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Effect of arrowroot (*Maranta* sp.) Food Products Supplementation in Diet Induced Hypercholesterolemic Mice

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Abstract

Food enriched with dietary fiber content has been associated to lower blood lipid profile. In this study, the flour, starch, and cookies from locally grown arrowroot plant, which is known to contain dietary fiber, were investigated for anti-lipidemic activity. Six groups of male ICR mice were fed with a high cholesterol diet for six weeks. At the end of six-week cholesterol induction, Group 1 received distilled water (dH20), Group 2 was fed with arrowroot starch (ARS), Group 3 with arrowroot starch cookies (ARSC), Group 4 with arrowroot flour (ARF), Group 5 with arrowroot flour cookies (ARFC), and Group 6 received simvastatin (SIM) for two weeks. Body weights were recorded before and after cholesterol induction and after arrowroot treatments. Lipid profile (total cholesterol, HDL, LDL, VDLD, triglycerides) of serum was measured at the end of the experiment. Also, livers were dissected, weighed, and evaluated for ballooning of hepatocytes, lipid inclusion (steatosis), and portal inflammation. After two weeks of treatment with arrowroot food products, a significant reduction of body weights on hypercholesterolemic mice were observed but the reduction was comparable with the group that received only distilled water. However, treatment with arrowroot starch significantly reduced the blood total cholesterol and the histological changes induced by a high cholesterol diet. Treatment with arrowroot starch and starch cookies significantly decreased steatosis, ballooning of hepatocytes, and resulted in the absence of portal inflammation in the liver—suggesting its hypolipidemic activity. Liver lipid profile and fecal lipid excretion analysis should be conducted to further support the result of this study.

Keywords: *arrowroot starch, arrowroot cookies, anti-lipidemic, hypercholesterolemia, dietary fiber*

Introduction

Hyperlipidemia is a metabolic syndrome characterized by elevated serum cholesterol (hypercholesterolemia) and lipoproteins (Surya *et al*., 2016). It is an important risk factor in the development of coronary heart disease and atherosclerotic lesions (Lopes *et al*., 2016). It was shown that among the factors that contribute to the development of hyperlipidemia is poor dietary habit (Tuzcu *et al*., 2017).

Dietary fiber is defined as the non-digestible carbohydrates and lignin that are present in plants (Mackowiak *et al*., 2016). In recent years, several studies have reported that a diet high in fiber has health benefits. These positive effects on health include reducing hyperlipidemia, modulating of blood lipid profiles, reducing cholesterol, and easing constipation (Mackowiak *et al*., 2016; Yangilar, 2013). Also, researches have shown that dietary fiber intake reduces the risk of developing coronary heart diseases, stroke, hypertension, diabetes, obesity, and gastrointestinal disorders, as well as enhances immune function in humans (Delcour *et al*., 2016). With this awareness, there has been a rapid development and consumption of food products with dietary fibers. However, the food products in the market usually utilize imported wheat grain. There is a need to develop an alternative source of food with dietary fiber and explore locally grown crops as a source of dietary fiber.

Arrowroot (*Maranta* sp.) is one of the locally grown tubers in the Bicol region (Malinis & Pacardo, 2012). Arrowroot rhizome is a good source of the

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production of starch. It is a rich source of starch besides other minerals and vitamins (Harmayani, 2011), and has been successfully used in food preparations (Miftakhussolikhah *et al*., 2016). However, it is one of the underutilized crops in the country. One of the possible reasons for its poor utilization is the lack of knowledge on its nutritive value. Studies from other countries have shown arrowroot to improve *Lactobacilli* production in the intestine (Harmayani *et al*., 2011), immunostimulatory activity *in vitro* and *in vivo* (Kumalasari *et al*., 2012), and antioxidant activity (Ramadhani *et al*., 2017; Saipriya *et al*., 2017). Investigations of the physicochemical quality and mineral content of arrowroot tuber from other countries have shown that it has sufficient amount of starch, dietary fibers, and other compounds (Harmayani *et al*., 2001; Kumalasari *et al*., 2012; Madineni *et al*., 2012). In this study, the anti-lipidemic effect of arrowroot flour, starch, and cookies was investigated.

Materials and Methods

Production of Arrowroot Powder and Food Products

Arrowroot rhizomes, flour, starch, and cookies were procured from Bicol University–Polangui Campus. The food products (cookies with flour and starch) were generated based on established protocols, with the addition of arrowroot flour or starch.

