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# EFFECT OF *LORANTHACEAE DAENDROPTHOE* SPECIES INFUSION ON SERUM TRANSAMINASE LEVEL AND HEPATIC NECROSIS IN RATS

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## ABSTRACT

*Loranthaceae daendropthoe is a herbal preparation which has been shown as cell antiproliferant and traditionally used as anticancer. The infusion of the plant is given via oral route, which is subsequently absorbed by gastrointestinal and metabolised in liver. Liver performs many essential functions, including the production of bile, regulation of plasma proteins and glucose as well as biotransformation of drugs and toxins. This study examined whether Loranthaceae daendropthoe makes liver tissue damage in animal model. Mouse as animal models were divided into two groups, the control group was given with aquademineralisata and treatment group was given with infusion of Loranthaceae daendropthoe. Serum transaminase, specific marker of hepatocellular necrosis and the histology of mouse liver were studied 17 days after the treatment with plant infusion. Data of serum transaminase and histology were compared between treatment and control groups. The result suggested that the infusion of Loranthaceae daendropthoe did not cause liver diseases.*

***Keywords**: Loranthaceae daendropthoe, infusion, serum transaminase, hepatic necrosis, SGOT, SGPT*

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## INTRODUCTION

The parasitic plants of the families Loranthaceae and Lauraceae, which can be found in Indonesia, have long been recognized as having capability in inhibiting the growth of cancer cells. Alkaloid laboratory examinations on parasitic plants as anticancer revealed empirical evidence of the capability of such plants, for example, parasites in tea and mango (Asmino, 1995). Duku parasite (*Loranthaceae daendropthoe* species) is a parasitic plant grows on the host of duku tree. This parasite had been suggested as having anticancer capability. Maceration as well as infusion of duku parasite had been found to inhibit myeloma cells in vitro (Roostantia et al, 1999; Ratna et al, 2000). Studies on the efficacy of *L daendropthoe* as anticancer in mouse had also been done. It was found that the administration of *L daendropthoe* infusion in rats infected with myeloma cells was able to eliminate these cells (Nuraini et al, 2000). As compared to methotrexate, a compound for anticancer chemotherapy, the inhibition capacity of methotrexate at concentration of 2.5 mg/ml was stronger as compared to 10% *L daendropthoe* maceration (Roostantia et al, 2000). In view of the side effects of methotrexate to various organs, such as bone marrow depression, hepatic fibrosis and cirrhosis, nausea, vomiting, and gastrointestinal ulcer, the use of *L daendropthoe* as anticancer should be developed further (Reynolds, 1993; Salmon et al, 1996).

Drugs or chemical substances in the body are subjected to various processes before reaching the targeted organs, producing efficacious effects, and finally, being excreted. Those substances undergo metabolism or biotransformation in hepatic organ. Similarly, *L daendropthoe*, after being absorbed and entered the circulation, it finally reaches the liver to undergo biotransformation process. Several drug substances may damage the function of the liver and result in hepatic tissue disorder or necrosis. *L daendropthoe* had been proved, both in vitro and in vivo, as having efficacy as anticancer. The result of this study will be very beneficial as until today cancer remains a disease that is not easily ovecome with therapy. To enhance the use of *L daendropthoe* as anticancer, further studies are basically needed. One of those studies concerns about the effect of *L daendropthoe* administration on hepatic function and tissue.

Hepatocellular damage can be detected in a number of methods. One of these methods, which is commonly used, is serum enzyme examination, i.e. the level of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). GOT is a mithocondrial enzyme commonly found in liver, heart, muscle and kidney. GOT value increases when there is acute cellular damage. The highest value is found in hepatocellular necrosis. GPT is a cytosolic enzyme. Its

absolute amount is less than that of GOT, although higher in heart and muscle. Its increase is significant in hepatic damage (Syaifullah, 1991).

