RESEARCH ARTICLE

Effects of *Peperomia pellucida* (L.) methanolic extract on total cholesterol levels and liver histology of diet-induced hypercholesterolemic rats

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ABSTRACT

Background: *Peperomia pellucida*, locally known in the Philippines as pansit-pansitan, is an annual herb with many ethnomedicinal properties.

Objective: The study was conducted to evaluate the antihypercholesterolemic property of *P. pellucida* methanolic extract (PPME) through measurement of its effects on total blood cholesterol level and liver histology of hypercholesterolemic rats.

Methodology: Thirty experimental male rats were divided into six groups: Group I was fed with standard diet; Group II was fed with High Cholesterol Diet (HCD) only; Groups III, IV and V were fed with HCD and treated with PPME at 200, 400 and 800 mg kg^{-1} bwt respectively; Group VI was fed with HCD and treated with atorvastatin; and, Group 7 was fed with 400mg/kg PPME. Total Blood Cholesterol (TBC) levels were monitored, liver histology was analysed, and results were compared with the control and atorvastatin-treated group.

Results: Administration of different doses of PPME in hypercholesterolemic rats significantly reduced total blood cholesterol similar to Atorvastatin, a known anticholesterolemic drug. Furthermore, PPME particularly at a concentration of 400mg kg^{-1} bwt was effective in ameliorating liver damages induced by high cholesterol diet as shown by qualitative and quantitative histological assessment.

Conclusion: This investigation suggests that PPME at a concentration of 400 mg/kg bwt is a potential antihypercholesterolemic agent. Further studies have to be taken to better the understanding on the mechanisms of actions of PPME on how it modulates liver damage in hypercholesterolemic conditions.

Keywords: *Peperomia pellucida, antihypercholesterolemia, liver, histology, high cholesterol diet*

Introduction

Hypercholesterolemia is the presence of high levels of cholesterol in the blood. The prevalence of high total cholesterol levels among Filipinos increased from 10.2% in 2008 to 46.9 % in 2015 [1,2]. It is estimated that high cholesterol causes 2.6 million deaths (4.5% of total) worldwide [3,4,5,6]. The increasing prevalence of hypercholesterolemia has become a serious issue in modern society. The modern lifestyle which includes minimal physical activity and a high-fat diet has contributed greatly to the increased prevalence of lifestyle-related diseases such as hypercholesterolemia [4,5,6]. Hypercholesterolemia can have deleterious effects which can cause various health problems [4,5,6,7]. In the past decade, increasing dependence on cholesterol-lowering drugs such as statins has been observed. Lipitor, a synthetic statin, is the most prescribed drug for hypercholesterolemia [8,9,10,11,12]. However, the use of statins has been highly debated due to the various adverse effects that have been reported such as myalgia, myopathy, and reduced cognition [6,7,8,9,10,11,12,13,14].

Providing basic health care has been a constant problem in developing countries. According to the World Health Organization, a large percentage of the world's population has inadequate or no access to sufficient health care services due to poverty [15,16]. WHO promotes the use of

traditional medicine because it allows all people to have access to basic health care that is accessible and affordable. In recent years, an interest in the use of herbal medicines as an alternative treatment is growing because of its proven quality, safety, and efficacy [15,16,17,18,19,20].

There is an abundance of medicinal plants in the Philippines. In 2007, the Philippine Institute of Traditional and Alternative Health Care (PITAHC) of the Department of Health (DOH) named the ten medicinal plants in the Philippines which should be prioritized for research and development. Included in the list is *Peperomia pellucida* (L.) HBK, commonly known as ulasimang-bato or pansit-pansitan.

P. pellucida has many uses in traditional medicine. It has been used as a topical medicine to treat acne, boils, abscesses, and eczema; used for the treatment of eye inflammation, conjunctivitis, renal problems, fatigue, and abdominal pain [20,21,22,23,24,25,26,27,28,29,30,31]. Despite the various medicinal claims associated with this plant, bibliographic data showed very limited studies about its antihypercholesterolemic effects [21,22,23,24,30,31]. Thus, this study aims to investigate the antihypercholesterolemic property of *P. pellucida* in diet-induced hypercholesterolemic rats through the measurement of total blood cholesterol levels and histology of the liver.

Methodology

Collection of Plant and Preparation of Methanolic Extract

Peperomia pellucida plants with a height of 8 to 10 cm were obtained from Isidro Farm, Gasan, Marinduque. These were brought to the Botany Division of the National Museum of the Philippines for authentication. The roots of the plants were removed and the remaining aerial plant parts were cleaned with distilled water and air-dried at room temperature in a 12/12 light and dark conditions.

The preparation of the methanolic crude extract was based on the study of Benjamin *et al.* [31]. The dried plant materials were grounded into powder using mortar and pestle. After this, the powdered materials (450g) were soaked in 1L 95% methanol for 48 hours. The solvent was then filtered through Whatman No. 1 filter paper. The filtrate was then evaporated under reduced pressure in a rotary evaporator to obtain the crude extract. A percent yield of 33% was obtained. The crude extract of *P. pellucida* was kept in a well-closed container labeled as PPME and immediately stored at -20 C.

Animals

Thirty male Sprague-Dawley rats 7 to 8 weeks old were obtained from the Industrial Technology Development Institute - Department of Science and Technology (ITDI-DOST) in Taguig. The rats were kept in individual plastic cages, with a dimension 24 x 40 x 20 cm and provided with beddings (autoclaved wood shavings) at the National Institutes of Health (NIH) Animal House in UP Manila. The rats were given one week to acclimatize at standard conditions for temperature (28±2 °C) and photoperiod (12h light-dark cycle) prior to actual treatment. The temperature was maintained with the use of an air conditioning unit. In order to maintain photoperiod, the experimental room was illuminated with fluorescent lights providing cool white light. The average illumination opposite of the cage was 6 ft-c. The dark light began at 9 pm while white light began at 9 am. Throughout the experiment, the animals were fed with rat pellets and autoclaved mineral water *ad libitum*. The rat pellets were composed of 23% crude protein, 3% crude fat, % crude fiber, 8% ash, and 2.5 % added minerals. Chew proof water bottles were provided per cage. This assured *ad libitum* access to water. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institute of Health (NIH) in UP Manila with IACUC protocol number 2014-020.