Experimental Animals

The study was conducted on four-week old male ICR mice obtained from the Bureau of Animal Industry (BAI), Quezon City. The animals were maintained under standard laboratory conditions with a photoperiod of 12 hours at the Bicol University College of Science Laboratory. The mice had free access to food and drink *ad libitum* throughout the study. They were acclimated to laboratory conditions for one week prior to the experiment proper. All procedures regarding the handling of the test animals were in accordance with the existing guidelines of the Philippine Association of Laboratory Animal Science (PALAS) for care and use of laboratory animals, Administrative Order 40 of the Bureau of Animal Industry, and Republic Act No. 8485 (PALAS CODE, 2002).

Induction of Hyperlipidemia

To stimulate hyperlipidemia, mice were induced of hypercholesterolemia, which was conducted based on the method described by Korou and colleagues (2010) with minor modifications. A high cholesterol diet was prepared daily by dissolving 2.5% cholesterol in coconut oil and using it to coat pellets. These coated pellets with cholesterol were provided to the animals every day while food remaining from the previous day was removed. Body weights were monitored before and after induction of hypercholesterolemia.

Induction of Hyperlipidemia

The antilipidemic activity was assessed by a method described by Suanasunsawat and co-workers (2008), with some modifications. Six groups of mice were established and fed with a high cholesterol diet for six weeks. After six weeks, Group 1 received distilled water (dH20), Group 2 were fed with arrowroot starch (AR) at a dose of 20 mg/kg, Group 3 with AR starch cookies (ASC) a*d libitum*, Group 4 with AR flour (ARF) at a dose of 20 mg/kg, Group 5 with AR flour cookies (AFC) *ad libitum*, and Group 6 received simvastatin (SIM) at a dose of 40 mg/kg. Body weights were monitored before and after hypercholesterolemia and treatments with arrowroot food products.

Blood Collection and Serum Lipid Measurement

After the experimental period, the mice were made to fast overnight, and blood samples were collected to determine the serum lipid profile. The samples were collected using capillary tubes introduced into the medial retro-orbital venous plexus under anesthesia (Zoletil®). The collected samples were brought to Centralink laboratory for determination of total cholesterol (TC), high density lipoprotein (HDL), low density lipoproteins (LDL), very low-density lipoproteins (VLDL), and triglycerides (TRI).

Histopathological Analysis

At the end of the eight-week period, the animals were euthanized and their livers were dissected and weighed. After weighing, the livers were fixed in 10% formalin and were brought to the Philippine Kidney Dialysis Foundation for slide preparation. The prepared slides were examined under a microscope to ascertain the presence of ballooning, steatosis, and portal inflammation. A score of 0 (absence) to 3 (severe lesion) was assigned to each parameter.

Statistical Analysis

All of the analyses were performed using the SPSS statistical software version 20. The data were analyzed using one-way analysis of variance (ANOVA), followed

by LSD (Least Significant Difference) for multiple comparisons. Body weight results were analyzed using t-test. Differences were considered significant when p-values were less than 0.05. Data were presented as mean ±SEM.

Results and Discussion

Effect on Body Weights and Liver Weight Index

At the end of the six-week induction of hypercholesterolemia, a statistically significant ($p<0.05$) increase was observed on the weights of all treated mice (Figure 1A), which indicated cholesterol induction. A high fat diet involving cholesterol is known to significantly elevate body weights by accumulation of adipose tissue (Woo *et al.*, 2017). A significant decrease on weights was observed after treatments with arrowroot products (Figure 1B). However, this reduction is comparable to the reduction of weight caused by distilled water. No significant effect was observed in the liver weight index (Table 1).