This study on the effect of *L daendropthoe* on hepatic function and tissue was carried out by measuring the level of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) as well as the histopathological appearance of hepatic tissue in rats after being given with *L daendropthoe* infusion in certain dose, method, and time in comparison to SGOT, SGPT, and histopathological appearance of hepatic tissue in rats receiving distilled water. This study was done to find whether the infusion of *L daendropthoe* could increase SGOT, SGPT, and hepatic tissue necrosis in rats. The general objective of this study was to identify the effect of *L daendropthoe* infusion on hepatic function and tissue in rats, while the particular objectives were to find the effect of *L daendropthoe* infusion on SGOT and SGOT level and histological appearance in rats. The benefit of this study was to identify the presence of hepatotoxic effect of *L daendropthoe* infusion, so that it can be used as alternative therapy for cancer.

## MATERIALS AND METHODS

This was an experimental study using post-treatment observation involving a control group. This study used male healthy wistar strain rats with bodyweight of 200 grams and aged 3 months. Each group comprised of 10 samples. Observed variables were independent variable, *L daendropthoe* infusion; dependent variables, comprising the levels of SGOT, SGPT and hepatic histopathological damage; and control variable, i.e., sex, cage, food and drink, bodyweight and age.

*L daendropthoe* infusion used in this study was the infusion of *L daendropthoe* leaf taken from the south of Sumatra in concentration of 10%. This percentage was used since in the previous study it was found that 10% was the lowest concentration that could provide antiproliferative effect on cancer cells. The age of the plant was one year old. All parts of the plant were not damaged from insect bites or other damaging elements. The plant grew in southern Sumatra in an area free from plant diseases. The part of the plant used for infusion was its leaves that met the sample criteria.

The infusion was made after the leaves was being grounded. Subsequently, stock solution of *L daendropthoe* infusion was made by scaling 10 g of *L daendropthoe* leaf powder, added with distilled water to reach the volume of 100 ml. The solution was boiled at 90-98 C for 25 minutes, stirred by using a sitter, and, afterwards, when the solution was still warm, it was filtered using a sheet of flannel. Distilled water was then added to the waste and filtered again until reaching a volume of 100 ml. It was the result of this filtering that presented as the infusion of 10% b/v with pH 6.8 - 7.3 (Department of Health, Republic of Indonesia, 2000). Other concentration of *L daendropthoe* was made to 40% by thickening the stock solution.

Either the dose, mode of administration, and repeat interval was carried out according to the study done by Nuraini et al (2000). The result of this study showed that *L daendropthoe* infusion was able to inhibit myeloma cells in rats infected with these cells. The dose given was 18.37 mg/kg rats bodyweight/times based on the conversion of human body surface area 145 mg/times to animal. The dose in human was determined according to empirical report from the users of parasitic plants in general (Asmino, 1995).

The conversion was from human with bodyweight of 70 kg to rats of 200 grams, resulting the value of 0.018 (Nuraini et al, 2000). The dose was given before meal and repeated every 120 hours (5 days) four times. The determination of repetition interval and number was also based on empirical report from the users of parasitic plants in general (Asmino, 1995). The administration of *L daendropthoe* infusion was done per oral using abdominal sonde of ± 5cm (Nuraini et al, 2000). Serum used in this study was that taken from rats blood, both from control and treatment groups. Hepatic organ was also taken from both groups.

Treatment and maintenance for experimental animal were done in the Laboratory of Biochemistry, Airlangga University School of Medicine, while SGOT and SGPT examination were measured using the method from Boehringer Mannheim program for automatic analysis. The examination was carried out in Regional Health Laboratory. Histopathological preparation making and examination of hepatic organ was done at the Laboratory of Anatomic Pathology, Airlangga University School of Veterinary Medicine.

The collected data were on the level of SGOT and SGPT from rats receiving *L daendropthoe* infusion and those receiving distilled water. To compare whether the SGOT and SGPT levels in both groups were significantly different or similar, we performed two means difference test wih independent t-test with a = 0.05. Data on rats hepatic damage scoring were analyzed using Wilcoxon test to compare treatment and control group with a = 0.05%.

