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RESEARCH ARTICLE

Hypoglycemic activity test on smooth pigweed (*Ammaranthus Hybridus L*) leaf water extract on male Wistar rats

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Abstract

Introduction: Type 2 diabetes mellitus consists of an array of dysfunction characterized by hyperglycemia. The activity of smooth pigweed (*Amaranthus hybridus L.*) leaves water extract on male Wistar rats. **Aims:** This research was started by supplying simplicia, making smooth pigweed leaves water extract, and testing the hypoglycemic activity of smooth pigweed leaves water extract on male Wistar rats. **Methods:** The glucose tolerance method was used to determine the hypoglycemic activity of smooth pigweed leaves water extract. Male white rats were divided into five groups of six rats each: a positive control group (0.5% of tragacanth suspension), a comparison group (Diabinese suspension at a dose of 22.5 mg/kg body weight (bw)), and three test groups at doses of 50 mg/kg bw, 100 mg/kg bw, and 150 mg/kg bw. **Results and conclusions:** The most significant hypoglycemic activity was seen with the dose of 150 mg/kg bw in comparison with the control group at 90 minutes.

Introduction

Diabetes mellitus is a non-contagious disease condition that arises from both relative and absolute insulin deficiency. Hyperglycemia is due to the absorption of glucose into inhibited cells and disrupted metabolism. Indonesian tropical forest medicinal plant species are used as raw materials in the herbal medicine industry, especially those against diabetes mellitus, such as smooth pigweed (*Amaranthus hybridus* L.), java plum (*Eugenia cumini* MERR), bilimbi (*Averrhoa Bilimbi* L.), and others. Knowledge of the medicinal and healing properties of a plant is generally based on natural clues or animal behaviour. For example, heart-shaped leaves cure cardiac disease, the yellow parts of a plant, such as turmeric and *temulawak*, are used in jaundice, sick animals that eat some types of plants indicate the plant is medicinal (Douglas *et al.*, 2000).

The traditional medicine industry and phytopharmacy have utilised various plant species as raw materials to treat several diseases, including fever, diarrhoea, malaria, hypertension, and thrush. (Anonim, 1989) Forest plants and gardens have many more medicinal potentials. Many people turn to alternative medicine for treating disease because they considered it safe with minimal side effects.

The study aims to determine the hypoglycemic activity of *Amaranthus hybridus* L water extract and the most effective concentration in lowering blood sugar levels in male Wistar rats.

Method

The study started with providing and characterising Simplicia, manufacturing smooth pigweed leaf extract,

and testing its hypoglycemic activity in male Wistar rats. Blood collection was carried out by cutting ± 0.5 cm of rat tail and measuring blood glucose levels in UV spectrophotometry (Evelyn, 1998).

The glucose tolerance method clinically used to diagnose diabetes was applied to determine the hypoglycemic activity of smooth pigweed leaf extract. (Gruben, 1981; Guyton & Hall, 1997) The male Wistar rats were divided into five groups of 6 mice, namely the positive control group (0.5% of tragacanth suspension), the comparison group (Diabinese suspension at a dose of 22.5 mg/kg body weight (bw)), and three test groups at doses of 50 mg/kg bw, 100 mg/kg bw, and 150 mg/kg bw, respectively (Hyne, K, 1987; Hoffbrand, 1996).

Materials

Ingredients

Test dosage (smooth pigweed leaf water extract), comparative substance (chlorpropamide 250 mg tablets), distilled water, standard glucose solution, suspended material (tragacanth), glucose reagent, NaCl 0.9%, alcohol 70%, oral glucose, gelatin, vanillin sulfate, dragendorf, NH4OH 10%, FeCl3, HCL 2N, alcohol, chloroform, Burchard Liebermann, ether, Mayer, Mg powder, filter paper (PT Eisai Indonesia, 1983).

Tools

Oral feeding tube, Eppendorf tube, centrifuge, scaled pipettes, UV-VIS spectrophotometry (Shimadzu UV type 1601), water stems, evaporation cups, crews, analytical balances (sartorius), 1 mL and 2.5 mL syringes, measuring glasses, stirring rods, ovens, rat scales, heaters, test tubes, micropipettes, desiccators, mortars, stampers, scissors, furnaces, glass funnels (Prof. Dr Arjatmo, 2016).

Experimental animals

Male, white, healthy Wistar rats, weighing around 200-300 g and aged about three months. The 30 rats were grouped randomly, using six rats per group: one positive control group, one comparison group, and three group tests (Roth, 1998).

Location and time of pick-up of smooth pigweed leaves (Amaranthus hybridus L)

Smooth pigweed leaves were collected from PT. Esai Sukabumi around January 2020, where the material was denominated in Herbarium Bogoriense, the botanical field of Biology Research Center-LIPI, Bogor. (Immaculata, 1991) The leaves were cleaned under running water, drained and dried by the wind away from direct sunlight. After they dried, the leaves were ground into powder (Immaculata, 1991).