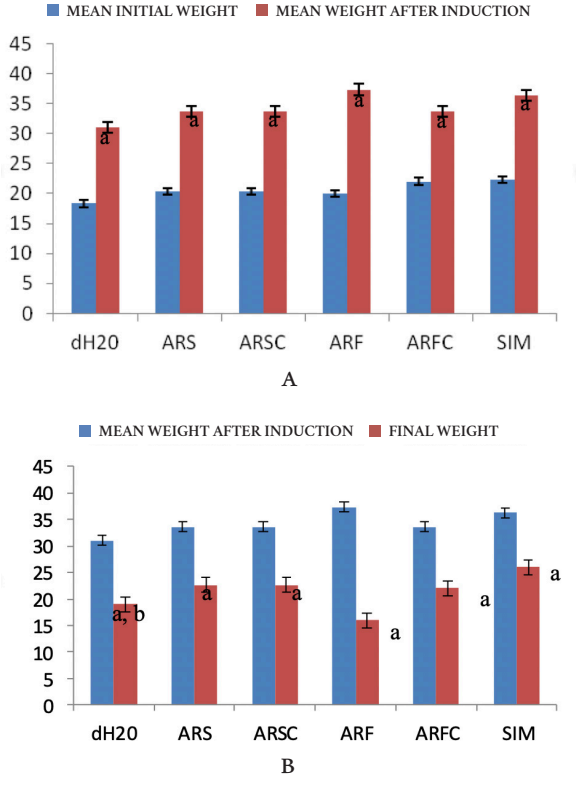

Figure 1. Body weights of mice at the beginning and after cholesterol induction (A) and final weights after treatments (B) with distilled water (dH20), arrowroot starch (ARS), arrowroot starch cookies (ARSC), arrowroot flour (ARF), arrowroot flour cookies (ARFC), and simvastatin (SIM). Values are represented as means standard error, $P< 0.05$. A: significantly different from mean initial weight. B: significantly different from SIM.

Table 1. Effect on the Liver Weight Index of Hypercholesterolemic Mice Fed with Arrowroot Food Products

Groups	Body weight	Liver weight	Liver weight Index^{ns}
dH20	26.00±0.58	1.92 ±0.10	7.40±0.54
ARS	22.67±1.45	1.98± 0.22	8.87±1.96
ARSC	22.67 ±1.85	1.92 ±0.17	8.47 ±0.56
ARF	23.00 ±1.67	2.14 ±0.08	9.28 ±1.27
ARFC	22.00 ±1.15	1.58 ±0.18	7.27±1.50
SIM	19.00 ±1.53	1.85± 0.28	8.35 ±2.08

(dH20) mice fed with high cholesterol diet and treated with distilled water; (ARS) mice fed with high cholesterol diet and treated with arrowroot starch; (ARSC) mice fed with high cholesterol diet and treated with arrowroot starch cookies; (ARF) mice fed with high cholesterol diet and treated with arrowroot flour; (ARFC) mice fed with high cholesterol diet and treated with arrowroot flour cookies; (SIM) fed with high cholesterol diet and received simvastatin. Values are represented as means ±standard error, P<0.05.

Effect on Serum Lipid Profile

The mice treated with arrowroot starch had a significantly reduced total cholesterol of hypercholesterolemic compared with those treated only with distilled water (Table 2). The rest of the treatments also reduced the total cholesterol, but the reduction was not statistically significant. This lowering activity of the total cholesterol caused by arrowroot starch suggests its antihyperlipidemic or hypolipidemic activity. As mentioned above, elevated cholesterol is a manifestation of hyperlipidemia and lower total cholesterol is associated with the reduction of the risk of coronary heart disease (Sharett *et al.*, 2011).

The very low-density lipoprotein (VLDL) and triglycerides levels were found to have decreased compared to the group treated with distilled water. However, this reduction was not statistically significant. No observable effect was seen in low density lipoprotein (LDL) level and arrowroot food products failed to elevate the high-density lipoprotein (HDL) cholesterol.

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Table 2. Serum Lipid Profile of Hypercholesterolemic Mice Fed with Arrowroot Food Products

Groups	Total cholesterol	HDL	Triglycerides	LDL	VLDL
dH20	180.04 ± 6.74	24.67 ± 9.08	1040.50 ± 7.67	64.19 ± 20.29	208.10 ± 1.53
ARS	140.17 ± 10.75*	21.05 ± 6.93	1017.33 ± 13.27	-84.35 ± 22.85	203.47 ± 2.65
ARSC	157.72 ± 7.26	16.01 ± 2.125	1023.15 ± 7.68	-62.92 ± 10.26	204.63 ± 1.54
ARF	165.30 ± 6.40	19.57 ± 6.01	1030.35 ± 7.53	-37.12 ± 21.42	206.07 ± 1.5
ARFC	152.43 ± 152.43	23.51 ± 4.39	1014.43 ± 42.69	-73.96 ± 1.79	202.89 ± 8.54
SIM	165.54 ± 24.36	23.90 ± 5.40	1029.16 ± 15.13	-52.73 ± 11.58	205.83 ± 3.02

Mice treated with distilled water (dH20); with arrowroot starch (ARS); with arrowroot starch cookies (ARSC); treated with arrowroot flour (ARF); treated with arrowroot flour cookies (ARFC); and mice that received simvastatin (SIM). Asterisk (*) indicates significant difference from distilled water. Values are represented as means ±standard error, P<0.05.

