

## Conference Paper

# The Effect of Papaya (*Carica papaya* L.) Leaf Extract to the Number of *Trypanosoma evansi* Steel in Liver and Kidney of Mice (*Mus musculus* L. 1758)

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## Abstract

The purpose of this study proved the activity of the ethanolic extract of papaya leaf as anti-trypanosomiasis agent, determined the dose of extract which having an effect in inhibiting growth of *T. evansi*, and evaluated the histopathology of liver and kidney infected by *Trypanosoma evansi* Steel. Male Balb-c mice were infected by  $3 \times 10^7$  *T. evansi* isolates. The native method was used to determine the level of parasitemia. The preparation was made using cytological smear method and paraffin method. The results showed that ethanolic extract of papaya leaf decreased the number of *T. evansi*. Ethanolic extract of papaya leaf at a dose of  $300 \text{ mg} \cdot \text{kg}^{-1}$  BW is the most influential dose to reduce the number of *T. evansi* in liver and kidney of mice. Liver reveals degenerative change varying such as congestion, lipid degeneration, vacuolar degeneration, necrosis, and cellular infiltration. Kidney reveals tubular degeneration, necrosis, cellular infiltration, and glomerulonephritis.

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**Keywords:** *Carica papaya* L.; kidney; liver; mice; *Trypanosoma evansi* Steel.

## 1. Introduction

Surra (trypanosomiasis) is a disease caused by infection with the haemo-protozoan belonging to the genus *Trypanosoma*. *Trypanosoma* is a parasite on the circulatory system, tissue fluid, and it can infect cells. Surra is endemic in Southeast Asia, and it is prevalent in Indonesia. *Trypanosoma evansi* Steel has a wide host range such as goats, sheep, pigs, dogs, cats and other wild animals. The disease is the most important single cause of economic losses such as slack up livestock growth, decreased livestock production, morbidity, and mortality [1]. In addition, Mackenzie et al. [2] mentions that *T. evansi* infection can cause immunosuppression or reduced immune response of the host or also called a clinical allergy.

Surra has a negative impact on the population of buffalo in the Philippines. Growth of buffalo population in endemic areas lower 50% compared with areas that are not. Economic losses due to this disease is estimated at up to 158 000 USD per year [3]. In Indonesia, surra was reported by Direktorat Kesehatan Hewan [4] occurred on Sumba,

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East Nusa Tenggara in 2010 to 2011. The result showed that 4 268 cattle was expressed trypanosomiasis while livestock deaths caused by this disease are reported as many as 1 760 cattle [4].

Control of *T. evansi* has been done by giving the antitrypanosomal (Suramin), but it is reported to have occurred Trypanosoma resistance. Research in Balai Penelitian Veteriner showed almost all isolates of *T. evansi* in Balivet resistant to isometamidium and most of the isolates were resistant to diminazene aceturate [5]. Therefore, control of the disease depends on the ability of the new drug which have a role as new anti-*T. evansi* to anticipate Trypanosoma resistance to all drugs.

*Carica papaya* (L.), commonly known as papaya, is a fruit crop cultivated in tropical and subtropical regions, and well known for its nutritional benefits and medicinal applications. Jimenez-Coello et al. [6] have used papaya seed extract as antitrypanosomal agent [6]. Papaya leaf was utilized as antitrypanosomal still not have been done. Therefore, it is necessary to evaluate the effects of papaya leaf extract to the number of *T. evansi* in the liver and kidneys of mice infected by *T. evansi* and determine the histopathological effect.

## 2. Materials and Methods

### 2.1. Processing of plant samples

The leaves were washed, cut into small piece, and dried for five days. The samples were ground into powder and stored before use for analysis. Papaya leaves extracted by maceration method using ethanol 96%. Extraction was done for  $3 \times 24$  h. The solvent was evaporated using a rotary vacuum evaporator at temperatures up to 40°C until the solvent does not evaporate again [7].

