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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the hypoglycemia effect of alkaloid compounds (Alkaloid crude extract) from oil free mahagony seeds (*Swietenia macrophylla*, King).
Study Design: Experiments of alkaloid compounds isolation and *in vivo* test for hypoglycemia effect.
Place and Duration of Study: Sample Mahagony seeds of species *S. macrophylla*, King obtained from Central Java concession plants between May 2013 and July 2013.
Methodology: The oil of mahagony seeds was isolated by maceration using n-hexane for 3 x 24 hours. Alkaloid compounds from oil free mahagony seeds was carried out using methanol and 10% acetic acid solutions. Test of hypoglycemia effect treated on wistar rats. Before treatment with alkaloid compounds, the rats were given no food for 24 hours. Negative control was aquadest

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treatment and positive control was glibenclamide treatment. The dose of testing material were 5 mg/kg.rat-wght., 10 mg/kg.rat-wght., and 20 mg/kg.rat-wght. Data collection was performed by measuring UV absorption at λ 630 nm of the blood serum at 30, 60, 90, 120, 150 minutes after the treatment.

Results: The test of hypoglycemia effect was t-test in 5% significancy level, and t- table value at 2.021, showed that significant differences of blood glucose level before and after treatment with alkaloid compounds of 5 mg/kg.rat-wght and 10 mg/kg.rat-wght.

Conclusion: Alkaloid compounds had the hypoglycemia effect.

Keywords: Alkaloid; mahagony seed; blood glucose.

1. INTRODUCTION

Mahogany (*Swietenia macrophylla*, King) is a tropical tree native Central America and South America and one of the largest species of the genus Swietenia and has a very important economic value. Mahagony seed has been using by people on the island of Java as a treat diabetes, high blood pressure, gout, eczema, fat bullets, and colds [1]. Diabetes mellitus is a group of symptoms that arise in a person, characterized by blood glucose levels that exceed normal values (hyperglycemia) due to the body lacks insulin. Diabetes mellitus is a chronic disease, which occurs when the pancreas did not produce enough insulin, or when the body could not effectively use the insulin that is produced. Diabetes mellitus is a disease in the world with a high incidence and high mortality, causes about 5% of all deaths globally each year. As many as 80% of people with diabetes live in developing countries and the majority of patients are middle age (45-64 years). Deaths due to diabetes increased by 50% in 10 years [2]. Mahagony seeds have been using such diabetes mellitus drugs based solely on experience from generation to generation, so it was necessary to explore the compounds contained therein. Research [3,4] concluded that the mahagony seeds contain alkaloid compounds, but these studies have not described the effects of alkaloid compounds to decrease blood glucose levels.

Based on the above, it can be concluded that although mahagony as a medicinal plant that widely available in Indonesia, but in-depth scientific research has not been doing so much. Research needs to be done to determine the content of alkaloid compounds that can be used effectively and efficiently, synthesized into other compounds more useful, or as a model for the synthesis of compounds. This study aims to isolate the alkaloid compounds from mahagony seeds and the effects to decrease blood glucose levels on the Wistar rat (*Rattus novergicus*).

2. METHODS

2.1 Collection of Plant Materials

Mahagony seeds of species *S. macrophylla,* King obtained from Central Java concession plants and identity defined in Section Pharmacognosy Laboratory of Biology, Faculty of Pharmacy, Gadjah Mada University Yogyakarta, Indonesia.

2.2 Isolation of Alkaloids Compounds

Alkaloid isolation procedure was a modification of that has been done [5,6]. Mahagony seeds are cleaned, dried, and blended to obtain powder. A total of 3000 g of mahagony seeds powder macerated with n-hexane for 24 hours (3 times) to take its oil. The mahagony residue as oil-free mahagony seeds were extracted with methanol at room temperature by maceration for 3 x 24 hours to take the compounds contained therein. The extract obtained was concentrated under reduced by pressure Buchii evaporator to obtain a dry extract. A total of 50 g of the dried methanol extracts placed in the separating funnel, added 50 mL of 10% acetic acid and 50 mL of diethyl ether, shaken, and separated. Phase of acid solution was taken and added 50 mL of 10% ammonia solution, added $CHCl_3$ and separated. The organic phase ($CHCl_3$ extract) contains primary, secondary, and tertiary alkaloids, which identified by Dragendorff Reagents gave orange precipitation, while the inorganic phase (alkaline solution) contains quaternary alkaloid. Further fractionation performed to obtain the pure alkaloid. Crude extract alkaloids detected by TLC and fractionated by liquid vacuum chromatography used polarity increasing eluent (n-hexane-EtOAc, EtOAc, EtOAc-MeOH). Fraction that still contains more than one compounds further fractionated successively used radial chromatography to obtain the pure compounds.

2.3 *In vivo* Test for Hypoglycemic Effect of Alkaloid Compounds

The test of hypoglycemia procedure was a modification of [7,8,9,10,11]. This study used white rats (Rattus novergicus) with an average age between 2-3 months and weighing between 150-200 grams. The rats were obtained from Faculty of Pharmacy, ethical clearance data and approval number handled by Faculty of Pharmacy, Gadjah Mada University. The research was done in the laboratory of Pharmacology Laboratory and Biological Sciences Laboratory, Faculty of Medicine, Gadjah Mada University Yogyakarta, Indonesia.

