



## **Cholesterol Lowering Effect Of Philippine Ashitaba (*Gynura Procumbens*) Crude Aqueous Leaf Extract In Hypercholesterolemic Mice**

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### **Abstract**

*Gynura procumbens*, a medicinal plant with various nutritive benefits, is being utilized as a source of total-cholesterol lowering herb in folk medicine. The Japanese variety of ashitaba has proven scientific basis in lowering blood cholesterol. But the Philippine variety lacks scientific study on its cholesterol lowering potential. Hence, this study focused on determining cholesterol lowering effect of *G. procumbens* (Philippine Ashitaba) crude aqueous leaf extract in hypercholesterolemic mice. Sixteen male BALB/c mice were given 0.5 mL of butter per day through oral gavage at 8:00 AM and 5:00 PM. On the third day, under general anesthesia, blood of one test animal was collected and subjected to Total Cholesterol (TC) determination to ascertain induction of hypercholesterolemia ( $TC \geq 250$  mg/dl). The fifteen remaining mice were assigned to positive control (n=5), negative control (n=5) and treatment (n=5) groups, which were administered with atorvastatin, distilled water, and crude aqueous leaf extract of *G. procumbens* via oral

gavage daily. On day 10, under pentobarbital anesthesia, blood samples were collected through cardiac puncture and TC levels were determined. The TC levels showed that the treatment ( $148 \pm 26$  mg/dl) and positive control ( $136 \pm 29$ ) groups were significantly decreased as compared to the negative control group ( $P < 0.05$ ). Fatty liver was observed in the negative control group while normal liver architecture was seen in the other two groups. The study showed that the crude aqueous leaf extract of *G. procumbens* (Philippine ashitaba) has cholesterol lowering effect on butter-induced hypercholesterolemia in mice. This plant, which is used as a vegetable can be a source of cholesterol-lowering nutraceutical.

**Keywords:** *G. procumbens*, Philippine Ashitaba, hypercholesterolemia

### **Introduction**

Obesity, diet, lifestyle, and genetic factors may lead to hypercholesterolemia, which may lead to other clinical diseases associated with atherosclerosis. In most industrial and developed countries, hypercholesterolemia has been

considered as one of the main factors causing chronic health diseases. Cholesterol, if observed at high levels is known to be toxic to the cells and has the ability to induce pro-inflammatory responses and promote cardiovascular diseases, peripheral vascular diseases and coronary artery diseases [1,2].

Moreover, it contributes to the development of non-alcoholic fatty liver diseases. When there is deposition of lipid in the liver, a fatty liver is usually observed. This condition causes dysfunction in the physiology of the liver [2,3]. In line with this, *Gynura procumbens*, a medicinal plant with various nutritive benefits, is utilized as a probable source of cholesterol-lowering medicinal plant.

In the past years, herbals drugs have been used as conventional medicines due to its economic affordability and non-toxicity with wide safety profile. Most studies related to the treatment of hypercholesterolemia have focused on the effects and mechanism of action of herbal medicines [4].

Philippine Ashitaba (*Gynura procumbens*) is a fast growing local herb widely distributed in Southeast Asia and is recently gained popularity for cultivation due to its purported medicinal property [5] and has been commonly used as a nontoxic alternative medicine in Indonesia for the treatment of kidney diseases, eruptive fevers, rash, hypertension, diabetes mellitus, and hyperlipidemia. The Philippine species has scarcity of studies on its purported medicinal property including the cholesterol lowering activity [1,6].

However, the pharmacological studies focused on providing evidences and mechanisms related to the therapeutic efficacy of *G. procumbens* are still limited, despite being routinely used as herbal medicine. Moreover, there are limited studies on the effects of *G. procumbens* on hypercholesterolemia [1, 7, 8]. Hence, the study aimed to determine the cholesterol-lowering effect

of *Gynura procumbens* (Philippine ashitaba) crude aqueous leaf extract in hypercholesterolemic mice.

### **Materials and Methods**

This study was approved by the Pamantasan ng Lungsod ng Maynila (PLM) - Unicersity Research Center and the PLM College of Medicine Research Publication Committee. It was registered to the Research Grant Administration Office, National Institutes of Health – University of the Philippines Manila. The animals were cared for according to the guidelines set by Philippine Animal Laboratory Science.

