

The Antihyperlipidemic Property of the Crude Methanolic Extract of *Antigonon leptopus* Hook & Arn. (*Polygonaceae*) Leaves in Triton X-Induced Hyperlipidemic Male Sprague-Dawley Rats

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ABSTRACT

Hyperlipidemia is a major factor that can promote atherosclerosis and atherosclerosis-related conditions. Cardiovascular disease (CVD) is the epidemic of modern civilization in which hyperlipidemia contributes significantly to its pathogenesis. This study aimed to investigate the antihyperlipidemic property of *Antigonon leptopus* leaves using the methanolic crude extract (ALME) in Triton-X-100 induced hyperlipidemic male Sprague-Dawley rats during seven-day treatment period. The efficacy of ALME 100, 200, 400mg/kg was compared to the standard simvastatin (10 mg/kg, p.o.) in Triton X-100-induced hyperlipidemic rats. Effect of ALME on serum lipid profile like Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein-Cholesterol (LDL-C), Very Low-Density Lipoprotein-Cholesterol (VLDL-C) and High-Density Lipoprotein-Cholesterol (HDL) were estimated. The results demonstrated that the ALME has an antihyperlipidemic potential. Different doses of ALME significantly reduced the TC, TG, LDL-C, and VLDL-C in Triton X-100-induced hyperlipidemic rats (p-value <0.05). The histopathological evaluation also revealed the positive effect of the plant extract, with ALME 100 mg/kg showing promising tissue-specific antihyperlipidemic effect.

Keywords

Antigonon leptopus; antihyperlipidemic; Cadena de amor; triton-x100.

1. INTRODUCTION

Hyperlipidemia is one of the major risk factors for cardiovascular disease that may lead to atherosclerosis, myocardial infarction, heart attack and cerebrovascular diseases. It is the term used to denote abnormal elevation of serum lipid concentrations including total cholesterol, triglycerides, and low-density lipoprotein cholesterol alongside with the decrease in high-density lipoprotein cholesterol. It is also known as dyslipidemia to describe the manifestations of different disorders of lipoprotein metabolism. Where excess LDL can build up in your blood vessels and deposit in the walls of blood vessels causing a buildup of

material called plaque [1]. Over time, this plaque could narrow blood vessels and reduces blood flow which could be referred to as atherosclerosis, leading to further development of atherosclerosis-associated conditions [2].

In addition, the development of atherosclerotic lesion is a characterization of atherosclerosis that could be a possible response to chemotactic stimuli provided either directly or indirectly by oxidized lipoproteins, and accumulation of cholesterol within macrophages [3]. Compared with native LDL, ox-LDL has been shown to have to promote the development of atherosclerosis, including uptake by the macrophage scavenger receptor [4]. Oxidative modification of low-density lipoprotein can induce intracellular production of reactive oxygen species (ROS) and lipid peroxidation (LPO) [5]. Lipid peroxidation products have been found to cause atherosclerotic lesions [5].

Further hyperlipidemia is associated with oxidative modification of LDL, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects [6]. This phenomenon takes place when the body's endogenous antioxidant defense mechanism from reactive oxygen species is deficient [7]. Thus, treatment with antioxidants can decrease the rate of lipid peroxidation, restore the body's antioxidant capacity, maintain the body's homeostasis, and possibly prevent or delay development of atherosclerosis and atherosclerosis-associated disease [8].

From that, experimental and epidemiological evidence suggest a strong relationship between atherosclerosis and elevated levels of plasma lipids. Hence, much attention is being given to primary and secondary prevention of hyperlipidemia.