Examination of the characteristics of smooth pigweed leaves

One gram of Simplicia was carefully weighed in a lidded scale bottle that had previously been heated at 105^{o}C for 30 minutes and maintained at this temperature (Ministry of Health and Social Welfare of Indonesian Drug Plant Invetaris, 2000). The simplicity in the bottle was flattened to form 5 -10 mm thick, then it was put in the drying room with the lid open and dried at a temperature of 105 ^{o}C until the weight remained. Every time before weighing, the bottle was let in a closed, cold state in the excavator at room temperature.

Determination of ash content

Approximately 2 g of Simplicia powder were inserted into the incandescent silicate crew at 500^{o}C, flattened slowly at 500^{o}C until the charcoal was depleted, then cooled and weighed. If the charcoal could not be removed, hot water was added then filtered through ash-free filter paper. Leftovers and filter paper were then heated in the silica crew until the weight was fixed, and then they were weighed. Ash levels were calculated against air-dried materials (Ministry of Health, 1995; Mutchler, 1991).

Making smooth pigweed leaves water extract

A total of 200 grams of smooth pigweed leaves were carefully weighed, wrapped in flannel cloth, added to 2 litres of distilled water, and boiled to half the volume. The procedure was repeated three times with 4 litres of filtrate. The extract was evaporated with the water handler until dry extract was obtained (Montgomery, 1993).

Comparative set-up

A total of 20 tablets were weighed. Their weight was 9.294 g; thus, the average weight of one tablet was 0.4647 g (Ministry of Health of the Republic of Indonesia, 1995). The tablet was finely ground in a mortar, weighed as much as 0.225 g, containing 121.04 mg of chlorpropamide, then dissolved in 50 mL of warm water. First, 0.25 grams of tragacanth was suspended in 50 mL of warm water while stirring, then 0.225 g of the comparative tablet was added to the suspension of the tragacanth little by little until they were perfectly mixed (PT Eisai Indonesia, 1983).

Testing hypoglycemia on smooth pigweed leaves water extract

Test animals were satisfied in advance for 18 hours then divided into five groups, namely the positive

control group, the comparison group, and three test groups: Dose 1 (50 mg/kg bw), Dose 2 (100 mg/kg bw), and Dose 3 (150 mg/kg bw). On the day of the experiment, all animals were weighed, identified, and placed into the restrictive box. Then, a blood test (t=0) was performed through the tail veins of the male Wistar rats. After it was ready, rats were given the test suspension orally. At the 30th minute, a snapshot of blood from the tail vein was taken, after which it was immediately given a glucose induction of 50% orally administered at a dose of 1 g/kg of body weight to all groups except the negative control group. After oral administration, blood tests were performed again at the 60th, 90th, 120th, 150th, and 180th minutes. Footage of blood accommodated in an Eppendorf tube was then centrifuged for 15 minutes at 3.500 rpm, and the clear supernatant was taken using a 20 micropipette. Blood serum was transferred into small tubes and added with 2 mL of glucose reagent to determine blood glucose levels. Measurement of blood glucose levels was carried out using a spectrophotometer at a wavelength of 546 nm.

Analysis

All data were evaluated statistically using ANOVA and t-tests using SPSS software. The decrease in blood glucose levels in the test group was measured by comparing the results obtained with the results from the positive control group.

Calculation

The formula for calculating blood glucose levels is as follows:

Blood glucose level = At/As x 100 mg/dl[13]

Description:

- A = Absorption at maximum wavelength
- At = absorption of test solution
- As = absorption of raw solution (standard glucose solution)

Application for ethical clearance

Ethical clearance was provided by the commission of research ethics for research involving living beings (humans, animals, and plants. Ethical clearance was submitted to the health research ethics committee of the Medical Faculty of Padjajaran University and Hasan Sadikin Bandung hospital. Ethical clearance was obtained by submitting the following documents: Form 1 (questions about complete and correct research), Form 2 (research information), Form 3 (letter of approval to participate in the study/informed consent), and Form 4 (the researcher's profiles and research proposal).

Results

Table I shows hypoglycemic activity test results of smooth pigweed by comparing one dose test group against the positive control group.

Table I: Hypoglycemic activity test results comparing one dose test group against the positive control group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Positive control	Dose 150 mg/kg bw	
0	85.38 ± 1.24	84.92 ± 2.82	0.763
30	87.61 ± 1.39	87.35 ± 1.35	0.304
60	103.27 ± 1.36	97.53 ± 0.87	0.689
90	115.89 ± 0.03	107.12 ± 1.03	0.465
120	122.58 ± 5.60	99.49 ± 1.66	0.0001
150	115.71 ± 2.74	94.37 ± 1.90	0.0001
180	113.62 ± 7.17	95.43 ± 2.86	0.002

Table II shows hypoglycemic activity test results of smooth pigweed by comparing two dose test groups against the positive control group.

