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DETERMINATION AND EVALUATION OF CRUDE EXTRACTS OF BATUAN (*GARCINIA BINUCAO* LINN. FAMILY: CLUSIACEAE) FRUITS AS ANTI-HYPERLIPIDEMIC ON HIGH CHOLESTEROL DIET – INDUCED SPRAGUE DAWLEY RATS

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ABSTRACT

Hyperlipidemia is a major risk factor for heart disease, and this is the leading cause of death. Many people are suffering from hyperlipidemia and this ailment is one of the most common diseases in the Philippines. Herbal medicine is widely used throughout the world, especially in the Philippines, because it is cheap and readily available. Because of this, the researchers studied the anti-hyperlipidemic effect of Batuan (*Garcinia binucao* Linn.) to find a new source that can help treating hyperlipidemia. This study aims to determine and evaluate the antihyperlipidemic effect of Batuan fruits in Sprague Dawley rats. Extraction of Batuan was conducted by Maceration method using 95% Ethanol as solvent. A sample of 5 rats per group was employed in the study. Distilled water served as negative group, atorvastatin served as positive group and 50 mg/kg ad 100 mg/kg of Batuan extract served as treatment group. The test results for the significant difference of between post-inducement and post-treatment within group showed that Distilled water is not significant for HDL, LDL, TG, VLDL, and TC. For Atorvastatin, it significantly decreases the count of LDL, TG, VLDL, and TC, and significantly increase the HDL count. For 50 mg/kg Batuan extract, it significantly decreases the LDL, TG, and, TC and significantly increase the HDL count. For 100 mg/kg Batuan extract, it significantly decreases the LDL, TG, andTC and significantly increase the HDL count during post treatment.

KEYWORDS: *Batuan as hyperlipidemia, Garcinia binucao and lipids, Antihyperlipidemia in Garciania binucao, Garcinia binucao in HDL & LDL.*

INTRODUCTION

Batuan is an evergreen tree developing to a greatest tallness of around 25 m(82 ft) with a trunk around 40 cm (16 in) in measurement. The leaves are oval to obovate around 5 to 12 cm (2.0 to 4.7 in) long and 4 to 7 cm (1.6 to 2.8 in) wide. The blossoms are rosy to rich white shade. The natural products are round berries, around 4 cm (1.6 in) in distance across with a delicious mash and various seeds. Overall, they grow up to a stature of around 25 meters. Having a place with the family Clusiaceae, these trees are found bounteously in woods that are situated in low heights, generally in Vietnam and the Philippines. Since the product of batuan has a very sharp taste, they are generally utilized as a sour specialist by the locals intheir food plans (Baca, 2016).

Since this plant is ordinarily developed in Asia, it is extremely pragmatic for us Filipinos to benefit from the diverse benefits. This plant can flourish in low and medium rises in damp and warm places. It can likewise flourish in concealed environments.

Many people are suffering from hyperlipidemia and this ailment is one of thecommon problems of the people here in the Philippines. Because of this, we need to find a source of which can help people's misery. The anti-hyperlipidemic property of Batuan (*Garcinia binucao*) fruits made us study this variety of plant to get a solution. There is a need also to study the High Cholesterol Diet- Induced Hyperlipidemia in Rats to be sure that the experiment conducted is safe. Users of the product are interested to know the benefits they can get out of the study.

Hyperlipidemia is a significant hazard factor for coronary illness, and this is the main source of death. Hyperlipidemia implies there is an excessive amount of cholesterol in the blood. Cholesterol is a waxy fat protein made by the liver. It is fundamental for solid cell films, mind working, hormone creation, and capacity of nutrients. Cholesterol turns into an issue when an excessive amount of terrible cholesterol or low-thickness lipoprotein (LDL) is delivered or ingested through unfortunate nourishments. Lipoproteins transport

cholesterol through the blood to the cells. An individual typically has no signs and manifestations. In familial or acquired, hyperlipidemia, there might be yellowish and greasy developments around the eyes or the joints (Nachtigal, 2014). According to Society Vascular Surgery, Hyperlipidemia implies your blood has an excessive number of lipids (or fats, for example, cholesterol and triglycerides.

Aside from Anti-hyperlipidemic effects, it has the highest natural content in the fruit Batuan (*Garcinia binucao*) with antioxidant ingredients to strengthen memory and help eliminate free radicals in the body as a result in the immune system. It can likewise assist with bringing down circulatory strain and, consequently, they are valuable for individuals with hypertension. It can likewise beapplied to the skin to mend bothered skin. We can likewise remove characteristic additives to endure food harm or damage.

OBJECTIVES

The main objective of the study is to determine and evaluate the Anti- Hyperlipidemic property of Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) fruits.

1. Identify the Anti-hyperlipidemic effect of Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) fruits on high-cholesterol diet- induced rats.
2. Evaluate the percentage yield of Batuan (*Garcinia binucao* LinnFamily:Clusiaceae) fruit extract.
3. Determine the biological activity of Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) extract in Sprague Dawley rats.
4. Compare the effectiveness of Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) extract with Atorvastatin as standard drug.

4.1. Negative Control (Distilled water) 4.2.Positive Control (Atorvastatin) 4.3.50 mg/kg of Batuan (*Garcinia binucao*) extract 4.4.100 mg/kg of Batuan (*Garcinia binucao*) extract. This research study is important to conduct to identify and observe the Anti-hyperlipidemic effect of Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) fruit on High Cholesterol Diet which will be the process in making induced hyperlipidemia in rats.

Statement of the Problem

The primary purpose of this study was to determine and evaluate the Antihyperlipidemic effect of Batuan (*Garcinia binucao* Linn) in Sprague Dawley Rats. This study aimed to answer the following.

1. How will Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) fruits beextracted?
2. What are the Phytochemical Properties of Batuan (*Garcinia binucao* Linn.Family: Clusiaceae)?
3. Will Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) exhibitanti- hyperlipidemic properties?

3.1. 50 mg/kg Batuan (*Garcinia binucao*)
3.2.100 mg/kg Batuan (*Garcinia binucao*)

4. Is there a significant difference between the positive control (atorvastatin), negative control (distilled water) and Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) extract?

4.1. Negative Control (Distilled water)
4.2. Positive Control (Atorvastatin)
4.3. 50 mg/kg of Batuan (*Garcinia binucao*) extract
4.4. 100 mg/kg of Batuan (*Garcinia binucao*) extract

5. At what dosage will Batuan (*Garcinia binucao* Linn. Family: Clusiaceae) extracts produce toxicity to Sprague Dawley rats?