The main principle of treating hyperlipidemic patients is to reduce the elevated lipid plasma levels of lipids which can decrease the occurrence of further cardiovascular or cerebrovascular disease. Current antihyperlipidemic therapy includes statins and fibrates where the former corrects the altered serum lipid concentration and the latter acts by enhancing the clearance of triglycerides rich lipoprotein [9]. These antihyperlipidemic drugs are extensively used as prophylactic agents to prevent atherosclerosis and atherosclerosis-associated disorders such as coronary heart disease [10]. Although these antihyperlipidemic synthetic drugs are effective agents, they have been associated with several side effects including stomach upset, nausea, vomiting, headache and dizziness [11]. It may also induce hepatotoxicity for those patients with liver damage and myopathy such as drug-induced rhabdomyolysis [12]. Besides, statins have been associated with an increased risk of developing Type 2 diabetes [2]. However, in many patients, contraindications and reduced tolerance hinder many patients in achieving their treatment goals. For this reason, the investigation on plant-based drugs serves as the basis of many scientific studies which can be a useful alternative strategy in treating hyperlipidemia of different etiology, as well as to prevent the progression of atherosclerosis.

Studies from different plants containing flavonoids, steroids, triterpenoids, saponins, phenol, and polyphenols are proven to have an antihyperlipidemic effect. *Psidium guajava L.* is one of these plants demonstrated hypolipidemic effect via leaf methanolic extract. Manikandan et al. [13] reported that *P. guajava* leaves extract possess anti-diabetic and hypolipidemic activities and the plant may be used as a hypoglycemic agent. Furthermore, the presence of flavonoids in *P. guajava*'s leaves play an essential role in the treatment of hyperlipidemia and atherosclerosis [14]. The present study stated that the methanolic extract of the leaves of the tree plants of *Amaranthus* reduces blood lipid concentration may be due to the presence of

steroids, flavonoids, and triterpenoids [9]. In another study, Akinpelu et al. [15] proved that ethanolic leaf extract of *Clerodendrum volubile* contained bioactive principles with hypolipidemic effect which were effective as curative agents than prophylactic agents.

Antigonon leptopus Hook. & Arn. locally known in the Philippines as "Cadena de Amor" is a climbing, somewhat woody, perennial vine, with stems that attains a length of 10 meters. *A. leptopus* belongs to family Polygonaceae and grows widespread in the Philippines. *A. leptopus* is mostly collected from the wild, and its different parts are used as folkloric medicine for the treatment of several diseases. For instance, the leaves are used to reduce swelling and diabetes, and the flowers are used to treat hypertension. It is also used as herbal medicine for pain and inflammatory reactions in the Philippines [16]. The isolated phytochemical constituent of the crude methanolic extract of *A. leptopus*' aerial parts include carbohydrates, glycosides, saponin, volatile oils, terpenes, tannins, flavonoids, and alkaloids, exhibit beneficial actions on human health [17]. The leaf methanolic extract *A. leptopus* was also positive to some of these phytochemicals [16]. Natural products with antioxidant activities may have potential activity against lipid peroxidation and its correlated diseases such as hyperlipidemia and atherosclerosis. Hence, an attempt has been made to investigate the antihyperlipidemic property of the methanolic crude extract of *A. leptopus* leaves in Triton-X induced hyperlipidemic male Sprague-Dawley rats thru the assessment of lipid parameters such as lipid profile and histopathological study of the liver in experimental rats. Moreover, the study also aimed to screen the potential of the crude extract as an alternative drug for hyperlipidemia in place of the expensive drugs in the market and to develop a therapy that is low-cost and readily accessible to Filipinos.

2. MATERIAL and METHODS

2.1 Collection and Authentication of Plant Material

Fresh leaves of *A. leptopus* were collected last November 24, 2016, in Brgy. Lumen San Jose, Batangas and authenticated by Mr. John Ray C. Callado at the National Museum – Botany Division located at Padre Burgos Ave, Ermita, Manila, 1000 Metro Manila.

2.2 Reagents and Materials

Triton-X 100, Tween-80, diethyl ether and methanol were purchased from Belman Laboratories whereas Selectra Pro XS Lipid Profile Analyzer was from ELITech Group Solutions.

