Hypolipidemic Activity of Ethanolic Extract of Philippine Mahogany Seed *(Swietenia macrophylla)*

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ABSTRACT

Hyperlipidemia is one of the most leading metabolic disorders that is vastly seen in this era of modernization and fast life. It can be acquired through having high calories intake, causing a deranged digestion and metabolism in our body that may be associated with a cluster of metabolic diseases (Kaimal, 2010). This metabolic condition is characterized by alterations occurring in serum lipid and lipoprotein profile due to elevated concentration of total cholesterol, low density lipoprotein, very low-density lipoprotein and triglycerides along with decrease in high density lipoprotein in the blood circulation (Thanga et al., 2013). At this time, the primary treatment for prevention of hyperlipidemia is dietary and improving lifestyles, followed by drug therapy if necessary. Many natural materials from plants have been used as an anti-hyperlipidemic drug. Furthermore, medicinal plants and their derivatives are promisingly gaining wide usage worldwide as they are a potential source of bioactive agents for medication.

INTRODUCTION

In the Philippines, increased prevalence of high total cholesterol among Filipinos is alarming, wherein it revealed that 51.5% of adults have already been suffering from borderline cholesterol for people at the age of 40 to 49 with the highest of 61.9% ages of 50 to 59 years old. This number has slightly decreased for people between the age of 60 to 69 at 58.9%, and even lower to people in their 70s at 57.5% when the older people are more cautious in their dietary concerns. Thus, every 1 out of 2 Filipinos has hyperlipidemia and they are not even aware of it. These people are subject to greater risk of heart disease and other illnesses (Alixandra Caole Vila). Last 2016, Vila cited that an organization called PATACSI (Philippine Association of Thoracic and Cardiovascular Surgeons, Incorporated), in partnership with the Department of Science and Technology's (DOST) Food and Nutrition Research Institute (FNRI) and with food and beverage firm Del Monte, found out that 46.9 percent of Filipinos aged 20 and up have borderline to high cholesterol. Being young at age, these people tend to lose track of their lifestyle choices by staying up late, eating fatty foods and working in the wee hours making them a candidate for having high levels of cholesterol.

High-fat diets and frequent feeding contribute to elevating the triglycerides (TG) levels, promoting lipid accumulation in the vessel wall. Thus, this phenomenon triggered cardiovascular diseases, such as hypertension, coronary heart diseases and stroke. Cardiovascular disease is one of the health problems in the community and the leading cause of death worldwide.

Philippine mahogany (Swietenia macrophylla) belongs to the family Meliaceae (Orwa et al., 2009). It is considered one of the first exotic timber trees planted, commonly used for the daily living. S. macrophylla seed is also claimed to have a medicinal significance against numerous diseases (Balijepalli et al., 2015). The major component of the S. macrophylla seed is limonoid or also known as tetranortriterpenoid, a modified triterpene. This component and its derivatives are said to be more diverse and abundant in Meliaceae family than in any other family (Paritala et al., 2015). Limonoids and their derivatives are the major constituents of S. macrophylla which are derived from tetracyclic triterpenes similar to euphol (H-20β) or tirucallol (H-20α) by a series of oxidative changes, interspersed with molecular rearrangements. Furthermore, S. macrophylla is abundant in several phytochemicals such as: saponin, alkaloids and flavanoids (Mursiti 2009). Moreover, saponins are naturally-occurring active glycosides found in plants. It has many pharmacological properties, such as anti-inflammatory, immunostimulant,

hypocholesterolemic, hypoglycemic, antifungal and cytotoxic activities (Marrelli, 2016). In addition, the methanol extract of swietenine, a tetranortriterpenoid saponin, can reduce the elevated cholesterol and triglyceride levels in diabetic rats (Moghadamtousi et al., 2013).

This study aims to determine the effectiveness of Philippine mahogany seed extract on hyperlipidemic rats. Specifically, the study aims to compare the pre-test and post-test of hypolipidemic activity of the different concentrations of extract between the standard control groups and experimental control groups. Also, to differentiate the pre-test and post-test examination of the lipid profile through hypolipidemic activity of Philippine mahogany seed extract.

MATERIALS AND METHODS

A total of dried 1000 grams of mahogany seeds were pulverized using Wiley mill and soaked in 5.0 L of 95% ethyl alcohol for 48 hours. The mixture was filtered and the filtrate obtained was concentrated using a rotary evaporator at 600C to obtain a semi-solid extract. This crude extraction of dried 1000 grams of mahogany seeds produced 3.9 L of ethanolic extract. The semi-solid extract was fractionated into 1000 mg/rat/day, 1500 mg/rat/day and 2000 mg/rat/day respectively.

A population of 15 male Sprague-Dawley rats (of the species Rattus norvegicus) weighing 170 to 200 grams were housed in cage shelters between 20-26ºC in accordance with the mandate of Animal Research Review Panel (2007). As commanded, lighting within cages during day hours was held at 25 lux and light intensity was fluorescent lights of about 25 to 36 watts with a low spectral intensity or wavelength. Rats were acclimated for 1 week with feed and drink by ad libitum. After a week, each rat was weighed and was divided into 5 groups.

