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RESEARCH ARTICLE

Effect of *Ehretia microphylla* on the blood cholesterol and weight of ICR mice (*Mus musculus*)

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ABSTRACT

Background: Simvastatin has been widely used in controlling blood cholesterol levels and weight; however, side effects such as muscle pain, muscle damage, liver damage, and hyperglycemia may occur. Studies have shown that intake of herbal medicines may be supplemental and may even be more effective than medicines available in the market. **Aims and Objectives:** The objectives of this study are to assess the effects of administration of aqueous leaf extract of *Ehretia microphylla* in ICR mice on blood cholesterol levels and weight. **Materials and Methods:** A total of 35 ICR mice were designated into five groups which are the negative control group, positive control group, low dose, mid dose, and high dose. All groups were fed with lard-coated feeds to increase blood cholesterol level and weight. The positive control group was treated with 2.0 ml of simvastatin, while the negative control remained untreated. The treatments, namely, the low dose, mid dose, and high dose were treated with 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the *E. microphylla* extract, respectively. **Results:** After 14 days of treatment, statistical analysis indicated that the *E. microphylla* extract had anticholesterolemic effect. There was a significant difference in the high-dose group. There was also no significant difference observed in the other groups, namely, the low dose and the mid dose. Furthermore, the positive group treated with simvastatin had a significantly lowered cholesterol level compared to the high-dose group. There are inconclusive results on the effect of *E. microphylla* on weight. **Conclusion:** *E. microphylla* has anti-hypercholesterolemic effects, which may be attributed to the different phytochemicals present in it.

KEY WORDS: Cholesterol; *Ehretia microphylla*; ICR Mice; *Tsaang gubat*; Weight

INTRODUCTION

Simvastatin, among others, is one of the drugs readily available to the public for cholesterol problems. It is hydrophobic and is derived from the fungal fermentation product of *Aspergillus terreus*[1] and is an inhibitor of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) functioning as a chief regulatory enzyme for cholesterol synthesis.[2] Simvastatin functions in competitive HMG-CoA inhibition, low-density lipoprotein (LDL), and triglyceride level reduction with its metabolism undergoing the cytochrome P450 3A4 pathway.[3] Simvastatin has a 5% circulation concentration and is administered as lactone, which is an inactive compound that needs to be hydrolyzed to produce the active form of simvastatin.[4] It is used to block the formation of cholesterol in the liver and increase the production of the receptors on liver cells that clean the bad cholesterol from the blood.[5] Simvastatin aims the hepatocytes and inhibits the enzyme HMG-CoA reductase that converts HMG-CoA into the cholesterol precursor mevalonic acid.[6] Furthermore, when simvastatin is bound to the active site, it alters enzyme conformation,

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specifically the active site, preventing HMG-CoA to obtain a functional arrangement. Conformational change of the active site strengthens simvastatin function, which is to inhibit HMG-CoA reductase, and when this enzyme is inhibited, it causes intracellular cholesterol level reduction.[4] When cholesterol level is decreased, protease activation occurs, which cleaves sterol regulatory element-binding proteins/SREBPs, translocated at nucleus region that fortifies LDL receptor gene expression, from the endoplasmic reticulum.[7] Cholesterol level decrease heightens hepatic LDL receptors, responsible for detecting lowering of circulating LDL and its precursors particularly very LDL and intermediate density lipoproteins. Simvastatin also inhibits apolipoprotein B-100 synthesis that causes a decrease in the production and secretion of triglyceride-rich lipoproteins.[4] This drug is known and proven to decrease the risk of heart attack for both men and women with both average and high levels of cholesterol, and for people who already had a heart attack, simvastatin can reduce the risk of dying and stroke as well.[5] Patients with heterozygous familial and non-familial hypercholesterolemia administered with about 10–40 mg of simvastatin orally once a day reduce plasma total and LDL-cholesterol concentration by about 30–45%.[1] Although there are a lot of beneficial effects, simvastatin intake may cause muscle pain, muscle damage, liver damage, and hyperglycemia/increased blood sugar.[8]