2.3 Extraction of Plant Material

The leaves of *A. leptopus* were dried under shade until it turned brown in color and reduced to coarse powder by using a mix grinder and were passed through the 40-mesh sieve. Five hundred grams (500g) of the powder were subjected to continuous hot extraction with methanol in Soxhlet apparatus [18]. After extraction, the extract was filtered with doubled layer cotton cloth and then with Whatman no.1 filter paper, and the pooled filtrate was evaporated to dryness in vacuo by rotary evaporator until all the solvent has been removed [19]. The percentage yield of methanolic extract of *A. leptopus* was calculated based on equation 1 [18].

$$\text{percentage of yield} = \frac{\text{weight of crude extract}}{\text{weight of dried ground plant}} \times 100 \quad (1)$$

2.4 Phytochemical Screening

Phytochemical screening was done by using Thin Layer Chromatography (TLC) with different color reaction reagents. This test was performed by applying the extract on a strip of pre-coated silica plates with the use of a capillary tube. The strip was air-dried and developed in a chamber with ethyl acetate. The chromatogram was air-dried and visualized according to Guevarra [20].

2.5 Preparation of Dose for Dried Extract and Control/Standard Drug

The methanolic extract of *A. leptopus* leaves was suspended in 10% Tween-80. Reference standard used was simvastatin 10mg/kg formulated into suspension in distilled water [21].

2.6 Animals and Diet

Male Sprague-Dawley rats (4-5 months old) weighing 150-250 grams were obtained from University of the Philippines-Manila National Institute of Health located at 623 Pedro Gil St., Ermita, Manila. The animals were maintained in a well-ventilated room with a 12-hour light and 12-hour dark condition at Lyceum of The Philippines University-Batangas' Animal House. The animals were acclimatized for seven days given standard pellet diet and water ad libitum.

2.7 Induction of Hyperlipidemia

After 18 hours of fasting, a single intraperitoneal injection of a freshly prepared solution of Triton-X-100 (100 mg/kg/BW) in saline solution was injected to induce hyperlipidemia in Sprague-Dawley. Serum cholesterol and triglycerides were measured after 72 hours of Triton-X-100 injection.

The animals were randomly divided into six groups with each group composed of six animals. The groupings were as follow:

Group 1: Normal Control – received normal diet

Group 2: Untreated Triton-X induced hyperlipidemia

Group 3: Triton X + Simvastatin (10mg/kg)

Group 4: Triton X + 100 mg/kg *A. leptopus* extract

Group 5: Triton X + 200 mg/kg *A. leptopus* extract

Group 6: Triton X + 400 mg/kg *A. leptopus* extract

2.8 Collection of Blood Sample and Biochemical Analysis

On the 8th day, retro-orbital sinus puncture was done for the collection blood samples under diethyl ether anesthesia after 12 hours of fasting (Untalan, et al., 2011) and allowed to clot for 30 minutes at room temperature. The collected blood was centrifuged for 20 minutes at 3000 rpm, and the serum was separated, and biochemical estimation of lipid parameters such as cholesterol, triglycerides, LDL, HDL, and VLDL were carried out [19].

The total cholesterol, triglycerides, and HDL-cholesterol were measured using Selectra Pro XS Lipid Profile Analyzer. Serum LDL cholesterol and VLDL cholesterol were estimated by using the following Friedewald's equation respectively:

$$LDL_{mg/dL} = (Total\ cholesterol - HDL) - VLDL \quad (2)$$

$$VLDL_{mg/dL} = \frac{Total\ serum\ Triglyceride}{5} \quad (3)$$

2.9 Histopathological Studies

At the end of the experimental study, animals were sacrificed, and the liver of each animal was removed, fixed in 10% buffered neutral formalin solution. After fixation, the samples were delivered to Hi-Precision Diagnostics Plus at San Roque, Rosario, Batangas for the preparation of slides. The slides were observed with an optical microscope at LPO (40x) noting microvesicular steatosis, intralobular inflammation, hepatic architecture changes in the microscopic features of the tissues were assessed by a registered veterinarian.

