



## Kidney Histopathology of Mice (*Mus musculus*) Infected with *Trypanosoma evansi* and Distributed Garlic Extract (*Allium sativum*)

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### Abstract

*T. evansi* is a blood parasite that is responsible for the occurrence of surra disease or also known as trypanosomiasis. The *T. evansi* cells are able to be damaged by the *Allicin* content of garlic. In the kidney itself, the parts that affect in the case of infection are namely tubules and glomerulus and can be identified through histopathology inspection by taking into account the level of damage. This research is aimed at studying the figure of mice kidney histopathology (*M. musculus*) that has been being infected by *T. evansi* and being distributed by garlic extract (*A. sativum*) with graded dose then compared to the distribution of commercial drug Tryponil. The samples used in this study were 30 mice with 6 treatment groups. P0 group was not infected by *T. evansi*, P1 group was infected by *T. evansi* without treatment, P2, P3, and P4 group respectively were infected by *T. evansi* and distributed garlic extract with 1,4 mg, 2,8 mg, and 5,6 doses respectively. In other hand, P5 group was distributed commercial drug namely Tryponil. The extraction method was through maserasi method. The distribution of the treatment was done in 3 days, euthanized and necropsied on mice in the purpose of organ harvesting for histology sampling with embedding method, blocking, and hematoxylin eosin coloring. The results showed that the closest to the distribution of commercial drug was the distribution of 5,6 mg dose (high dose), where both the treatments showed the result that the damage was not much, then followed by the distribution of 2,8 mg dose (fair dose) and 1,4 mg dose (light dose).

**Keywords:** Garlic, Histopathology, Kidney, *T. evansi*

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### Introduction

*T. evansi* is now included to one of blood parasites that spreads across the world with high risk infection to animals and human so that it is considered as zoonosis disease and currently has no method to control it effectively (Wardhana & Sawitri, 2018). Livestock in Indonesia has not released yet from surra disease that is caused by *T. evansi*. The potential of surra

infection and other *Trypanosoma spesies* are adequately high in Indonesia (Novita, 2019). Currently, the treatment for *T. evansi* infection still uses commercial medication. The cost to spend for this kind of treatment is still classified expensive, thus, it is necessary to find alternative medication such as herbal medication (Wahyuwardani et al. 2018)

Garlic with scientific name *A. sativum* is spread across the world and used as medicinal plants for medication and prevention to certain disease. Garlic is also been reliant to alleviate pain by increasing antioxidant in kidney (Hashemi *et al.*, 2019). The most important chemical composition in Garlic (*A. sativum*) is in its organosulphur compound like *Allicin* (Mikaili *et al.*, 2013). It is great possibility that vital substance in parasite cell like Trypanothione reductase is resisted through disulfide bond forming by garlic (*A. sativum*) so that it is assumed to be effective as anti-parasite agent (Krstin *et al.*, 2018).

Mice are one of test animals that are sensitive to detect surra disease caused by *T. evansi* (Fahrimal *et al.*, 2013). Rodents like mice are currently becoming animals that are dominantly used as laboratory animals. Mice have good characteristics to be used in laboratory regulation and genetically very close to human (Hau & Steven, 2011).

Kidney functions in the process of blood filtration (Dyce *et al.*, 2010). One of the methods to find out physiological aspect and biochemistry of kidney function in test animal is by using investigation towards kidney that has been separated from the body. The advantage of this method is the possibility of making controllably variable modification, omitting systemic effects but preserving anatomy, function, and biochemistry (Rigali & Veronica, 2009). Based on research done by Wahyuwardani *et al* 2018, mice kidney that has been infected by *T. evansi* experiences histopathology alteration namely the existence of wound like edema, bleeding, and infiltration of inflammatory cell.

Based on the above situation, it is recognized that *T. evansi* can attack kidney and generate some histopathology wound. It is considered important to conduct a research to study the effect of Garlic (*A. sativum*) extract distribution towards kidney histopathology figure with mice test animal (*M. musculus*) as the medication attempt to *T. evansi* infection.