Ehretia microphylla, known in the Philippines as *Tsaang gubat*, is a BFAD registered drug and folkloric shrub used as an analgesic, anti-inflammatory, antidiabetic, antimicrobial, and antispasmodic usually to cure cough and diarrhea.[9] It is considered as a dicto shrub or herb with a height of 1–4 m and having oblong-obovate leaves in clusters that are fixed in an alternate fashion. Leaves of *Tsaang gubat* are toothed near the apex while narrow and pointed at the base, exhibiting netted venation and stipule absence. *E. microphylla* stems are hispid in nature, meaning that stems are veiled with bristles that manifest buds and shoots leading to inflorescences. Flowers can either be solitary or axillary comprising of 2–4 flowers situated on a shared pedicel with green linear-lobed sepals and white oblong-lobed corolla having 4–5 stamens and corresponding filaments. *Tsaang gubat* fruits display a red or yellow color with a round shape, 4-seeded characteristic, fleshy external, and stony internal. Seeds are generally circular, hard, and white or translucent in color with green leafy cotyledons.[10] *Tsaang gubat* contains rosmarinic acid and microphyllone, which contributes in controlling allergies.[11] Furthermore, *E. microphylla* is composed of triterpenes such as a-amyrin, b-amyrin, and baurenol and other phytochemicals, namely, flavonoids, alkaloids, tannins, saponins, terpenoids, and glycosides

Flavonoids are polyphenolic compounds, which are obtained in vegetables and fruits. It has beneficial functions such as antimicrobial, antioxidant, photoreceptor, feeding repellant, and visual attractor.[12] Among these uses, flavonoids are known as antioxidants, which decrease the formation of free radicals through scavenging free radicals by hydrogen donation.[13] Flavonoids also activate important defense enzymes battling oxidative stress and electrophilic toxicants producing prooxidant activity.[14] On the other hand, alkaloids are composed of nitrogen heterocyclic rings, which are found in 15–20% vascular plants.[15] Alkaloids are water soluble due to its negative oxidation state and act as medicine for diabetes, cancer, and malaria. Alkaloids act as antibiotics, interference to hydrolytic enzymes, and inhibitors of translation, damaging membranes, microtubules, and microfilaments.[16] Also found in plants are tannins which are polyphenolic compounds, that bind and precipitate proteins, cellulose, starch, and minerals, generating decomposition resistant and insoluble products.[17] Furthermore, tannins have antimicrobial and antioxidizing characteristics since it hampered superoxide radical growth as well as bacteria and fungi growth due to lipid peroxidation defense and ester linkage hydrolysis, respectively.[18] Saponins, known as plant glycosides, are composed of polycyclic aglycone that can either be a triterpenoid or a choline steroid fastened through ether bond on a sugar side chain or C3.[19] Saponins have a foaming characteristic due to its water-soluble side chain and non-polar sapogenin, another term for aglycone, hence acting as a antibacterial agent.[20] Terpenoids are anti-inflammatory that fights against cancer and malaria.[21] Cardiac glycosides prevent sodium-potassium pump activity through changing monovalent ion transport against electrochemical gradient.[22] As such, cardiac glycosides are given to treat atrial arrhythmia, heart failure, and interferon-beta obstruction.[23]

Although widely distributed in Asia, *E. microphylla* is deficient in the number of researches on it. This study aimed to assess the effects of *E. microphylla* on the weight and blood cholesterol levels. On the other hand, simvastatin is efficacious in maintaining weight and is much more effective than placebo-controlled diets, however, also has side effects just like any other drug.[5] Results of this study will aid people to choose which is the more suitable weight loss option for them, whether natural through plant extracts and herbs or the traditional usage of medicinal weight loss pills.