2.10 Statistical Analysis

The results were presented as means \pm Standard Error of the Mean. The data were analyzed using independent student t-test to compare the baseline data. Paired t-test was applied to test the changes within groups for statistical significance. A two-tailed probability value of $p < 0.05$ was considered significant.

3. RESULTS

3.1 Crude Methanolic Extract

After continuous hot extraction, the methanolic extract was then concentrated on a rotary evaporator yielding brownish green, semi-solid extract amounting to 19.2259 g (refer Figure 1) with a total percentage yield of 15.38%.



Figure 1. Crude methanolic extract of *A. leptopus* leaves

3.2 Phytochemical Screening

A total of 1mg of the methanolic extract of the *A. leptopus* were dissolved in 1ml of methanol. The Thin-Layer Chromatography (TLC) plate was eluted with ethyl acetate in a development chamber. The spotting was observed under ultraviolet of wavelength 254 nm and 365 nm. The results of the phytochemical screening were tabulated as in Table 1.

Table 1. Phytochemical screening

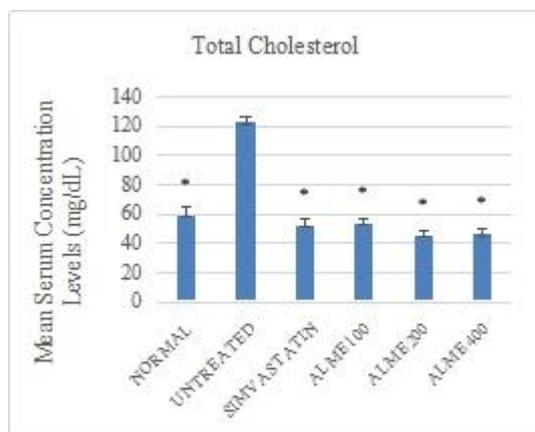
Phytochemical Constituent	Presence
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Alkaloids	+
Anthraquinones	+
Anthrone	+
Carotenoids	+
Coumarin	+
Flavonoids	+
Indoles	+
Phenols	+
Sapogenins	+
Saponins	+
Steroids	+
Steroid Glycoside	+
Tannins	+
Terpenes	+

Note: (+) positive, (-) negative

3.3 Effect of ALME (100, 200, 400 mg/kg BW) on Total Cholesterol (TC)

After statistical treatment, it was found out that there is a significant decrease in the total cholesterol after the treatment of different doses of extract and the standard drug (p-value=0.000). Bonferroni pairwise comparison test was used to assess the differences between groups. Based on the result shown in the graph in Figure 2, there is a significant decrease in the total cholesterol of the normal group (59.36 ± 5.77), simvastatin (52.57 ± 3.65) and the three doses of *A. leptopus*; 100 mg/kg extract (53.31 ± 3.67), 200 mg/kg (45.47 ± 3.83) and 400 mg/kg (46.132 ± 3.43) when compared to the untreated group intoxicated by triton-X (122.71 ± 4.47) (p-value=0.000). When the three doses of the *A. leptopus* were compared with the standard drug, simvastatin, it showed that there are no significant differences in the decrease in the total cholesterol of the animals intoxicated with triton-x (p-value= 1.000). Also, it is noted that all dose of the extract showed comparable effect, hence even at low concentration, the *A. leptopus* extract can exhibit lowering of total cholesterol which is also comparable to the standard drug, simvastatin (p-value= 1.000).

