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ORIGINAL ARTICLE

The effect of celery ethanol extract (*Apium graveolens l.*) against fatty liver in rat model of hyperlipidemia

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ABSTRACT

Fatty liver is a disorder of liver characterized by the accumulation of fat liver cells. The incidence of fatty liver in Indonesia is 30%. Fatty liver can be caused by hyperlipidemia. Hyperlipidemia triggers an increase of oxidative stress that leads to fatty liver. Treatment is usually carried out using statins, but long-term consumption of statin may lead to several side effects. Other treatment that can lower cholesterol levels traditionally is celery. This study aimed to determine the effect of ethanol extract of celery on the microscopic appearance of the liver induced by hypercholesterolemia in experimental rats. This was an posttest control group experimental on 25 rats that were divided into 5 groups: negative control group, positive control group, and three (3) treatment groups. Each treatment group was given a high-fat diet for 14 days, followed by a provision of high-fat diet with different celery extract doses of 37.5 mg/KgBW/day, 75 mg/KgBW/day, and 150 mg/KgBW/day. After the treatment completed, the rats were sacrificed and dissected, and their liver was for histopathological preparations. Data on the in the average fat score between the control group and the three treatment groups were collected and analyzed using one way ANOVA if they were normally distributed with the Kruskal Wallis test as the alternative test. To determine whether there was a significant difference between two treatment groups, a post-hoc statistical test was performed. A significant change was observed in the histological presentations of the liver between control and treatment groups ($p < 0.5$). There was a marked improvement of cytoplasm, sinusoid and cell nucleus in the treatment groups compared to positive control group with a dose of 37.5 mg/KgBW/day as the most effective celery ethanol extract to improve liver fat due to fat accumulation.

Keyword: Celery Ethanol Extract, Fatty Liver, Hyperlipidemia, Histopathological, Rat

INTRODUCTION

Hyperlipidemia is an increase in lipids in the blood that takes the form of increased cholesterol (hypercholesterol) and triglycerides (hypertriglycerides) levels characterized by increased serum

levels of Low Density Lipoprotein (LDL) in fasting blood plasma.[1] Hyperlipidemia is one of the risk factors of non-communicable diseases including cardiovascular disease and fatty liver.[2,3] Fatty liver has become a world problem with a prevalence of 10-15%, with Indonesia having a prevalence of 30%.[4] Fatty liver is a disorder of the liver characterized by accumulation (steatosis) in the liver accompanied by signs of damages to the hepatocytes. The liver is the largest organ in the human body that functions in fat metabolism, one of which is synthesizing cholesterol.[5,6] The degree of hepatic fatty damage can be determined by the Kleiner scoring method and observation on the presence of vacuoles in the hepatocyte cytoplasm. Fatty liver occurs by an increase in cholesterol which cannot be balanced by very low-density lipoprotein (VLDL), resulting in the accumulation of fat in the liver. Hyperlipidemia indicates the presence of free radicals in the body, which result in oxidative stress. Increased oxidative stress will lead to the decrease in activity of the enzyme Lipoprotein Lipase (LPL), leading to VLDL accumulation in the liver and fat infiltration around hepatocytes.[7]

Treatment of hyperlipidemia can be done in a modern and traditional ways. There are several drugs that can lower blood cholesterol levels, including simvastatin, one of the statin class drugs. The maximum dose of simvastatin is 80 mg/day. If consumed continuously in a long term, simvastatin can causes several side effects including gastrointestinal disturbances, rhabdomyolysis, myopathy, acute renal failure, and dementia.[8] As some side effects of statins are quite dangerous, studies on the treatment of hyperlipidemia using celery as an alternative have been carried out.[9,10,11,12]

Celery (*Apium graveolens L.*) is a plant from the Apiaceae family which is rich in antioxidants, such as tannins, flavonoids, limonene, selinene, vitamins A, and Vitamin C. Celery has been used as traditional medicine as it can reduce lipid levels and blood pressure.[13,14,15] Based on a previous study in Semarang, Indonesia, by Umarudin et al., the ethanol extract of celery at a dose of 75 mg/KgBW/day effectively lowers cholesterol levels. However, they did not assess the histopathological appearance of fatty liver objectively.[14] This study aimed to determine the effect of ethanol extract of celery on the microscopic appearance of the liver induced by hypercholesterolemia in experimental rats.