MATERIALS AND METHODS

Plant Collection and Processing

About 7 kg *E. microphylla* leaves were obtained from the Bureau of Plant Industries, Quirino Avenue, Manila, Philippines. Identification and authentication of collected samples were done at the Herbarium, De La Salle University, Manila, Philippines, with an identification number of DLSUH 3151.

The leaves were washed with tap water, air-dried for 3 days, and powdered using a mechanical blender. 500 g of powdered

Ehretia leaves were soaked in 2.5 L of distilled water for 72 h. The solution was filtered through a muslin cloth, and the resulting filtrate was filtered for the second time using a Whatman No. 1 filter paper. The filtrate was then freeze-dried and lyophilized to obtain dark brown powder. Powdered extracts were then weighed and stored in an airtight and waterproof container at 4°C. *E. microphylla* extracts were reconstituted in distilled water for the administration of treatment through oral gavage to the subjects.

Animal Procurement

Twenty-five 5-week-old male ICR mice were acquired from the Food and Drug Administration, Alabang, Muntinlupa, Philippines. They were housed individually in standard sized cages served fed daily food and water. Before the experiment, all mice were acclimatized for 2 weeks and a half for adaptation at the DLSU Animal House with a temperature of 23°C and 55% humidity at a 12 h light:12 h dark cycle.

Induction of Hypercholesterolemia and Administration of Treatment

After the acclimatization phase, all subjects were fed with lard-coated feeds for the duration of 28 days. Twenty-five mice were randomly distributed to five groups ($n = 5$). Different treatments were administered to the ICR mice for 14 days through oral gavage after induction of hypercholesterolemia [Table 1].

Determination of Weight and Cholesterol Levels

The subjects were weighed and blood cholesterol was determined using an Easy touch glucose, cholesterol, and uric acid meter (Bioptik Technology, Inc., Taiwan) during baseline, after feeding with lard-coated feeds (28th day), and after treatment (42nd day).

Data Analysis

One-way analysis of variance (ANOVA) was used to determine significant differences between each treatment groups. Significant differences were then compared using Tukey's test to at $P < 0.05$. All statistical analysis was performed using STATA version 12.

RESULTS

The obtained mean and standard deviation for the weight and blood cholesterol of the different groups are plotted in line graphs as shown in Figures 1 and 2.

Based on the analysis, the weights of the different groups are not significantly different at baseline (day 0). It can be seen graphically that there is an increase in the weights of all the groups after feeding of lard-coated feeds for 28 days. After such, treatment administration resulted in significant differences in the effects on weight. *Post-hoc* analysis showed that all the treatments are significantly different, except for the positive control and high dose. It may show that 1000 mg/kg of *E. microphylla* extracts have the same effect as simvastatin on weight.

Based on statistical analysis, it shows that the cholesterol levels at baseline (day 0) are within the normal range of 63–174 mg/dL.[24] Blood cholesterol was increased after administration of lard-coated feeds for 28 days. Afterward, analysis after administration of the different treatments

Table 1: Experimental design of the study

Group	Treatment (day 28–42)
Negative control	None
Positive control	Simvastatin
Low dose	250 mg/kg *E. microphylla*
Mid dose	500 mg/kg *E. microphylla*
High dose	1000 mg/kg *E. microphylla*

E. microphylla: Ehretia microphylla

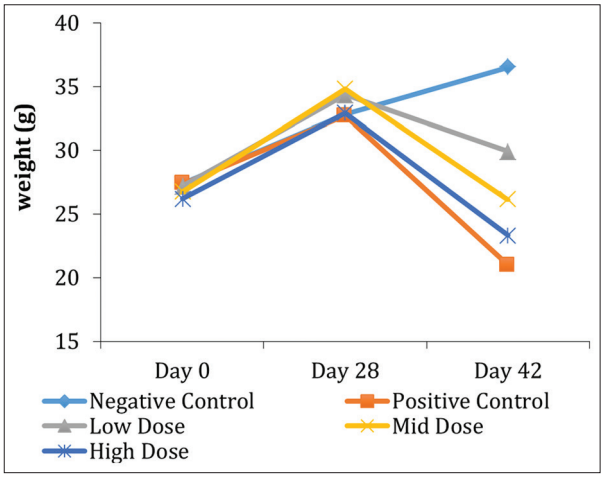

Figure 1: Line graph showing the mean weight of each group at baseline, day 28, and day 42