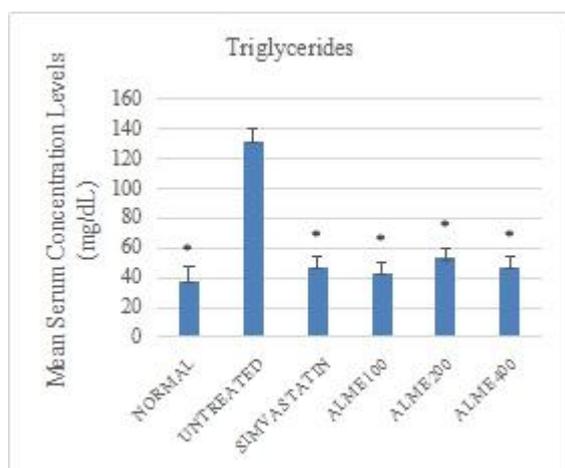


Note : Data are expressed as means \pm SEM (n=6). * (p < 0.05) vs untreated group

Figure 2. Effects on Serum TC

3.4 Effect of ALME (100, 200, 400 mg/kg BW) on Triglycerides (TG)

After statistical treatment, it was found out that there is a significant decrease in triglyceride levels after the treatment with different doses of extract and the standard drug (p-value= 0.000). Bonferroni pairwise comparison test was used to assess the differences between groups. Results in Figure 3 showed that there is a significant difference in the triglycerides of the normal group (37.33 ± 9.87), simvastatin (47.15 ± 6.71) and the three doses of *A. leptopus*; 100 mg/kg extract (43.54 ± 7.24), 200 mg/kg (53.08 ± 6.66) and 400 mg/kg (46.64 ± 7.03) when compared to the untreated group intoxicated by triton-X (132.00 ± 8.396) (p-value= 0.000). When the three doses of the *A. leptopus* were compared with the standard drug, simvastatin, it showed that there are no significant differences in the decrease in the triglycerides of the animals intoxicated with triton-x (p-value= 1.000). Furthermore, it is noted that all dose of the extract showed comparable effect, hence even at low concentration, the *A. leptopus* extract can exhibit lowering of triglycerides which are also comparable to the standard drug, simvastatin (p-value= 1.000).



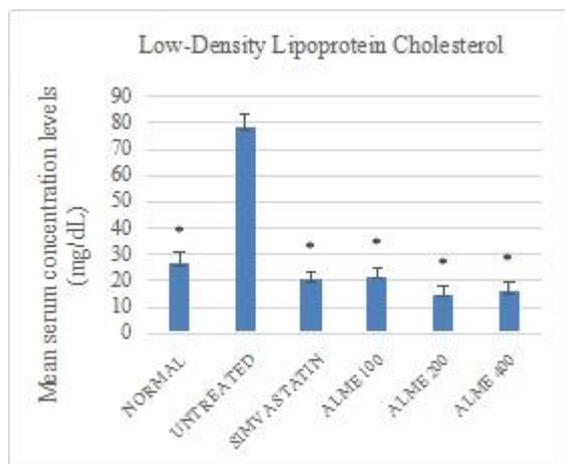
Note: Data are expressed as means \pm SEM (n=6). * (p < 0.05) vs untreated group

Figure 3. Effect on Serum TG

3.5 Effect of ALME (100, 200, 400 mg/kg BW) on Low-Density Lipoprotein-Cholesterol (LDL-c)

After statistical treatment, it was found out that there is a significant decrease in LDL-c after the treatment of different doses of extract and the standard drug (p-value= 0.000). Bonferroni pairwise comparison test was used to evaluate the differences between groups. Figure 4 presented the effects on serum LDL-c. Result showed that there is a significant difference in LDL-c of the normal group (29.93 ± 3.90), simvastatin (20.662 ± 2.92) and the three doses of *A. leptopus*; 100 mg/kg extract (21.75 ± 3.05), 200 mg/kg (14.82 ± 3.04) and 400 mg/kg (16.24 ± 3.06) when compared to the untreated group intoxicated by triton-X (78.563 ± 4.42) (p-value= 0.000). When the three doses of the *A. leptopus* were compared with the standard drug, simvastatin, it showed that there are no significant differences in the decrease in the LDL-c of the animals intoxicated with triton-x (p-value= 1.000). Besides, it is noted that all dose of the extract showed comparable effect, hence even at low concentration, the *A.*

leptopus extract can exhibit lowering of LDL-c which is also comparable to the standard drug, simvastatin (p-value= 1.000).