METHODOLOGY

Research Design

This research employs an experimental design with quantitative approach, andaims to determine and evaluate the antihyperlipidemic activity of ethanolic extract of Batuan (*Garcinia binucao Linn*. Family: *Clusiaceae*) on high cholesterol diet- inducedSprague Dawley rats.

Chemical and Reagents

All of the chemicals and reagents that are used in this study are analytical grade and some reagents were provided by St. Dominic College of Asia.

Plant Material

The fruit, Batuan (*Garcinia binucao* Linn. Family: Clusiaceae), were collected from Iloilo City and taxonomically identified and authenticated from the Bureau of Plant Industry in Quirino Ave, Malate, Manila. The fruits were air dried at room temperature for 30 days.

Extraction Method

Extraction was the initial step in isolating the ideal characteristic items from the crude materials. As indicated by the extraction rule the technique that was utilized is the dissolvable extraction which includes ethanol.

Solvent extraction is the most generally utilized technique. The extraction of natural products progresses through the following stages: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to- solid ration, the extraction temperature and extraction duration will affect the extraction efficiency (WenShang, Q., GenLi, L., CaiYe, W., 2018).

The collected *G. binucao* fruits were completely washed with refined water and air dried at room temperature. Dried examples were then squashed utilizing a blender until consistently powdered. Fifty grams of the powdered examples were absorbed 500 mL of 95% ethanol for 72 hours and sifted utilizing Whatman channel paper grade no. 41. The filtrate was evaporated under reduced pressure through rotary evaporation (Banito, Dizon,

Laurena, & Castillo, 2016).

Phytochemical Screening
Phytochemical test for Tannins, Saponins, Alkaloids and Flavonoids were performed according to procedure described by Krishnamoorthy, Nagappan, Sereen, & Rajendran (2014). Phytichemical test for Phenols was performed according to procedure described by Mamta, & Jyoti (2012). Mayer's test was done for test Alkaloids. Ferric chloride test was done for test of Tannins and Phenols. Froth test wasdone for Saponins.

Ferric Chloride Test for Tannins
To 1mL of test solution, add 2mL of ferric chloride test reagent. A brownish green or blue black solution or precipitate was taken as an evidence for the presence of tannins (Krishnamoorthy, Nagappan, Sereen, & Rajendran ,2014).

Froth Test for Saponins
To 1mL of exract, add 2 mL of distilled water and shaken vigorously and allowed to stand for 10mins. There is the development of foam on the surface of the mixture. Then shake for 10 mins., it indicates the presence of saponins. (Krishnamoorthy, Nagappan, Sereen, & Rajendran, 2014).

Test for Flavonoids
To 1mL of test solution, add 2mL of 1% aluminium solution. An appearance of yellow solution indicates the presence of flavonoids. (Krishnamoorthy, Nagappan, Sereen, & Rajendran, 2014).

Ferric Chloride Test for Phenols
To the test solution, add few drops of 5% ferric chloride solution. A dark green, blue-black, or blue-green solution indicates the presence of phenol (Mamta, & Jyoti, 2012).

Mayer's Test for Alkaloids
To 1mL of extract, add 2mL of conc. HCL then add few drops of Mayer's reagent. Positive test shows green color or white precipitate. (Krishnamoorthy, Nagappan, Sereen, & Rajendran, 2014).

Selection for Test Animal
Healthy twenty five (25) Sprague-dawley rats of either gender, each weighing 150 – 200 g was used. The rats were maintained under standard laboratory conditions. The source of rats is from the University of the Philippines- Diliman. The reason for selecting rats as test animals is that rats are used in evaluating various pharmacological effects including but not limited to antihyperlipidemic activity. The rats were acclimated for seven (7) days in a temperature of 25±1°C and a relative humidity of 50±5%. Plastic cages with steel cover and autoclaved wood shavings were used as beddings of the rats.

Experimental Design
The rats were given Altromin 1324 and Wilkins distilled water as their standardmaintenance diet. Coconut oil was given to the rats as it serves as high cholesterol inducer. The rats were given free access to water and food. After giving the *G. binucao*extract. The rats were divided into four (4) groups each containing five (5) Sprague Dawley rats. First group (Group A) was served as negative control and received a high-cholesterol and high-glucose diet and there is no treatment administered. The second group (Group B) served as a positive control group and received atorvastatin. The third group (Group C) served as a treatment group and received 50mg/kg of batuan extract. The fourth group (Group D) also served as a treatment group and received 100mg/kg of batuan extract (Alamgeer, Ghuffar, Ahmad, & Mushtaq, 2014).

Table 2: Different Experimental Groups.

Group No.	Treatment
Group A (negative control)	Distilled water
Group B (positive control)	Atorvastatin
Group C (Treatment control)	50 mg/kg of Batuan extract
Group D (Treatment control)	100 mg/kg of Batuan extract

Blood Sample Collection for AnimalTail Vein
The tail vein procedure can be used to collect blood from mice and rats. Distal end of the tail is cleaned with warm water (Stewart, 2017). According to Stewart (2017), alcohol should not be used as it will cause vasoconstriction. Stewartalso states that warming the tail before the procedure with a warm washcloth or a heating pad will increase blood flow. The distal tip of the tail is amputated with a sterile surgical blade or a sharp pair of sterile surgical scissors (NIH.gov, 2010). However, no more than 1 mm of tail tissue should be removed from a mouse or 2 mm from a rat (NIH.gov, 2010). The blood collection tube is positioned under the clipped part of the tail. Stroking the tail or squeezing the tail from the base to the distal end can increase blood flow but can also increase the contamination of the sample with other cells and other tissue products (Stewart, 2017).

Hyperlipidemic Tests Procedures
Blood samples were taken from the tail vein, and the sera were subjected to laboratory analysis for lipid profiling Triglycerides (TG), Total Cholesterol (TC), Lipoprotein Cholesterol (LDL-C), (HDL-C) High Density Lipoprotein and Very low Density Lipoprotein (VLDL-C) levels (Evanghelista, 2018).

Estimation of Total cholesterol (TC) CHOD-PAP
This method was used for the estimation of serum

cholesterol. In this method, the following were pipetted into the reaction vessel using a micropipette. Test samples (T): 0.02 ml serum, 2.00 ml reaction solution; the standard sample (S): 0.02ml standard and 2.00 ml reaction solution, while for the blank sample (B): 0.02ml DW and 2.00ml reaction solution. The mixture was mixed well and incubated for 10 minutes at +20 to 25c or 5 minutes at 37c. The absorbance was read at 505/670 nm against the reagent blank (Ghori , Khan , Alam & Abrar, 2015).