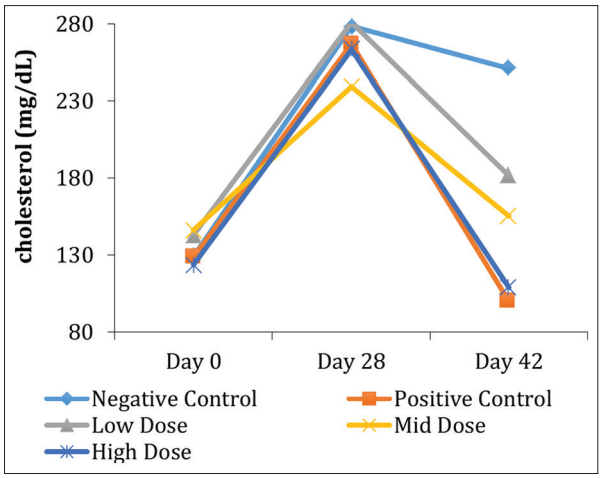

Figure 2: Line graph showing the mean cholesterol of each group at baseline, day 28, and day 42

showed that significant differences are seen at day 42. Moreover, all the treatment groups are not different with each other except the positive control and high dose and the low and mid dose. This shows that *E. microphylla* may be effective as well in lowering cholesterol levels.

DISCUSSION

After the different treatments were administered for 28 days and after administration of lard-coated feeds, the positive control and *E. microphylla* groups lost weight that may either be due to mechanism action of the drug or presence of different phytochemicals present in the plant. Specifically, tannins have antioxidant properties by cleaving ester linkages through hydrolysis.[17] Cleaving ester linkages by hydrolysis are synonymous to breaking down of large aggregates into smaller subunits, and ergo energy is used up and not excessively stored lowering contributing mass in the body.[25] Furthermore, assessment of the different groups shows that the high dose of *E. microphylla* may have the same effect as simvastatin. Since *E. microphylla* is a natural supplement, toxic effects may be lesser and may not be observed at all.

When assessing the cholesterol levels, administration of *E. microphylla* extracts resulted in lower blood cholesterol, which is similar to the levels of simvastatin. Specifically, statins may have the ability to lower blood cholesterol by reducing its capacity to synthesize in the liver by inhibition of HMG-CoA reductase.[4] In more recent years, pharmacotherapy using natural therapy has been used to limit the different unwanted side effects. Among the different natural therapy, health products containing flavonoids have been used for weight reduction and cholesterol-lowering activities.[26] The positive effects of *E.* microphylla on cholesterol and weight may be attributed to the presence of different phytochemicals, specifically flavonoids, which have been shown in other studies in laboratory animals[27] and humans.[28]

This study shows that *E. microphylla* may be effective in lowering weight and blood cholesterol in hypercholesterolemia-induced mice. Furthermore, it was seen that *E. microphylla* at high dose may be able to have the same effects as the positive control, simvastatin. Finally, since *E. microphylla* is a natural remedy, it may have lesser side effects when compared with the use of simvastatin on prolonged administration.

CONCLUSION

The primary objective of the study was to determine whether *E. microphylla* is capable of having cholesterol-reducing and weight loss properties when given to ICR mice. After the experiment, it was confirmed that *E. microphylla* possesses a cholesterol and weight lowering characteristic with an optimal dose of 1000 mg/kg for it to function meanwhile. The favorable findings of this study concerning *Tsaang gubat's* cholesterol lowering property may be further characterized and analyzed to further understand its mechanism of action.

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