The rats must be in the same condition in the initial time. All rats were fed the same food and drink as well as adapted for 1 week. Then 24 hours before research the rats were fasted in a way not fed but were given just drinking water. The rats were randomly divided into five groups, each group consisting of six rats (There were 30 rats at all). There were group I (distilled water treatment as a negative control), group II (glibenclamide treatment as a positive control), group IIIA (alkaloid treatment 5 mg/ kg.rat-wght), group IIIB (alkaloid treatment 10 mg/ kg.rat-wght), and group IIIC (alkaloid treatment 20 mg/ kg.rat-wght). Giving distilled water, glibenclamide, and alkaloid compounds used a stomach sonde. The rats must be fasted for 24 hours in a way not fed but were given drinking enough water for 24 hours before treatment. The first day for group I, second day for group II, and so on. At the first, the tail of rats cut to take the rat blood (t_o). Then, the rats were given 2 mL of 17.5% glucose solution. Giving distilled water for group I, glibenclamide for group II, and alkaloid compounds for group III, used a stomach sonde. The rats blood was taken 30, 60, 90, 120, and 150 minutes after treatment with the testing material. The blood glucose levels measured used o-toluidine method. As much as 0.1 ml of blood was taken, plus 1 mL of trichloroacetic acid, and then centrifuged for 5 minutes. As much as 0.5 mL of supernatant was taken and put into a test tube. While it made a comparison is 0.1 mL of standard glucose solution (100 mg/100 mL) was added with 1 mL of trichloroacetic acid. As much as 0.5 mL of this mixture was taken and put into a test tube. The blank solution was made of 0.5 mL of trichloroacetic acid. The three tubes each containing supernatant, standard solution, and the blank solution was added 2 mL o-toluidine, heated in a boiling water bath for 8 minutes, and read the absorbance for each tube at λ 630 nm. Blood glucose levels determined by the formula:

Blood glucose levels = $(A_{sampel}-A_{blanko} / A_{standar}-A_{blanko})$ x 100 mg/100 mL

2.4 Statistic Analysis

Blood glucose levels were analyzed by t test: t = B / Sb

Where in B was the difference between each pair of observations and Sb was standard error. Statistical analysis was carried out manually.

3. RESULTS

Data was collected before and after glucose treatment, and at minute 30, 60, 90, 120, and 150 after administration of testing material (alkaloid compounds).

The average, percentage of blood glucose levels decreasing, and t value were shown in Table 1.

Table 1. The average, percentage of blood glucose levels, and t value

No	Pair	Average blood glucose levels (mg)	Blood glucose levels decreasing (%)
1	Group I	104,17	
	Group II	49, 60	52,39
2	Group I	104,17	
	Group IIIA	158,23	-51,90
3	Group I	104,17	
	Group IIIB	84,28	19,09
4	Group I	104,17	
	Group IIIC	111,87	7,39

4. DISCUSSION

In this study, the test animals such as male wistar rats with reasoning that the type of rats were relatively resistant to various treatment and not temperamental, which expected to persist during the study. Statistical test of the data obtained by the "paired sample test", a useful test to perform test of two related samples or so-called "paired samples", in this case compared blood glucose levels before and after the rats were treated with testing materials, with a significance level of 5%, degrees of freedom 41, and the t value was 2,021. The t-value of Group I-II was 6,667; Group I-IIIA was -8,149; Group I-IIIB was 2,931; and Group I-IIIC was -1,140. The t test calculation results for each group showed a difference between the effects of alkaloid compounds to decrease the rat's blood glucose levels. Based on the t value calculating, a significant difference was in group IIIA and IIIB. The group IIIC caused no significant difference.

The average of blood glucose levels showed potent sequence of testing materials to decrease the blood glucose levels on the rats, which in group II > group IIIB.

Results of calculation of the average and the t value showed that the potent of alkaloid compounds to decrease the blood glucose levels (compared to the negative control) was alkaloid of 10 mg/ kg.rat-wght (group IIIB). While alkaloid of 5 mg/kg.rat-wght (group IIIA), although caused the significant difference but not potent to decrease the blood glucose levels. Group IIIC decreased the blood glucose levels but caused no significant difference.

The results showed that alkaloid 10 mg/kg.rat-wght. decreased the blood glucose levels. In this case there were disorder that when the potent was alkaloid 10 mg/kg.rat-wght to decrease the blood glucose levels, should the alkaloid 20 mg/kg.rat-wght that contain more testing material had the highest potent, but in fact was not. This was probably caused by the optimum dose was 10 mg/kg.rat-wght, so the adding of the testing material didn't cause the higher potent, event lower potent. The experimental results indicate that the drug at a dose that was too low or too high will cause ineffectiveness. So, drug consumption must be wisely and according to the doctor receipt. Alkaloid compounds function as well as antifeedant. The results of antifeedant activities were dose-dependent. The antifeedant effect of erythrinaline alkaloids may be useful for crop protection [12]. A decrease in blood glucose in rats caused by secondary metabolit compounds contained in natural materials, rich in antioxidants that could neutralize free radicals and might help lower blood glucose levels and overcome the fatigue caused by blood glucose levels were not balanced [13,14,15]. Research of [15] concluded that ethanol extracts from various parts of the plant showed antihyperglycemic. Among 14 plant extracts tested, Soga (bark and flower parts) showed very large fenolik content. Antihyperglycemic activity of plant extracts was considered much better than acarbose antidiabetic drugs. Alkaloid compounds from oil free mahagony seeds had the hypoglycemia effect to the rats. Secondary metabolites in mahogany seeds interacted and decreased the blood glucose levels.

5. CONCLUSION

The conclusion of this research as follows:

1. Isolation of alkaloid compounds of mahagony seed can be carried out used methanol-acetic acid solution.
2. Alkaloid compounds had the hypoglycemia effect to the rats.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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