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# Effect of *Tinospora cordifolia* Extract on the Liver Histopathology of Balb/c Mice Infected with *Salmonella typhimurium*

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**Abstract :** Typhoid fever is caused by *Salmonella typhimurium*. *Tinospora cordifolia* is a plant species that has a function as a natural immunomodulation. The extracts of *T. cordifolia* modulate the functions of hepatic protective and immune stimulatory. Hepatic protective herb increases the activity of the Kupffer cells. The aim of the research is to know effects of *T. cordifolia* extract on the liver histopathology of Balb/c mice infected *S. typhimurium.* This study was conducted a month in 2015 at Microbiological and Biomedical Laboratories, Faculty of Medicine, University of Brawijaya. There were 5 treatment namely negative control group, a positive control group that injected with *S. typhimurium*, mice group were injected with *S. typhimurium* and treated with 0.225, 0.375, and 0.75 mg /day *T. cordifolia* extract. Liver histopathology was done by light microscope observation in liver paraffin which stained with Hematoxylin-Eosin. The results showed that the positive control has nodular granulomata composed of principal on macrophages with many lymphocytes and few neutrophils. In addition, nodular lesion size was reduced in the treatment group compared to control positive and the presence of polynuclear leukocyte cells was replaced by mononuclear leukocytes. It can be concluded that *T. cordifolia* extract has protection effect and reduce the damage that caused by *S. typhimurium* in the liver.
**Keywords:** Hematoxylin-Eosin staining, liver, leukocyte, *S. typhimurium*, *T. cordifolia* extract.

## Introduction

Typhoid fever is one of serious health problem, especially in the developing countries. This disease is caused by *Salmonella typhimurium*. It is facultative intracellular bacteria that can live and even multiply in macrophages, as well as resistant to enzymes in lysosomes. In addition, it has the ability to prevent and inhibit phagolysosome fusion that is hard to kill. One way to destroy and eliminate the bacteria is to use an immune stimulant. It will spur the function of macrophages for killing through the respiratory burst[1]. Research has shown that several medicinal inhibits plants grow the bacterial pathogens[2].

*Tinospora cordifolia* extracts are widely used as a system of traditional medicine for the treatment[3]. The extracts of *T. cordifolia* modulate the functions of hepatic protective and immune stimulatory in mature rats. It has been studied extensively for its adaptogenic activity. The aqueous extract has significant adaptogenic activity on a variety of biological, physical and chemical stressors in a different model of animals[4]. Hepatic protective herb increases the activity of the Kupffer cells in a chronic liver disease model using carbon clearance test as a parameter[5].

The change of liver occurs from endotoxin of *Salmonella* and the reaction of immunity against germs so that hepatocytes cell is reversible. The use of the light microscope in the liver and the description of fat degeneration will be seen as well as the swelling of the cell as the first manifestation. It is caused by the displacement of extra water into the intracellular. The liver will experience hyperemia, it is softer and swollen. Cloudy swelling occurs in the first week of infection. Degeneration of ballooning and vacuolization of hepatocyte cells will occur. The proliferation of Kupffer cells, lymphocytes, and neutrophil will appear among hepatocyte cells as well as the formation of focal typhoid nodule[6]. The aim of the research is to know the effects of *T. cordifolia* extract on the liver histopathology of Balb/c mice infected *S. typhimurium.*

## Materials and Methods

This study was conducted a month in 2015 at Microbiological and Biomedical Laboratories, Faculty of Medicine, University of Brawijaya. This research used male mice strain Balb/c were 6-8 weeks old, body weight 20-30 grams, healthy, no anatomical abnormalities, and had undergone adaptation for a week. The materials needed are *S. typhimurium* bacteria, *T. cordifolia* extract solution, canada balsam, liver of mice, paraffin blocks, microtome, light microscope, sterile physiological saline, alcohol series (75%, 85%, 95%, 99%, and 100%), HE staining, formalin buffer, xylol, and sterile aquadest.