Each group was composed of 3 rats, group 1 was assigned as the negative control, group 2 was the positive control, treated with atorvastatin, group 3 was the hyperlipidemic which was given with 1000 mg/kg of ethanolic extract, group 4 was the hyperlipidemic which was given with 1500 mg/kg of ethanolic extract, and group 5 was the hyperlipidemic which was given with 2000 mg/kg of ethanolic extract. For 7 days, groups 2, 3, 4 and 5 was treated by a single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/kg) in physiological saline solution after an overnight fasting for 18 hours (Gundamaraju et al., 2014). Then, the ethanolic extract with a concentration of 1000 mg/kg, 1500 mg/kg and 2000 mg/kg were given orally for 7 days (Sa'adah, 2017). The standard control group was given a basalt feed only.

Blood serum was collected prior to testing, after the induction of Triton X-100 and after the treatment of the ethanolic extract of S. macrophylla seeds. Baseline and pre-test blood sample were collected by retroorbital method. According to the guidelines on blood collection of Michigan Medicine Confluence (Nowland, 2017), no more than 10% of the blood volume should be removed at one sampling which means that the blood volume that was obtained varied from each rat weight. 1.0 to 1.6ml of blood was extracted in a rat with an average weight of 170 grams to 200 grams. (Stewart et.al., 2018) For the post-test, cardiac puncture was done to obtain larger amounts of blood. The serum samples should not be hemolyzed and the extracted blood was directly dripped to the gel-separator tube. Serum was separated from the blood cells by centrifugation at 3000 rpm for 10 minutes. And after all blood collections, the rats underwent cervical dislocation euthanasia (University of Texas at Austin, 2013).

For measuring the total cholesterol, and triglycerides, an enzymatic colorimetric CHOD-PAP method was used. A 10 µl of blood serum was added to 1000 µl of CHOD-PAP reagent. It was properly mixed and was incubated for 10 min at 20-25°C by using a stat fax. Measuring of HDL-C was done by the LDL, VLDL and chylomicrons precipitation method. Blood serum of 500 µl was added to 1000 µl of HDL-reagent, then was mixed and incubated for 10 minutes at room temperature. After that, it was centrifuged for two minutes with a speed of 10,000 rpm. Supernatant was separated from the precipitate. Supernatant of 100 µl was added to 1000 µl of cholesterol reagent, then was mixed and incubated for 10 minutes at 20-25°C. Absorbance will be measured at wavelength (λ) 540 nm. Measuring of LDL-C was done using the Friedewald equation: [LDL-chol] = [Total chol] - [HDL-chol] - ([TG]/5)) (Quispe, 2017).

This research involved dealings with an animal, which was the oral administration of the extracted plant's seed and extraction of appropriate blood needed for this study. The researchers provided the evidence of approval for animal experimentation from Institutional Animal Care and Use Committee (IACUC). The researchers also ensured that the process and procedure of the experiment was reviewed and was under the direct supervision of the assigned adviser and qualified veterinary professional who conducted the collection of blood and testing of control groups. This was to guarantee that all actions are appropriate and humane.

The analysis of the following data was based on the inquiry that has been used by Sa'adah (2017) in his research. The data of the total cholesterol, triglycerides, LDL-C, and HDL-C level were analyzed using ANOVA, a test that was used to compare the data from three experimental groups which were treated with 1000 mg/kg, 1500 mg/kg and 2000 mg/kg of ethanolic extract. Furthermore, paired t-test was used in comparing the pre-test and post-test of each experimental group to identify the significant difference of each sample before and after the treatment of Philippine mahogany seed extract.

RESULTS AND DISCUSSION

Rats which were induced with Triton x-100 during the pre-test caused increase in the lipid profile. After one week, the rats were treated with atorvastatin (positive control group) and the ethanolic extract of *Swietenia macrophylla* with different concentrations (experimental groups). The lipid profile of the rats such as Triglycerides, Total cholesterol, LDL-C decreased and the HDL-C increased. Levels of triglycerides with the concentration of 1000 mg/kg had significantly reduced at 31.2% while HDL-C increased for about 224.6%. Concentration of 2000 mg/kg in total cholesterol, showed increased reduction at 42.39% and 79.6% for LDL-C. Furthermore, pre-test and post-test showed significant results in triglycerides, total cholesterol, HDL-C and LDL-C. This reduction may be due to increase metabolism or utilization of lipid by activating the decrease production of lipids by inhibiting the HMG CoA enzyme (See Table 1.)