Induction of Hypercholesterolemia

Hypercholesterolemia was induced by feeding the rat with a high cholesterol diet (HCD). It was prepared using 5 grams of butter and 8 grams of commercial pellet [32]. To ensure that the appropriate amount of HCD diet was consumed by the animals, monitoring how much food each animal consumes per day was done. Pellets were usually ground into a powder and HCD diet was incorporated to only about half of the amount of food to ensure that they will consume the administered HCD diet. The other half was given as soon as the food with HCD was consumed. After this, treatment with *P. pellucida* extracts followed. In order to confirm hypercholesterolemia, 1 mL blood sample was collected from the saphenous vein of the rats and were then sent to the laboratory for testing. The total cholesterol of 120mg/dL was considered hypercholesterolemic.

Experimental Setup

The thirty acclimatized male Sprague-Dawley rats were divided into six groups with five individuals as follows: Group 1 was fed with standard diet; Group 2 was fed with

HCD; Groups III, IV, and V were fed with HCD and administered with *P. pellucida* methanolic extract (PPME) at 200, 400 and 800 mg kg^{-1} bwt, respectively. Based on previous researches done, the optimal dosage of PPME is 400 mg kg^{-1} bwt. The researchers chose 200 as a lower dosage and 800 as a higher dosage. Both of these are considered safe since previous studies also utilized these concentrations [31]. PPME extract was administered through oral gavage by reconstituting the extract at the desired consideration using distilled water. Group VI was fed with HCD and treated with atorvastatin (Pfizer Lipitor®) at 10 mg kg^{-1} bwt. An established protocol exhibited the effectiveness of atorvastatin which was dissolved in distilled water and was administered through oral gavage [34]. The thirty rat specimens received daily treatment which lasted for 14 days. After 14 days, the rats were subjected to overnight fasting. Blood samples were then collected from the saphenous vein of all the groups of rats. Total cholesterol was measured using EasyTouch® Blood Cholesterol Meter Test Kit that provides measurement at a range of 80-300mg/dL.

Total cholesterol and weight of the rats were monitored on Day 7, Day 14, and Day 28 which corresponds to before administration of HCD, after administration of HCD, and after administration of the extract. After treatment, they were subjected to overnight fasting, then blood samples were collected from the periorbital sinus of the mice. To sacrifice the mice, they were anesthetized with 30 mg/kg Zoletil injected intraperitoneally. They were sacrificed via cervical dislocation, and their livers were excised.

Histological Analysis

The livers that have been excised out of the 30 rats were prepared for gross and histological analysis. The livers were fixed in a 10% formalin solution before sending them to the laboratory. The fixed tissues were sent to High Precision Laboratory for histological preparation. The livers were cut at 2-3 µm using a rotary microtome and stained with hematoxylin & eosin. The resulting slides were then analyzed under the light microscope (Olympus CX23 Binocular). They were analyzed blindly by three researchers and a veterinary pathologist. A modified Knodell scoring system was also employed to provide a semi-quantitative assessment of the observed histological features.

Statistical Analysis

Total blood cholesterol and body weight values were presented as mean ± standard error of the mean (SEM). To compare the groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed. On the other hand, mean scores were subjected to Kruskal-Wallis to determine if there is any difference between the means of the group. A Dunn's Multiple Comparison Test was used to determine which of the means are different with p set at <0.05. Data were analyzed using Graphpad (version 6.0).

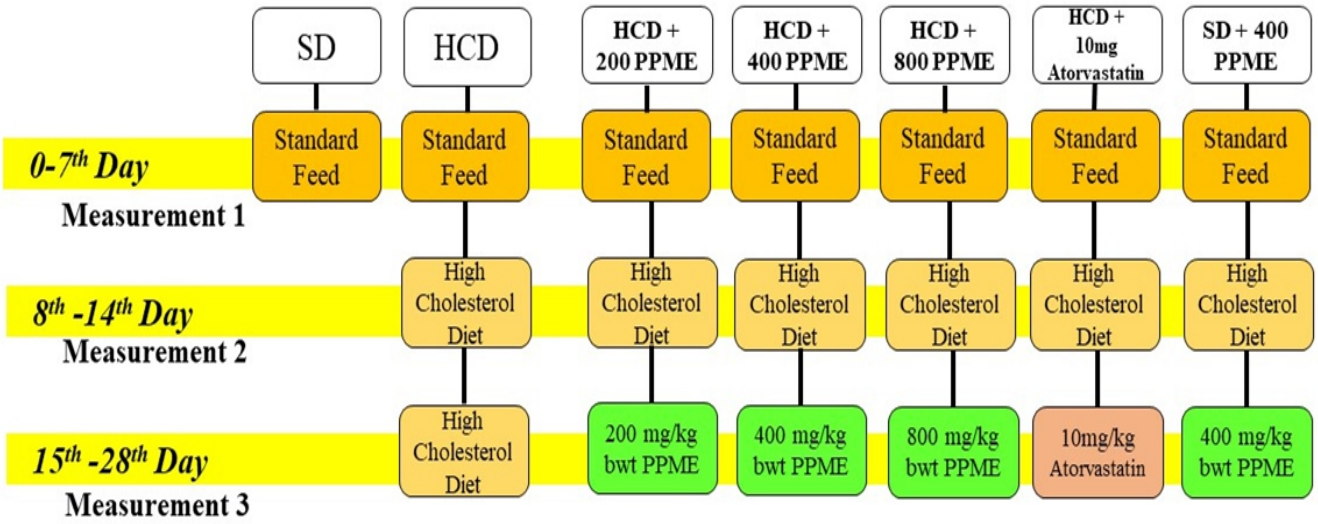

Figure 1. *Graphical illustration of the experimental set-up.*

Table 1. *Modified Knodell Scoring System*

Necroinflammatory Stages	Score
None: Hepatic lobules are polyhedral with varied sizes; each lobule has sinusoids originating at the portal vein; portal vein are branched and converged between hepatic plates; central vein and hepatic sinusoids are devoid of blood; hepatic plates are made up of small hepatocytes cells; hepatic cells have non-granulated cytoplasm, nuclei are euchromatic with one/two prominent nucleoli and intact nuclear membrane	0
Blood congestion in some of central veins and sinusoids of the liver; Microvesicular steatosis (<50%); multinucleation; pyknosis and hyperchromatism in liver cells	1
Microvesicular steatosis (>50%); occasional ballooned zone 3 hepatocytes; scattered rate intra-acinar polymorphonuclear cells + intraacinar lymphocytes; no mild portal chronic inflammation, occasional disintegration of the collagenous supporting tissue of the lobules	2
Macrovesicular steatosis; more obvious ballooning of hepatocytes (predominantly zone 3) intra-acinar polymorphonuclear cells noted, may be associated with zone 3 pericellular fibrosis; portal and intra-acinar chronic inflammation noted, mild to moderate	3
Panacinar steatosis; ballooning and disarray obvious, predominantly in zone 3; intra-acinar inflammation noted as scattered pmn's, pmn's associated with ballooned hepatocytes + mild chronic inflammation; portal chronic inflammation mild or moderate, not marked	4

Results

Effects of PPME on body weight

All rats remained alive and healthy throughout the period of study. The body weight of rats was monitored on days 7, 14, and 28 respectively. Figure 2 shows the mean body weight on days 7, 14, and 28 which correspond to before administration of HCD, after administration of HCD, and after administration of PPME. After 14 days of treatment with PPME, a significant increase in body weight was noted between rats fed with HCD and those treated with atorvastatin compared to the control group. On the other hand, a minimal increase in body weight was noted to groups administered with PPME.