## **Materials and Methods**

This research was conducted on February to March 2021 in Ruang Laboratorium Terpadu Klinik Hewan Pendidikan of Hasanuddin University for extraction and Balai Veteriner Banjarbaru for sample processing. The type of this research is laboratory experiment research that was conducted to observe kidney histopathology figure of mice (*M. musculus*) that has been infected by *T. evansi* and distributed garlic extract (*A. sativum*). Besides, this research is categorized as descriptive research because it gives description about kidney histopathology figure of mice that is observed. The populations of this research are male mice in interval age of 2 – 3 months and in interval weight of 20-40 grams. The treatment distributed is distributing garlic extract (*A. sativum*) and *Tryponil*. The samples of this research are about 30 mice with six treatment groups that are divided into treatment 0 with no *T. evansi* infection, treatment 1 with *T. evansi* infection and no medication, treatment 2 with *T. evansi* infection and garlic extract distribution at 1,4 mg dose, treatment 3 with *T. evansi* infection and garlic extract distribution at 2,8 mg dose, treatment 4 with *T. evansi* infection and garlic extract distribution at 5,6 mg dose, treatment 5 with *T. evansi* infection and garlic extract distribution at 7 mg/kg dose.

The tools used in this study were lab coats, mice cages, feed and drink containers, minor surgical instruments (*Scalpel, blade, stainless steel* tray, anatomical tweezers, scissors), scales,

camera, funnel, measuring cup, stirrer, large container, blender, rotary evaporator, stationery, light microscope, micropipette, spatula, tissue processor, paraffin bath, microtome, paraffin section, flotation bath, incubator and hotplate.

The materials used in this study were garlic obtained from traditional markets in Makassar City, South Sulawesi, 96% ethanol, 10% formalin, male mice weighing 20-40 grams, mouse feed, masks, latex gloves, PCR tube, cover glass, object glass, nucleic tube, 1 ml syringe, picric acid, phosphate-buffer saline glucose (PBSG) solution, tissue, organ tube, gauze, micropipette tip, aluminum foil, cassette tissue, xylol, alcohol, paraffin, aquades, a solution of hematoxylin and eosin.

The research method began with the preparation stage, namely providing mice obtained from mouse breeders in South Sulawesi and the preparation of isolates obtained from the blood of cows with *T. evansi* infection in the South Kalimantan area with a buntok isolate code made on March 13, 2020 which has gone through the inoculation process. . The implementation phase began with the making of garlic extract using the maceration method as was done in the research by Putranti *et al.*, (2019). *T. evansi* infection in mice was performed intraperitoneally with a dose of 0.3 ml. checking the growth of parasites in the blood of mice was carried out the day after infection. Checking was done by taking 1 mill micron of blood from the mice and making blood smear preparations. Garlic extract and *Trypanil* were distributed after the parasitemia level of *T. evansi* reached  $10^4$  which was at 3<sup>rd</sup> day. The distribution of the extract was done orally according to the dose and the distribution of *Trypanil* was done through intraperitoneal. Euthanasia was done to the mice from all over the treatments by fracturing the neck bone of the mice and after necropsy, the organ was stored in 10% formalin solution. The making of kidney histopathology sample was begun with trimming process to coloration with *Hematoxylin eosin*. The microscopic observation was done under microscope with 40 times and 100 times magnification and the alteration of histopathology was observed.

## Results and Discussion

### The Expansion of *T. evansi* in Blood

The target of medication in this research is stock which is usually infected by *T. evansi*, but in this case, mice were used because they were considered to be sensitive enough to detect surra disease caused by *T. evansi* (Fahrimal *et al.*, 2012). Isolates of *T. evansi* used in this research were from Balinese cow in South Kalimantan that were confirmed infected by *T. evansi*. These isolates were made using inoculation method. Before used, *thawing* process was conducted and PBSG solution was added as the inspection toward live mortality of *T. evansi* parasite under electron microscope was conducted as well. Isolates of *T. evansi* was injected to mice intraperitoneally. *T. evansi* could go through mucosal tissue on inner organ then undergo vena to the heart. The heart would then pump blood that has been contaminated by *T. evansi* and distributed to the entire body including kidney (Yahya *et al.*, 2017)