### 2.2. Phytochemicals analysis

The analysis for alkaloids, flavonoids, saponins, tannins, and steroids was carried out according to standard methods [7].

### 2.3. Study design

This study is an experimental research using completely randomized design (CRD) with five treatment variables and five replications for each treatment. All mice were then divided randomly into five groups, each consisting of five mice with different treatment for each group, namely:

- Group Po (negative control) untreated infection *T. evansi* and ethanol extract of papaya.
- Group P1 infected by *T. evansi*, but not given the ethanol extract of papaya.
- Group P2 infected by *T. evansi* and ethanol extract of papaya  $75 \text{ mg} \cdot \text{kg}^{-1}$ .

- Group P<sub>3</sub> infected by *T. evansi* and ethanol extract of papaya 150 mg · kg<sup>-1</sup>.
- Group P<sub>4</sub> infected by *T. evansi* and ethanol extract of papaya 300 mg · kg<sup>-1</sup>.

## 2.4. Trypanosoma evansi isolate

*T. evansi* isolate used in the present study was obtained from Balai Veteriner Banjarbaru. Twenty-five Balb-C mice were infected with *T. evansi* by injecting with 0.2 mL,  $3 \times 10^7$  parasites by intraperitoneal route. Tail clipping collected the blood of infected mice and daily examined beginning from the second-day post inoculation. Infected mice blood showing a high parasitaemia was collected directly from the heart. Determination of categories parasitaemia observed under a microscope with a magnification of 400x is classifying as absent (-), slight (+) (1 to 5 Trypanosomes/field), moderate (++) (6 to 15 Trypanosomes/field), severe (+++) (15 to 25 Trypanosomes/field), and very severe (++++) (> 25 Trypanosomes/field).

## 2.5. Animal treatment

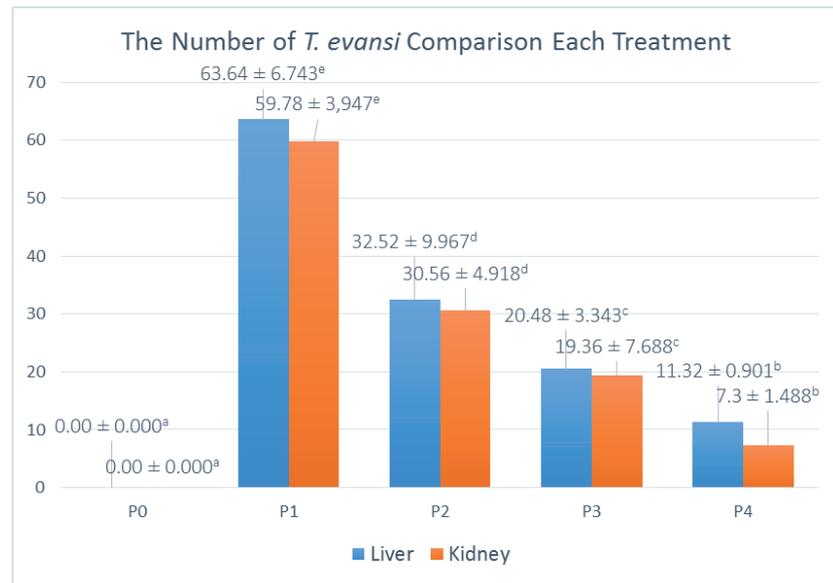
Extract administered orally as much as 0.3 mL. The treatment is given to mice that have been categorized as having very severe parasitaemia by category (+4) in the previous inspection using native methods. The treatment was done for three days. On 4 d after treatment, all of the mice were sacrificed to take the liver and kidneys.