Effects of PPME on total cholesterol level

The occurrence of hypercholesterolemia in test rats was determined by measuring the total cholesterol level of the rats after seven days of feeding with a high cholesterol diet. Rats with total cholesterol greater than 120mg/dL after one week were considered hypercholesterolemic. Figure 3 shows the mean total cholesterol level of rats before and after the administration of HCD and extract. Compared to the control group, the total blood cholesterol of HCD group increased. An apparent decrease in the total cholesterol level of rats treated with PPME and atorvastatin was noted.

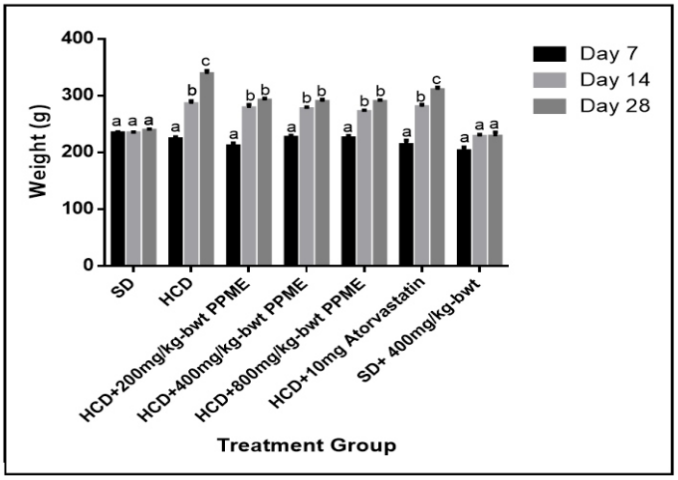

Figure 2. Effects of administration of P. pellucida methanolic extract (PPME) on body weight of rats. *Values were expressed as Mean ± SEM. Differences in letter denotes significant difference in weight at p<0.05 using Tukey's post hoc test.*

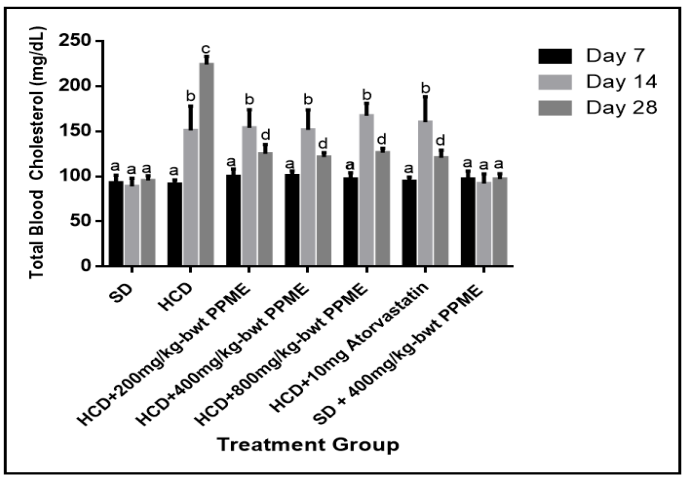

Figure 3. Effects of administration of P. pellucida methanolic extract (PPME) on total blood cholesterol of rats. *Values were expressed as Mean ± SEM. Differences in letter denotes significant difference in blood cholesterol at p<0.05 using Tukey's test.*

Effect of PPME on liver histology

The hepatic lobules of rats from the standard diet (SD) group showed normal architecture of the liver. Liver parenchyma consists of branching and anastomosing cords of hepatocytes radiating from the central vein. Blood sinusoids which separate the hepatic cords were lined by flat endothelial cells (Figure 4A). The polygonal hepatocytes possess the characteristic acidophilic cytoplasm with basophilic bodies and single, central, and vesicular nucleus with prominent nucleoli (Figure 5A). The portal vein together with the bile ducts and the branches of the hepatic artery was evident at the portal tracts (Figure 6A).

Mice fed with a high-fat diet exhibited severe macrovesicular and microvesicular steatosis in the hepatic lobules. Mononuclear cellular infiltration was also observed within the hepatic lobules (Figure 4B). Hepatocytes exhibited vacuolated cytoplasm and possess a highly pyknotic nucleus. Blood sinusoids were indistinct due to ballooning (Figure 5B). The hepatic portal triad was congested and dilated. Further, lymphocytic infiltration along the portal triad was evident. Hepatocytes near the portal triad were vacuolated and hyperplasia of cells lining of the bile duct were noted (Figure 6B).

Group III or those administered with 200 mg/kg –bwt PPME exhibited moderate steatosis in hepatic lobules. A reduction in the size of the central vein was noted (Figure 4C). A minimal number of hepatocytes displayed ballooning and erythrocytes infiltration were observed within the sinusoids (Figure 5C). Further, a significant reduction in the size of the hepatic portal triad was noted. Incidences of steatosis were also observed in the hepatocytes near the portal area (Figure 6C).

Histopathology of liver sections of rats administered 400 mg PPME provided more protection against diet-induced damages seen in the liver. Branching and anastomosing cords of polygonal hepatocytes with vesicular nuclei were seen radiating from the central vein similar to liver sections of rat fed with a standard diet (Figure 4D). The cytoplasm of the hepatocytes was acidophilic and blood sinusoids lined with endothelial cells were distinct (Figure 5D). A minimal cellular infiltration was seen along the portal area and minimal macrovesicular steatosis was evident. Slight fibrosis on the hepatic vein was also noted. (Figure 6D).

Sections of the liver from rats administered with 800 mg/kg –bwt PPME showed a reduction in the size of the central vein. The surrounding hepatic sinusoids were enlarged due to the infiltration of erythrocytes (Figure 4E). Mild macrovesicular steatosis was observed and hepatocytes were irregular in shape (Figure 5E). Slight fibrosis on the lining of the hepatic portal vein was also seen (Figure 6E).