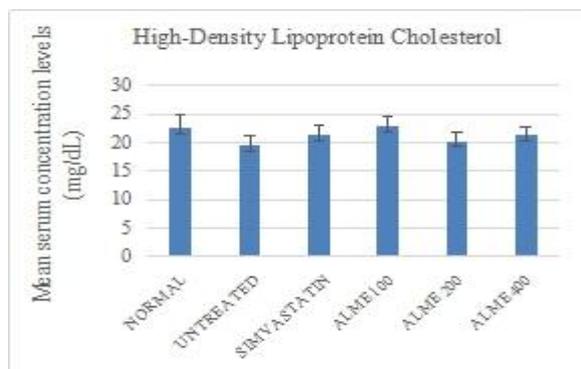


Note : Data are expressed as means \pm SEM (n=6). * (p < 0.05) vs untreated group

Figure 4. Effect on Serum LDL-c

3.6 Effect of ALME (100, 200, 400 mg/kg BW) on High-Density Lipoprotein-Cholesterol (HDL-c)

After statistical treatment, it was found out that there is no significant increase in HDL-c after the treatment of different doses of extract and the standard drug (p-value= 1.000). Bonferroni pairwise comparison test was used to measure the differences between groups. With reference to Figure 5, there is no significant difference in the HDL-c of the normal group (22.53 ± 2.37), simvastatin (21.45 ± 1.56) and the three doses of *A. leptopus*; 100 mg/kg extract (22.920 ± 1.66) 200 mg/kg (20.24 ± 1.60) and 400 mg/kg (21.24 ± 1.42) when compared to the untreated group intoxicated by triton-X (19.49 ± 1.78) (p-value= 1.000). When the three doses of the *A. leptopus* were compared with the standard drug, simvastatin, it showed that there are no significant differences in the increase in the HDL-c levels of the animals intoxicated with triton-x. (p-value= 1.000).



Note : Data are expressed as means \pm SEM (n=6). * (p < 0.05) vs untreated group

