EFFECT OF *Archidendron pauciflorum* IN DIET ON HEMATOLOGY, GLUCOSE, LIVER FUNCTION, AND WEIGHT INDUCED-RATS WITH HIGH-FAT DIET

Hilmi Al Wahid[1], Aziiz Mardanarian Rosdianto[2], and Ronny Lesmana[2]

[1]Study Program of Veterinary Medicine, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

[2]Physiology Division, Department of Biomedical Science, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Abstract

A. pauciflorum is known to have various pharmacological activities, including antimicrobial, antioxidant, antidiabetic, antidiarrheal, anticancer, and anti-gastric activity. In-vivo studies were conducted to determine the therapeutic and metabolic effects of A. pauciflorum. This research aimed to study the Effect of A. pauciflorum added to the diet in rats. The treatment group was divided into 4 to six mice each. The treatment group consisted of normal control, a negative control, and two test groups. The normal control group was given a normal diet (NCD), and the negative control group was given a high-fat diet (HFD) diet containing palmitic fatty acid to be overweight. Both test groups were given a normal diet and preparations containing A. pauciflorum (NCD-JK), and high-fat diets were fed with A. pauciflorum (HFD-JK). The treatment was carried out for nine weeks. Adding A. pauciflorum to feed can increase hemoglobin, RBC, WBC, glucose, and body weight caused by the content of Se in A. pauciflorum, which can protect cells from free radicals and affect the composition of gut microbiota. However, the administration of A. pauciflorum did not affect the platelets, ALT, and AST levels, which might be due to the lack of Se content in A. pauciflorum.

Keywords*: Archidendron pauciflorum, hematology, glycemia, liver function, body weight*

Korespondensi: Ronny Lesmana, Physiology Division, Department of Biomedical Science, Faculty of Medicine, Universitas Padjadjaran, Bandung, 45363, Indonesia. email: ronny@unpad.ac.id

INTRODUCTION

As many as 49.5% of the Indonesian population still use traditional medicine in the form of herbs, 4.5% consume traditional medicine every day, and the rest consume it occasionally[1]. These traditional medicines can be in the form of their potion of traditional medicine or made in the industry. On the other hand, people in developed countries are trying to reduce their dependence on medical drugs and use herbal ingredients derived from plants, which is the cause of the increasing demand for medicinal plants[1,2].

One of the diseases that can be treated using herbal plants is metabolic syndrome. Metabolic syndrome describes metabolic disorders like hypertension, obesity, insulin resistance, and cardiovascular disease. Insulin resistance is one of the predisposing factors for atherosclerosis[3]. It causes microvascular damage caused by lipids, oxidized Low-Density Lipoprotein (LDL) particles, and free fatty acids that activate the inflammatory process and trigger disease. This inflammation is responsible for all atherosclerotic processes, from early endothelial dysfunction to the formation of atherosclerotic plaques[4]. According to the Global Burden of Disease Study (GBD), atherosclerosis is a 30% cause of death globally and is the number one cause of death in several countries. In 2012 around 7.4 million people died from atherosclerosis, which occurred in several developing countries with low to middle-income levels.

Medicinal plants used to treat atherosclerosis include Archidendron pauciflorum (local name jengkol). A. pauciflorum has various therapeutic effects such as cleansing the blood, treating diarrhea, antioxidant, antimicrobial, inhibiting hypertension, inhibiting the respiratory disease, lowering blood sugar levels, and increasing appetite and weight[5]. It contains selenium which functions to regulate selenoproteins in the body. Selenoproteins consist of glutathione peroxidases (GPX) which play a role in detoxifying intracellular hydrogen peroxide, thereby protecting cells from lipoprotein and deoxyribonucleic acid (DNA) damage. At the same time, thioredoxin reductases (TXR) regenerate thioredoxins, thereby balancing the redox status of cells[6].

Based on the background, A. pauciflorum is known to have many activities and properties. However, the literature searches in internationally reputed journals are also very limited for in vivo studies. So that further research was conducted on the Effect of A. pauciflorum added to feed on the hematological profile, glycemia, liver function, and body weight in test animals.

MATERIAL AND METHODS

The Research Ethics Committee approved this study of Universitas Padjadjaran, Jatinangor, Indonesia (Approved number 356/UN6.KEP/EC/2022).

Animals and treatment

Twenty-four male (8-12 weeks) Wistar rats (Rattus norvegicus) were obtained from PT. Biofarma Parongpong and kept at Padjadjaran University (Dipatiukur, 40132), were used in this study. Before any treatment, all animals were kept for one week in the laboratory conditions of temperature (24±25°C), relative humidity (55±5%), and a 12h light/dark cycle[7,8]. They received a nutritionally standard diet, and water was supplied ad libitum. All animal protocols conformed to the NIH Guide for the Care and Use of Laboratory Animals.