Table 1. Comparative analysis of the hypolipidemic effect of Philippine Mahogany Seed extract in terms of different concentrations and standard control group after pre-test and post-test

Parameters	Test	Negative Control	Positive Control	1000 mg/kg	1500 mg/kg	2000 mg/kg
Triglycerides	Pre	66.4000	90.7667	94.3667	92.5667	87.9000
	Post	66.8333	64.0667	64.9333	67.4333	76.9667**
Total Cholesterol	Pre	85.6667	152.9333	152.5667	154.6667	173.6333
	Post	82.2333	86.4333	90.5667	92.5000	100.0333**
LDL-C	Pre	35.5200	114.2467	112.8933	115.8400	138.6867
	Post	29.1333	31.0767	28.2667	37.0800	28.2733**
HDL-C	Pre	36.8663	20.5333	20.8667	20.3000	17.3667
	Post	39.7333	42.4000	49.2667	41.9333	56.3663**

Among all concentrations, the dosage of 2000 mg/kg showed a marked decreased in its triglycerides, total cholesterol and LDL-C and a significant increase in its HDL-C compared with other concentrations and positive control. Triglycerides from 87.9000 decreased to 76.9667, Total Cholesterol from 173.6333 to 100.0333, LDL-C from 138.6867 to 28.2733 and HDL-C that increased from 17.3667 to 56.3667. This is because of the higher concentrations of phytochemical constituents present in this dosage. According to Francis (2002), a naturally occurring surface active glycoside which is saponin can be found in many plants and one of the major components of *S. macrophylla* is limonoid, a modified triterpene. Alkaloids and saponins were also the most abundant constituents present and it has been reported that this has the capability of reducing blood levels of cholesterol and aids in a variety of diseases (Paritala et al.,

2015) thus, exhibited by having a remarkable hypolipidemic activity on all the parameters of the lipid profile.

The comparative values of pre-test and post-test of the control group and experimental groups based on the lipid profile of the rats are shown on Table 2. The computed significance level for the pre-test and post-test indicates that there was no significant difference between the pre-test and post-test of both groups. The results from the study imply that the ethanolic extract from the seed of *S. macrophylla* is an alternative solution to lower the lipid profile of hyperlipidemic rats. The positive control used in the present study is Atorvastin. And according to Taldone (2008), atorvastatin is a member of the drug class known as statins and this is used for lowering cholesterol levels. Thus, this proves that the ethanolic extract and atorvastatin are both effective to lower the lipid profile with the same level of effectiveness.

Table 2. Comparison of the pre-test and post-test between the positive control group and experimental groups

Parameters	Test	Groups	Sig	Remarks
Triglycerides	Pre-test	Positive control Experimental Groups	.193	Not significant
	Post-test	Positive control Experimental Groups	.236	Not significant
Total Cholesterol	Pre-test	Positive control Experimental Groups	.114	Not significant
	Post-test	Positive control Experimental Groups	.441	Not significant
LDL-C	Pre-test	Positive control Experimental Groups	.381	Not significant
	Post-test	Positive control Experimental Groups	.070	Not significant
HDL-C	Pre-test	Positive control Experimental Groups	.109	Not significant
	Post-test	Positive control Experimental Groups	.102	Not significant

Statistical Method: Analysis of Variance (T- test)
Level of significance (a) = <0.05

Paired t-test was used between the pre and post-test examinations of the serum lipid profile of rats. This includes triglycerides, total cholesterol, HDL-C and LDL-C. With this, table 3 showed that ethanolic treatment of Philippine mahogany (*Swietenia macrophylla*) seed with different concentrations can significantly ($p<0.05$) decrease elevated levels of lipid profile. According to the one study conducted, the findings extracted by *S. macrophylla* shows a potential on reducing serum lipid profile because of its major components, saponins and tritertepenes (Maiti et al., 2007). These properties are known to be hypolipidemic which modulates the expression of many genes associated with lipid metabolism and hence regulates hyperlipidemia associated (Song et al., 2011). In accordance with the result, the researchers believed that the component of *S. macrophylla* has the ability to reduced lipid levels.

Table 3. Comparison of the pre-test and post-test after treatment of Philippine mahogany seed extract

Tests (Pre and Post)	Sig. (2-tailed)	Remarks
Triglycerides	0.000	Significant
Total Cholesterol	0.000	Significant
HDL-C	0.000	Significant
LDL-C	0.000	Significant

Level of significance (a) = <0.05

CONCLUSION

Lipid-rich diet that was given to Sprague Dawley rats for seven days caused a significant increase in total cholesterol, triglycerides and LDL-C and significant decrease in HDL-C levels. After the intake of ethanolic extract of mahogany seeds with different concentrations, the total cholesterol, triglycerides and LDL-C significantly decrease and the HDL-C went back to its normal level. This only proves that the phytochemical constituents such as alkaloids, saponins and triterpenes were found to be an effective hypolipidemic agent. A higher decrease in total cholesterol, triglycerides, LDL-C levels, and the higher increase in HDL-C were observed in hyperlipidemic rats which were treated with 2000 mg/kg of the ethanolic extract of Philippine mahogany seed (*Swietenia macrophylla*). This only shows that this dosage (2000 mg/kg) is the most effective dosage for improvement of hyperlipidemia among all concentrations that were used in this study.

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