

Effect of Leaves and Stems Extract of Ciplukan (*Physalis Angulata* L.) on The Severity of Steatosis in Wistar Rat Liver Induced by Egg Yolk and Propylthiouracil

**Adi Nugraha¹, Ety Sari Handayani², Dewa Nyoman Murti Adyaksa³, Miranti Dewi
Pramaningtyas^{4*}**

AFFILIATIONS

1. Faculty of Medicine,
Universitas Islam
Indonesia, Yogyakarta,
Indonesia
2. Department of Anatomi,
Faculty of Medicine,
Universitas Islam
Indonesia, Yogyakarta,
Indonesia
3. Department of Anatomical
Pathology, Faculty of
Medicine, Public Health,
and Nursing, Universitas
Gadjah Mada, Yogyakarta,
Indonesia
4. Department of Physiology,
Faculty of Medicine,
Universitas Islam
Indonesia, Yogyakarta,
Indonesia



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ABSTRACT

*The increasing habit of consuming high-fat foods and a sedentary lifestyle increases the incidence of hepatic steatosis. There are currently no approved medical drugs to prevent and treat steatosis. The leaves and stems of the ciplukan plant (*Physalis Angulata* (L.)) are rich in flavonoid compounds and phenolic acids which have preventive and curative effects on steatosis. This research aimed to determine the impact of ciplukan leaves and stems extract on the severity of steatosis in the liver of Wistar rats induced by egg yolk and propylthiouracil. This research consisted of four groups: first group induced by egg yolk and propylthiouracil for six weeks (n=7), second group induced egg yolk and propylthiouracil plus simvastatin 0.18 mg/200 gBW for six weeks (n=7), third group induced by egg yolk and propylthiouracil plus ciplukan extract 400 mg/kgBW for six weeks (n=7), fourth group induced by egg yolk and propylthiouracil plus ciplukan extract 800 mg/kgBW for six weeks (n=7). The severity of steatosis was determined based on histopathological observations by an anatomical pathology specialist. The first group had four preparations with moderate degree of steatosis. The second group had three preparations with moderate degree of steatosis. The third group had two preparations with moderate degree of steatosis. The fourth group had two preparations with moderate degree of steatosis. The statistical test results showed no significant difference in severity between the treatment groups (p=0.692). The administration of ciplukan leaves and stems extract can prevent a decrease in high-density lipoprotein levels in Wistar rats induced by egg yolk and propylthiouracil. However, no significant influence was found on the severity of hepatic steatosis.*

KEYWORDS:

Dyslipidemia, Liver, Steatosis, Ciplukan, Extract

CORRESPONDING AUTHOR:

Miranti Dewi Pramaningtyas
miranti.dewi@uii.ac.id

INTRODUCTION

The increasing trend of consuming high-fat foods and leading a sedentary lifestyle in society presents new challenges for Asian countries. This is related to the rising prevalence of various health disorders, including non-alcoholic fatty liver disease (NAFLD). The incidence of this disease in Asia varies greatly, ranging from 5% in Singapore to 30% in Indonesia¹. This condition begins with the

accumulation of intrahepatic triglycerides, known as steatosis, reaching $\geq 5\%$ of liver weight. This buildup is linked to an imbalance between the liver's input and output of fatty acids², leading to an increased risk of hepatocellular carcinoma³. Therefore, preventive measures and treatment for steatosis are necessary to prevent the progression to severe conditions.

To date, the prevention and treatment of steatosis are limited to lifestyle changes alone. This is because there are no approved medical drugs to treat people with fatty liver disease⁴. Therefore, research on herbal medicines has great potential for development. One potential herbal plant that could be used is the *ciplukan* plant (*Physalis Angulata* (L.)). The plant is rich in bioactive compounds such as flavonoids and phenolic acids. Both types of compounds can be found in the leaves and stems of the *ciplukan* plant. The flavonoid compounds contained in the leaves and stems of this plant include myricetin, quercetin, rutin, and kaempferol^{5,6}. Meanwhile, the phenolic acid compounds found in the leaves and stems of this plant are chlorogenic acid and p-coumaric acid⁷. In several studies conducted on experimental animals, these compounds were found to have the ability to prevent and repair steatosis through various mechanisms.