## RESULTS

A study has been carried out involving 20 rats, which were divided into two groups. One group served as control group, and another group as treatment group. The first group received distilled water only, while the latter received *L daendropthoe* infusion. The levels of SGOT and SGPT enzymes in both groups were measured with the result as follows:

Table 1. The result of GOT enzyme measurement in rats' serum

| No | Control | Treatment |
|---|---|---|
| | GOT u/l | GOT u/l |
| 1 | 74 | 72 |
| 2 | 76 | 136 |
| 3 | 72 | 162 |
| 4 | 52 | 204 |
| 5 | 54 | 154 |
| 6 | 58 | 166 |
| 7 | 62 | 118 |
| 8 | 102 | 100 |
| 9 | 86 | 164 |
| 10 | 98 | 94 |
| Mean | 73.4 | 137 |

Table 2. The result of GPT enzyme measurement in rats' serum

| No | Control | Treatment |
|---|---|---|
| | GOT u/l | GOT u/l |
| 1 | 74 | 72 |
| 2 | 76 | 136 |
| 3 | 72 | 162 |
| 4 | 52 | 204 |
| 5 | 54 | 154 |
| 6 | 58 | 166 |
| 7 | 62 | 118 |
| 8 | 102 | 100 |
| 9 | 86 | 164 |
| 10 | 98 | 94 |
| Mean | 73.4 | 137 |

The result of histopathological examination of rats liver was based on the damage resulting from hepatic cells degeneration in control and treatment group receiving *L daendropthoe* infusion. The result of statistical analysis was as follows: the difference of blood SGOT level in control and treatment group can be seen in Figure 4. The difference of blood SGPT level in control and treatment group can be seen in Figure 5.

Table 3. The result of histopathological examination scoring of rats liver in control and treatment groups.

| No | Control | Treatment |
|---|---|---|
| 1 | 0 | 0.2 |
| 2 | 0.1 | 0.2 |
| 3 | 0 | 0.2 |
| 4 | 0.1 | 0.2 |
| 5 | 0 | 0.2 |
| 6 | 0.1 | 0.3 |
| 7 | 0 | 0.3 |
| 8 | 0.1 | 0.5 |
| 9 | 0.1 | 0.2 |
| 10 | 0.1 | 0.5 |

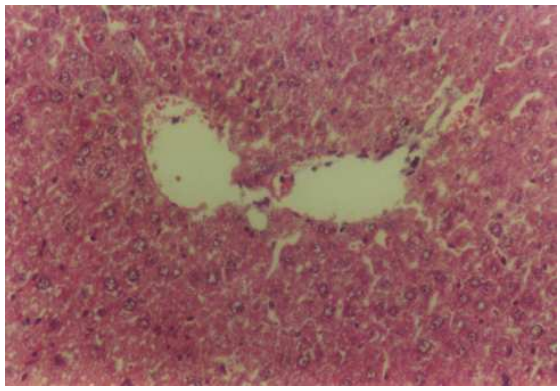

Figure 1. The result of histopathological examination in normal rats liver in control group. The liver ells appear normal, no necrosis is found (magnification 100 x).

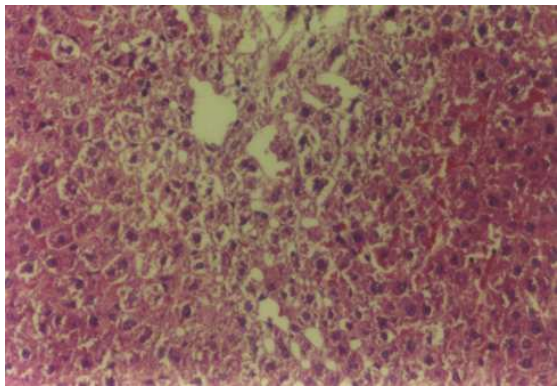

Figure 2. The result of histopathological examination of rats liver cells shows a grade 1 damage in group receiving *L daendropthoe* infusion (magnification 100 x).