## 2.6. Cytological preparation

Cytological preparations of liver and kidney were conducted using a smear method with Giemsa staining [8]. Observation was done with a magnification of 1000x. The average number of 10 field of view averaged to obtained the average number of *T. evansi* each mouse. The average number of *T. evansi* each mouse combined and averaged to obtained the average number of *T. evansi* each treatment group. The data obtained were processed with SPSS 20.0 for Windows and data distribution curve seen with the test One Sample Kolmogorov-Smirnov and Levene homogeneity test. If the observed data meet the requirements of parametric analysis, the data were analyzed using ANOVA (Analysis of Variance) and continued with Duncan test. If the observed data does not meet the requirements of parametric analysis, the data were analyzed using Kruskal-Wallis test followed by Mann-Whitney test. Significance level used was  $\alpha = 0.05$ .

## 2.7. Histological preparation

Small pieces of tissue samples were collected in 10% formalin. Histological preparations of liver and kidney were conducted using a paraffin method [9]. Thin sections of 5  $\mu\text{m}$  thickness were cut and stained with routine haematoxylin and eosin (HE) staining for recording the histopathological change. Qualitative observations were conducted



**Figure 1:** Diagram of the number of *T. evansi* comparison each treatment.

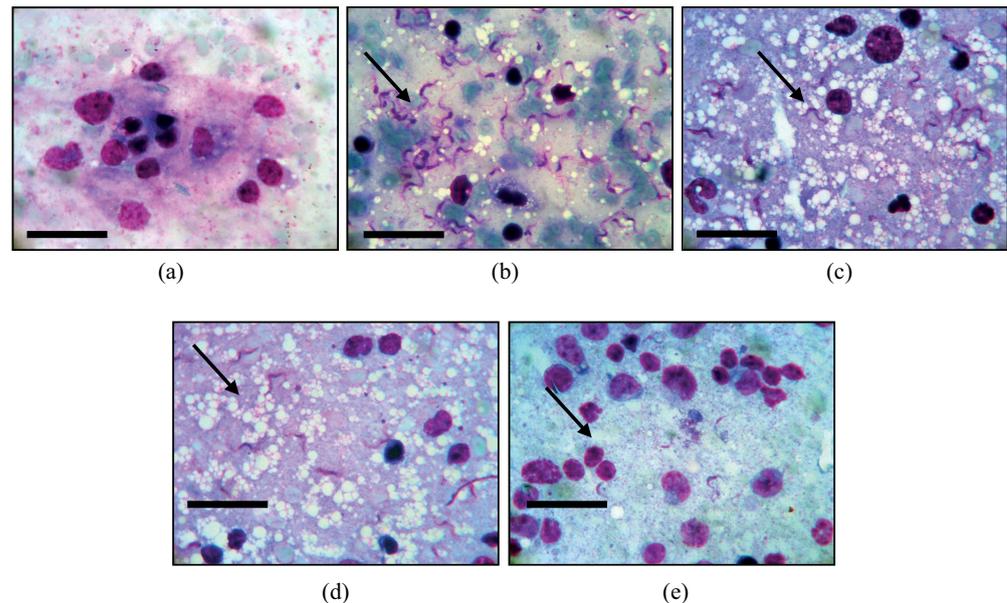
to the liver, including congestion, necrosis, fatty degeneration, and vacuolar degeneration. While the kidney was observed for tubular degeneration, necrosis, cellular infiltration, and glomerulonephritis [10].

### 3. Result and Discussion

The mean, standard deviation and the significance of the number of *T. evansi* in the liver and kidneys of mice after treated with ethanol extract of papaya leaf on each treatment are presented in diagram form in Figure 1. The ethanol extract of papaya leaves decreased the number of *T. evansi* both the liver and kidneys. Number of *T. evansi* in the P1 group is the largest number as compared to other treatment groups while the P4 group had the least number of *T. evansi* compared with the other treatment groups (P1, P2, and P3) (see Figure 1).