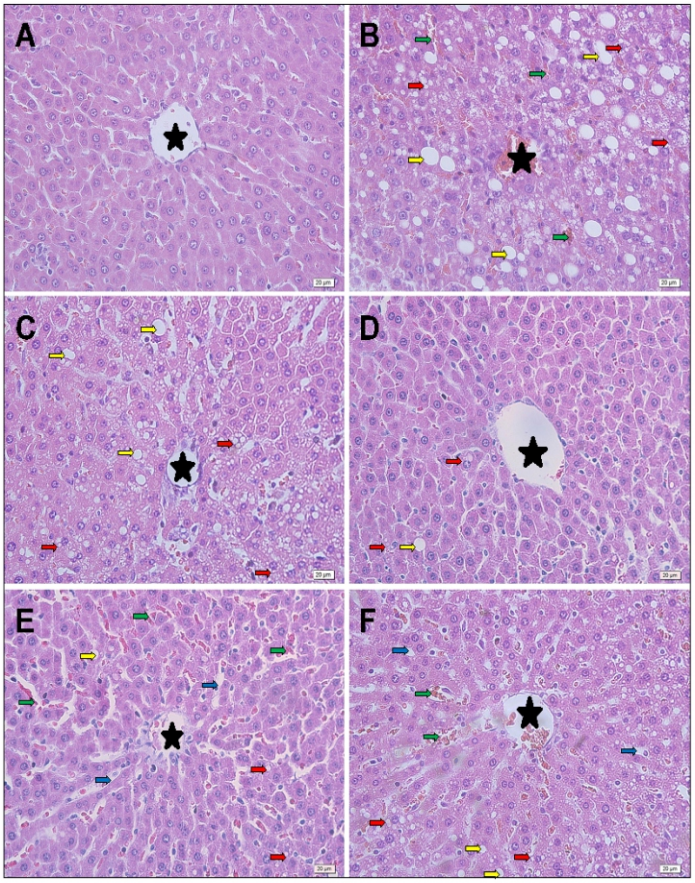

Figure 4. Representative photomicrographs of rat liver showing the central vein and hepatic plates of Groups I-VI. *(A) The liver section of Group I fed with standard diet showing a well-defined and intact central vein (star) with normal hepatocytes (green thin arrow) arranged in cords and separated by sinusoids (black thin arrow). (B) The liver section of Group II fed with HCD showing severe macrovesicular (yellow arrow) and microvesicular (red arrow) steatosis, hepatocyte ballooning (orange arrow) blood congestion (green) and significant reduction in the size of the central vein (C) The liver section of Group III fed with HCD and administered with 200 mg/kg -bwt PPME shows moderate microvesicular steatosis and mild macrovesicular steatosis. (D) The liver section of Group IV fed with HCD and treated with 400 mg/kg -bwt PPME showing an almost similar morphology with Group I. (E) The liver section of Group V fed with HCD and administered with 800 mg/kg -bwt PPME shows moderate sinusoid dilatation (thick green arrow), blood congestion and mild steatosis. (F) The liver section of Group VI fed with HCD and administered with 10 mg/kg atorvastatin shows mild blood congestion and macrovesicular steatosis and moderate microvesicular steatosis. (H&E 400x).*

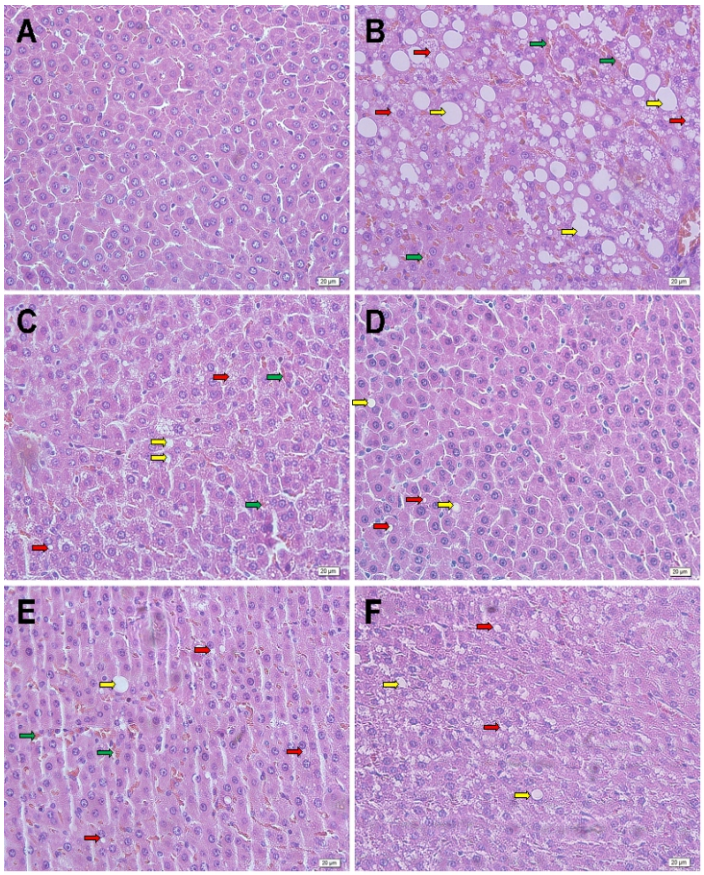

Figure 5. Representative photomicrographs rat liver showing the liver parenchyma of Groups I-VI. *(A) The liver section of Group I fed with standard diet showing large and polyhedral hepatocytes (green arrow) wiltspherical and centrally located nuclei; (B) The liver section of Group II fed with HCD showing irregularly-shaped hepatocytes with severe macrovesicular (yellow arrow) and microvesicular (red arrow) steatosis and blood congestion (green arrow); (C) The liver section of the Group III fed with HCD and administered with 200 mg/kg –bwt PPME showing irregularly-shaped hepatocytes, mild microvesicular (red arrow) and macrovesicular (yellow arrow) steatosis and blood congestion (green arrow); (D) The liver section of the group fed with HCD and administered with 400 mg/kg –bwt PPME showing normal liver parenchyma; (E) The liver section of the group fed with HCD and administered with 800 mg/kg –bwt shows parenchyma with mild steatosis and moderate blood congestion; and, (F) The liver section of the group fed with HCD and treated with 10 mg/kg atorvastatin showing irregularly shaped hepatocytes with mild steatosis. (H&E 400x).*