Estimation of Triglycerides (TG)

GPO-PAP method was used to estimate the serum triglycerides. For this 0.01ml of serum was taken in a test tube (T) in which 1ml reaction solution was added. In another test tube (S) 0.01ml standard and 1ml reaction solution were added. The solution was mixed well and incubated at +20 to 25C for 10 min. The absorbance of standard and test against reagent blank was read at 505 (500-540 nm) (Ghori, Khan, Alam & Abrar, 2015).

Estimation of High density lipoprotein (HDL)

CHOD-PAP method was used to estimate the serum HDL cholesterol level. CHOD-PAP method (Henry, 1974) was used to estimate the serum HDL cholesterol level. For this 2ml if serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10min at +15 to 25c and then centrifuged for 15min at 4000rpm. Within 2hr after centrifugation, the clear supernatant was used for the determination of HDL-C. One ml of the supernatant was taken in a test tube (T) and 1 ml of reaction solution was added to it. In another test tube, 0.1 ml DW was taken and 1ml reaction solution (B) was added. The mixtures were mixed thoroughly, incubated for 10min (Ghori,Khan, Alam & Abrar, 2015).

Estimation of Low density lipoprotein (LDL)

LDL cholesterol was estimated by using Firedwald's (1972)formula as follows.

LDL in mg% =total cholesterol−HDL−C−Triglyceride 5 (Ghori, Khan, Alam & Abrar, 2015).

Estimation of Very low density lipoprotein (VLDL)

VLDL cholesterol was estimated by using the following formula: VLDL in mg % =Triglyceride 5 (Ghori, Khan, Alam & Abrar, 2015).

Histopathological studies

At the end of the study period, animals from both the experimental groups were sacrificed. The liver and aorta were dissected out, washed, 5µm thick section slides were prepared and stained with hematoxylin-eosin and examined by light microscopy (Ghori, Khan, Alam & Abrar, 2015).

Acute Toxicity Study

The acute toxicity of Blanco (*Garcinia binucao* Linn.) fruit extract was performed in five (5) Sprague Dawley rats according to the Organizational for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals and as stated in the guideline, test substances that have a known dose thatcause marked pain and distress due to corrosive or severely irritant actions shall notbe given to animals. For the selection of animal species, it is said that healthy younganimals of commonly used laboratory strains should be used. According to the guideline, the temperature in the experimental animal room should be 22 °C (± 3°C).

The relative humidity of the housing and feeding conditions should be at least 30% and does not exceed 70% other than during room cleaning the aim should be 50-60% (OECD 425). The animals were kept in a cage for at least 5 days prior to dosing for them to be able to adjust to the laboratory conditions. According to the guideline, for the administration of doses, the test substance should be administered in a single dose by gavage using a stomach tube. It is also stated that the animals should be fasted before dosing (rat: the food except water should be held back overnight). It is also stated that the time interval between treatment groups can be determined by its onset, duration, and severity of toxic signs. The animals were observed individually after 30 minutes, and periodically for the first 24 hours, with special attention for the first 4 hours, and daily for a total of 14 days. A single dose of 5mg/kg; 50mg/kg; 300mg/kg; 2000mg/kg; and 3000mg/kg of the extracts of *G. binucao* were administered through oral gavages. (OECD 425)

Statistical Analysis

The data were compared using Analysis of Variance (ANOVA). A sample size of five (5) rats per group can produce a statistical significance when calculated based on the E value (df of ANOVA) while utilizing the least number of animals for the procedure. The E value is calculated with the following equation.

E value= total number of animals-total number of groups

Thus,

E value= 20

rats - 4 groups Evalue- 16 (Charan, & Kantharia, 2013).

PRESENTATION, INTERPRETATION, AND ANALYSIS OF DATA /DISCUSSION OF RESULTS

1. **How will Batuan (*Garcinia binucao* Linn. Family. Clusiaceae) fruits be extracted?**

Batuan (*Garcinia binucao)* fruit was collected from a farm in Iloilo city, Philippines. The fruits were washed with distilled water to remove unwanted substances such as dirt and other extraneous substances. The fruits were air dried at room temperature for 30 days and grinded using a blender until consistently powdered. The extractionthat was performed was maceration. 50 grams of the powdered fruits were absorbed 500 mL of 95% ethanol for 72 hours and filtered using Whatman channel paper grade no. 41.The filtrate was evaporated to dryness under reduced pressure using a rotary evaporator.

2. What are the Phytochemical Properties of Batuan (Garcinia binucao Linn. Family: Clusiaceae)?

Table 3: Phytochemical Test Results of Batuan(*Garcinia binucao* Linn. Family: Clusiaceae).

Phytochemicals	Results
Tannins	+
Saponins	-
Flavonoids	+
Phenols	-
Alkaloids	-

The secondary metabolites such as Tannins, Saponins, Flavonoids, Phenols, and Alkaloids were determined in Ethanolic extracts of Batuan (*Garcinia binucao*) using confirmatory tests by Vergeji, & Sukendar (2016). Phytochemical Screening and Antioxidant Activity of Edible Wild Fruits in Benguet, Cordillera Administrative Region, Philippines is a peer-reviewed journal served as one of the bases for the results of phytochemical screening of ethanolic extract of Batuan fruits. *Garcinia binucao* Crude Ethanolic Extract Prevents Alcohol-Induced Neurotoxic Effect on Learning, Short-term Memory, and Motor Functions in Drosophila is another published journal that was used as one of the bases for the phytochemical screening.

The phytochemical analysis shows that Batuan (*Garcinia binucao* Linn. Family: Clusiaceae*)* contains Tannins, and flavonoids. According to Gato, Kadowaki, Hashimoto & Yokoyama (2012), tannin- rich fiber from young Persimmon (*Diospyros kaki*) fruits had bile acid-binding properties. Bile acid- binding agents can lower blood cholesterol levels and have been used clinically in treating hypercholesterolemia (Gato, Kadowaki, Hashimoto & Yokoyama, 2012).

According to Zeka, et al (2017), oral administration of flavonoids quercetin glycoside rutin to streptozotocin-induced diabetic rats has been shown to be able toreduce lipid levels in plasma and tissues. Zeka, et al (2017) also stated that it increases plasma HDL cholesterol due to an observed reduction in activity of (HMGCoA) reductase, increases the plasma Lecithin Cholesterol Acyltransferase (LCAT). Flavonoids lower the risk of atherosclerosis, one of the diseases caused by hyperlipidemia, by protecting LDL cholesterol from free radical damage (Szalay, 2015).