The first mechanism involves the suppression of the lipogenesis process and stimulation of beta-oxidation of fatty acids, as carried out by myricetin, quercetin, chlorogenic acid, and p-coumaric acid. The second mechanism entails the reduction of tumor necrosis factor- α (TNF- α) formation and reactive oxygen species (ROS). This mechanism can be achieved by rutin or quercetin. The third mechanism is the inhibition of fatty acid uptake by

the liver, commonly done by myricetin and kaempferol^{8–13}.

Therefore, the researcher aims to investigate the influence of giving extracts of *ciplukan* leaves and stems on the severity of steatosis in the livers of Wistar rats induced by egg yolks and propylthiouracil.

METHODS

This study is laboratory experimental research using a post-test randomized control group design, comparing the severity of steatosis between treatment groups. The research was conducted at the Pathology Anatomy Laboratory, Faculty of Medicine, Gadjah Mada University, for approximately 6 months.

Extracts of the *ciplukan* plant leaves and stems are the compounds used in this research. These extracts are made from powdered *ciplukan* leaves and stems obtained from a traditional herbal medicine store in Sleman City, which are then dissolved in 70% ethanol. The solution is then concentrated using a rotary evaporator, resulting in a thick ethanol extract. This extract is then administered to Wistar rats using an oral gavage according to predetermined doses.

The population of this study consists of male Wistar rats (*Rattus norvegicus*) aged 2–3 months with a body weight of 150–280 grams. The rats were maintained in an animal room under a controlled environmental condition. The sample size for this

study was calculated using the Charan formula, resulting in a minimum of 4 rats per group and a maximum of 6 rats per group¹⁴.

In this study, there were four treatment groups, including: the first group, as a control, induced using egg yolk and propylthiouracil (PTU) for six weeks ($n = 7$); the second group induced using egg yolk and propylthiouracil (PTU) with the addition of simvastatin 0.18 mg/200 gBW for six weeks ($n = 7$); the third group induced using egg yolk and propylthiouracil (PTU) with the addition of 400 mg/kgbw of ciplukan extract for six weeks ($n = 7$); and the fourth group induced using egg yolk and propylthiouracil (PTU) with the addition of 800 mg/kgbw of ciplukan extract for six weeks ($n = 7$). The dosage determination aligns with the research of Afriyeni and Surya (2019) and Nugroho, Soelistijo, and Nugraha (2021).

Organ harvesting was carried out after the entire group had received treatment for six weeks. Before organ harvesting, mice were fasted for one day. The termination process was carried out when the mice were under ketamine anesthesia. A horizontal incision is made in the abdomen, followed by the extraction of the liver. The organ is then cleaned, soaked in formalin solution, and stored in the freezer.

Histopathology liver specimen preparation is taken from the terminated rats. The extracted liver is then cleaned and treated with a fixative for at least

12 hours. After that, the organ is dehydrated with various alcohol concentrations, cleaned with xylene, and then embedded in paraffin. The tissue is then cut to a thickness of 4-5 μm and stained with hematoxylin-eosin (HE) dye¹⁵.

The specimen readings are done by an anatomic pathology specialist from the Department of Anatomic Pathology, Faculty of Medicine, Public Health, and Nursing at Gadjah Mada University. The readings are done by observing the entire specimen area (the whole slide). The observation is done at low magnification (40x–100x) and high magnification (400x)^{16–18}. Then, the percentage of steatosis is quantified and converted into levels of severity according to criteria: healthy (<5%), mild (5–33%), moderate (34–66%), and severe (>66%)³.

In this research, the high-density lipoprotein (HDL) levels of rats were also examined. The examination was carried out on blood that had been centrifuged at a speed of 3000 rpm for 10 minutes. The serum was then analyzed at central university laboratory of Gadjah Mada University.

The data from this study are analyzed using the Kruskal-Wallis test and post hoc analysis. This analysis will be conducted using statistical software.

This study has passed ethical review by the Faculty of Medicine, Public Health, and Nursing at Gadjah Mada University Ethics Commission based on the issuance of the ethical review clearance letter number: KE/FK/0669/EC/2021.

RESULT AND DISCUSSION

In this study, there were 28 rats divided into four treatment groups. As the study progressed, four rats died, leaving 24 rats for liver organ extraction and lipid profile testing. This number meets the minimum required sample size for this research.