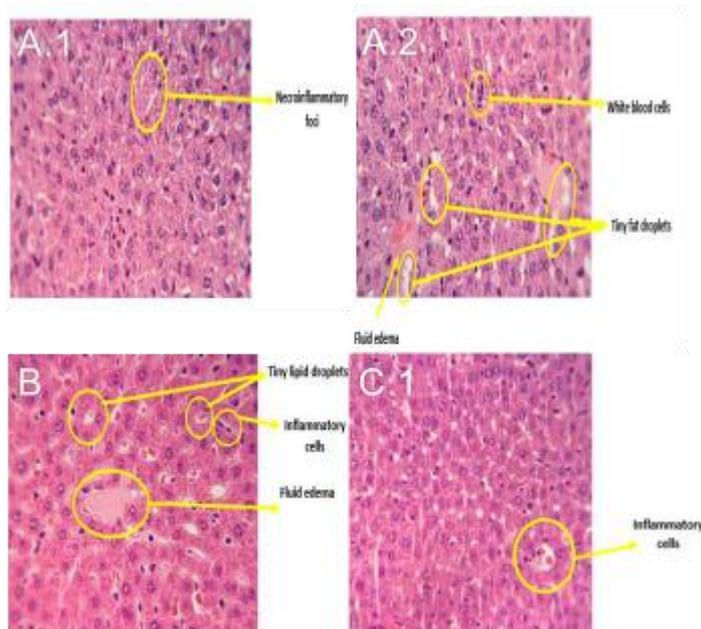
Figure 5. Effect on Serum HDL-c

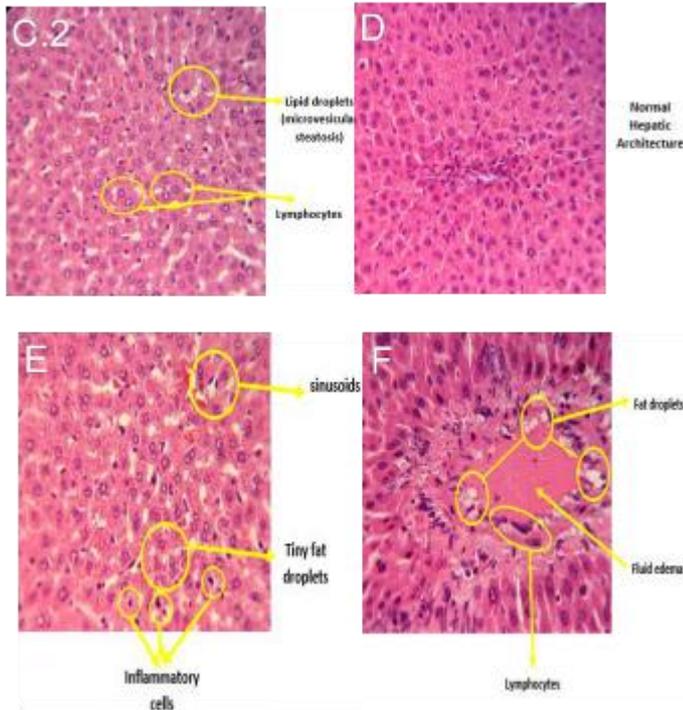
3.7 Effect of ALME (100, 200, 400 mg/kg BW) on Very Low-Density Lipoprotein-Cholesterol (VLDL-c)

After statistical treatment, it was found out that there is a significant decrease in VLDL-c after the treatment of different doses of extract and the standard drug (p-value= 0.000). Bonferroni pairwise comparison test was used to assess the differences between groups. Result showed that there is a significant difference in VLDL-c of the normal group (7.47 ± 2.05), simvastatin (9.42 ± 1.35) and the three doses of *A. leptopus*; 100 mg/kg extract (8.68 ± 1.51), 200 mg/kg (10.60 ± 1.33) and 400 mg/kg (9.38 ± 1.41) when compared to the untreated group intoxicated by triton-X (26.40 ± 1.73) (p-value= 0.000). When the three doses of the *A. leptopus* were compared with the standard drug, simvastatin, it showed that there are no significant differences in the decrease in the VLDL-c of the animals intoxicated with triton-x (p-value= 1.000). Moreover, it is noted that all dose of the extract showed comparable effect, hence even at low concentration, the *A. leptopus* extract can exhibit lowering of VLDL-c which is also comparable to the standard drug, simvastatin (p-value= 1.000).

3.8 Histopathological Analysis

The results of the histopathological analysis are shown in Figure 6 followed by the explanation of the figure as tabulated in Table 2.





Note : A: Normal Control Group, B: Untreated Triton X-induced Hyperlipidemic Group, C: Triton X + Simvastatin Group, D: Triton X + ALME 100mg/kg, E: Triton X + ALME 200mg/kg

F: Triton X + ALME 400mg/kg

Figure 6. Histopathology of the liver

Treatment Group	Findings	Interpretation
Normal Control Group	Sections of specimen consist of few necro inflammatory foci scattered in the hepatic lobule (Figure 6 A.1) and scattered numerous fat droplets with mixture of inflammatory cells and mild intralobular inflammation (Figure 6 A.2).	The existing inflammation may be due to environmental uncontrolled conditions
Untreated Triton X-induced Hyperlipidemic Group	Scattered numerous tiny lipid droplets (microvesicular steatosis) with few inflammatory cells (Figure 6 B).	The presence of lipid droplets that may indicate hyperlipidemia due to inhibition of lipoproteins in the liver.
Triton X + Simvastatin Group	Few sections with inflammatory cells and mild intralobular inflammation (Figure 6 C.1) and scattered few tiny lipid droplets (microvesicular steatosis)	Upon giving the standard drug treatment, it shows that it only lessens the inflammation and lipid