Figure 1. Isolate of *T. evansi* which has been dissolved in PBSG solution. Note: *T. evansi* (Black Arrow, 10x10)

After the infection process in the mice was completed, the mice's blood was checked every day by the blood smear method. The process of giving the extract and *Tryponil* was conducted after the amount of parasitemia of *T. evansi* reached a value of  $10^4$ , namely on day 3 post infection, where this value of  $10^4$  was able to provide changes to the organs, so that the effectiveness of the extract and *Tryponil* could be clearly observed. This is in line with research from Subekti *et al.*, (2013), which stated that the LD<sub>50</sub> of *T. evansi* was  $10^4$ /rat. LD<sub>50</sub> is the cumulative mortality of 50% of mice tested.



Figure 2. *T. evansi* in Checking of Blood Test Results. Note: *T. evansi* (Black Arrow, 40x10).

Blood was taken from the mice on the tail, then dripped onto an *object glass* and examined under a microscope. The image above shows the presence of *T. evansi* around erythrocytes or red blood cells. The morphology of *T. evansi* on the blood smear shows the presence of a nucleus in the middle and is complemented by the presence of a small posterior *kinetoplasm* (Kumar *et al.*, 2012).

Table 1. The calculation of the expansion of *T. evansi* before and after treatment

Treatment	Before Treatment	After Treatment
P0	-	-
P1	$9 \times 10^4 \times 10 = 9 \times 10^5$	$16 \times 10^4 \times 10 = 16 \times 10^5 = 1,6 \times 10^6$
P2	$54 \times 10^4 \times 10 = 54 \times 10^5 = 5,4 \times 10^6$	$33 \times 10^4 \times 10 = 33 \times 10^5 = 3,3 \times 10^6$
P3	$23 \times 10^4 \times 10 = 23 \times 10^5 = 2,3 \times 10^6$	$16 \times 10^4 \times 10 = 16 \times 10^5 = 1,6 \times 10^6$
P4	$21 \times 10^4 \times 10 = 21 \times 10^5 = 2,1 \times 10^6$	$4 \times 10^4 \times 10 = 4 \times 10^5$
P5	$50 \times 10^4 \times 10 = 50 \times 10^5 = 5 \times 10^6$	-

\*Parasitemia values of *T. evansi* in blood before and after treatment. (Source: Parasite Lab of Balai Veteriner Banjarbaru)

The parasitemia value of *T. evansi* in peripheral blood increased sharply within 2-4 days and was then usually followed by death (Subekti *et al.* 2014). Based on table 6 above, it could be seen that there was a decrease in the level of parasitemia of *T. evansi* after the inspection for 3 days. Seen in treatment 1, the value of parasitemia increased because the mice used were infected with *T. evansi* but were not given any treatment. In contrast to treatments 1,2,3,4 and 5 which were treated with garlic extract and the commercial drug *Tryponil* showed a decrease in the number of parasitemia. The most significant changes occurred in the 4th and 5th treatments where the parasitemia value decreased from before being given treatment because the *Alllicin* content in garlic was able to damage cells and decrease the expansion of *T. evansi* in blood (Zainal-Abidin and Mohd, 2011).

### The Result of Microscopic Observation of Mice Kidney

Based on the research conducted to 30 mice with 5 treatment groups, the samples were taken namely 5 kidneys of each treatment and necropsy then was conducted and observed microscopically with the results as follow:

Table 2. The Result of Microscopic Observation of Mice Kidney

Treatment Groups	Microscopic Appearance
P0	No alteration
P1	The size grew up, the color turned dark
P2	The color turned pale, the size grew up
P3	The kidney grew up, hemorrhage
P4	The color turned pale, hemorrhage in some parts
P5	Hemorrhage, the size was consistent

Microscopic Observation of Mice Kidney (Source: Parasite Laboratory of Banjarbaru Veterinary Center)