METHODS AND SUBJECT

This study was conducted at the Veterinary Laboratory of the Faculty of Medicine, Universitas Padjadjaran and the Anatomical Pathology Laboratory of the Faculty of Medicine, Jenderal Achmad Yani University. Ethical approval was obtained from the Universitas Padjadjaran Ethics Committee through the issuance of the ethical clearance number 881/UN6.KEP/EC/2021 dated October 18, 2021.

Research Design

This study applied the post-test control group experimental design by observing and documenting the effect of celery ethanol extract, which was given in 3 different doses in 3 different treatment groups, on the histopathological condition of male Wistar rat livers. The changes observed were then compared to positive controls based on the Kleiner's scoring.

Research Materials

The research materials used for the treatment were standard pellets for rats, high fat diet feed, water for the rats to drink, celery ethanol extract, and propylthiouracil. The materials for the preparation of the test preparations were celery, water, 0.9% physiological NaCl, paraformaldehyde solution, and ethanol.

Research Subject

This study used male Wistar rats. The experimental animals were adapted to the Animal Laboratory of Universitas Padjadjaran for 7 days, fed *ad libitum*. They were then divided into 5 groups: negative control group (NC), positive control group (PC), treatment group 1 (T1), treatment group 2 (T2), and treatment group 3 (T3) to determine the effect and effectiveness of celery ethanol extract. The negative control group, received standard food of 20-25 g/rat/day and water of 10 ml/100gBW/day while the positive control group received uncontrolled high-fat diet (HFD) of 20-25g/rat/day, 3 ml tube feeding, and 0.01% PTU. The treatment groups received celery extract dose of 37.5 mg/KgBW/day (T1), 75 mg/KgBW/day (T2), and 150 mg/KgBW/day (T3) after 14 days of feeding with HFD and PTU.

To determine the sample size for each group, the frederer formula was used and a minimum number of samples of five (5) rats was set, leading to a total number of rats needed for this study of 25. Experimental samples were adjusted based on the research design of Completely Randomized Design (CRD).

Research Object

This study used green celery. The celery used was obtained from Lembang, West Java, Indonesia and was extracted at the Research and Innovation Laboratory of Institut Teknologi Bandung.

High-Fat Diet Formulation

The High Fat Diet was obtained from the animal laboratory of the Faculty of Medicine, Universitas Padjadjaran. The composition of the non-tube high fat diet consisted of 8,000 grams of standard feed, 15 duck egg yolks, 25,00 grams of flour, 1,000 grams of goat fat, 7,500 mg of coconut oil, and aquadest to be sufficient for 20 rats for 7 days. Tube high-fat diet consisted of 2 grams of quail eggs, 2 ml of lard per rat. In addition, 100 mg PTU was dissolved in 10 ml of distilled water to get 0.01% PTU.[11.16]

Preparation of Celery Ethanol Extract

Celery ethanol extract was produced at the Research and Innovation Laboratory of Institute Teknologi Bandung (ITB), Indonesia. The leaves and stems of celery were used for the extraction. The celery was rinsed under running water, then cut into pieces, dried, and ground using a blender to get powder consistency, which was then filtered through the paper strain. This powder is then immersed in 96% ethanol for 24 hours in a percolator. Using a rotary evaporator, the ethanol was evaporated until the extract becomes thick, then the extract was dried by heating water at 60^0C.[16]

Experimental Animal Treatment

The study used a sample of 25 rats which were acclimatized for 7 days. The rats were given food and drink and weighed regularly to ensure their fit into the inclusion criteria. After weighing, group one was given standard food and drink; while groups two to five were fed a high-fat diet of 20-25 g/rat/day (non-tube) and 3 mL (tube), along with a PTU drug at a dose of 0.01% for 14 days. After 14 days, the experimental rats in the treatment groups three, four, and five were given high fat diet and graded doses of celery ethanol extract. Rats were terminated on day 35.