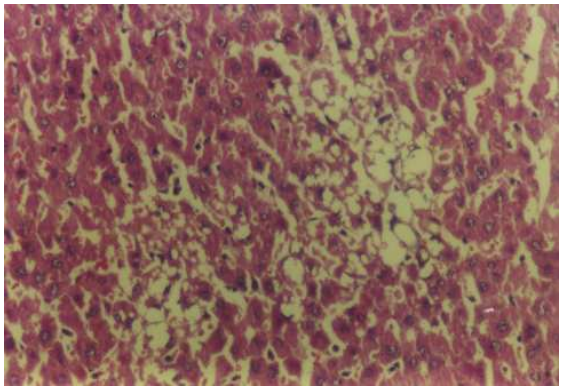

Figure 3. The result of histopathological examination of rats liver cells shows a grade 2 damage in group receiving *L daendropthoe* infusion (magnification 100 x).

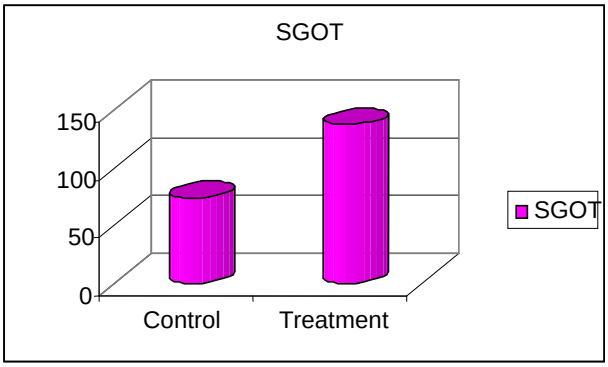


Figure 4. The difference of mean SGOT level in rats blood between control and treatment groups.

Table 4. Mean, standard deviation, and probability of SGOT level in control and treatment group.

| | Control | Treatment |
|---|---|---|
| X | 73.4 | 137.0 |
| SD | 17.6 | 40.5 |
| p | 0.000 | 0.000 |

Statistical analysis of scroring results from histopathological examination in rats liver revealed significant difference between control and treatment group (p = 0.005).

## DISCUSSION

GOT and GPT are aminotransferase enzymes that catalyzed transfer from alpha amino group from aspartate and alanin to alpha keto group from ketoglutaric acid to produce oxaloacetic and pyruvic acid highly needed in cytric acid cycle (Giannini et.al.,2005). Both enzymes exist in the liver in a high concentration. GOT is widely spread to the heart, skeletal muscle, kidney, brain, and red blood cells. GPT is also found in lower concetration in skeletal muscle and kidney. Hepatic damage, either acute or chronic, finally leads to an increase of aminotransferase enzyme in the serum, while the increase of serum GPT level is a more specific remark of hepatic damage (Giannini et al, 2005).

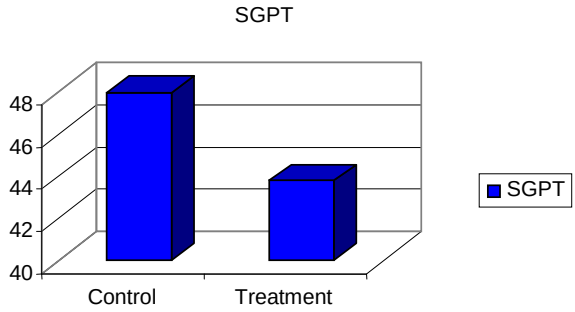


Figure 5. The difference of mean SGPT level in rats blood between control and treatment groups.

Table 5. Mean, standard deviation, and probability of SGPT level in control and treatment group.

| | Control | Treatment |
|---|---|---|
| X | 48.0 | 43.8 |
| SD | 11.6 | 8 |
| p | 0.358 | 0.358 |

The magnitude of the increase in aminotransferase enzyme can be classified into three levels. The increase is mild if it is less than five times. An increase of 5 - 10 times is regarded as moderatem while more than 10 is significant as compared to the upper limit of normal value (Giannini et al, 2005). These data were also confirmed by other authors who found that hepatic cellular damage due to toxicant or drug materials resulted in an increase of transaminase serum as much as 8 - 500 times (Carerro et al, 2004).