#### **Plant and Extract Preparation**

The plant was submitted for authentication to the Department of Botany, College of Science, De LaSalle University, Taft Avenue, Manila, Philippines. The plants were sourced from the Bureau of Plant Industry in San Andres, Manila, Philippines.

The leaves were dried under shade for 7 days. The dried leaves were pulverized using a mortar and pestle. The pulverized sample was soaked in 1 liter of distilled for 24 hour at 4°C. The resulting solution was filtered using Whatman filter paper No. 5. The filtrate was lyophilized. The lyophilized sample was reconstituted using distilled water to create a 500 mg/mL stock solution, stored in an amber bottle, and refrigerated at 4°C until further use.

#### **Animal Use and Care**

All mice used were sourced from the Animal Laboratory of the National Institutes of Health – University of the Philippines Manila. All mice were male with body weigh of 20-24 grams and individually caged. Litter beddings change every three days. Environmental temperature set at 20°C with 12-hour light and dark cycle and humidity of 72%. Standard animal pellets of 5 grams per day were given and water ad libitum. No mice died during the two weeks of acclimatization prior the commencement of the research experiment. All animals were euthanized at the

conclusion of the study and were disposed of according to established institutional protocol.

#### Induction of Hypercholesterolemia in mice

Fifteen (15) male BALB/c mice were given 0.5 mL of butter per day through oral gavage. After the third day, blood of one test animal was collected under anesthesia and subjected to Total Cholesterol (TC) determination to ascertain induction of hypercholesterolemia ( $TC \geq 250$  mg/dl).

#### Administration of Treatment

The fifteen remaining mice were assigned to positive control (n=5), negative control (n=5) and treatment (n=5) groups, which were administered with atorvastatin (40 mg/Kg BW, distilled water (0.5mL), and crude aqueous leaf extract (250mg/Kg BW from a stock solution of 500mg/Kg BW) of *Gynura procumbens*, respectively, via oral gavage daily for ten days.

#### Determination of Total Cholesterol (TC)

On day 10, blood samples were collected through cardiac puncture under pentobarbital anesthesia and TC levels were determined using the Lieberman Buchard method.

#### Statistical Analysis

The results were presented in mean  $\pm$  SD and subjected to statistical analysis using unpaired Student t-test using CYSTAT. Significance was set at  $P < 0.05$ .

#### Results and Discussion

Previous studies have established that the aqueous extract of *G. procumbens* has antioxidant properties. This findings can be ascertain to its high antioxidant content and significant free radical scavenging activity which can essentially prevent the oxidation of lipids and promote protection from damage by these free radicals [8]. *G. procumbens* also contain active chemical constituents such as tannins, terpenoids, sterol glycoside, saponins and flavonoids [2]. Specifically, flavonoids and saponins, which are some of the most common components of *G.*

*procumbens* extracts [6], have been reported to be responsible for the hypolipidemic effects. The presence of these bioactive compounds is able to lower cholesterol absorption by the inhibition of cholesterol micellar solubility [3].

Table 1. Serum levels of Total Cholesterol among the different groups

Groups	Serum Total Cholesterol	P<value
Negative Group	256 $\pm$ 30	
Treatment Group	148 $\pm$ 26	*P<0.05
Positive Group	136 $\pm$ 29	*P<0.05

\* Negative Group vs. Treatment and Positive Groups; P value for Treatment Group vs. Positive Group was  $>0.05$

In the study, it was observed from the Total Cholesterol (TC) levels that the treatment (148  $\pm$  26 mg/dl) and positive control (136  $\pm$  29) groups were significantly decreased as compared to the negative control group ( $P < 0.05$ ) as shown in Table 1. The study showed that the crude aqueous leaf extract of *Gynura procumbens* (*Ashitaba*) has cholesterol lowering effect on butter-induced hypercholesterolemia in mice.

Therefore, the results were in line with the findings by a study by Zhang and Tang [9], wherein the extract of *G. procumbens* is found to significantly reduced the serum cholesterol and triglyceride levels. Hence, *G. procumbens* can be a source of cholesterol-lowering nutraceuticals.

#### Conclusion

From the results of the study, it can therefore be established that *G. procumbens* can be a source of cholesterol-lowering nutraceutical, and that the mechanism by which it lowered the cholesterol levels may

be due to the presence of bioactive compounds such as flavonoids and saponins, in its crude aqueous extracts.

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