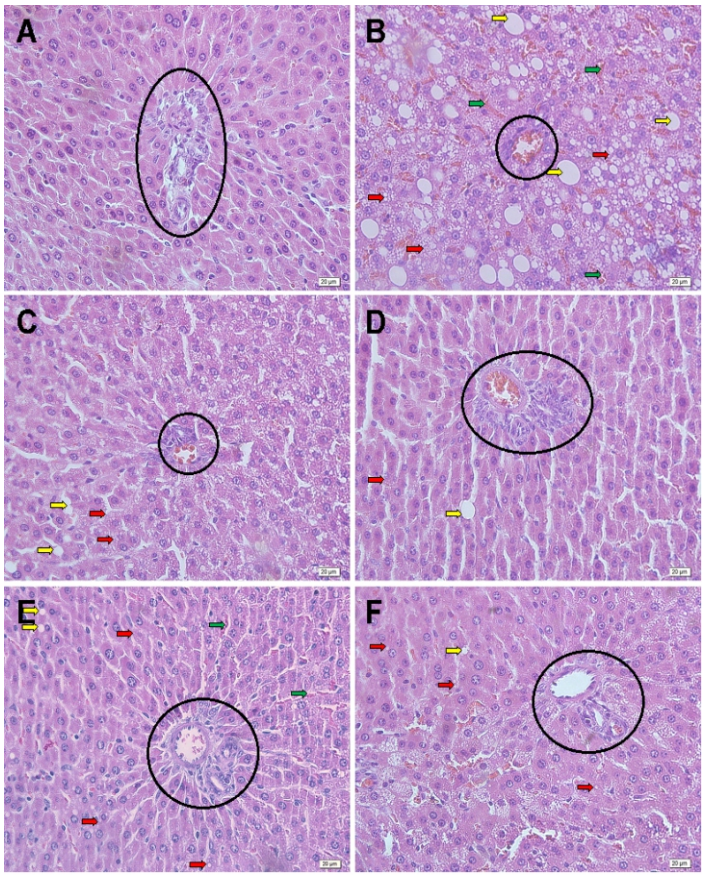

Figure 6. Representative photomicrographs of rat liver showing the portal triad of Groups I-VI. *(A) The liver section of Group I fed with standard diet showing a normal portal triad (encircled) with regularly shaped periportal hepatocytes arranged in cords; (B) The liver section of the Group II fed with HCD showing an indistinct portal triad with severe steatosis (red and yellow) and blood congestion (green arrow). (C) The liver section of the Group II fed with HCD and administered with 200 mg/kg –bwt PPME showing diminished portal triad and moderate steatosis. (D) The liver section of the Group IV fed with HCD and administered with 400 mg/kg -bwt PPME showing portal triad with portal vein exhibiting blood congestion. (E) The liver section of Group V fed with HCD and administered with 800 mg/kg PPME showing portal triad with moderate microvesicular steatosis and blood congestion in hepatic vein; (F) The liver section of the group fed with HCD and adinistered with 10 mg/kg atorvastatin showing a normal portal triad with mild macrovesicular steatosis and moderate microvesicular steatosis. (H&E 400x).*

The liver sections from Group VI or those treated with atorvastatin showed moderate damage as evidenced by the presence of blood congestion in the sinusoids, sinusoid dilatation, hepatocyte ballooning, as well as microvesicular and macrovesicular steatosis along the hepatic lobule (Figure 4F). The hepatocytes lack the characteristic polygonal shape (Figure 5F). Also, the portal triad appears to be normal (Figure 6F).

Quantitative analysis of the histological scores generated by using the modified Knodell's index is presented in Figure 7. A lower mean score indicates lesser liver damage. Results revealed that rats administered with 400mg/kg bwt PPME and those administered with HCD+400mg/kg bwt PPME and HCD+800mg/kg bwt PPME were not statistically different with rats fed with standard diet hence have liver histology that is nearly identical with

that of the control group. This means that PPME at 400 and 800 mg/kg was effective in mitigating HCD-induced liver damages. On the other hand, rats fed with HCD+ 10mg atorvastatin and HCD + 200 mg/kg bwt were not statistically significant with the HCD group. This means that atorvastatin and 200 mg/kg PPME were not effective enough to alleviate liver damage.

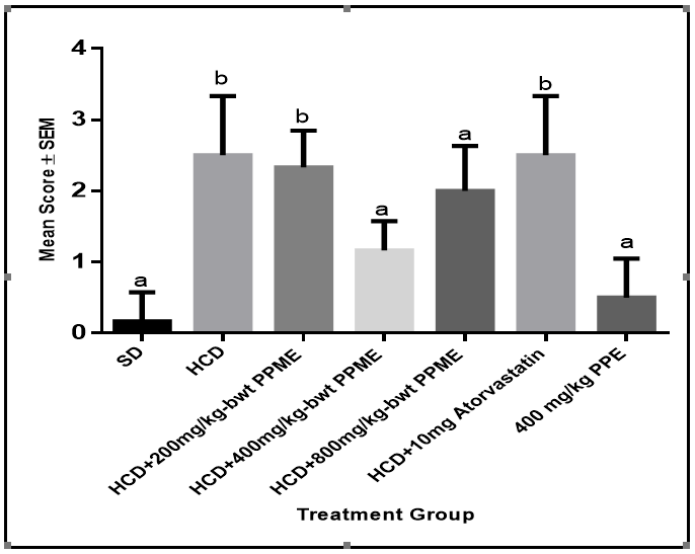

Figure 7. Mean score of hepatic tissue samples from different treatment groups. *Differences in letter denote significant difference at p<0.05 using Dunn's Multiple Comparison Test.*

Discussion

Hypercholesterolemia has been implicated in the pathophysiology of atherosclerosis, coronary heart disease, and myocardial ischemia [34,35,36]. Lowering cholesterol levels may decrease the risk of the above-mentioned diseases thus, enormous efforts have been done to achieve this goal [34,35,36,37]. Statins have been used as a first-line treatment because of its capacity to reduce cholesterol synthesis however, evidence of its clinical efficacy is low. Moreover, the therapy cost and side effects cannot be ignored [10,12,13,14]. The need to urgently seek a new, safe, and cholesterol-lowering drug is therefore important. The use of natural products with hypocholesterolemic effect is believed to be useful in reducing the risk associated with increased cholesterol level [17,18,19]. In this study, it has been demonstrated that PPME especially at 400mg/kg - bwt can ameliorate diet-induced liver damage at the same time lower the cholesterol level of hypercholesterolemic rats. The similar effect of PPME with atorvastatin in lowering cholesterol levels and protecting the liver against diet-induced injuries means that it can be an alternative to counteract the detrimental effects brought about by high cholesterol diet.