There were 5 treatment namely negative control group, a positive control group that injected with *S. typhoidrium*, mice group were injected with *S. typhimurium* and treated with 0.225, 0.375, and 0.75 mg /day *T. cordifolia* extract. There are some steps in histopathology test include tissue processing and HE staining. Procedures in tissue processing are the liver was taken with aseptic technique incorporated into the liquid fixation buffered formalin for 24 - 48 hours, fixed in buffered formalin for 24 - 48 hours, dehydrate with alcohol series of: 75%, 85%, 95%, 99%, and 100% respectively for 1.5 hours, the clearing using xylol for 1.5 hours, paraffin impregnating and embedding (manufacture of blocks), cut tissue using a microtome with the thickness of 3 -7 mm. After that, HE staining and mounting the cover glass were done by placed pieces of tissue on a glass slide (given albumin and glycine), entered into xylol I, II, and III respectively for 1 minute, entered into a series of alcohol 100%, 99%, 95%, 85%, and 75% respectively for 2 seconds, rinsed with flowing water, dip into Hematoxillin Mayer for 5 - 10 minutes, rinsed with running water for 5 - 10 minutes, dip into eosin for 1 - 2 minutes then rinsed with water, entered into a series of alcohol 75%, 85%, 90%, 100%, 100%, 100% in moderation, entered into Xylol I, II, III, IV, and V in moderation, covered with glass slide with a given canada balsam.

## Result and Discussion

*S. typhimurium* endotoxin and the immune response against *S. typhimurium* caused injury of the hepatocyte surrounded the area of inflammation. Picture fatty degeneration accompanied by swelling of the cell at the first appearance of manifestation. Hepatic also experienced hyperemia, softer, and swell and abscess formation. Cloudy swelling occurs in the first week of infection. The proliferation of Kupffer cells, lymphocytes, and neutrophils appear between cells hepatocytes accompanied by the formation of nodules focal necrosis typhoid that seems small center with many mononuclear cells[7].

The histology picture is one of the parameters. It is used to determine the level of damage and tissue repair. In the intracellular bacterial infection in the liver tissue of mice looked as atypical lymphocytes or activated with the characteristics of larger size and reactive cytoplasm become wider, more color blue or gray, oval core, lobe shape and some are children nucleus with coarse chromatin. *T. cordifolia* extract as immunodulator that minimizes damage to the liver tissue.