Table II: Hypoglycemic activity test results comparing two dose test groups against the positive control group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Positive control	Dose 150 mg/kg bw	
0	85.38 ± 1.24	85.35 ± 2.70	0.982
30	87.61 ± 1.39	85.05 ± 3.28	0.136
60	103.27 ± 1.36	93.17 ± 1.52	0.0001
90	115.89 ± 0.03	104.06 ± 2.45	0.0001
120	122.58 ± 5.60	96.59 ± 1.37	0.0001
150	115.71 ± 2.74	94.52 ± 1.76	0.0001
180	113.62 ± 7.17	96.57 ± 1.14	0.003

Table III shows hypoglycemic activity test results of smooth pigweed by comparing three dose test groups against the positive control group.

Table III: Hypoglycemic activity test results comparing three dose test groups against the positive control group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Positive control	Dose 150 mg/kg bw	
0	85.38 ± 1.24	82.72 ± 0.85	0.004
30	87.61 ± 1.39	85.23 ± 1.94	0.025
60	103.27 ± 1.36	86.46 ± 1.32	0.0001
90	115.89 ± 0.03	96.14 ± 2.71	0.0001
120	122.58 ± 5.60	85.57 ± 2.00	0.0001
150	115.71 ± 2.74	84.79 ± 1.63	0.0001
180	113.62 ± 7.17	93.65 ± 2.24	0.002

Table IV shows the results of the hypoglycemic activity test of smooth pigweed by comparing the three-dose test groups against the comparison group.

Table IV: Hypoglycemic activity test results comparing three dose test groups against the comparison group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Comparison group	Dose 150 mg/kg bw	
0	79.30 ± 14.93	82.72 ± 0.85	0.590
30	87.02 ± 2.01	85.23 ± 1.94	0.282
60	76.55 ± 1.44	86.46 ± 1.32	0.0001
90	73.41 ± 2.11	96.14 ± 2.71	0.0001
120	54.60 ± 1.76	84.57 ± 2.00	0.0001
150	72.50 ± 5.67	84.79 ± 1.63	0.001
180	72.01 ± 4.49	93.65 ± 2.24	0.0001

Discussion

In this study, a test on the characteristics of Simplicia and phytochemical filtering of smooth pigweed leaf (*Amaranthus hybridus* L.) was carried out. When examining simplicial characteristics, the total ash content was 17.7 %, and drying shrinkage was 2.5 %. The result of the phytochemical analysis showed that smooth pigweed leaves contain flavonoids, saponins, tannins, alkaloids, steroids, and triterpenoids (Montgomery *et al.*, 1993).

One component of smooth pigweed that can lower blood glucose levels is flavonoids, in accordance with the literature. The selection of the test group, Dose 1 (50 mg/Kg BW), Dose 2 (100 mg/Kg BW), Dose 3 (150 mg/Kg BW), was adjusted to use in the community (Sulistina G. Ganiswarna *et al.* 1995).

At minute 0 (t=0), the results showed that the initial blood glucose levels of the rat did not provide a noticeable difference between the control group and the test group.

At the 30th minute (t=30), no statistically noticeable difference was shown because the number of efficacious substances applied at doses of 50 mg/kg bw, 100 mg/ kg bw, and 150 mg/ kg bw did not affect blood glucose levels.

At the 60th minute (t=60), there was no noticeable difference of all three groups (50 mg/ kg bw, 100 mg/ kg bw, and 150 mg/ kg bw) compared to the control group.

At the 90th minute (t=90), the three groups showed a noticeable difference when compared to the control group because the number of efficacious substances that were applied had an effect on blood glucose levels.

At the 120th minute, the three groups showed a statistically noticeable difference when compared to the control group.

This study also used comparison tablets Diabenese 22.5 mg/ kg bw (containing chlorpropamide 121.04 mg) to see the equality of some variations of the spinach test given. In the third trial, the test group showed a lower hypoglycemic activity when compared to the comparison group (Diabenese), indicating there was no equivalence between the test dose and the comparison group (Toro Gelson, 1976).

Conclusion

The smooth pigweed plant contains flavonoids, saponins, tannins, alkaloids, steroids, and triterpenoids. At the characteristic examination of Simplicia, the ash content was 17.7 % and the drying shrinkage 2.5 %. All test doses given were 50 mg/ kg bw, 100 mg/ kg bw, and 150 mg/ kg bw, which produced a lowering of blood glucose levels. The highest drop in blood glucose levels was at the dose of 150 mg/ kg bw at the 90th minute (t = 90) and the 120th minute (t = 120). Furthermore, it was followed successively by the doses of 100 mg/ kg bw and 50 mg/ kg bw. Hypoglycemic activity for a dose of 150 mg/ kg bw is still lower when compared to that of the comparison group (Diabinese tablets containing 250 mg of chlorpropamide at a dose of 22.50 mg/ kg bw).

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