

THESIS

**THE HEPATOPROTECTOR EFFECT OF OREGANO
(*Origanum vulgare*) LEAF EXTRACT ON
HISTOPATHOLOGICAL CHANGES OF MICE (*Mus
musculus*) LIVER INDUCED BY RHODAMIN B**



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UNIVERSITY OF AIRLANGGA
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APPROVAL

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DECLARATION

Hereby, I declare that in this thesis entitled

**THE HEPATOPROTECTOR EFFECT OF OREGANO (*Origanum vulgare*)
LEAF EXTRACT ON HISTOPATHOLOGICAL CHANGES OF MICE
(*Mus musculus*) LIVER INDUCED BY RHODAMIN B**

There is no other work ever published to obtain a college degree in a certain college and to my knowledge there is also no work or opinion ever written or published by others, except those in writing referred to this paper and mentioned in the references.

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SUMMARY

The use of dyes in the culinary and food industries is common to increase the attractiveness of the food itself. Regulation of the Minister of Health of the Republic of Indonesia No. 239/MenKes/Per/V/85 has regulated certain dyes which are declared as hazardous materials, one of which is Rhodamine B. The use of Rhodamine B is still found widespread, this is shown by a study by Tjiptaningdyah and Sucahyo in 2017 which stated that in 20 kinds of snacks marketed in schools in Surabaya, 30 percent or as many as six kinds of snacks were identified as containing synthetic dye which is Rhodamine B. Rhodamine B contains chlorine compounds (Cl^-) which is a free radical compound that is dangerous and highly reactive. The metabolism of Rhodamine B will produce by-products in the form of free radicals, and the presence of free radicals will induce the formation of ROS (Reactive Oxygen Species) and a state of oxidative stress so that it can initiate lipid peroxidation which can cause DNA strands to break and oxidize all membranes and biological tissues resulting in organ damage. Liver is the main organ that is attacked by ROS (Reactive Oxygen Species), especially parenchyma cells. This is because parenchyma cells are primary cells that are injured due to oxidative stress induction in the liver. A number of cytokines such as $\text{TNF-}\alpha$ can be produced in Kupffer cells induced by oxidative stress, causing cell death (necrosis) and inflammation.

Oregano (*Origanum vulgare*) leaves are herbal plants known for their antimicrobial and antioxidant properties, this is supported by the high content of flavonoids and phenolic acids that can maximize antioxidant abilities. The mechanism for preventing free radicals by flavonoids is slowing down the formation of ROS, breaking down ROS and regulating protection with antioxidants. The hydroxy groups in flavonoids have high reactivity as hydrogen donors and will stabilize free radicals, so as to prevent damage to liver tissue.

This study aims to examine the ability of Oregano leaf extract as a hepatoprotector in preventing liver cell damage in the form of degeneration, necrosis, and portal inflammation in mice liver induced by Rhodamine B. The mice (*Mus musculus*) used were male and weighed 20 – 30 grams, divided into five groups, each group consisting of four mice. Before treatment, the mice were adapted for seven days, then given Oregano leaf extract from the 1st to the 24th day and given Rhodamine B induction from the 4th to the 24th day. The group consisted of a negative control group (given CMC-Na 1% and aquadest), a positive control group (given Rhodamine B 3.5 mg/20 g BW and aquadest), a T1 treatment group (Oregano leaf extract at a dose of 100 mg/kg BW + induction Rhodamine B 3.5 mg/20 g BW), treatment group T2 (Oregano leaf extract dose 200 mg/kg BW + induction Rhodamin B 3.5 mg/20 g BW), and treatment group P3 (Oregano leaf extract at 400 mg/kg BW + induction of Rhodamine B 3.5 mg/20 g BW). On the 25th day, all mice were sacrificed and the liver was taken for histopathological examination with Haematoxylin-Eosin staining.

The results of histopathological examination of the liver tissue of mice induced by Rhodamine B in the positive control group showed hepatocyte damage in the form of degeneration, necrosis, and portal inflammation. Based on this study, administration of Oregano leaf extract at doses of 100, 200, and 400 mg/kg BW can prevent hepatocyte damage which is characterized by a lower degree of damage based on the results of scoring the histopathological changes of liver tissue in the form of degeneration, necrosis, and portal inflammation. Based on the Kruskal-Wallis test followed by the Mann-Whitney test, the best dose of Oregano leaf extract in preventing damage to hepatocytes was 400 mg/kg BW.

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Alya Luthfana Zakiyah

ABSTRACT

This research was conducted to examine the hepatoprotector effect of Oregano (*Origanum vulgare*) leaves extract on the histopathological changes of liver induced by Rhodamine B in mice (*Mus musculus*). Twenty male mice were divided into five groups and were administered via oral gavage with different treatments for 24 days. The groups consisted of C- (CMC Na 1% + aquadest), C+ (Rhodamine B 3.5 mg/20 g BW), T1, T2, and T3 (100, 200, and 400 mg/kg BW of Oregano leaves extract respectively + Rhodamine B 3.5 mg/20 g BW). The histopathological changes of liver were examined by using Knodell Scoring method. Then, the data were analyzed using Kruskal-Wallis and then continued with using Mann-Whitney test. The result of statistical analysis showed significant difference ($p < 0.05$) between treatment groups for degeneration of hepatocytes, necrosis of hepatocytes, and portal inflammation examination. This research showed that Oregano leaves extract could protect and prevent the damage of mice liver from the destructive effect of Rhodamine B. The effective dose of Oregano leaves extract on this research was 400 mg/kg BW.

Keywords: *Origanum vulgare*, Rhodamine B, liver, *Mus musculus*

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Abbreviation

Ppm	: Parts per million
ROS	: Reactive Oxygen Species
SOD	: Superoxide Dismutase
CAT	: Catalase
GPx	: Gluthathione Peroxidase
BHA	: Butyl Hydroxyl Anisole
BHT	: Butyl Hydroxy Toluen
TBHQ	: Tertiary butylhydroquinone
PUFA	: Polyunsaturated Fatty Acids
AST	: Aspartate Aminotransferase
ALT	: Alanine Aminotransferase
ALP	: Alkaline phosphatase
IARC	: International Agency for Research Center
IUPAC	: International Union of Pure and Applied Chemistry
ATP	: Adenosine Triphosphate
HE	: Haematoxylin Eosin
BW	: Body weight
SPSS	: Statistical Product and Service Solution
OLE	: Oregano Leaf Extrac