculation of *T. evansi* (10^3/ml)	Trypanocidal (7 mg/kgBW)	Garlic extract
1	K0	5	-	-	-
2	K1	5	v	-	-
3	K2	5	v	v	-
4	K3	5	v	-	100 mg/BW
5	K4	5	v	-	150 mg/BW
6	K5	5	v	-	200 mg/BW

Table 1. is a list of the groups of test mice according to the experimental design. K0 is a normal control group, which is a group without treatment. K1 is the group that became a negative control, namely the group infected with *T. evansi* and not given treatment. K2 is the positive control group, namely the group infected with *T. evansi* and given antiparasitic. Meanwhile, K3 to K5 are groups infected with *T. evansi* and given treatment using garlic extract with their respective doses as written in the table.

In this study, the average number of leukocytes in mice K0, K1, K2, K3, K4 and K5 after being treated for 3 consecutive days can be seen in Table 2. The average number of leukocytes in the untreated group (K0) at the beginning of the study was 9.88±0.74 x10^3/µl and at the end of the study it was 11.43±2.93 x10^3/µl and was still in the range of normal leukocyte counts of 4.5 – 11.3 x 10^3/µl (Ihedioha JI et al, 2012). While the average number of leukocytes in the group of mice infected with *T. evansi* and given trypanocidal (K2) there was a fairly high increase. At the beginning of the study the average number of leukocytes was 9.74±4.68 x 10^3/µl and at the end of the study it was 17.10±5.21 x 10^3/µl and in the group of mice infected with *T. evansi* but not treated (K1) there was also an increase in the average number the mean leukocyte count was also quite high, namely at the beginning of the study it was 13.46±4.61 x 10^3/µl and at the end of the study it was 19.24±6.69 x 10^3/µl. In the group of mice given garlic extract at a dose of 100 mg/kg BW (K3), the average number of leukocytes decreased. At the beginning of the study the value was 10.00±2.46 x 10^3/µl and at the end of the study it was 9.32±3.82 x 10^3/µl. Likewise in the group of mice who were given garlic extract at a dose of 150 mg/kg BW (K4). At the beginning of the study, it was 9.72±5.33 x 10^3/µl and at the end of the study it was 9.15±3.91 x 10^3/µl. Meanwhile in the group of mice infected with *T. evansi* and given garlic extract at a dose of 200 mg/kg BW (K5) there was an increase in the average value of leukocytes, but the value was not too high. At the beginning of the study the value was 10.00±1.69 x 10^3/µl and at the end of the study it was 13.44±7.52 x 10^3/µl.

Table 2. Total White Blood Cell (WBC) of Research Mice (x10^6/µL)

	K0	K1	K2	K3	K4	K5
Before infection *T. evansi*	9.88±0.74	9.74±4.68	13.46±4.61	10.00±2.46	9.72±5.33	10.00±1.69
After 3 days of treatment	11.43±2.93	17.10±5.21	19.24±6.69	9.32±3.82	9.15±3.91	13.44±7.52