The result of microscopic observation of mice kidney showed that there was an alteration to something better about size and color of kidneys in each treatment given except on normal control. The alteration occurred was the size grew up and the color turned dark on P1. On garlic extract distribution with graded dose, the alteration was nearly the same namely on P2 where the color turned pale and the size grew up, on P3 where the kidney grew up and hemorrhage, on P4 where the color turned pale and hemorrhage. While on P5, hemorrhage occurred but the size of the kidney was consistent. This was in line with the research done by Yahya *et al.*, (2017) that *Trypanosoma* infection can cause degenerative alteration on kidney and as well, the distribution of foreign substance into the body like plant extract can give toxic effect on tissue.

### Kidney Histopathology Observation

#### P0 Group

Based on histopathology observation of mice kidney which was not infected by *T. evansi* and without treatment, it was found that there was no alteration with histopathology figure as follow:

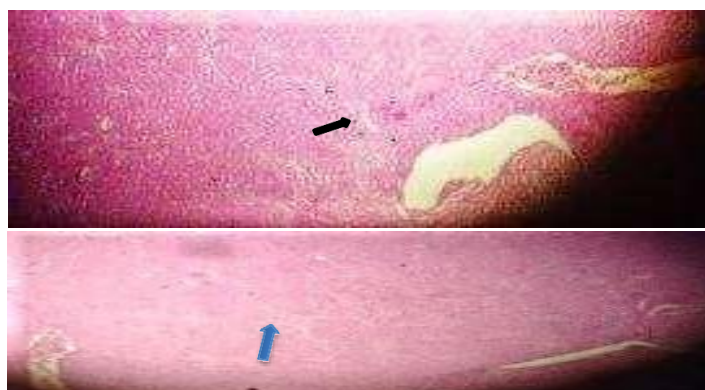


Figure 3. Histopathology of mice kidney that was not infected by *T. evansi* and without treatment (HE, 10x10).

In the figure above, the tubules (black arrows) were still tight and there was no enlargement (*hyperplasia*) of the interstitial cells (blue arrows). This means that there was no significant microscopic alteration so that it could be said that there was no damage to the microscopic appearance of the mice kidney in the P0 group, according to the results of macroscopic observations that there was no alteration in the kidney in this treatment group. Kidneys



are said to be normal if there is no visible necrosis and cell infiltration (Sugihartini & M. Alif, 2016).

### P1 Group

Based on histopathology observation of mice kidney which was infected by *T. evansi* and without treatment, it was found that there were alterations in its glomerulus with histopathology figure as follow:

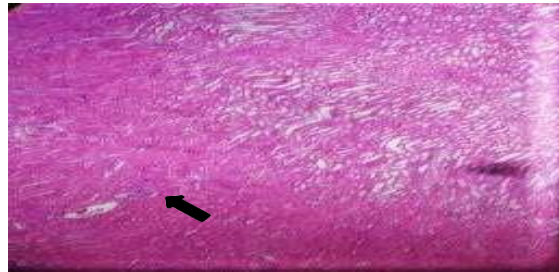


Figure 4. Histopathology of mice kidney that was being infected by *T. evansi* and without treatment (HE, 10x10).

In the figure above, it was seen that a microscopic alteration occurred in glomerulus anatomy namely glomerulus enlargement marked by the glomerulus that almost covered kapsula bowman indicating that there was an inflammatory reaction in kidney as the result if infiltration of lymphocytes cell and macrophage around blood vessel of the glomerulus that explained that *T. evansi* was pathogen on mice (Wahyuwardani *et al.*, 2018). This was also in line with the microscopic appearance where the kidney experienced growing and the color turned pale. The alterations on kidney was primarily caused by toxic produced by parasite and immune complex accumulation that damaged the structure and function of kidney (Bal *et al.*, 2012).