3. Will Batuan (*Garcinia binucao* Linn, Family: Clusiaceae) exhibitanti- hyperlipidemic properties?

The anti-hyperlipidemic properties of Batuan extract was determined by analyzing the different lipid parameters such as High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Triglyceride (TG), Very low-density lipoprotein (VLDL), and Total cholesterol (TC). HDL, also called the ―good cholesterol‖, is responsible forthe return of lipoprotein and cholesterol from peripheral tissues to the liver for excretion process (Walker & Whittlesea, 2012). LDL is considered as bad cholesterol because it takes cholesterol to the arteries and will stay in the artery walls (McDermott, 2018). If there is too much cholesterol in the arteries it may lead to accumulation plaque known as atherosclerosis, that can increase risk of blood clots in arteries, reduce blood flow oxygen to major organs (McDermott, 2018). VLDL contains higher triglyceride that LDL. VLDL is also considered as bad cholesterol just like LDL because cholesterols are being build-up on the artery wallsand can clog the arteries and lead to stroke or heart attack (McDermott, 2018). Triglycerides are a type of fat that are essential in facilitating the transfer of energy from food into body cells (Mims, 2018). High triglyceride may be a sign of metabolic syndrome- combination of high blood pressure, high blood sugar, low HDL, and high triglycerides (High triglycerides, n.d.). Having higher HDL than LDL, VLDL, and TG is preferred because HDL can prevent a person having cardiovascular diseases.

Table 4: Average Count for Baseline.

Group	HDL	LDL	TG	VLDL	TC
Distilled Water	68.60	95.60	113.00	20.40	186.80
Atorvastatin	66.20	98.00	101.00	16.20	184.40
50 mg/kgBatuan	68.80	104.40	96.40	20.40	192.48
100 mg/kgBatuan	66.20	97.60	100.20	13.00	183.84

Table 4 shows the average count for baseline. It shows the condition of the rats before inducement and giving treatment.

Table 5: Average Count for Post-Inducement.

Group	HDL	LDL	TG	VLDL	TC
Distilled Water	28.00	172.60	178.40	47.80	236.28
Atorvastatin	47.60	145.20	177.60	6.60	228.32
50 mg/kg Batuan	51.80	135.20	187.80	22.00	224.56
100 mg/kg Batuan	53.60	145.40	190.60	10.20	237.12

Table 5 shows the average count for post-inducement of rats. The rats were given Altromin 1324 and Wilkins distilled water as their standard maintenance diet. Coconut oil was given to rats and serves as high cholesterol inducer.

Table 6: Average Count for Post-Treatment.

Group	HDL	LDL	TG	VLDL	TC
Distilled Water	23.60	185.00	175.00	50.40	243.60
Atorvastatin	64.80	94.80	94.80	18.60	178.56
50 mg/kg Batuan	65.80	105.80	101.60	15.80	191.92
100 mg/kg Batuan	66.80	94.80	95.00	17.00	180.60

Table 6 shows the average count for post-treatment. It shows the results of different lipid profiles, which include HDL, LDL, TG, VLDL, and TC, after giving the treatment to rats. The treatment includes Atorvastatin, 50 mg/kg Batuan extract, and 100 mg/kg Batuan extract. Atorvastatin served as positive control group, while 50 mg/kg and 100 mg/kg of Batuan extract served as treatment control groups.

Table 7: Test for Significant Difference Between Post-Inducementand Post- Treatment Within Groups.

Group	HDL	LDL	TG	VLDL	TG	Significance
Atorvastatin	.000	.000	.000	.031	.000	Significant
50 mg/kgBatuan	.001	.000	.000	.245	.000	Significant except forVLDL
100 mg/kgatuan	.002	.000	.000	.344	.000	Significantexcept for VLDL

Table 7 shows the test for significant difference betweenpost-inducement and post- treatment within groups. Table 7 compares if there is change or difference between post- inducement and post-treatment of lipid profiles of the different treatments.

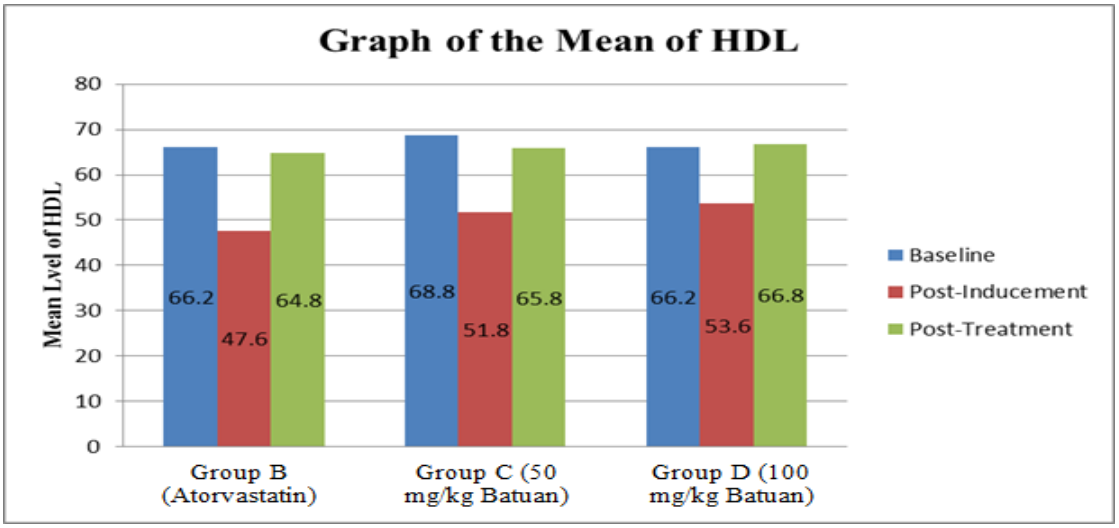
3.1 50 mg/kg Batuan (*Garcinia binucao*) extract

50 g/kg Batuan extracts show anti hyperlipidemic effect as shown in table 7. The computed p-value in the post induction compared to the post treatment is less than .05 alpha level in HDL, LDL, TG, and TC. This shows that there is a significant difference. Hence, 50 mg/kg of Batuan significantly increases the HDL and significantly lowers the LDL, TG, and TC during post treatment. High triglyceride and total cholesterol levels are the primary concerns of patients diagnosed with hyperlipidemia and must be monitored. The 50 mg/kg ethanolic concentration of Batuan shows effectiveness in treating hyperlipidemia as it lowers total cholesterol and triglyceride. However, the computed p-value for VLDL is greater than .05 alpha level. This means that there is no significant difference. Thus, 50 mg/kg of Batuan shows no improvement in the VLDL count during post-treatment.

