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The Effect of Ashitaba (*Angelica keiskei* (Miq.) Koidz.)) Sap on the Total Cholesterol Levels of Cisplatin-Induced Wistar Rats

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Abstract

Cisplatin is a platinum-based anticancer drug that, in long-term use, causes nephrotoxicity due to oxidative stress and increases total cholesterol and triglycerides in animal models. *Angelica keiskei* (Miq.) Koidz., (*A. keiskei*) or Japanese celery ashitaba, has been reported for its antioxidant and nephroprotective activity. This study aims to determine the activity of *A. keiskei* sap on total cholesterol levels of cisplatin-induced Wistar rats. The sap of *A. keiskei*- was freeze-dried until a yield of 3.62% w/v was obtained. The fat content in *A. keiskei* sap powder was obtained at 7.36%. A total of 60 g of *A. keiskei* sap powder was macerated with 96% ethanol solvent (1:10) for 5 x 24 h until the ethanol extract of *A. keiskei* sap (ASEE) of 82.08% w/w was obtained. The pharmacology activity was conducted on male Wistar rats, which were divided into 5 groups, namely normal (treated with CMC Na 0.3%), negative (nephrotoxicity induced with cisplatin 5 mg/kg BW), positive (nephrotoxicity induced with cisplatin 5 mg/kg BW and treated with quercetin 20 mg/kg BW), and two test groups which were nephrotoxicity induced with cisplatin 5 mg/kg body weight and treated with ASEE 1000 mg/kg BW, and ASEE 1500 mg/ kg BW. It was found that neither dose of ASEE altered the total cholesterol levels in cisplatin-induced male Wistar rats and could maintain the cholesterol levels in the normal range.

Keywords: *Angelica keiskei* sap, cholesterol, cisplatin

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Introduction

Cisplatin is one of the most potential and widely used drugs for the treatment of various types of cancer such as testicular, ovarian, breast, bladder, lung, cervical, and several others.[1] Cisplatin works by cross-binding purine bases by interfering with the mechanism of deoxyribonucleic acid (DNA) formation, thereby causing DNA damage.[2] In using cisplatin, the process of mitosis does not occur, causing cytotoxic and apoptosis.[3]

Long-term use of cisplatin and excessive doses can cause toxicity including nephrotoxicity, ototoxicity, hepatotoxicity, hematological, gastrointestinal, and metabolic disorders, e.g., abnormal cholesterol levels.[4–6] Nephrotoxicity on rat-induced cisplatin has been reported for an increase in total cholesterol and triglyceride levels.[7] Metabolic testing such as total cholesterol levels can be used as a biomarker of nephrotoxicity caused by kidney injury due to cisplatin use.[5]

Angelica keiskei (Miq.) Koidz., or Japanese celery ashitaba, contains flavonoids such as quercetin and luteolin, polyphenols, and chalcone compounds including xanthoangelol, 4-hydroxyderricin, and isobavachalcone.[8,9] Flavonoid compounds found in *A. keiskei* have been reported for their activity as antioxidants.[10] The reported pharmacological activities of *A. keiskei* include nephroprotective, antiobesity, antidiabetic, anti-inflammatory, antitumor, and anti-hyperlipidemia.[10–16] This study aims to provide the effect of ethanol extract of *A. keiskei* sap (ASEE) on the cholesterol levels of cisplatin-induced Wistar rats to ensure its safety for dyslipidemia patients.

Methods

1. Equipment

The equipment used was a freeze dryer (Ihanil, Vac 8), rotary evaporator (IKA RV 10), Soxhlet apparatus, desiccator, centrifuge (Hettich EBA 280), multimode reader (Tecan, Infinite 200PRO NanoQuant), glassware (Pyrex), oral sonde, spuit (Terumo), restrainer, and rat cages.

2. Materials

The sap of *A. keiskei* was collected from Mount Rinjani, Sembalun, Lombok-Indonesia. Taxonomic determination (Number.2847/ITI.C11.2/TA00/2023) was conducted by a certified botanist at the Bandung Institute of Technology (ITB), ethanol 96% (pharmaceutical grade), carboxymethyl cellulose sodium CMC Na (pharmaceutical grade), hexane (Merck), cisplatin (PT. Kalbe Farma - Indonesia), quercetin (Sigma Aldrich, CAS No.849061-97-8), ketamine (Ket-A-100 Agrovet market), Cholesterol kit (Linear Chemicals, SLU).