Preparation of Specimen

Liver that had been removed was then placed in a tube containing 10% formalin preservative liquid. The tube containing the liver sample was submitted to the analyst for processing according to standard histological methods with Hematoxylin-Eosin staining. The preparation was divided into eight processes of fixation, trimming, dehydration, clearing, blocking, cutting, staining, and closing.

Specimen Observation

After the preparation was ready, histopathological observations were carried out under a light microscope with 400X magnification in five fields of view. The method used for reading was the semi-quantitative eyeballing method Assessment. of liver damage was performed using the Kleiner scoring as described in table 1.[17]

Table 1 Fatty Liver Histopathological Scoring

Lesion	Score
Lesion < 5%	0
Lesion 5%-33%	1
Lesion 33-66%	2
Lesion >66%.	3

Data Analysis

This study assessed the number of cells that experience degeneration due to the presence of fat in the liver. Data obtained was analyzed statistically with one way ANOVA test if the data were normally distributed. If the data were not normally distributed, the Kruskal Wallis test was used as the alternative test. To determine whether there was a significant difference between the two treatment groups, a post-hoc statistical test was carried out.

RESULTS AND DISCUSSION

Histology of Rat Liver

Microscopic view of the liver was the main focus of this study and was observed based on the presence of vacuoles in the hepatocyte cytoplasm, sinusoidal changes, and shifts in the hepatocyte nucleus.

Observations were made on the hepatocytes where the portal vein extends to the central vein. This is due to the blood supply from the digestive system to the liver via the portal vein. Thus, the hepatocyte damage will be found in the central vein area.[7]

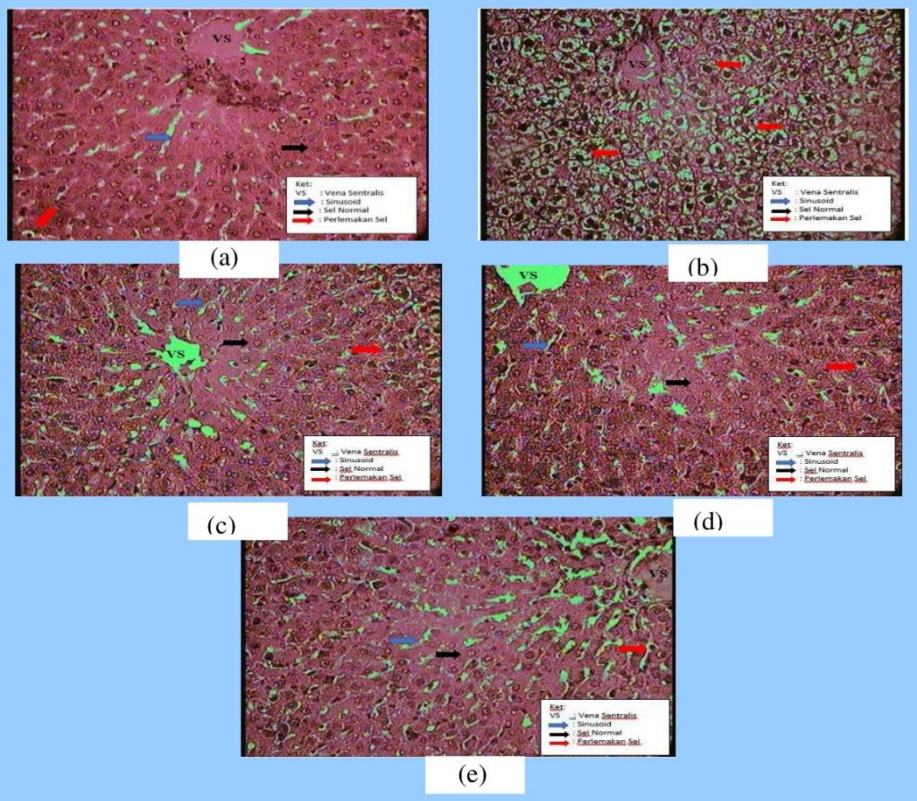

Figure 1. Histopathological image of the liver of rats treated with H&E staining at 400x magnification. VS (centralis vein), blue arrow (sinusoid), black arrow (normal cell), red arrow (cell fat). (a) Negative Control; (b) Positive Control; (c) Group T1; (d) Group T2; (e) Group T3