3.2 100mg/kg Batuan (*Garcinia binucao*) extract

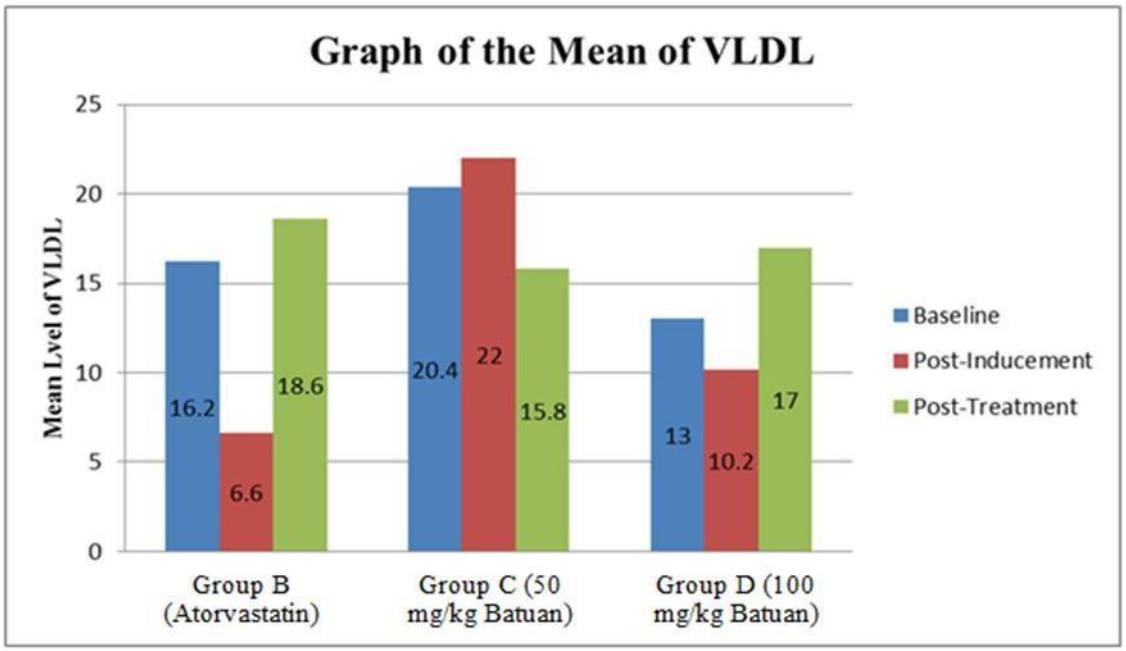
100 mg/kg Batuan extracts also exhibit Antihyperlipidemic effects as shown in Table 7. Comparing the post-inducement and post-treatment of 100 mg/kg of Batuan, as shown in table 7, the computed p-value is less than .05 alpha level in High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Triglyceride (TG), and Total Cholesterol (TC). This would mean that there is a significant difference or change in the lipid profile, thus significantly decreasing the LDL, TG, and TC and significantly increasing the HDL during post treatment.

The result means that 100 mg/kg Batuan extracts exhibit antihyperlipidemic effect since it lowers the TG and TC. However, the computed p-value for VLDL is greaterthan .05 alpha level. This means that there is no significant difference and no changes on the VLDL levels are shown.



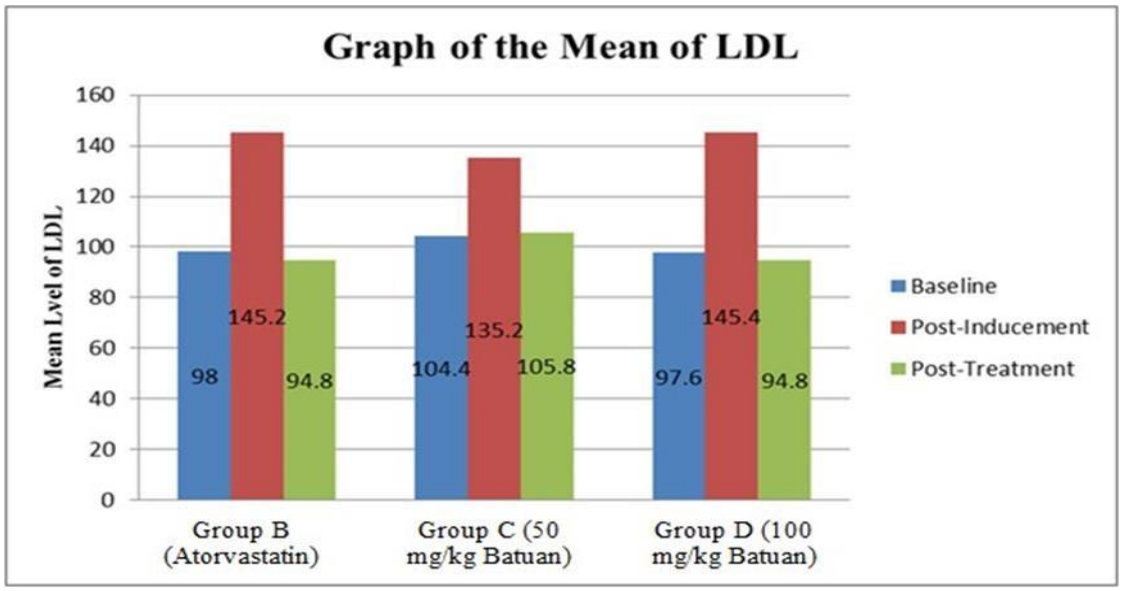
Graph 1 Mean of HDL of Atorvastatin, 50 mg/kg, and 100 mg/kg.

The concentration of Batuan (Garcinia binucao) plays an important role in determining its effectiveness as an Anti Hyperlipidemic agent. As shown in Graph 1, 50 mg/kg Batuan ethanolic extract, 100 mg/kg Batuan ethanolic extract, shows almost the same level with atorvastatin in increasing HDL when comparing the post- inducement and post-treatment. This means that 50 mg/kg Batuan extract and 100 mg/kg Batuan extract can help in increasing HDL. Increased HDL is good, since HDL is known as good cholesterol. Based on the result, 100 mg/kg may be used as an antihyperlipidemic agent.