3. Procedure

3.1 Extraction

Approximately 2L of *A. keiskei* sap was freeze-dried at a temperature of -80°C to obtain yellow sap powder *A. keiskei*. The sap powder was macerated in 96% ethanol solvent (1:10) for 5 x 24 hours. The filtrate was collected and evaporated at a temperature of 60°C, with 85 rpm for 1 hour until a thick extract of ASEE was obtained.[13]

3.2 Determination of Fat Content on Sap Powder A. keiskei

Approximately weight of 1g of the sap powder was wrapped in filter paper and put into a Soxhlet flask. The hexane solution was added to the Soxhlet flask and the mixture was heated for 6 hours, then was evaporated at 105°C until a crude fat was obtained. The flask containing the crude fat was cooled in a desiccator and weighed (Soxhlet method: SNI 01-2891-1992).

$$\% \text{ fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100\% \quad (17)$$

3.3 Animal Acclimatization

The experiment was carried out on 25 male Wistar rats (*Rattus norvegicus*), aged between 6-8 weeks, and weighed between 200-220 g. The study was approved by the Research Ethics Committee of Padjadjaran University (Document No. 1241/UN6.KEP/EC/2023). The rats are placed in plastic tub cages with net-shaped wire lids and cage mats using clean rice husks which are replaced every 2 days. Animals were given a light-dark cycle for 12 hours and given standard feed and ad libitum drinking water. The standard pellet feed used low fiber (5%), protein (20%), and fat (5–10%).

3.4 The Effect of ASEE on the Total Cholesterol Level of Wistar Rats

The male Wistar rats were distributed randomly into five groups (5 rats each), and were treated as follows:

- The normal group was orally given a 0.3% CMC Na suspension for 10 days.
- The negative group was given CMC Na 0.3% orally for 10 days.
- The positive group was given 20 mg/kg of quercetin BW orally for 10 days.
- Treatment 1 group was given an ASEE dose of 1000 mg/kg BW orally, 1 time a day for 10 days.
- Treatment 2 was given an ASEE dose of 1500 mg/kg BW orally, 1 time daily for 10 days.

All groups, except the normal group, were nephrotoxicity-induced using cisplatin 5 mg/kg BW intraperitoneally on day 7.

At the end of the experiment, rats were sequentially sedated with ketamine at a dose of 100 mg/kg BW.[18] Blood samples of as much as 3 mL were taken in the caudal vein of the mouse's tail. Each blood sample obtained from each mouse was collected into a plain sample vial with a capacity of 4 mL that was clearly labeled.[19]

The serum was separated by centrifugation at 11,000 rpm for 10 minutes to measure the total cholesterol level. 10 µL of serum and standard 1 mL of reagent each were added and incubated for 10 min at room temperature. Absorbance was measured at 500 nm using a multimode reader. The absorption results were calculated using the following formula:

$$\text{Cholesterol Total } \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{A Sample}}{\text{A Standard}} \text{ x C Standard}$$

3.5 Data Analysis

Data analysis was performed using GraphPad Prism 8.1.2. The difference in the nephroprotective activity ratio of test animals was analyzed using the one-way analysis of variance (ANOVA) test at a confidence level of 95%. The Bonferroni test determined the mean significant difference between each group with $p < 0.05$.

Results and Discussion

1. Extraction and Fat Content

The sap of *A. keiskei* used in this study was obtained from the stem part of the plant. The characteristics of *A. keiskei* sap include liquid, solid yellow, and slightly sticky. From the freeze-drying process, dry powder of *A. keiskei* sap was obtained by 72.4 g with a yield of 3.62% w/v. This result was smaller than other studies on *A. keiskei* sap obtained a yield of 4.20% w/v.[13] The *A. keiskei* sap powder has a yellow and specific odor. The maceration yielded 49.25 g (82.08% w/w) ASEE.

A. keiskei contains several phytonutrients, including protein, sugar, and electrolytes such as calciums, ferric, pottasium, magnesium, and natrium.[20] A smaller fat content of 7.36% was obtained in our ASEE compared to a previous study, which was 12%.[21] It was reported previously that the sap of *A. keiskei* contains flavonoids, polyphenols, and chalcone compounds.[8,9,13] Thus, confirming its potential pharmacology activity is necessary.

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A small increase in total cholesterol levels was observed in the rats treated with ASEE, although not significantly when compared to the negative group (cisplatin 5 mg/kg BW) (Fig.1). In the positive group, quercetin 20 mg/kg BW could reduce the total cholesterol levels, although not significantly when compared to the negative group (cisplatin 5 mg/kg BW) (Fig.1). However, the success of the induction is proven by comparing the data of the negative control group with that of the normal group.