In the experiment, a high cholesterol diet which consists of unsalted butter was used to induce hypercholesterolemia in selected groups of rats. The butter used for the high cholesterol diet contains 30mg of cholesterol, 8g of saturated fat, 12g of total fat, and 0.5g of trans fat for every 15g of butter [37]. Diets high in saturated fat and cholesterol promote weight gain as well as increased concentration of low-density lipoprotein (LDL) cholesterol [7,32,33,34,38]. Indeed, rats fed with HCD had a significant increase in body weight as well as total cholesterol that exceeded the baseline level. Similar results were also seen in various studies [32,38,29,40]. Rodents fed with HCD increase their food intake resulting to rise in body weight which leads to elevated total cholesterol, low-density lipoprotein and triglycerides level in their blood [32,38,29,40,41].

Administration of PPME at various concentrations to hypercholesterolemic rats resulted in a significant decrease in total cholesterol levels. This may be attributed to the action of its active compounds [21,24] Phytochemical analysis of *P. pellucida* showed that saponins and tannins are its most abundant constituents [60]. Various studies have already shown that both tannins and saponins have pronounced antihypercholesterolemic effects. Saponin reduces plasma cholesterol levels by binding to cholesterol to form insoluble complexes to be excreted through the bile [21,22,42,43,44,45]. Tannin, on the other hand, enhances reverse cholesterol transport and reduces intestinal cholesterol absorption [46,47]. Because of these, cholesterol reabsorption is lessened, preventing high blood cholesterol levels [42,45,46,47].

There was no significant difference in the mean total cholesterol levels between groups treated with PPME. This suggests that all the dosages (200 mg/kg, 400 mg/kg, and 800 mg/kg) are similarly effective in lowering total cholesterol levels in the blood. However, results showed that the highest percentage of decrease in total blood cholesterol levels was exhibited by the group treated with 400 mg/kg plant extract. On the other hand, although there was a significant decrease in the total cholesterol levels of the experimental groups, reduction to normal level was not achieved. This may be attributed to the short duration of the study as longer exposure to the plant extract may further reduce the blood cholesterol levels of the rats. Studies have shown that extract administration should be done at least four weeks to restore the normal cholesterol level

[40,42,44,45]. In addition, the age of the rats may have affected the total blood cholesterol levels. According to Parini *et al.* [48], growth hormones found in younger male rats (7-8 weeks) may influence plasma cholesterol levels.

Similar results were also observed in hypercholesterolemic rats treated with atorvastatin. Statin drugs such as atorvastatin are used along with proper diet to lower low-density lipoproteins and triglycerides in the blood. Its mechanism of action involves inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A Reductase A (HMG-CoAR) thereby reducing cholesterol biosynthesis [8,9,10,11,12,13,14].

The primary organ that metabolizes ingested cholesterol is the liver [49,50,51,52]. Continuous consumption of high cholesterol diet can therefore cause changes in various organs of the body especially the liver. Based on the results, the liver of rats fed with HCD developed severe steatosis, lobular and portal inflammation, blood congestion, and hepatocyte injury in the form of ballooning and apoptosis. These lesions are hallmarks of non-alcoholic fatty liver disease (NAFLD) which results from increased levels of free cholesterol in hepatocytes and non-parenchymal hepatic cells [51,52,53,54]. Dietary sources and derangement in numerous steps of hepatic cholesterol homeostasis which includes activation of cholesterol biosynthetic pathways increased cholesterol de-esterification, and attenuation of cholesterol export may contribute to increasing levels of free cholesterol in the liver [32,34,36]. Enhancing the level of free cholesterol will not only lead to NAFLD but even hinder the ability of the liver to combat free radicals by suppressing the antioxidant activity resulting in the accumulation of free radicals and subsequent increase in oxidative stress in the livers of rats fed with HCD [34,36,42,50,51]. This, therefore, explains the greater damage seen in the liver section of rats fed with HCD.

We also compared the efficacy of Atorvastatin in improving diet-induced liver damage. Based on the results, liver sections of rats fed with HCD and treated with 10 mg/kg atorvastatin exhibited severe liver damage. Metabolic disorders such as hypercholesterolemia are associated with metabolic inflammation which can disrupt HMG-CoAR mediated cholesterol synthesis, resulting in intracellular lipid accumulation and eventually leads to inflammatory stress [50,51,52]. Under these circumstances, it was observed that atorvastatin increased serum ALT and AST levels as well as aggravate steatosis, inflammation, and fibrosis [13,14]. This only indicates that atorvastatin will not improve but even worsen liver injury under inflammatory stress.

Meanwhile, liver sections of rats treated with PPME showed improved liver histology compared to HCD group. This means that PPME was able to protect the liver against diet-induced injuries however, 400mg/kg-bwt concentration provided more protection than 200 and 800 mg/kg-bwt concentration. Rats administered with 400mg/kg bwt PPME showed almost similar histology with rats fed with a standard diet as seen in the quantitative analysis of histological scores. The occasional blood congestion, steatosis, hepatocyte ballooning, and the absence of fibrosis show that it was able to prevent the progression of NAFLD. This coincides with our former study whereby PPME at 400mg/kg bwt concentration was effective enough in alleviating trichloroethylene induced liver damage by elevating SOD and CAT and lowering TT and TrxR activities under oxidative stress [31].

The ability of *P. pellucida* to alleviate cholesterol levels and diet-induced liver injuries can be attributed to the many active compounds that it contains [42,44,45,54,55]. Aside from tannins and saponins, numerous bioactive components of *P. pellucida* has also been shown to have anti-hypercholesterolemic potential. Phytol, a diterpene that accounts for 37.88% of the compounds extracted from PPME showed that it has significantly lowered triglycerides and low-density lipoprotein (LDL) of hypercholesterolemic rats [29,54]. Sesamin, another active compound extracted from *P. pellucida* has also demonstrated a hypocholesterolemic effect by lowering the very low-density lipoprotein (VLDL) and LDL cholesterol concentration [29,54]. Further, piperine which is a type of alkaloid extracted among Piperacae family has also been shown to significantly reduced a high-fat diet-induced weight gain, lowered TC and LDL cholesterol concentrations, and protected against the development of NAFLD by decreasing the accumulation of hepatic triglycerides [55,56,57]. In addition, rats that were fed with high-fat diet and supplemented with piperine showed a significant reduction in the HMG CoA reductase activity in the liver which suggests the cholesterol-reducing property of piperine [57].