Animals were randomly divided into four groups of 6 rats: the standard control group (NCD) (control group), a standard diet with 10% A. pauciflorum (NCD-JK) (48.3% carbohydrate, 24.89% protein, 9.7% fat), high-fat diet (HFD) (negative control group) and high-fat diet with 10% A. pauciflorum (HFD-JK) (14.2% carbohydrate, 17.16% protein, 51.58% fat). Daily feeding was ±40 g/ rat and ±500 mL of drinking water/ cage. Food intake and animal weight were measured every day. The treatment was carried out for nine weeks[8].

Hematology analysis

Blood samples were collected from the animal through tails. The collected samples were immediately placed in a prepared ethylenediaminetetraacetic acid (EDTA) tube, kept on ice, and sent to the laboratory. Hematological analysis was carried out using a VetScan HM5 consisting of hemoglobin, red blood cell, white blood cell, and platelet levels[9].

Serum analysis

Blood samples were collected from the animal through tails. The collected samples were immediately placed in a prepared centrifuge tube, kept on ice, and sent to the laboratory. Then it was centrifuged at 10000 RPM, and the serum was taken using a micropipette. The serum is then tested using the DiasSys glucose test kit to determine the glucose level and Randox test kit ALT and AST.

Statistical analysis

Data from the study results comprised the average hematological profile, glycemia, liver function, and body weight of rats in each treatment group compared with the control group. The above data were tested for normality to see standard data distribution using the Kolmogorov-Smirnov test. Furthermore, the data analysis process with one-way ANOVA for different tests with a confidence level of 95%. If there is a significant difference, the Bonferroni test is carried out to see the difference between animal treatments. If the data is not normally distributed and not homogeneous, the Kruskal Wallis nonparametric test is carried out, followed by Dunn's further test. All statistical analyses were performed using Prism 8 (GraphPad Software).

RESULT

The effect of A. pauciflorum on hematology

Hemoglobin levels in the NCD-JK group increased but not significantly ($p>0.05$), and in the HFD-JK group, it was no significant change ($p>0.05$). However, red blood cells (RBC) levels in the NCD-JK test group were significantly higher ($p<0.05$). White blood cell (WBC) levels in the HFD and HFD-JK groups were significantly higher ($p>0.05$). Platelet levels in the NCD-JK and HFD-JK groups were not significantly increased ($p>0.05$) (Table 1).

Table 1. The effect of Se supplementation on hemoglobin, RBC, WBC, and platelet

	NCD	HFD	NCD-JK	HFD-JK
Hemoglobin (g/dL)	16.28 ± 0.58	13.78 ± 0.34	17.17 ± 0.32	13.87 ± 0.19
RBC (10^{12}/L)	10.09 ± 0.18	9.73 ± 0.22	**10.87 ± 0.18[a]**	9.81 ± 0.14
WBC (10^9/L)	20.06 ± 1.53	30.03 ± 2.73	21.91 ± 0.97	38.23 ± 3.51
Platelet (10^9/L)	715.00 ± 24.42	643.00 ± 83.20	718.6 ± 7.63	663.8 ± 45.16

Results are expressed as mean ± SEM. Values significantly different are in bold, a—$p < 0.05$, b—$p < 0.01$, c—$p < 0.001$

The effect of A. pauciflorum on serum

Blood glucose levels in the NCD-JK group were significantly higher ($p<0.05$) compared to the NCD group, but the increase was still within the standard threshold (60-130 mg/dL). The tests carried out on ALT, and AST levels did not significantly change ($p>0.05$) (Table 2).

Table 2. The effect of Se supplementation on glucose, ALT, and AST

	NCD	HFD	NCD-JK	HFD-JK
Glucose (mg/dL)	63,83 ± 6,63	135,89 ± 12.87	**99,47 ± 6,93[a]**	107,72 ± 3,73
ALT (U/L)	3,65 ± 1,08	1,90 ± 1,17	7,89 ± 1,52	8,47 ± 0,90
AST (U/L)	23,38 ± 0,33	20,82 ± 1,24	22,20 ± 1,70	21,94 ± 0,23

Results are expressed as mean ± SEM. Values significantly different as compared to control are in bold, a—$p < 0.05$, b—$p < 0.01$, c—$p < 0.001$

The effect of A. pauciflorum on weight and feed consumption

The increased body weight in the NCD-JK and HFD-JK groups was significantly higher ($p<0.005$). Meanwhile, the amount of feed consumption in the NCD-JK group was significantly higher ($p<0.05$). HFD-JK group did not significantly change in this study ($p>0.05$) (Table 3).