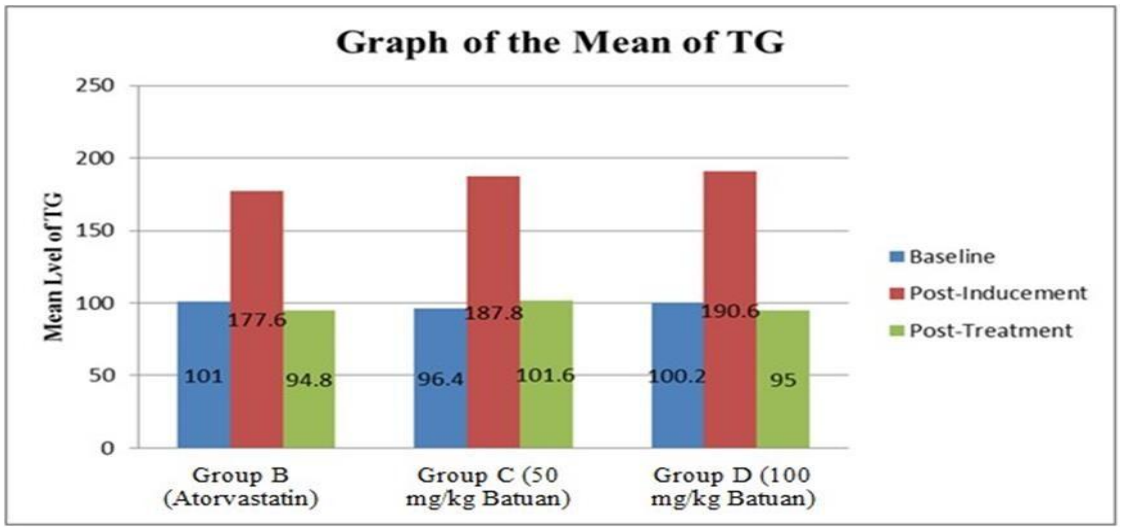


Graph 2: Mean of VLDL of Atorvastatin, 50 mg/kg, and 100 mg/kg.

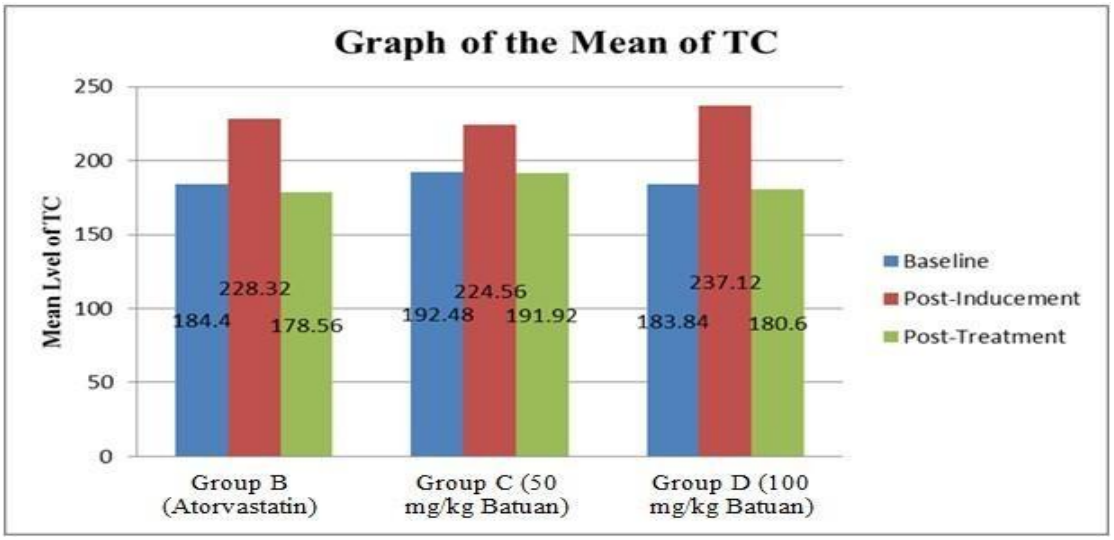
For Graph 2, atorvastatin and 100 mg/kg Batuan extract have lower post inducement than 50 mg/kg Batuan extract. This means that atorvastatin and 100 mg/kg Batuan extract did not receive the same inducement process or they have different levels of cholesterol than 50 mg/kg Batuan extract. As a result, atorvastatin and 100 mg/kg Batuan extract were not able to decrease the VLDL during post-treatment. For 50 mg/kg Batuan extract, the VLDL was decreased during post treatment. It means that50 mg/kg Batuan extract can help in lowering the VLDL.



Graph 3: Mean of LDL of Atorvastatin, 50 mg/kg, and 100 mg/kg.



Graph 4: Mean of TG of Atorvastatin, 50 mg/kg, and 100 mg/kg.



Graph 5: Mean of TC of Atorvastatin, 50 mg/kg, and 100 mg/kg.

Graphs 3, 4, and 5 show that atorvastatin, 50 mg/kg Batuan extract, and 100 mg/kg Batuan extract decreases the LDL, TG, and TC count of rats when comparing the post-inducement and post-treatment. As shown in Graphs 3, 4, and 5 atorvastatin and 100 mg/kg Batuan have the same level of LDL, TG, and TC. This means that they have the same effect in lowering the LDL, TG, and TC. Graph 3 also shows that 50 mg/kg Batuan extract has higher LDL, and TC than atorvastatin and 100 mg/kg. This means that the higher the dose, the lower the LDL, TG, and TC level. Decreased levels of LDL are good because it is bad cholesterol. The result shows that 50 mg/kg and 100 mg/kg Batuan extract have effects close or similar to atorvastatin, making it a possible substitute or alternative to atorvastatin.

Several studies have shown that Garcinia plant species have significant effect in treating hyperlipidemia. According to the study conducted by Sarma, et al. (2016), *Garcinia pedunculata* polyphenol-rich extract can lower TC, TG, and LDL, hence, G. *pedunculata* treatment has efficient way to cure hyperlipidemia. In another study, *Garcinia mangostana*, showed that it has a potential to be an anti-obesity drugs since it can lower the LDL and TG level (Abuzaid, Sukandar, Kurniati, & Adnyana, 2017). As shown in the results of our study, *Garcinia binucao* has a potential to be an antihyperlipidemic drugs because it can lower the LDL, TG, and TC level and it can also increase the level of HDL.

4. Is there a significant difference between the positive control (atorvastatin), negative control (distilled water), and Batuan (*Garcinia binucao* Linn. Family:Clusiaceae) extract?

Table 8: Means & Standard Deviation of Different Groups.