Conclusion

This study demonstrated the antihypercholesterolemic property of the crude methanolic extract of *P. pellucida* in rats by measuring the total cholesterol of rats and the histological examination of the liver using a modified scoring system.

Measurement of total cholesterol showed that *P. pellucida* may have the potential in regulating blood

cholesterol as it significantly lowered the total plasma cholesterol among hypercholesterolemia diet-induced rats. Findings from liver histological examination suggest that diet-induced hypercholesterolemia can lead to several hepatic injuries such as blood congestion, microvesicular steatosis, macrovesicular steatosis, lobular inflammation, and hepatocellular ballooning associated with fatty liver disease. In the treatment groups, less damage was observed as compared to the control groups.

This investigation suggests that PPME at a concentration of 400 mg/kg bwt is a potential antihypercholesterolemic because of its ability to effectively lower the total blood cholesterol levels in the blood and ameliorate hepatic tissue injuries resulting from diet-induced hypercholesterolemia.

Acknowledgement

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References

1. Food and Nutrition Research Institute (FNRI). (2013) 8th National Nutritional Survey, FNRI Department of Science and Technology.
2. Rutherford JN, McDAde TW, Feranil A, Adair L, Kuzawa C. (2010) High prevalence of low HDL-c in the Philippines compared to the US: Population differences in association with diet and BMI. E-Journal of Asia Pacific Journal of Clinical Nutrition 19(1):57-67.
3. Khoo KL, Tan H, Liew YM, Deslypere JP, Janus E. (2003) Lipids and coronary heart disease in Asia. Atherosclerosis 169(1):1-10.
4. Yi SW, Yi JJ, Ohrr H. (2019) Total cholesterol and all-cause mortality by sex and age: A prospective cohort study among 12.8 million adults. Scientific Reports 9 (1596).
5. Grundy SM, Cleeman JI, Merz CNB, *et al.* (2004). Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. Journal of the American College of Cardiology 44(3):720-732.
6. Lloyd-Jones D, Adams R, Carnethon M, *et al.* (2009) Heart disease and stroke statistics—2009 update a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 119(3):480-486.
7. Bahadur S, Ullah H, Hafizullah M, Gul AM, Awan, ZA. (2011) Prevalence of hyperlipidemia in obese and non-obese coronary artery disease patients. Journal of Postgraduate Medical Institute (Peshawar-Pakistan) 25(1).
8. Gotto AM. (2002) Statins: Powerful drugs for lowering cholesterol advice for patients. Circulation 105(13):1514-1516.
9. Loch A, Bewersdorf JP, Kofink D, Ismail D, Abidin IZ, Veriah RS. (2017). Generic atorvastatin is as effective as the brand-name drug (LIPITOR®) in lowering cholesterol levels: A cross-sectional retrospective cohort study. BMC Research Note 10:291.
10. Schaefer EJ, McNamara JR, Tayler T, *et al.* (2004) Comparisons of effects of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin) on fasting and postprandial lipoproteins in patients with coronary heart disease versus control subjects. The American Journal of Cardiology 93(1):31-39.
11. Armitage J. (2007) The safety of statins in clinical practice. The Lancet 370(9601):1781-1790.
12. Donnell J, Ahuja GD. (2005) Statins including Baycol: Questionable cholesterol control. In: Drug Injury: Liability, Analysis, and Prevention (2nd ed.) Tucson, AZ: Lawyers & Judges Pub. Co., pp. 469-477.
13. Thompson PD, Clarkson P, Karas RH. (2003) Statin-associated myopathy. JAMA: The Journal of the American Medical Association 289(13):1681-1690.
14. Wu W, Chao L, Yang P, *et al.* (2016) Inflammatory stress sensitizes the liver to atorvastatin-induced injury in ApoE-/- mice. Plos One 11(7):e0159512.
15. Leach B, Paluzzi JE, Munderi P. (Eds.) (2005) UN millennium development library: Prescription for healthy development: Increasing access to medicines. Earthscan.
16. World Health Organization. (2013) WHO traditional medicine strategy 2014-2023.
17. Rates SMK. (2001) Plants as source of drugs. Toxicon 39(5):603-613.
18. Rout SP, Choudary KA, Kar DM, Das L, Jain A. (2009) Plants in traditional medicinal system-future source of new drugs. International Journal of Pharmacy and Pharmaceutical Sciences 1(1):1-23.
19. Lahlou M. (2013) The success of natural products in drug discovery. Pharmacology & Pharmacy 04(03):17-31.
20. Balunas MJ, Kinghorn AD. (2005) Drug discovery from medicinal plants. Life Sciences 78(5):431-441.
21. De Fátima Arrigoni-Blank M, Dmitrieva EG, Franzotti EM, Antoniolli AR, Andrade MR, Marchioro M.