Effect on Liver Histology

Accumulation of adipocytes in the liver (hepatic steatosis), ballooning of hepatocytes, and vascular dysfunction are common outcomes of a high fat cholesterol diet and are often observed in hyperlipidemia (Woo *et al*., 2017; Saleem *et al*., 2017). In this study, hematoxylin-eosin stained liver samples obtained from groups fed with high cholesterol diet showed signs of ballooning of hepatocytes, steatosis, and slight portal inflammation (Figure 2A). Treatment with arrowroot products significantly reduced ballooning of hepatocytes and a significant reduction of steatosis or lipid inclusion was observed. Also, portal inflammation was not seen except with arrowroot flour (Figure 2B-E and Table 3). These observations show that the administration of arrowroot food products significantly suppresses lipid deposition caused by the high cholesterol diet, thereby offering protection against hyperlipidemia.

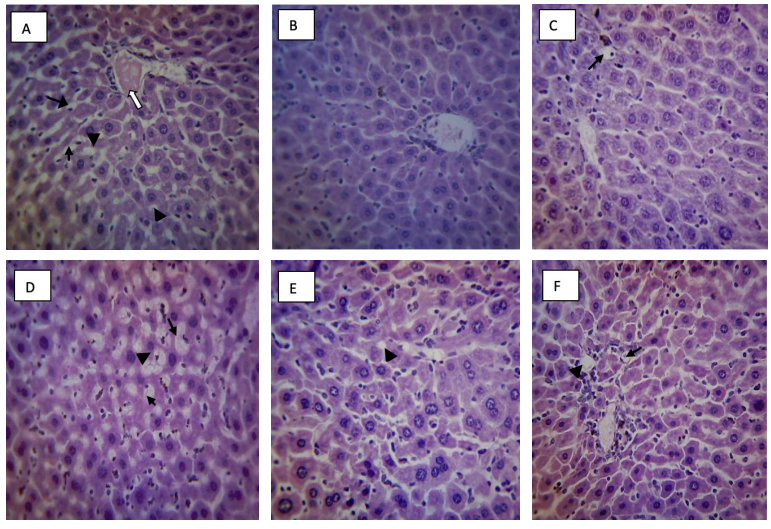

Figure 2. Representative photographs of livers of hypercholesterolemic mice treated with distilled water (A), arrowroot starch (B), arrowroot starch cookies (C), arrowroot flour (D), arrowroot flour cookies (E), and simvastatin (F). Arrows indicate ballooning, arrowhead steatosis, and white arrow for portal inflammation. H&E stain. 400x

Table 3. Histological Observations of Hepatic Tissue

Groups	Ballooning	Steatosis	Portal Inflammation
dH20	1.67±0.33	2.67±0.58[c, d]	0.67±0.33
ARS	1.0±0.00[a]	0.33±0.33[a,b]	0.00±0.00[a,b]
ARSC	1.00±0.00[a]	1.33±0.58[a,b,c]	0.00±0.00[a,b]
ARF	1.00±0.00[a]	2.33±0.33[c, d]	0.67±0.33
ARFC	1.00±0.00[a]	2.33±0.33[c, d]	0.00±0.00[a,b]
SIM	0.67±0.33[a]	1.67±0.23[a,b]	0.00±0.00[a,b]

3, severe; 2, moderate; 1, mild; 0 no changes in histology (scores). Values are represented as means standard error, P<0.05. a: significantly different from dH20 group; b: significantly different from ARF group; c: significantly different from ARS group; d: significantly different from SIM group.

It is well established that lifestyle, especially having a high fat diet, is a major factor in the development of hyperlipidemia. Food products with high dietary fiber content have been associated with reducing hyperlipidemia (Yangilar, 2013). In this study, only the arrowroot starch reduced the total cholesterol and, together with arrowroot starch cookies, decreased the histological changes caused by a high cholesterol diet. Also, it is noteworthy that although the other arrowroot food products failed to restore the disturbed serum lipid lipoproteins, the administration of arrowroot protected or reduced the histological damage caused by the high cholesterol diet. These imply that the actions of arrowroot products might be in liver lipid synthesis as arrowroot is known to have good antioxidant activity (Saipriya *et al*., 2017); it might act on HMG CoA reductase enzymes. This enzyme limits the production of cholesterol in the liver or decreases the assimilation of cholesterol in the intestine (Saleem *et al*., 2017) and, thus, lowering the hepatic fat content and cholesterol level.

Conclusion and Recommendations

The administration of arrowroot starch significantly reduced the serum cholesterol in mice. Also, together with arrowroot starch cookies, arrowroot starch reduced the histological changes induced by the high cholesterol diet. Thus, the starch may have the ability to lower cholesterol levels. Longer treatments (three to four weeks) or an increase in concentrations are recommended to further establish the antilipidemic activity of arrowroot food products.

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