*Trypanosoma evansi* infected mice intraperitoneally into the body and found in the blood. It is due to *T. evansi* able to penetrate the mucosal tissue in the internal organs and through a vein to the heart [11]. The heart pumps the blood which is infected by *T. evansi* to all organs of the body. It is evidenced by the existence of *T. evansi* in the liver (see Figure 2) and kidneys (see Figure 3) of mice. The growth of *T. evansi* increased after it infected to the mice (*Mus musculus* L.). However, the number of *T. evansi* decreased along with increasing doses of papaya leaf extract.

Analysis of the number of *T. evansi* in the liver of mice with Kruskal-Wallis and Mann-Whitney prove the effects of ethanol extract of papaya leaf against the number of *T. evansi* in the liver of mice. Results of the analysis of the number of *T. evansi* in the kidneys of mice with ANOVA and Duncan also prove the effect of ethanol extract of

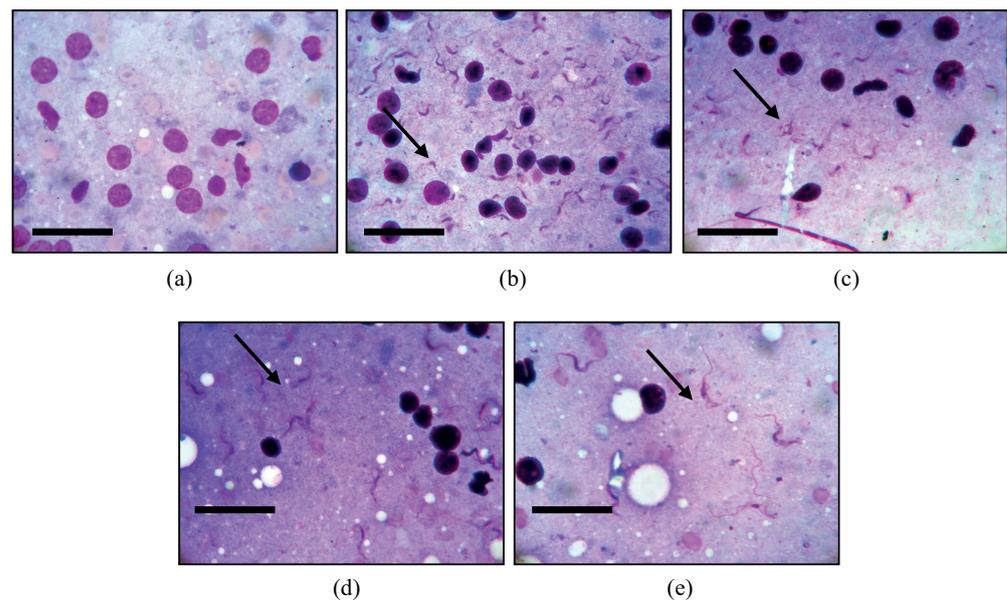


**Figure 2:** (a) Hepatic smear of negative control; (b) Hepatic smear of mice was infected by *T. evansi*; (c) Hepatic smear of mice was infected by *T. evansi* and ethanol extract of papaya  $75 \text{ mg} \cdot \text{kg}^{-1}$ ; (d) Hepatic smear of mice was infected by *T. evansi* and ethanol extract of papaya  $150 \text{ mg} \cdot \text{kg}^{-1}$ . (e) Hepatic smear of mice was infected by *T. evansi* and ethanol extract of papaya  $300 \text{ mg} \cdot \text{kg}^{-1}$ . (→) *T. evansi*; scale  $30 \mu\text{m}$ ; Giemsa staining; magnification 1300x.

papaya leaf against the number of *T. evansi* in the kidneys of mice. Number of *T. evansi* in the P1 group has a significant difference to the other treatment groups for P1 group only infected by *T. evansi* without being given papaya extract. There are caused by *T. evansi* grown rapidly without any factors which inhibited it. The body of the mice produced antibodies to fight invaders, but it is not capable of inhibiting the growth of *T. evansi* which had grown a lot. When compared with P1 group, P2 and P3 groups have a significant difference. This shows that doses of  $75 \text{ mg} \cdot \text{kg}^{-1}$  and  $150 \text{ mg} \cdot \text{kg}^{-1}$  decreased the number of *T. evansi*, but not yet approaching normal conditions. The P4 group had significant differences in all treatments including Po group (negative control). The average score of the number of *T. evansi* at P4 group had the least number of *T. evansi* compared with P2 and P3. This shows that the ethanol extract of papaya dose of  $300 \text{ mg/kg}$  was able to decrease the number of *T. evansi*, but still not approaching normal conditions.