		(Figure 6 C.2).	droplets.
Triton X + ALME 100 mg/kg		Sections of specimen show normal hepatic architecture in clear hepatic lobule, radial liver cell cord and clear hepatic sinusoids (Figure 6 D).	No significant findings and normal structure were exhibited.
Triton X + ALME 200 mg/kg		Scattered numerous tiny fat droplets with mixture of inflammatory cells and mild intralobular inflammation (Figure 6 E).	Multiple productions of tiny fat droplets indicate that the extract at a dose of 200 mg/kg produces damaging effects to the liver.
Triton X + ALME 400 mg/kg		Sections of the specimen have few fat droplets. Mild portal inflammation which consists of few lymphocytes and fat deposits attached to the venous wall (Figure 6 F).	Presence of portal inflammation and fat deposits indicates that at 400mg dose the liver cannot compensate the extract anymore

Table 2. Histopathological results

3.9 Discussion

The study used the crude methanolic extract of *A. leptopus* leaves to screen the antihyperlipidemic property which was almost comparable to effects of the standard simvastatin drug.

The results are discussed under the lipid profile in serum. Treatment of Simvastatin and ALME 100, 200, 400mg/kg in triton-x 100 induced hyperlipidemic rats, significantly decreased the serum levels of TC, TG, LDL-c, and VLDL-c.

Observed hypolipidemic activity of all the doses could be attributed to the phytochemical constituent present in the plant which may act as synergistic and enhance the activity of other compounds. Where several studies reported that saponins increase the lipoprotein lipase activity might result from the rapid catabolism of LDL-cholesterol through its hepatic receptors for final elimination in the form of bile acids where this could be a possible cause for the reduction in lipid concentrations of the experimental rats [22]. Flavonoids are known hypolipidemic activity, which is being used as cancer-prevention and cardioprotective, attributable to its antioxidant property [19]. Tannins have been reported to elicit antihyperlipidemic property in rats. It is reported that not only functional phenolic compounds, but also other potent components such as fiber, that are responsible for the lipid-lowering action [23]. It has also been reported that anthraquinones can decrease blood lipid levels by the inhibition of cholesterol synthesis. Hence, flavonoids, steroids, saponins and anthocyanin, a heterogeneous group of ubiquitous plant polyphenols have exhibited a variety of pharmacological activities, including well hypolipidemic property. Therefore, the present study confirms the significant antihyperlipidemic potential of *A. leptopus* owing to its ability to reduce the level of total cholesterol, triglycerides, and LDL. The antihyperlipidemic activity may be attributed to some of its active principles.

4. Conclusion

In conclusion, this study showed that ALME exerted antihyperlipidemic potential, where lipid profiles parameters were markedly reduced by ALME treatment of hyperlipidemic rats. Through histopathological evaluation, ALME 100 mg/kg showed promising tissue-specific antihyperlipidemic effect. Thus, the hypolipidemic properties of ALME may be due to phytochemical constituents present in the plant, each with a single or diverse range of biological activities. Further biochemical and pharmacological investigations are in progress to isolate and identify the active compounds in *A. leptopus*. The researchers also highly recommend the study for further analyses, screening, purification, and isolation should be done on an individual phytochemical constituent on the methanolic leaves extract to ascertain the mechanism of action of its antihyperlipidemic property.

5. ACKNOWLEDGMENTS

The authors would like to show their heartfelt gratitude to the entire Faculty of Pharmacy, Omar Villalobos, Sheryl Aguila, Zerenie Castillo, Roi Martin Pajimna and Aaron Cobeng, and Nica Villanueva for their utmost support. The authors would also like to acknowledge the departmental laboratory technologist, Dianne Fajarillo for her assistance towards the actualization of this work.

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