GROUP		LIPID PARAMETE RS	BASELINE	POST-INDUCEMENT	POST-TREATMENT
		HDL	68.6	28	23.6
		LDL	95.6	172.6	185
	Mean	TG	113	178.4	175
		VLDL TC	20.4 186.8	47.8 236.28	50.4 243.6
NEGATIVE (Distilledwater)	Std Deviation	HDLLDL TG VLDL	1.5166 3.8471 10.8858 10.0150	6.5955 15.0266 19.1128 6.2209	3.0496 15.9687 11.9373 6.0663
		TC	2.8531	12.0744	16.4773
	Mean	HDL	66.2	47.6	64.8
		LDL	98	145.2	94.8
		TG	101	177.6	94.8
		VLDL	16.2	6.6	18.6
POSTIVE		TC	184.4	228.32	1778.56
(Atorvastatin)		HDL	4.969909	3.507136	4.549725
	Std Deviation	LDL TG VLDL	8.3666 8.154753 9.284396	12.17374 13.2778 4.929503	3.114482 2.683282 8.414274
		TC	5.758472	13.26695	4.236508
	Mean	HDL	68.8	51.8	65.8
		LDL	104.4	135.2	105.8
		TG	96.4	187.8	101.6
		VLDL	20.4	22	15.8
50 mg/kg		TC	192.48	224.56	191.92
Batuan extract		HDL	3.420526	7.120393	4.207137
	Std Deviation	LDL TG VLDL	5.458938 5.412947 6.107373	1.643168 7.52994 6.63325	5.01996 7.503333 10.54514
		TC	7.650621	7.639895	8.600698
	Mean	HDL	66.2	53.6	66.8
		LDL	97.6	145.4	94.8
		TG	100.2	190.6	95
		VLDL	13	10.2	17
100 mg/kg		TC	183.84	237.12	180.6
Batuan extract		HDL	5.974948	6.618157	2.863564
	Std Deviation	LDL TG VLDL	3.781534 7.918333	5.07937 3.435113	2.774887 3.535534

			10.88577	10.61603	11.24722
		TC	7.377533	3.830405	2.024846

Table 8 shows the mean and standard deviation results of the different lipidparameters (HDL, LDL, TG, VLDL, and TC) during baseline, post- inducement, ad post-treatment of rats.

Table 9: Test for Significant Difference Between Groups.

Compared Group		HDL	LDL	TG	VLDL	TC	Significance
DistilledWater	Atorvastatin	**.000***	**.000***	**.000***	**.000***	**.000***	Significant
	50 mg/kg	**.000***	**.000***	**.000***	**.000***	**.000***	Significant
	100 mg/kg	**.000***	**.000***	**.000***	**.000***	**.000***	Significant
Atorvastatin	50 mg/kg	.711	**.045***	.194	.635	**.037**	Significant except for HDL, TG, VLDL
	100 mg/kg	.462	1.000	.969	.786	.737	Not Significant
50 mg/kg	100 mg/kg	.711	**.045***	.207	.839	.073	Not significant except for LDL

Table 9 shows the test for significant difference between groups. Table 9 compares a treatment to another treatment whether the difference is significant or not.

4.1 Negative Control (Distilled water)

For Distilled water compared with Atorvastatin, 50 mg/kg of Batuan extractand 100 mg/kg of Batuan extract the computed p-value is less than .05 alpha level in HDL, LDL, TG, VLDL and, TC. This means that there is a significant difference.

Hence, distilled water shows better result of significant differences in lowering theLD, TG, VLDL, and TC and increasing the HDL compared to the other groups. Multiple factors such as not being able to control the lipid profile levels of the rats during Post induction and randomized selection on the gender of the Sprague Dawley rats as per methodology, are the possible reasons why distilled water shows better effects compared to atorvastatin or the ethanolic crude extract of the plant sample which also shows Anti hyperlipidemic effects.

4.2 Positive Control (Atorvastatin)

For Atorvastatin compared with 50 mg/kg of Batuan extract, the computed p-value for LDL and TC is less than .05 alpha level. This means that there is a significant difference. Hence, the LDL and TC count of 50 mg/kg Batuan extract issignificantly higher than Atorvastatin in post-treatment. Thus stating that atorvastatin is better in lowering LDL and TC count. However, the computed p-value for HDL, TG, and VLDL is greater than .05 alpha level. This means that there is no significant difference. Hence, the computed HDL, TG, and VLDL for Atorvastatin in post-treatment is not significantly different compared to 50 mg/kg Batuan extract.

For Atorvastatin compared with 100 mg/kg of Batuan extract the computed p-value is greater than .05 alpha level in HDL, LDL, TG, VLDL, and TC. This means that there is no significant difference.

This means that there is no significant difference in the HDL, LDL, TG, VLDL, and, TC of Atorvastatin compared to 100 mg/kg Batuan extract in post-treatment and has almost identical effects in altering the lipid profile.

4.3 50 mg/kg Batuan (*Garcinia binucao*)

For 50 mg/kg of Batuan compared with 100 mg/kg of Batuan, the computed p-value is greater than .05 alpha level in HDL, TG, VLDL, and TC. This means thatthere is no significant difference. Hence, the computed HDL, TG, VLDL, and TC for 50 mg/kg Batuan extract in post-treatment is not significantly different compared to 100 mg/kg Batuan and have shown similar results in decreasing the TG, VLDL, AND TC.

4.4 100 mg/kg Batuan (*Garcinia binucao*)

As stated above, 100 mg/kg Batuan (Garcinia bucao) has similar effects when compared to atorvastatin, having no significant difference as its computed p value has a computed p-value of greater than .05 alpha level. 100 mg/kg vs 50 mg/kg has similar effects across the lipid profile except for the LDL on which 50 mg/kg Batuan extract has more effectiveness on lowering.

Table 8: Does Blanco (*Garcinia binucao*) has significant difference withatorvastatin?.

Compared Treatments		P-value	Interpretation	Analysis
Atorvastatin	50mg/kg	0.045	Significant	Accept alternativehypothesis
	100 mg/kg	.462	Not Significant	Accept nullhypothesis
50mg/kg	100mg/kg	.711	Not significant	Accept nullhypothesis

The analysis revealed that there was a significant difference between atorvastatin and 50 mg/kg. Comparison between atorvastatin and 100 mg/kg of the sample has no significant difference and has same effect. The analysis also revealed that there is no significant difference between atorvastatin and 100 mg/kg.

5. At what dosage will Batuan (Garcinia binucao Linn. Family: Clusiaceae) extracts produce toxicity to Sprague Dawler rats?

The acute toxicity of Batuan (*Garcinia binucao* Linn.) fruit extract was performed in five (5) Sprague dawley rats. The relative humidity of the housing and feeding conditions should be at least 30% and does not exceed 70% other than during room cleaning the aim should be 50-60% (OECD 425). The animals were observed individually after 30 minutes, and periodically for the first 24 hours, with special attention for the first 4 hours, and daily for a total of 14 days. A single dose of the extracts of Batuan was administered through oral gavages. No rats died when administered with 2000 mg/kg and 5000 mg/kg Batuan extract.