Based on the Figure 1, the number of *T. evansi* at liver a bit more than the number of *T. evansi* in the kidneys in each treatment group. This is thought to be caused by blood flow to the heart more than the blood flow to the kidneys. *T. evansi* is flagellates that live in the bloodstream. According to Pearce [12], liver received blood from the artery hepatica that carries oxygen-rich blood from the lungs, and the portal vein carries nutrient-rich blood to the hepatocytes. While the kidneys receive blood only from the renal artery [12].

Papaya leaves contain secondary metabolites that are thought to contribute inhibiting the growth of *T. evansi*. According to Verpoorte and Alfermann [13], secondary metabolites are the metabolites that are not essential for the growth of organisms,



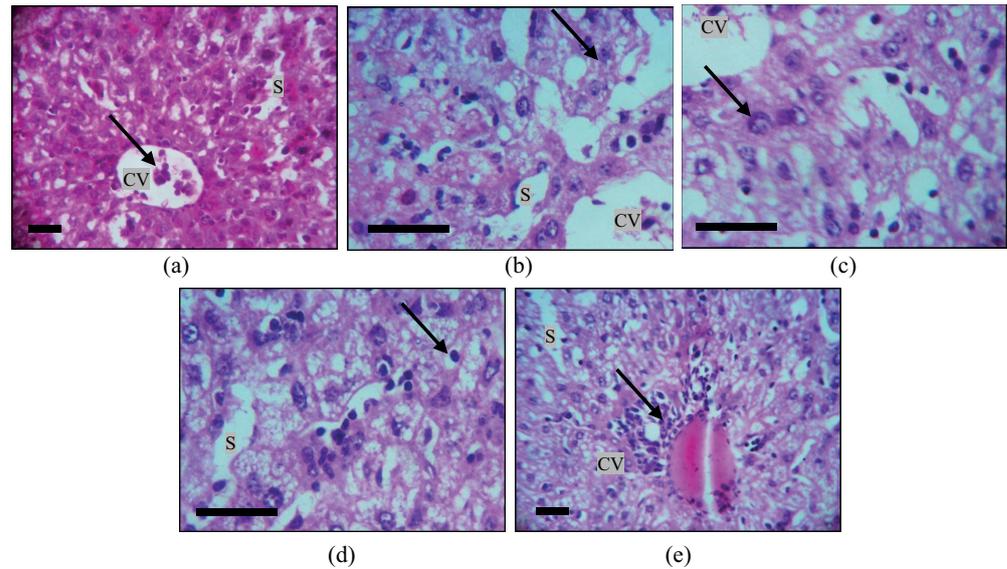
**Figure 3:** (a) Renal smear of negative control; (b) Renal smear of mice was infected by *T. evansi*; (c) Renal smear of mice was infected by *T. evansi* and ethanol extract of papaya 75 mg · kg<sup>-1</sup>; (d) Renal smear of mice was infected by *T. evansi* and ethanol extract of papaya 150 mg · kg<sup>-1</sup>; (e) Renal smear of mice was infected by *T. evansi* and ethanol extract of papaya 300 mg · kg<sup>-1</sup>; (→) *T. evansi*; scale 30 μm; Giemsa staining; magnification 1 300×.