Table 3. The effect of Se supplementation on feed consumption and wight gain

	NCD	HFD	NCD-JK	HFD-JK
Feed Consumption (g)	5303.5 ± 2.90	2376 ± 1.35	**8615 ± 4.71[c]**	**4851 ± 2.8[c]**
Weight gain (g)	33 ± 2,34	4,33 ± 3,25	**68 ± 5,53[c]**	**50.17 ± 3,06[c]**

Results are expressed as mean ± SEM. Values significantly different as compared to control are in bold, a—$p < 0.05$, b—$p < 0.01$, c—$p < 0.001$

DISCUSSION

RBC levels in the NCD-JK group increased significantly ($p<0.05$). It can be caused by A. pauciflorum containing Se, an essential trace element needed by the body[10]. Periodic supplementation of Se in A. pauciflorum can increase selenoenzymes such as glutathione peroxidase, selenoprotein-P, and thioredoxin

reductase[11–14]. Glutathione peroxidase protects Hb against reactive oxygen species (ROS) in erythrocytes. ROS can interfere with the process of erythropoiesis. In erythropoiesis, the synthesis and accumulation of Hb occur, characterized by the maturation of erythrocytes. Hb accumulation can potentially generate excessive ROS and causes oxidative stress. Hemoglobin A (HbA) is a tetramer consisting of two globin subunits attached to a heme moiety. Free globin is structurally unstable, very sensitive to oxidative stress, and tends to be denatured, which has the potential to produce ROS through reactions catalyzed by Hb and Fe bonds. The globin aggregates not bound to the erythrocyte membrane form Heinz bodies and eventually cause hemolysis due to membrane damage and exposure to excessive oxidative stress[13,15,16]. A similar study has shown that the administration of Se supplementation in humans can increase hemoglobin concentration, selenoprotein P (SEPP), and plasma GPx activity, which may affect the secretion of hepcidin[17,18].

WBC levels in the HFD-JK group increased but not significantly ($p>0.05$), and all groups showed a state of leukocytosis. A similar study has shown that the administration of a high-fat diet can increase white blood cell levels caused by inflammation of the bone marrow[19]. HFD can cause infected bone marrow will produce excessive blast cells, which can cause leukocytosis[20]. The Se content in A. pauciflorum is thought to increase the blood cell count. It may be related to the increase in antioxidant enzymes (glutathione peroxidases), which provide additional protection against the action of free radicals, which is a fundamental fact for increased cell survival[21,22].

The unchanged platelet levels were similar to the results of a study conducted by[23] that supplementation of 300 g Se in adults did not affect platelet levels in plasma. However, Se deficiency could affect it because Se is required to develop precursor cells in the bone marrow. Therefore, the non-effect of platelet levels after the administration of Se supplementation in this study may be due to the lack of Se content in A. pauciflorum.

HFD-JK glucose levels were not significantly changed ($p<0.05$), which indicated that A. pauciflorum did not affect blood glucose levels. Short-term HFD feeding can increase glucose due to increased hepatic glucose production and cause an increase in insulin secretion, which compensates for hepatic insulin resistance. This insulin secretion can be mediated by increased gastric inhibitory polypeptide secretion (GIP). Increased insulin secretion stimulates the development of peripheral insulin resistance, mitochondrial dysfunction, and obesity in response to overfeeding, suggesting a role for insulin and GIP in the development of peripheral insulin resistance and obesity[24–26]. Se supplementation in A. pauciflorum has beneficial results such as decreased levels of hemoglobin A1C (HbA1c), fasting plasma glucose (FPG), serum insulin, and homeostasis model of assessment of insulin resistance (HOMA-IR), HOMA-B, and increased quantitative insulin sensitivity check index (QUICKI). However, the Effect of Se is affected by various factors, including levels of Se supplementation, and depends on the health condition of the animal[27].

There was no change in ALT and AST levels after nine-week treatment. These results differ from study results[28] that feeding HFD can cause oxidative stress and affect the liver's metabolism, which causes changes in liver weight, fat deposition, inflammation, and fibrosis accompanied by increased plasma activity of liver enzymes. In addition, it can cause differences in the kits used so that they have different test results.

The Se content influences the increase in body weight in A. pauciflorum consumed by rats. The gut microbiota can use Se to express selenoproteins to aid in the absorption of nutrients in the gut[29,30]. Administration of Se supplements affects the function and metabolism of the gut microbiota. It affects gut microbiota composition and colonization, alters the uniqueness of microbial diversity, and affects microbial composition[30]. Se supplementation significantly reduces Doreasp and increases levels of microorganisms that have a potential protective effect against colitis and intestinal barrier disorders (such as Turicibacter and Akkermansi). Dorea sp is one of the most prevalent gut microbiota species that provides hydrogen and carbon dioxide in the gut with these effects. Se can optimize the intestinal flora to protect it from dysfunction and optimize intestinal absorption[31]. High feed consumption is probably due to the zinc (Zn) content of A. pauciflorum[10]. It regulates growth hormone (GH) gene receptors, increases sensitivity to endogenous growth hormone (GH), increases physiological GH secretion without altering GH response to stimulation of pharmacological activity, and through direct non-GH-mediated effects on insulin-like growth factor-synthesis, binding protein 3 (IGFBP-3), synthesis of insulin-like growth factor-1 (IGF-1), and stimulates the taste buds and affects appetite[32,33].

In conclusion, adding A. pauciflorum to feed can increase hemoglobin, RBC, WBC, glucose, and body weight caused by the content of

Se in A. pauciflorum, which can protect cells from free radicals and affect the composition of gut microbiota. However, the administration of A. pauciflorum did not affect the platelets, ALT, and AST levels, which might be due to the lack of Se content in A. pauciflorum.

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