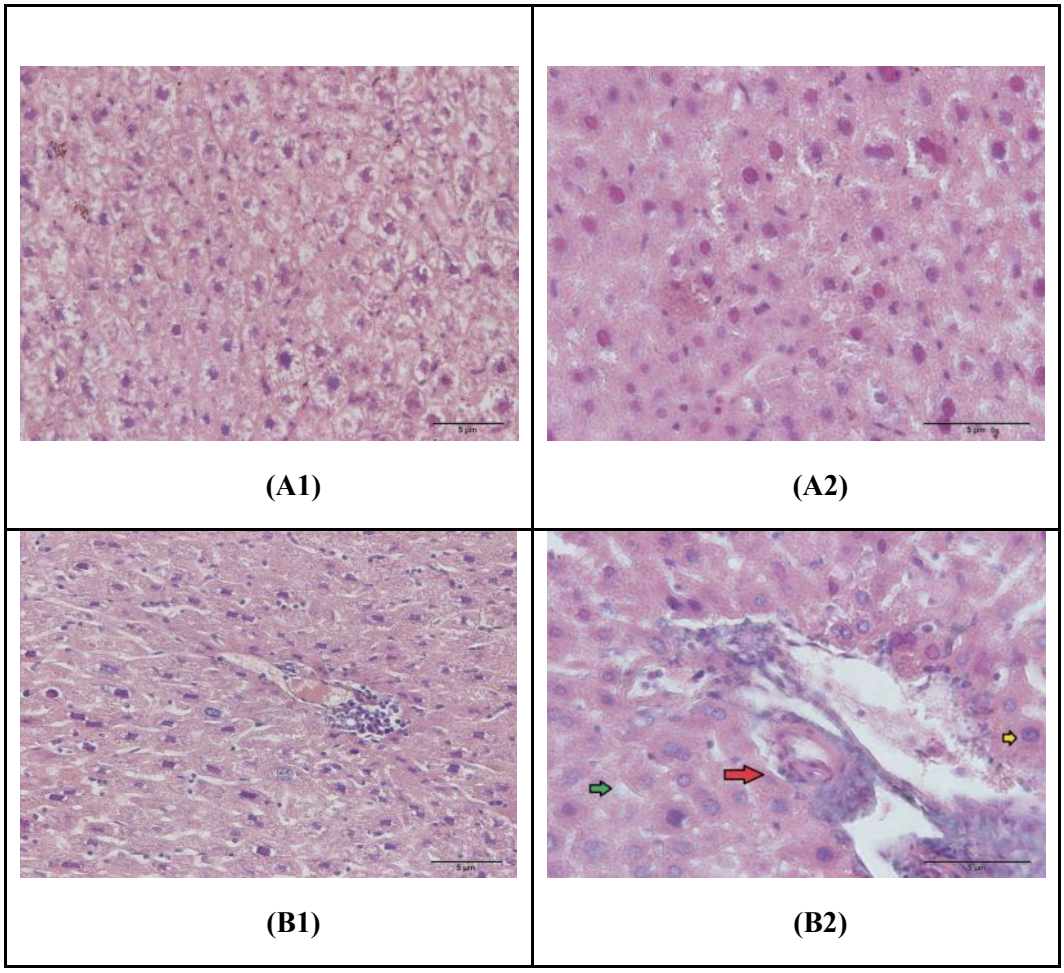


**Figure 1. The histological observation of liver in different magnification: 400x (A1 and B1); 600x (A2 and B2). Description: (A). The negative control group of mice (healthy); (B). The positive control group of mice (injected with S. typhimurium). Green arrow (vacuole); red arrow (granuloma) and yellow arrow (lymphocytes).**

Based on the result of the study, it showed the improvement of liver tissue in the mice group treated with 0.225, 0.375, and 0.75 mg/day *T. cordifolia* extract. Figure 1 showed histological liver in negative control and picture (A2) showed histological liver in the positive control (injected positive *S. typhimurium*). Based on Figure 1, the positive control has nodular granulomata composed of principal on macrophages with many lymphocytes and few neutrophils. Infiltration of macrophages led necrosis was getting an increase in proportion by *S. typhimurium* injection. Granuloma of mice in the positive control was large, due to a number of macrophages and epithelial cells formed in large quantities greater multinucleated cells. In addition, some nodular granulomata were gathering together to form nodular granulomata epithelium by the hepatic parenchyma[8].

Based on Figure 2 (C, D, E), it appeared that nodular lesion size was reduced compared to control treatment and the presence of poly nuclear leukocyte cells were replaced by mononuclear leukocytes. Nodular lesions histologically were seen as a transitional form between solids abscesses and lesions granulomata. The poly nuclear leukocyte cell infiltration was seen in the liver parenchyma, but nodular lesions are not visible and only find a collection of small-sized lesions are small[9].