2. The Effect of ASEE on Total Cholesterol Levels of Wistar Rats

A small increase in total cholesterol levels was observed in the rats treated with ASEE, although not significantly when compared to the negative group (cisplatin 5 mg/kg BW) (Fig. 1). In the positive group, quercetin 20 mg/kg BW could reduce the total cholesterol levels, although not significantly when compared to the negative group (cisplatin 5 mg/kg BW) (Fig. 1). However, the success of the induction is proven by comparing the data of the negative control group with that of the normal group. The use of cisplatin in excess doses leads to nephrotoxicity.[22] Poor kidney function generates lipid metabolism disorders, including increased total cholesterol, triglycerides, and changes in lipoprotein composition, which can later develop into vascular disease.[23]

Rats treated with cisplatin 5 mg/kg BW intraperitoneally did not experience an increase in cholesterol when compared to the normal group. The dose used for induction should likely be higher. A previous study described that cisplatin at a dose of 5 mg/kg BW was reported to accumulate mostly in the inner cortex and corticomedullary junction of the rat kidney, which is the location of proximal and distal tubules (on day 5). However, when a lethal dose was used (16 mg/kg BW) cisplatin was detected in renal columns (on day 3).[24,25] Moreover, cisplatin at a dose of 20 mg/kg BW can inhibit fatty acid oxidation in animal models.[6]

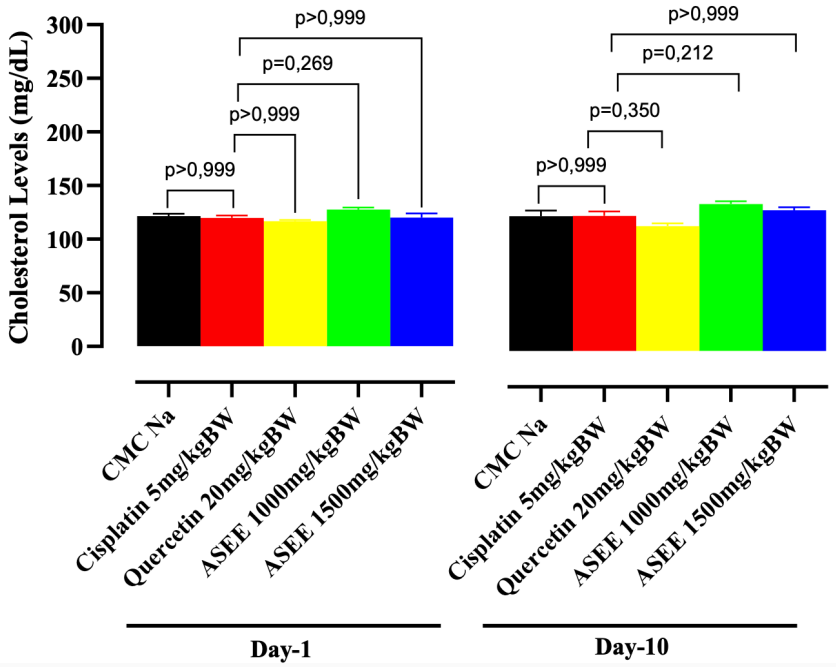

Figure 1. The Statistical Analysis Result on the Effect of ASEE on the Serum Total Cholesterol Level of Cisplatin-Induced Rats at Day 1 and Day 10

Table 1. The Effect of ASEE on the Serum Total Cholesterol Level of Cisplatin-Induced Rats at Day 1 and Day 10

Groups	Total Cholesterol (mg/dL)	
	Day-1	Day-10
Normal (CMC Na 0.3%)	123.23 ±0.80	125.60 ±2.89
Negative (Cisplatin 5 mg/kg BW)	119.86 ±2.35	125.83 ±2.39
Positive (Quercetin 20 mg/kg BW)	116.76 ±1.05	116.60 ±2.48
Treatment 1 (ASEE 1000 mg/kg BW)	127.62 ±1.97	137.05 ±2.43
Treatment 2 (ASEE 1500 mg/kg BW)	120.11 ±2.65	131.18 ±2.74

In this study, neither dose of ASEE altered the total cholesterol levels of cisplatin-induced male Wistar rats. They maintained the cholesterol levels in the normal range (<200 mg/dL). Conversely, a previous study reported that treatment with *A. keiskei* sap dose of 1000 mg/kg BW resulted in increased total cholesterol levels significantly compared to normal groups.[26] Interestingly, a clinical trial described that adult participants consuming *A. keiskei* (Chalcurb®) 220 mg/capsule showed no significant changes in total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and overall glucose levels (fasting glucose and HBA1c).[12]

Previous studies described that isoflavones, flavones, and flavanones could reduce blood cholesterol levels by inhibiting cholesterol synthesis and increasing LDL receptor expression.[27,28] It was suggested that this pharmacology activity may resulted from the effect of flavonoids on SREBP-2.[29–32]

Conclusion

The ethanol extract of dry sap powder of *Angelica keiskei* (Miq.) Koidz. (ASEE), or Japanese celery ashitaba, collected from Mount Rinjani, Sembalun, Lombok-Indonesia, contained 7.36% of fat. In this study, both doses of ASEE (1000 mg/kg BW and 1500 mg/kg BW) did not alter the total cholesterol levels of cisplatin-induced male Wistar rats and maintained the cholesterol levels in the normal range (< 200 mg/dL). It is obvious that ASEE is safe to be consumed by patients with dyslipidemia, however, further studies on the molecular pathway affected by ASEE are interesting to explore.

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Conflict of Interest

The authors declare no conflict of interest.

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