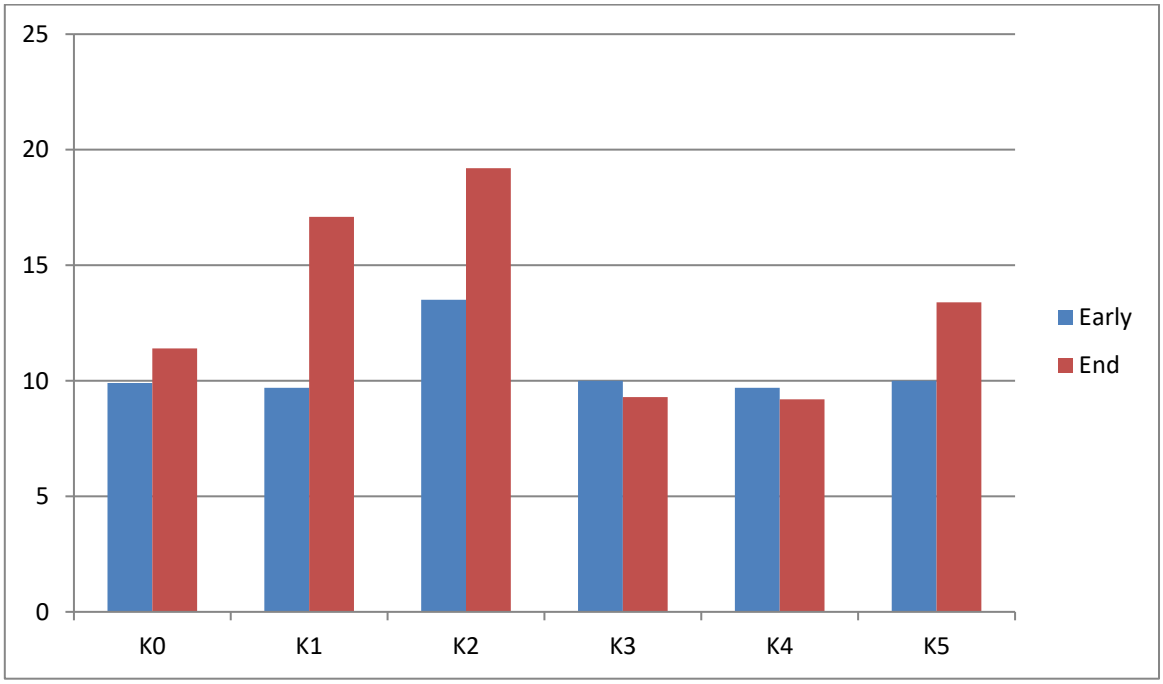

Figure 1. Changes in the mean number of white blood cells (x10^6/µL) before infection with *T. evansi* (Early) and after treatment (End)

Figure 1 shows the appearance of the average number of white blood cells during the observation of the six groups of mice. The average white blood cell count on K0 did not appear to have changed significantly. Meanwhile, in K1 and K2, there was a high increase in the average number of white blood cells. The average number of leukocytes in the K1 and K2 groups of mice showed an increase, so it can be assumed that there was an immune response that appeared to fight the *T. evansi* infection that occurred. Meanwhile, the K3 and K4 groups of mice showed a decrease in the average number of leukocytes and in the K5 group of mice there was a slight increase in the average number of leukocytes. This indicates that there is no body immune response to the *T. evansi* infection that occurs, or there has been a neglected infection because the agent has been successfully inactivated or killed due to the activity of the given garlic extract.

Leukopenia is reported in every event of trypanosomiasis and is associated with decreased myelopoiesis (Sivajothi S, et al. 2015), but this leukopenia is found when the infection has lasted more than 3 weeks (Jenkins, G.C. and Facer, C.A, 1985). In this study, mice infected with *T. evansi* experienced an increase in the average number of leukocytes, this indicates that the body's normal reaction occurs to form leukocytes when infection occurs. If the infection goes

on for some time, there may be a decrease in the average number of blood leukocytes. This can occur with the assumption that the agent successfully suppresses the process of leukocyte formation.

In the treatment group of mice that were given garlic extract at a dose of 100 mg/kg BW (K3) and a dose of 150 mg/kg BW (K4) there was a decrease in the average number of leukocytes. Meanwhile, with a dose of 200 mg/kg BW, the increase was not too high. This indicates that garlic at the beginning of administration does not stimulate the formation of leukocytes or that garlic is able to suppress the growth of *T. evansi*, so that the body has no interest in forming the body's defense cells. This is related to garlic's ability to inhibit the growth of a wide range of microbes, including viruses, bacteria, protozoa, and fungi (Nok et al., 1996; Zhang, 1999; Pizorno and Murray, 2000; Yin et al., 2002; Hernawan). UE and Setyawan AD, 2003). Oxidized garlic will form allicin and then allicin is a precursor for the formation of allyl sulfide, including diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl sulfide (DAS), metallyl sulfide, dipropyl sulfide, dipropyl disulfide, allyl mercaptan, and allyl methyl sulfide (Gupta and Porter, 2001). Compounds that can inhibit the growth of Trypanosoma are DADS (Nok et al., 1996).

Several studies have shown that garlic extract is effective against several protozoa including *Opalina ranarum, Balantidium entozoon*, *Entamoeba histolytica*, *Trypanosoma* sp., *Leishmania* sp., *Leptomonas* sp., and *Crithidia* sp. (Reuter et al., 1966). It is suspected that allicin, ajoene, and organosulfide which are derived compounds from garlic are the effective antiprotozoal compounds (Bayan, L. et al. 2013).

Cellular immunity is the body's main way of fighting *T. evansi* infection in mice, not a humoral immune response. So that data from the average number of leukocytes alone cannot provide specific information about the immune status of the animal, so it is necessary to calculate the number of each type of leukocyte cell (Aboderin, FI and V.O Oyetayo, 2006).

Conclusion

In this study, it was shown that infection with *Trypanosoma evansi* in mice caused an increase in the average number of leukocytes at the beginning of infection. The administration of garlic extract (*Allium sativum* L) at the beginning of the infection caused neither the formation nor an increase in the average number of white blood cells in the mice infected with *T. evansi*.

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