Table 1. Kruskal-Wallis Analysis of High-Density Lipoprotein (HDL) in Rats

Group	HDL (mean $\pm$ SD)	p-value
I	27.70 $\pm$ 0.59 mg/dL	<0.001
II	69.64 $\pm$ 1.74 mg/dL	
III	44.26 $\pm$ 0.63 mg/dL	
IV	61.03 $\pm$ 0.95 mg/dL	

Post hoc analysis: KI-KII $p < 0.001$, KI-KIII $p = 0.199$, KI-KIV $p = 0.006$, KII-KIII $p = 0.004$, KII-KIV $p = 0.116$, KIII-KIV $p = 0.186$.

Based on the results of serum lipid profile tests, the average level of high-density lipoprotein (HDL) was significantly higher in the group given additional simvastatin and ciplukan extract induction compared to the control group ($p < 0.001$). Meanwhile, the difference in average HDL levels appeared significant in the group given additional simvastatin induction ($p < 0.01$) and ciplukan extract 800 mg/kgbw ($p = 0.06$) (Table 1).

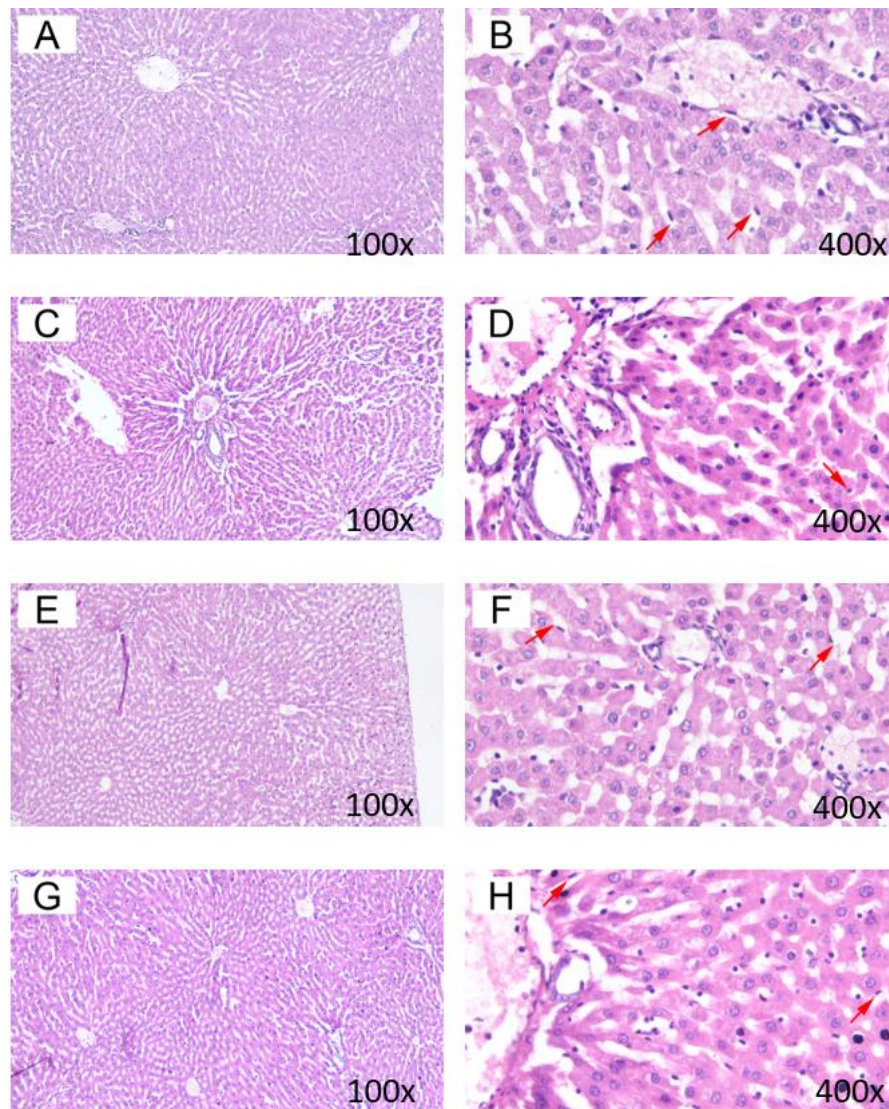


Figure 1: Histopathological overview of the liver with H&E staining. Groups I (A and B), II (C and D), III (E and F), and IV (G and H). A red arrow denotes the existence of steatosis.