SUMMARY OF FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

Summary of Findings

Based on the research work, it was found that Batuan could be successfully extracted using ethanol extract with a significant anti-hyperlipidemic activity. The researchers also found out that it has a short-term general toxicity in rats.

DISCUSSION

Many people are suffering from hyperlipidemia and this ailment is one of the most common diseases in the Philippines. Hyperlipidemia is described as having abnormally high concentrations of triglycerides and total cholesterol in the body. Because of this, the researchers studied the anti-hyperlipidemic effect of Batuan to find a new source that can help treating hyperlipidemia. Since Batuan is ordinarily developed in some areas of the Philippines, it is extremely pragmatic for us Filipinos to get benefit from the plant. Atorvastatin, a drug commonly used in treating hyperlipidemia, lowers cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, thus preventing the formation of the major building block of the cholesterol molecule. Documented studies regarding the anti-hyperlipidemic effects genus *Garcinia* is the main basis of the study and is thereforeproven with this study.

The extraction that was performed in this research was Maceration. Batuan fruits were washed with distilled water to remove unwanted substances such as dirt and other extraneous substances. The fruits were air dried at room temperature for 30 days and grinded using a blender until consistently powdered. 50 grams of the powdered fruits were absorbed 500 mL of 95% ethanol for 72 hours and filtered using Whatman channel paper grade no. 41.The filtrate was evaporated to dryness under reduced pressure using a rotary evaporator.

Phytochemical studies regarding the plant sample shows that it contains polyphenols such as Flavonoids and tannins. Multiple studies state that the genus garcinia and family clusiaceae have antihyperlipidemic activity due to the polyphenols present in plants. Studies regarding flavonoids suggest that it reduces blood cholesterol levels through inhibition of cholesterol synthesis and increase of LDL receptor expression and is said to inhibit the HMG COA reductase, a mechanism of action commonly utilized by statin drugs (Zeka, et al., 2017). It is also stated that tannins are laso responsible for treating hyperlipidemia, as phenolic compound can inhibit LDL oxidation, retard/prevent foam cell formation, and further minimize the possible damage of vessels caused by oxidized LDL (Amarowicz, 2016).

Batuan ethanolic extract exhibits anti-hyperlipidemic effect that is similar to Atorvastatin as shown in the result of Test for significant difference for between post-inducement and post-treatment within groups. 50 mg/kg of Batuan significantly increases the HDL and significantly lowers the LDL, TG, and TC during post treatment. High triglyceride and total cholesterol levels are the primary concerns of patients diagnosed with hyperlipidemia and must be monitored. The 50 mg/kg ethanolic concentration of Batuan shows effectiveness in treating hyperlipidemia as it lowers total cholesterol and triglyceride. However, 50 mg/kg of Batuan shows no improvement in the VLDL count during post- treatment. 100 mg/kg Batuan extracts also exhibit Antihyperlipidemic effects as shown in Table 7. 100 mg/kg Batuan extracts exhibit antihyperlipidemic effect since it lowers the TG and TC. However, there is no significant difference and no changes on the VLDL levels in the results of 100 mg/kg.

Tests for significant difference between groups for post treatment results show that Distilled water (negative control group) shows better result of significant differences in lowering the LD, TG, VLDL, and TC and increasing the HDL compared to the other groups. Multiple factors such as not being able to control thelipid profile levels of the rats during Post induction and randomized selection on the gender of the Sprague

Dawley rats per methodology are the possible reasons why distilled water shows better effects compared to atorvastatin or the Ethanolic crude extract of the plant sample which also shows Anti hyperlipidemic effects.

For Atorvastatin compared with 50 mg/kg of Batuan extract, the LDL and TC count of 50 mg/kg Batuan extract is significantly higher than Atorvastatin in post-treatment. Thus stating that atorvastatin is better in lowering LDL and TC count. There is no significant difference in HDL, TG, and VLDL levels between atorvastatin and 50 mg/kg batuan extract. Hence, the computed HDL, TG, and VLDL for Atorvastatin in post-treatment is not significantly different compared to 50 mg/kg Batuan extract. For Atorvastatin compared with 100 mg/kg of Batuan, extract there is no significant difference in the HDL, LDL, TG, VLDL, and, TC in post-treatment and has almost identical effects in altering the lipid profile.

A single dose of the extracts of Batuan was administered through oral gavages. No rats died when administered with 2000 mg/kg and 5000 mg/kg Batuanextract.

CONCLUSIONS

Hyperlipidemia is a major risk factor for heart disease and this is the leadingcause of death. It is one of the risk factors that contribute to the prevalence of coronary heart diseases and antihyperlipidemic agents, such as statin, was used to treat hyperlipidemia as a current therapy. Herbal medicine is widely used throughout the world especially in the Philippines because it is cheap and readily available. Theadministration of ethanol extract of Batuan extract to Sprague Dawley rats showed significant anti-hyperlipidemic effect with short term general toxicity. The results revealed that Batuan's (*Garcinia binucao*) post-treatment produces anti hyperlipidemic effects that are comparable to the standard drug atorvastatin, as the sample lowers both LDL and TG levels of the rats: two parameters that are mainly observed in patients with hyperlipidemia. The concentration of the plant sample is an important factor because as shown in the results, the higher the concentration the better the results. As shown in results, atorvastatin and 100 mg/kg Batuan have the same level of LDL, TG, and TC. This means that they have the same effect in lowering the LDL, TG, and TC. The resultsalso showed that 50 mg/kg Batuan extract has higher LDL, and TC than atorvastatinand 100 mg/kg. This means that the higher the dose, the lower the LDL, TG, and TClevel. Decreased levels of LDL are good because it is bad cholesterol. The result shows that 50 mg/kg and 100 mg/kg Batuan extract have effects close or similar to atorvastatin, making it a possible substitute or alternative to atorvastatin.

Limitations and Recommendations

In the light of the conclusions, the following recommendations are hereby advanced:

1. Further phytochemical work to identify and analyze the chemical compounds present in Batuan fruits.
2. Further characterization of ethanolic extract of Batuan (*Garcinia binucao*)fruits.
3. Performed more extensive studies such as chemical and structural analysis of the Batuan (*Garcinia binucao*) ethanolic extract.
4. Determine the gender and weight of the rats before performing the post- inducement. The weight of the rats should be the same or almost the same.
5. The level of HDL of the rat should be controlled in post induction treatment.
6. Use higher concentration of Batuan (*Garcinia binucao*) ethanolic extract.

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