Constituents	Reagent	Result
Alkaloid	Wagner	+
	Meyer	+
	Dragendorf	+
Flavonoid	HCl + Mg	+
Saponin	H <sub>2</sub> O	+
Tanin	FeCl <sub>3</sub>	+
Steroid	(CH <sub>3</sub> CO) <sub>2</sub> O + H <sub>2</sub> SO <sub>4</sub>	+

TABLE 1: Phytochemical analysis.

and it is found in the unique structure between species and serves to defend themselves from unfavorable environmental conditions. Papaya leaves contain alkaloids, flavonoids [14], saponins [15], tannins [16], and steroids. This is evident after phytochemical test of the ethanol extract of papaya as shown in Table 1.

Alkaloids could inhibit the growth of *T. cruzi* in epimastigote phase [17]. Alkaloid has trypanocidal activity which causes a partial lysis in trypomastigotes phase. This is supported by Cowan [18] which states that carpain (alkaloid contained in papaya) has antiprotozoal effect. Flavonoids used to treat diseases caused by protozoa [19]. Flavonoids containing hydroxyl groups that influence the degree of oxygenation that may indirectly increase antiprotozoal activity through the inhibition of respiration [20]. The substances contained in plants, especially flavonoids have antitypanosomal activity [21, 22]. Derivatives flavonoids expressed can suppress the growth of *T. cruzi* significantly [23].

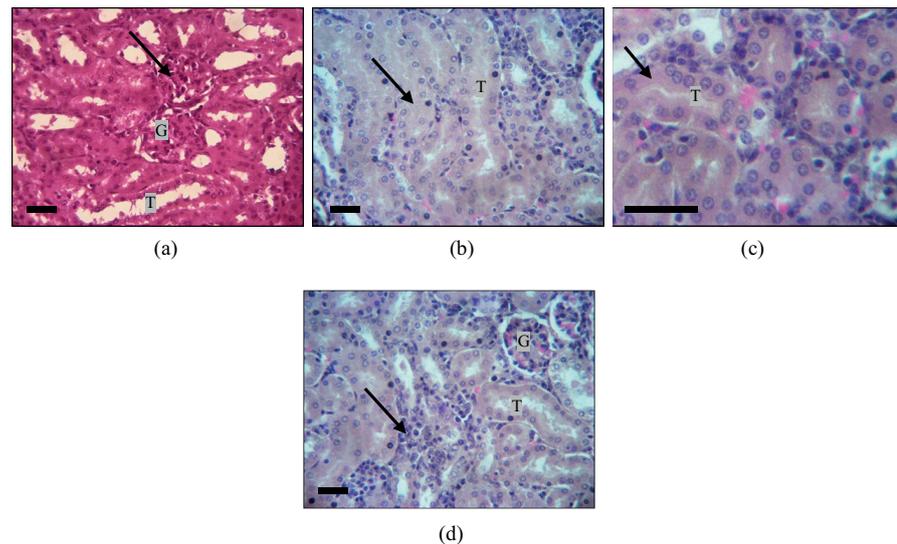


**Figure 4:** Histopathological of Liver (a) Congestion (520x); (b) Fatty degeneration (1300x); (c) Vacuolar degeneration (1300x); (d) Necrosis (1300x); (e) Cellular infiltration (520x); S = sinusoid; CV = central vein; thickness 5  $\mu\text{m}$ ; scale 30  $\mu\text{m}$ ; HE staining.

Saponin has able to decreased the number of protozoa [24] and reacted with cholesterol in the cell membrane of protozoa that cause cell rupture and lysis [25]. This could lead to disruption of metabolic processes and inhibits the growth of protozoa. Saponins are glycosides that have the aglycone a steroid. Saponins and steroids disrupted the ion balance of microorganisms through inhibition of the Na + pump. Saponins stimulate specific antigen DTH (Delayed Type Hypersensitivity) and proliferation of T cells that play a role in cellular immunity [26]. It is also supported by Talib et al. [27] research reported that saponins from *Sapindus rarak* DC. seed methanol extract reduced 57% of protozoa. Saponins decreased the index number of *T. cruzi* and enhanced survival in mice given saponin [28]. The presence of tannins in the body of organisms are increased stimulation of phagocytic cells and anti-infection activity [18]. Tannins could disable an enzyme that works inside the body of *T. evansi* and disrupts the process of protein transport through the cell membrane, preventing the activity of protease and inhibit growth. Adelodun et al. [29] proved that the tannin contained in *Uvaria chamae* P.Beauv. capable of extending the long life of mice infected by *T. brucei* as well as reduce the level of parasitaemia.