(2004) Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae). Journal of Ethnopharmacology 91(2):215-218.
22. Abere TA, Agoreyo FO, Eze GI. (2013) Phytochemical, antimicrobial and toxicological evaluation of the leaves of *Peperomia pellucida* (L.) HBK (Piperaceae). Journal of Pharmaceutical and Allied Sciences 9(3):1637-1652
23. Sussa FV, Damatto SR, Alencar MM, Mazzilli BP, Silva PS. (2013) Natural radioactivity determination in samples of *Peperomia pellucida* commonly used as a medicinal herb. Journal of Environmental Radioactivity 116:148-151.
24. Theresa-Ibibia EK. (2012) Phytochemical and antimicrobial analyses of extracts of *Peperomia pellucida* (L). Journal of Pharmacy Research 5(5).
25. Wei LS, Siong JYF, Syamsumir DF. (2011). Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Medica Iranica 49(10).
26. Khan MR, Omoloso AD. (2002) Antibacterial activity of *Hygrophila stricta* and *Peperomia pellucida*. Fitoterapia 73(3):251-254.
27. Bayma J, Arruda MSP, Müller AH, Arruda AC, Canto WC. (2000) A dimeric ArC 2 compound from *Peperomia pellucida*. Phytochemistry 55(7):779-782.
28. Tarigan IM, Bahri S, Saragih A. (2013). Aktivitas antihiperurisemia ekstrak etanol herba suruhan (*Peperomia pellucida* (L.) Kunth) pada mencit jantan. Journal of Pharmaceutics and Pharmacology 1(1):37-43.
29. Gini TG, Jothi J. (2013) Preliminary phytochemical screening of Whole Plant Extracts of *Peperomia pellucida* (Linn.) HBK (Piperaceae) and *Marsilea quadrifolia* Linn. (Marsileaceae). International Journal of Pharmacognosy and Phytochemical Research 5(5):200-214.
30. Raghavendra HL, Rashith Kekuda TR. (2018) Ethnobotanical uses, phytochemistry and pharmacological activities of *Pepperomia pellucida* (L) Kunth (Piperaceae) - A review. International Journal of Pharmacy and Pharmaceutical Sciences 10(2):1-8.
31. Benjamin KS, Gaspi SAD, Matibag JLR, Co, EL, Sia Su GL. (2013) Enzyme activity and histopathology of rat liver treated with crude methanolic extract of *Peperomia pellucida* (L.) HBK. Journal of Biological Sciences, 13(4).
32. Matos SL, de Paula H, Pedrosa ML, *et al.* (2005). Dietary models for inducing hypercholesterolemia in rats. Brazilian Archives of Biology and Technology 48(2):203-209.
33. Huang C, Cen C, Wang C, Zhan H, Ding X. (2014) Synergistic effects of colchicine combined with atorvastatin in rats with hyperlipidemia. Lipids in Health Diseases 13(67):10-1186.
34. Nelson RH. (2014) Hyperlipidemia as a risk factor for cardiovascular disease. Primary Care 40(1):195-211.
35. Zarate A, Manuel-Apolinar L, Saucedo R, Hernandez-Valencia M, Basurto, L. (2016) Hypercholesterolemia as a risk factor for cardiovascular disease: Current controversial therapeutic management. Archives of Medicinal Research 47(7):491-495.
36. Bessone F, Razori MV, Roma MG. (2019) Molecular pathway of non-alcoholic fatty liver disease development and progression. Cellular and Molecular Life Sciences 76(1):99-128.
37. Fonterra Food Services (2015). Anchor Unsalted Butter.
38. Sarna LK, Wu N, Wang P, Hwang SY, Siow YL, Oh K. (2012). Folic acid supplementation attenuates high fat diet induced hepatic oxidative stress via regulation of NADPH oxidase. Canadian Journal of Physiology and Pharmacology 90(2):155-165.
39. Engel S, Tholstrup T. (2015). Butter increased total and LDL cholesterol compared with olive oil but resulted in higher HDL cholesterol compared with a habitual diet. The American Journal of Clinical Nutrition 102(2):309-315.
40. Milewska M, Sinska B, Gromadzka-Ostrowska J. (2007) Hypercholesterolemic diets containing different common fats and rats plasma lipids. Rocz Panstw Zakl Hig 58(1):15-21.
41. Yang R, Wang C, Ye H, *et al.* (2019). Effects of feeding hyperlipidemia rats with symbiotic oats-frozen yogurt on serum trigllycerides and cholesterol. Food Science and Nutrition 7(3):1096-1103.
42. Odetola AA, Nwanne CM, Mafoluko GA, Oyagbemi AA. (2008) The effect of dietary supplementation with *Peperomia pellucida* on body weight, serum lipids and glucose in normal and nutritionally induced hypercholesterolemia in rats. Nigerian Journal of Natural Products and Medicine 12(1).
43. Olaleye MT. (2007) Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. Journal of Medicinal Plants Research 1(1):9-13.
44. Sheikh H, Sikder S, Paul SK, Hasan AR, Rahaman MM, Kundu SP. (2012) Hypoglycemic, Anti-

inflamatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae). International Journal 4(1).

45. Hamzah RU, Odetola AA, Erukainure OL, Oyagbemi AA. (2012) *Peperomia pellucida* in diets modulates hyperglyceamia, oxidative stress and dyslipidemia in diabetic rats. Journal of Acute Disease 1(2):135-140.
46. Gato N, Kadowaki A, Hashimoto N, Yokohama S, Matsumoto K. (2012). Persimmon fruit tannin-rich fiber reduces cholesterol levels in humans. Annals of Nutrition and Metabolism 62(1):1-6.
47. Tebib K, Besancon P, Rouanet JM. (1994) Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. Journal of Nutrition 124(2):2451-2457.
48. Parini P, Angelin B, Rudling M. (1999) Cholesterol and lipoprotein metabolism in aging reversal of hypercholesterolemia by growth hormone treatment in old rats. Arteriosclerosis, Thrombosis, and Vascular Biology 19(4):832-839.
49. Arguello G, Balboa E, Arrese M, Zanlungo S. (2015) Recent insights on the role of cholesterol on non-alcoholic fatty liver disease. Biochimica et Biophysica Acta (BBA): Molecular Basis of Disease 1852(9):1765-1777.
50. Postic C, Girard J. (2008) The role of lipogenic pathway in the development of hepatic steatosis. Diabetes Metabolism 34:643-648.
51. Thomas Brown G, Kleiner DE. (2016) Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Metabolism 65(8):1080-1086.
52. Green CO, Wheatley AO, Hanchard B, et al. (2011) Histopathological alterations in organ structures of hypercholesterolemic rats fed Ortanique peel polymethoxylated flavones. Basic and Applied Pathology 4:71-77.
53. Fernando DH, Forbes JM, Angus PW, Herath CB. (2019) Development and progression of non-alcoholic fatty liver disease: The role of advance glycation end products. International Journal of Molecular Science 20(20):5037.
54. Alvez NSF, Setzer WN, da Silva JKR. (2018) The chemistry and biological activities of *Pepperomia pellucida* (Pipperaceae): A critical review. Journal of Ethnopharmacology 232:90-102.
55. Kim KJ, Lee MS, Jo K, Hwang JK. (2011) Piperidine alkaloids from *Piper retrofractum* Vahl. protect against high-fat diet-induced obesity by regulating lipid metabolism and activating AMP-activated protein kinase. Biochemical and Biophysical Research Communications 411(1):219-225.
56. Shah SS, Shah GB, Singh SD, *et al*. (2011). Effect of piperine in the regulation of obesity-induced dyslipidemia in high-fat diet rats. Indian Journal of Pharmacology 43(3):296.
57. Vijayakumar RN, Nalini N. (2006) Lipid–lowering efficacy of piperine from *Piper nigrum* L. in high-fat diet and antithyroid drug-induced hypercholesterolemic rats. Journal of Food Biochemistry 30(4):405-421.
58. Olotu MCB, Dy AES. (2015) Teratogenic effects of *Pepperomia pellucida* metanolic crude extract on *Danio rerio* embryos. Undergraduate Thesis. Department of Biology, University of the Philippines Manila.