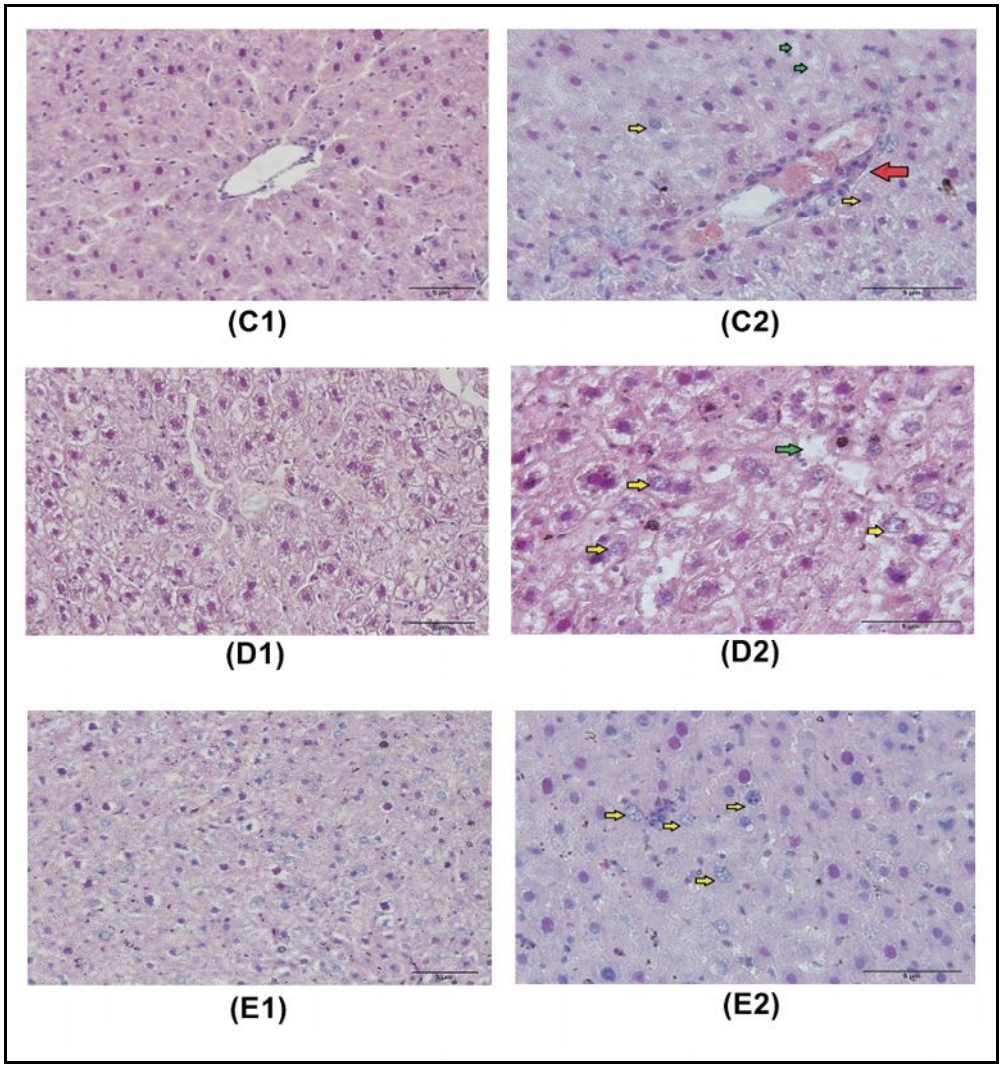


**Figure 2. Histological features of liver in different magnification: 400x (C1, D1, E1) and 600x (C2, D2, E2). Description: Groups of mice were injected with S. typhimurium and 0.225(C), 0.375 (D), and 0.750 (E) mg/day T. cordifolia.**

*T. cordifolia* extract was able to activate macrophages, which is characterized by the increasing ability of macrophage phagocytosis compared to the control group. Macrophages as phagocytes have an effective function. They will be activated the microbial cells, cytokines, and other stimulation. The natural immune response began with the introduction of bacterial components such as LPS and DNA. It is followed by the capture and destruction of the bacteria through phagocytic cells. This role is performed by macrophages, NK cells, and neutrophils.

*T. cordifolia* has an effect on the nonspecific immune response by increasing macrophage phagocytosis, and activation of NK cell. In this study, showed that the treatment group was different compared to the positive control group. From these result, it can be concluded that the *T. cordifolia* extract increase phagocytosis index by activating the NADH oxidase. In addition, *T. cordifolia* extract therapy only decreases the size and number of lesions cell population PMN cells to mononuclear cells. *T. cordifolia* extract induces damage to the kidney and liver[10]. Based on the research results, the dose of 500mg/kg extract therapy of *T. cordifolia* decrease the serum levels of creatinine, urea levels in the blood as alkaline phosphatase indicated in histopathology analysis. The kidney and liver preparations HE Balb/c mice show lymphocyte infiltration and differences in tubular form on pain control mice[11]. *T. cordifolia* has an effect on nonspecific immune response. *T. cordifolia* extract has protection effect and reduces the damage that caused by *S. typhimurium* in the liver. It showed with nodular lesion size was reduced in the treatment group compared to control treatment and the presence of poly nuclear leukocyte cells were replaced by mononuclear leukocytes.

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