Based on the results of the histopathology slide analysis, it was found that there was evidence of steatosis in all treatment groups (Figure 1). In the first group, there were four samples with moderate severity and two samples with mild severity. In the second group, there were three samples with moderate severity and four samples with mild severity. In the third group, there were two samples with moderate severity and three samples with mild severity. Meanwhile, in the fourth group, there were two samples with moderate severity and four samples with mild severity. However, despite the varying severity levels of steatosis in each group, there was no significant difference statistically ($p = 0.692$) (Table 2).

Table 2. Kruskal-Wallis Analysis of Steatosis Severity Level

Criteria	Group	N	p-value
Steatosis	I	6	0.692
	II	7	
	III	5	
	IV	6	

In this study, the researchers wanted to find out the effects of giving extracts of ciplukan leaves and stem on the severity of steatosis in the livers of Wistar rats induced with dyslipidemia. The induction of dyslipidemia in this study was done by feeding the rats with egg yolks (as a high-fat food) and propylthiouracil (PTU) through an oral gavage for six weeks.

Based on the lipid profile examination results, the induction of dyslipidemia with egg yolks and PTU showed positive outcomes. This was evidenced by the decrease in the average HDL levels in the group that only received egg yolk and propylthiouracil to

below 40 mg/dL. These results are consistent with the research by Anindita Maulida Tara Gayatri, Andina Putri Aulia, and Mohamad Riza (2023) and also the study by Rahayuningsih et al. (2015), which showed a decrease in the average HDL levels in rats induced with a high-fat diet and PTU without any intervention provided.

From the histopathological observations, it can be said that the induction of dyslipidemia in this study could also trigger the formation of hepatic steatosis. This is because signs of steatosis were found in all examined specimens. The presence of steatosis can occur due to the high-fat content in egg yolks, which can trigger an increase in fatty acid uptake by hepatocyte cells. Additionally, PTU as an anti-thyroid medication that works by reducing the production of triiodothyronine (T3) hormones can trigger an increase in the levels of sterol regulatory element-binding protein 1c (SREBP-1c) and a decrease in the levels of carnitine palmitoyl transferase-1 (CPT-1). The increase in SREBP-1c levels and the decrease in CPT-1 levels affect the increase in de novo lipogenesis processes and the decrease in fatty acid oxidation¹⁹⁻²².

The histopathological observations also showed differences in the severity of steatosis between the non-intervention group and the intervention group. In this study, Group I, or the non-intervention group, had the most moderate degree compared to the other groups. These findings are consistent with the research by Ma, Gao, and Liu (2015) and Xia et al. (2016), which indicate that steatosis is more

common in groups induced by high-fat diets without any intervention.

Although there were differences in the severity of steatosis observed in histopathology, statistically, these differences were not significant ($p = 0.692$). The statistical test results indicate that the active ingredients in the *ciplukan* leaves and stems extract, such as myricetin, chlorogenic acid, and others, still do not have a significant effect on the severity of hepatic steatosis in this study. These findings differ from the research by Xia et al. (2016), which shows that myricetin has a significant effect on preventing or treating steatosis through changes in the PPAR signaling pathway. The results also differ from the study by Ma, Gao, and Liu (2015), which states that chlorogenic acid can significantly prevent or treat steatosis by inhibiting the PPAR γ pathway.

This study has a limitation in the form of less representative observation results. This is due to suboptimal pre-analytical conditions, namely improper and prolonged organ storage. This results in widened hepatic sinusoids, making it difficult to distinguish from the appearance of steatosis.

CONCLUSION

In this study, the administration of *ciplukan* leaves and stems extract can prevent a decrease in high-density lipoprotein levels in Wistar rats induced by egg yolk and propylthiouracil. However, no significant influence was found on the severity of hepatic steatosis. Furthermore, to improve future research, further studies need to be conducted with

the administration of *ciplukan* leaves and stems extract over a longer period of time.

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