### P2 Group

Based on histopathology observation of mice kidney which was infected by *T. evansi* and distributed garlic extract at 1,4 mg dose, it was found that there were alterations in its glomerulus and interstitial cell with histopathology figure as follow:

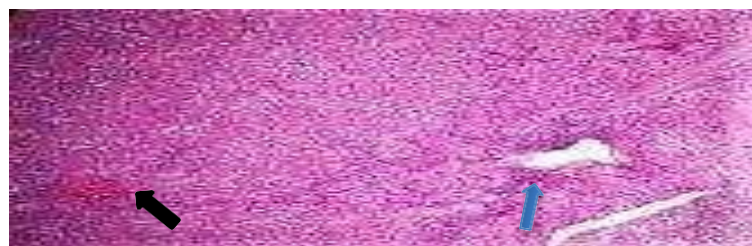


Figure 5. Histopathology of mice kidney that was infected by *T. evansi* and distributed garlic extract at 1,4 mg dose (HE, 10x10).

In the figure above, it could be seen that on the microscopic appearance, there was hemorrhage in the glomerulus in the renal cortex in 1 spot (black arrow) and there was filtration of inflammatory cells (blue arrow), which could indicate that there was inflammation of the kidney due to infection by *T. evansi* (Wahyuwardani *et al.*, 2018). Distributing garlic extract in treatment had an effect on decreasing the expansion of *T. evansi* according to research by Zainal-Abidin and Mohd (2011) that *Allicin* or *Diallyl thiosulfinate* (C3 H5 SS (O) C3 H5) in garlic can cause cell damage in hemoflagellate cells such as *T. evansi*, so the damage caused was not too heavy compared to the treatment that was not given the treatment.

### P3 Group

Based on histopathology observation of mice kidney which was infected by *T. evansi* and distributed garlic extract at 2,8 mg dose, it was found that there were alterations in its tubules with histopathology figure as follow:



Figure 6. Histopathology of mice kidney that was infected by *T. evansi* and being distributed garlic extract at 2,8 mg dose (HE, 10x10).

In the figure above, it could be seen that on the microscopic appearance, there were hemorrhages at several points of the kidney tubules (blue arrows), this indicated that an inflammatory reaction occurred in the kidney due to infection of *T. evansi* (Wahyuwardani *et al.*, 2018). Alterations in the renal tubules could be caused by the renal tubular epithelial cells having high metabolic activity (Osborne, 1972). The distribution of garlic extract in this treatment group showed that the damage formed was decreasing and only at 1 point. The *allicin* content in garlic caused a highly oxidative intracellular environment in *T. evansi* which caused oxidative damage and cell death (Zainal-Abidin & Mohd, 2011).

### P4 Group

Based on histopathology observation of mice kidney which was infected by *T. evansi* and distributed garlic extract at 2,8 mg dose, it was found that there were alterations in its tubules with histopathology figure as follow:

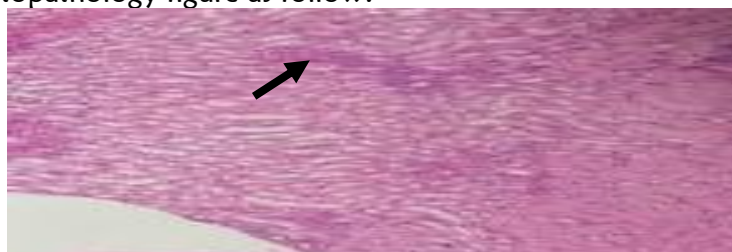


Figure 7. Histopathology of mice kidney that was infected by *T. evansi* and distributed garlic extract at 5,6 mg dose (HE, 10x10).

The figure above shows the presence of several inflammatory cell infiltrations in the tubules (black arrows). Inflammatory cell infiltrations that appeared were caused by a toxin produced by *T. evansi* that triggered an inflammatory reaction in the tubules (Ghaffar *et al.*, 2017). The use of garlic extract in this treatment was considered to have a fairly good repair effect, where the damage formed was lighter than the other treatments. The dose in this treatment was the highest dose distributed, meaning that the *Allicin* content in garlic was able to decrease the expansion of *T. evansi* (Zainal-Abidin and Mohd, 2011).