This study found a significant increase of SGOT between control (x = 73.4 ug/l) and treatment group (x = 137 ug/l). The SGPT level in control and treatment group that received *L daendropthoe* infusion the difference was found despite statistically insignificant. Based on the classification, the GOT increase in group receiving *L daendropthoe* infusion was mild, could not be regarded as pathological, particularly the SGOT level

as this enzyme can also be found in other organ. In this study, the significant increase was found only in SGOT level. It was possible that the increase of this enzyme resulted from outside hepatic process, while SGPT level, the more specific marker of hepatic cell damage, did not increase.

A study by Outtara et al showed that rats given with CCL4 (a hepatotoxic substance) has serum transaminase level of 8000 times higher compared to control. Another study in human revealed that hepatic damage induced by drug increased transaminase serum as high as 37 times and returned to normal as the drug was withdrawn (Carrascosa, 1997). Similarly, in corosive chemical substance intoxication, the increase of serum transaminase level is more than 100 times and the histopathological appearance showed massif hepatic cell necrosis (Kamijo et al., 2000). In comparison to that research, the increase of transaminase enzyme in this study was still in normal range.

Drug or chemical substance-induced hepatic cell damage occurs in various mechanisms. Those substances should be subjected to biotransformation in the liver to become non-toxic metabolites. The enzyme Cytochrome P450 changes certain drug substances into reactive metabolite, resulting in hepatic disorder. This metabolite has a covalent binding with molecules in the body and induce cytotoxic process, mutation, and the formation of new antigen. Cytotocix process leads the cells to be subjected to necrosis and apoptosis, bringing about either acute or chronic hepatitis. In mutated cells, there may be carcinogenesis/teratogenesis that results in hepatocellular adenoma carcinoma, while new antigen formation induces immunological reaction. Biotransformation process may also result in free radical that causes membrane lipid peroxidation, which, in turn, causes damage in cellular membrane (Carerro et al, 2004).

By observing cellular intactness as indicated by the presence of secreted enzymes if necrosis occurred, this study investigated whether *L daendropthoe* did not produce reactive metabolite that induces damage in hepatic cells through cytotoxic mechanism. The possibility that *L daendropthoe* could also produce reactive metabolite that result in mutation process and the formation of new antigen was, however, not investigated in this study. The result of this study showed that the enzyme level, particularly SGPT, did not indicate a significant increase. It implied that the result of biotransformation of *L daendropthoe* in the liver did not produce cytotoxic reactive metabolite that results in the necrosis of hepatic cells.

There was a statistically significant difference in histopathological damage between control and treatment group. This difference confirmed that the administration of *L daendropthoe* infusion could induce hepatic cells damage. Although it was statistically significant, the extent of damage was still less than 25%. A study on liver necrosis due to paracetamol by Lilik et al (2000) showed that 90% of its histopathological appearance exhibited necrosis grade 2 and 3, indicating an extensive necrosis. However, this study found that *L daendropthoe* infusion caused necrosis grade 1. The liver damage in this study was statistically significant, but not accompanied with significant icrease of serum transaminase enzyme level. This was because the damage in ¾ of hepatic tissue occasionally showed normal level of serum transaminase enzyme (Lilik et al., 1998).

## CONCLUSIONS

In conclusion, *L daendropthoe* infusion does not increase GPT enzyme level. However, it does increase GOT enzyme level in rats blood serum, although the increase remains in normal range. The infusion of *L daendropthoe* result in the appearance of hepatic cell necrosis at histopathological examination, even though the necrosis is still mild. It is suggested to conduct further studies on chronic hepatotoxic effect of *L daendropthoe* infusion and its effect on the formation of other reactive metabolites.

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