*T. evansi* need glucose and oxygen for growth and development that lead to degenerative changes which disrupted the host body's metabolic processes. It can cause cell damage due the toxins produced by *T. evansi* or immune reaction. Degenerative change in liver due to infection of *T. evansi* may include congestion, fatty degeneration, vacuolar degeneration, and necrosis, while degenerative change on kidneys such as glomerulonephritis, necrosis, cellular infiltration and tubular degeneration [10].

Congestion, fatty degeneration, vacuolar degeneration, necrosis and cellular infiltration is the degenerative change found in the liver (see Figure 4). In the treatment P1 group (positive control) was found all kinds of cell damage. This condition is thought to be caused by the inability of the immune system to inhibit the activity of *T. evansi*



**Figure 5:** Histopathological of Kidney (a) Glomerulonephritis (520x); (b) Tubular degeneration (520x); (c) Necrosis (1300x); (d) Cellular infiltration (520x); G = glomerulus; T = tubular; thickness 5  $\mu\text{m}$ ; scale 30  $\mu\text{m}$ ; HE staining.

which takes up glucose and oxygen needed by hepatocytes. P2 group (dose of  $75 \text{ mg} \cdot \text{kg}^{-1}$ ) still shows the same conditions as the P1 group. While the P3 group (a dose of  $150 \text{ mg} \cdot \text{kg}^{-1}$ ), fatty degeneration was not found. This is probably caused by dosing extract more so inhibit infection of *T. evansi* and reversible nature of fatty degeneration, the cells will return to normal if the factors causing the damage is inhibited. In the P4 group (a dose of  $300 \text{ mg} \cdot \text{kg}^{-1}$ ), fatty degeneration and vacuolar degeneration was not found. This is probably because the dose of the extract that much more can inhibit the activity of *T. evansi* and the cells returned to normal.

Glomerulonephritis, tubular degeneration, necrosis and cellular infiltration were found in all treatment groups (see Figure 5). This means increasing doses of papaya leaf extract ethanol does not affect renal histopathology. However, it still needs further research to determine quantitative histopathological conditions due to infection with *T. evansi* and ethanol extract of papaya.

*Trypanosoma evansi* infections can cause degenerative change in the liver and kidneys. Administration of foreign substances in the body such as the papaya leaf extract can also provide known toxic effects to the tissue. However, the treatment group showed that papaya leaf extract led to a decrease in the ability and the inhibition of *T. evansi* to breeding, so that the number of parasites can be suppressed. Therefore, it is necessary to conduct further research on specific compounds that can suppress the growth of *T. evansi* and active the immune system to inhibit the activity of these parasites. The specific compound that is used is not expected to adversely affect to the tissues of the body so that it can restore the body back to normal.

## 4. Conclusion

The ethanol extract of papaya had proven to decrease the number of *Trypanosoma evansi* in the liver and kidneys. The dose of  $300 \text{ mg} \cdot \text{kg}^{-1}$  of papaya leaf extract is the most able to decrease the number of *T. evansi* on the liver and kidneys than doses of  $75 \text{ mg} \cdot \text{kg}^{-1}$  and  $150 \text{ mg} \cdot \text{kg}^{-1}$ . Liver revealed degenerative change varying such as congestion, fatty degeneration, vacuolar degeneration, necrosis and cellular infiltration. Kidney revealed glomerulonephritis, tubular degeneration, necrosis and cellular infiltration.

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