### P5 Group

Based on histopathology observation of mice kidney which was infected by *T. evansi* and distributed commercial drug, it was not found that there was no significant alteration with histopathology figure as follow:

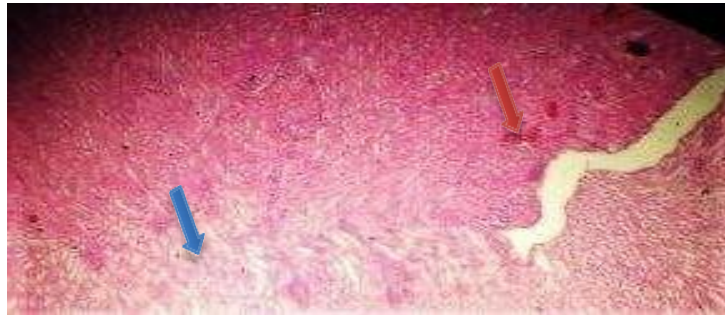


Figure 8. Histopathology of mice kidney that was infected by *T. evansi* and distributed commercial drug (HE, 10x10).

In the figure above, there was no significant change. There was no change in the tubules (blue arrows), but there were hemorrhagic spots on the interstitial cells (red arrows). The alterations on interstitial cell occurred as the result of an inflammatory reaction in the kidney infected by *T. evansi*. There were no significant alterations found in other parts of the kidney after the distribution of commercial drug as the research of Wahyuwardani *et al.*, (2018) where in his study, there were no histopathology alterations in the mice kidney that had been being infected by *T. evansi* and were then being distributed commercial drug.

### Level of Kidney Damage

Table 3. Level of kidney damage after treatment

Treatment Groups	Level of Damage	Description
P0	Normal	The tubules were tight, no enlargement on the glomerulus -
P1	Heavy	Enlargement on glomerulus +++
P2	Fair	Hemorrhage on glomerulus +++, there were inflammatory cell infiltrations ++
P3	Fair	Hemorrhage on renal tubules ++
P4	Light	infiltrations on inflammatory cell on tubules ++
P5	Light	The tubules were consistent -, hemorrhage on interstitial cell +

#### Notes:

- = No alteration found on histopathology observation
- + = the alteration was light and only in one spot
- ++ = Light alteration found  $\geq 25-30\%$  heavy but in one spot
- +++ = Heavy alterations found multivocal or  $\geq 30-50\%$

\*Level of Damage of Mice Kidney Histopathology (Source: Wahyuwardani *et al.*, 2018).

The qualitative data on the level of kidney damage showed that in the treatment group 1, the level of kidney damage was 30-50%. In the treatment group 2, the alteration was fair namely bleeding in the glomerulus and infiltration of inflammatory cell. In treatment group 3, fair damage was 25-30% in hemorrhagic renal tubules. In the treatment group 4, the level of damage to the tubules was 25-30%. In treatment group 5, the level of damage to interstitial cells and tubules did not change. The highest level of damage was in the



treatment group 1 where it was without treatment and the damage formed in the glomerulus was caused by toxin produced by parasites and the accumulation of immune complex that damaged kidney structure and function (Bal *et al.*, 2012). While the lowest level of damage was in the treatment group 4 and 5 where it was the use of high-dose garlic extract which gave the same effect as the administration of commercial drug for *Trypanosoma* infection because the distribution of garlic extract caused damage to hemoflagellate cells such as *T. evansi* (Zainal-Abidin & Mohd, 2011) as well as the distribution of commercial drugs proved to be able to prevent the expansion of *T. evansi* (Wahyuwardani *et al.*, 2018).

### Conclusion and Recommendation

According to the result of the research conducted, it is concluded that garlic extract is effective to decrease the damage on kidney caused by *T. evansi* infection by distributing 5,6 mg dose (high dose) where this dose has the closest result to the distribution of commercial drug.

The recommendation for the future research will be a conduction of long term medication to obtain a maximum result. It is also necessary to conduct a research about the availability of garlic extract in gel.

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