In normal rats, the hepatocytes near the central vein form a radially arranged plate and the central nucleus and sinusoids are clearly visible. The histopathological changes in the liver in this study are illustrated in Figure 1 part (b). The image shows that the hepatocytes close to the central vein are fatty with a shift in the cell nucleus and the sinusoids appear to be irregular. Fatty is characterized by the presence of clear vacuoles in the cytoplasm, and this condition is found in every observation field.

The results of the histopathological image of the rat liver showed that on the microscopic view, the T1, T2 and T3 groups had a better image than the positive control group. In the T1, T2 and T3 groups, normal hepatocyte cytoplasm, repair of sinusoids, and hepatocyte nuclei were found more.

Effects of Celery Ethanol Extract on Average Fatty Liver

Statistical results show that the data were normally distributed and homogeneous, hence the one-way ANOVA test was used for analysis. Results of this one-way ANOVA test are listed in table 1, showing significant results (p-value≤0.05). In T1, T2, and T3 groups, there was a decrease in the average amount of fatty liver when compared to the positive control. The mean difference between the positive control and the treatment groups demonstrated that the celery ethanol extract could reduce liver fat in rats.

Table 2. Average rat fatty liver cells by group

Group	Mean ±SSD	P - value
Negative control	0,400±0,141	
Positive control	2,520±0,558	
Treatment 1	0,960±0,456	0,00
Treatment 2	0,920±0,641	
Treatment 3	0,600±0,469	

Description:
Treatment 1: Given HFD and Celery Extract dose of 37.5 mg/KgBB/Day
Treatment 2: Given HFD and Celery Extract at a dose of 75 mg/KgBB/day
Treatment 3: Given HFD and Celery Extract at a dose of 150 mg/KgBW/day.
Significance <0.05: significant difference.

The results of the Post Hoc Tukey test showed that there was an effective repair of rat liver fat between the two groups. The results showed that the negative control had an average value of 0.400 with a standard deviation of 0.141, indicating that the average lesions in the negative control was <5% clinically without fattening with a score of 1. The positive control had an average of 2.520 with a standard deviation of 0.558, which indicated an average lesions of > 66%, clinically fattening s with a score of 3. T1, T2, and T3 groups presented a decrease in the average fattiness compared to the positive control, with the average value that was close to the negative control, which was 0.600 in T3, with a standard deviation of 0.469.

There was a decrease in the average number between the positive control and the treatment group because the rat hepatocytes were suspected to be affected by the celery's antioxidants. Celery is a group of polyphenols which contain flavonoids, tannins, and vitamin C that act as reducing agents and free radical scavengers. The function of flavonoids in celery is to inhibit HMG-CoA reductase so that the HMG-CoA is not converted to cholesterol. Vitamin C in celery can reduce oxidative stress; thereby increasing the LPL enzyme activities. The increase in the LPL enzyme could convert VLDL into IDL

so that the accumulation of fat in the liver can be reduced, resulting in histopathological improvement of fatty liver in the liver. [7,18,19,20]

The most effective dose is determined from the smallest dose that can reduce the condition significantly. Celery ethanol extract at a dose of 37.5 mg/KgBW/day is able to significantly improve fatty liver. Thus, a dose of 37.5 mg/KgBW/day of celery ethanol extract is deemed to be the most effective dose to repair fatty liver.

CONCLUSION

Changes in the histopathological image of the liver of rats is shown to be induced by a high-fat diet and improvement in the hepatocyte cytoplasm and sinusoids are found in rats that receive of 37.5 mg/KgBW/day, 75 mg/KgBW/day, and 150 mg/KgBW/days celery extract when compared to the positive control. The dose of 37.5 mg/KgBW/day as the effective dose to improve the histopathological image of rat livers.

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DECLARATION OF INTERESTS

We hereby declare no conflict of interest.

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