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Research Article

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Anti-obesity Activity of *Broussonetia luzonicus* (Moraceae) leaves

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ABSTRACT

Obesity is currently a major threat to the health of people all over the world. This condition is a risk factor for cardiovascular diseases, diabetes, and certain types of cancer. This study concerned with the use of Broussonetia luzonicus leaves to prevent the development of obesity in male Swiss mice. Broussonetia luzonicus leaves were collected from Nueva Vizcaya, Philippines. The leaves were air-dried and extracted using a percolator and the concentrated under reduced pressure to obtain the crude methanolic extract. Obesity was induced by feeding them the mice with high caloric diet. Twenty-five mice were divided into 5 groups namely, negative control, positive control which is orlistat, 1000mg/kg, 1500 mg/kg, and 2000 mg/kg of the crude methanolic extract of B. luzonicus leaves. The crude methanolic extract was administered using an oral gavage for 28 days, weights were collected every 7 days, and in the last day, the epididymal fats were collected, stained and viewed under a microscope. The size of the adipocytes were measured using the software image J version 1.49. The size of the adipocytes were statistically analyzed using ANOVA with scheffe as the post-hoc analysis. The results showed that the weight measurements were inconsistent which may be due to the young age of the test animals. The reduction of adipocyte size indicated that there is no significant difference among the positive control, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg dose of the methanolic extract from which can be concluded that the methanolic extracts of B. luzonicus leaves is just as effective as orlistat in decreasing adipocyte size.

Keywords: *Broussonetia luzonicus, Alukon, Orlistat,* Adipocyte size, Methanolic extract, Anti-obesity, Moraceae, Leaves

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INTRODUCTION

Obesity is an excessive accumulation of fats due to numerous factors such as sedentary lifestyle and consumption of high caloric foods which causes an imbalance in the energy expenditure and energy intake of an individual. At the cellular level, it is the increase in the size of adipocytes or fat cells due to the accumulation of fats in the cytoplasm[1]. Obesity is linked to a metabolic syndrome which is known to cause hypertension, insulin resistance, and hyperlipidemia[2]. In chronic situations it can cause atherosclerosis which can lead to heart attack, ischemic stroke, and death. Aside from cardiovascular diseases, obesity can also cause osteoarthritis, diabetes, and different forms of cancer such as breast, colon, and endometrial cancer.

According to WHO[3], the number of obesity cases has doubled since 1980. In 2014, there were more than 1.9 billion adults who were overweight, out of which, 600 million were obese. These values were measured using the Body Mass Index (BMI) which is a simple ratio of the height to the weight of the individual which measures the amount of body fat. A BMI value which is greater than or equal to 25 indicates overweight and a BMI value greater than or

equal to 30 indicates obesity. However, BMI is just an estimation of body fat for it does not indicate how much of the weight is fat and how much of the weight is muscle. A relatively measurement method is the Body Adiposity Index (BAI) which indicates the percentage of fats in the body. Another way to measure body fat is by using a fat caliper.

Obesity can be managed by lifestyle changes such as increasing physical activities, eating more fruits and vegetables, and decreasing intake of fats and sugars. Today, there are agents sold in the market which assist patients in achieving healthy body weight called anti-obesity drugs. These agents are divided into 2 categories: Appetite suppressants or anorexic agents such as sibutramine and phentermine which acts on the central nervous system by suppressing appetite or by inducing thermogenesis consequently leading to lipolysis and pancreatic lipase inhibitors such as orlistat which prevent the metabolism and absorption of fats in the gastrointestinal tract[4].Additional use of anti-obesity drugs coupled with lifestyle modification can be beneficial in managing obesity. However, these agents are known to have undesirable adverse side effects. Sibutramine was withdrawn from the market in 2010 due to a significant increase in the risk of myocardial infarction and strokes[5]. Another study showed that sibutramine can cause frequent genotoxic damage in Swiss mice[6]. Orlistat was also reported to cause fecal incontinence, flatus, oily discharge, evacuation, soft stools, and some reports of hepatotoxicity[7]. Hence, there is an urgent need to discover safe and effective anti-obesity agents.

*Broussonetia luzonicus*is a tree which is commonly found in the Northern region of the Philippines. This tree belongs to the family Moraceae. It is locally known as "*Himbabao*" in *Tagalo*g(which is the native language of Manila and its surrounding provinces in the Philippines) and "*Alukon*" in *Ilocano* (the native language of the northern regions of the Philippines). The leaves of this tree are commonly eaten as vegetables.

2. Statement of the Problem

The problem that this research sought to resolve was whether the methanolic extract from the leaves of *Broussonetia luzonicus (*Moraceae) has anti-obesity activity.

3. Significance of the Study

Plants have been a source of treatment and prevention of various diseases. A number of drug products in the market today have been isolated or partially synthesized from plant or animal sources. These drugs provide effective therapeutic action and are relatively safe for use. Currently, the potential of natural products for the management of obesity is still under development but and it may offer an effective and safer alternative compared to its synthetic counterparts[8].

4. Review of the Literature

Previous studies of other *Broussonetia spp.* have reported the following activity. Broussonetinine A and B which was isolated from the bark of *Broussonetiakazinoki* have been known to have greatly inhibited α-glucosidase[9]. Another study reported that Broussonone A which was isolated from the bark of *Broussonetiakazinoki* have pancreatic lipase inhibitory activity[10]. Phenolic compounds from *Broussonetiapapyrifera* leaves possess antioxidant and estrogen biosynthesis-inhibiting properties[11]. Currently, there are only two studies regarding *B. luzonicus.* The phytochemical screening of the methanolic extract of *Broussonetia luzonicus* leaves indicated the presence of carbohydrates, reducing sugars, flavonoids, tannins, alkaloids, and sterols[12]. Another study reported that the DCM extract of *B. luzonicus* leaves afforded epitaraxerol, lupenone, squalene, β- carotene, vitamin K and β-sitosterol[13]. However, there is still no study regarding the anti-obesity activity of *B. luzonicus*. This study therefore, seeks to determine the anti-obesity activity of *B. luzonicus* leaves.

Secondary metabolites from plants have been a source of medicinal products. The constituents of the methanolic extracts of *Broussonetia luzonicus* leaves contain carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, and sterols[12]. Another study of the DCM extract *B. luzonicus* leaves indicate the presence of epitaraxerol, lupenone, squalene, β- carotene, vitamin K and β-sitosterol[13]. Flavonoids such as lupenone has been reported to effectively inhibit adipocyte differentiation and expression of adipogenic marker genes in 3T3-L1 preadepocytes, this resulted in the significant reduction in lipid accumulation and expression of adipogenic marker genes[14]. Another phenolic compound Broussonone A showed non-competitive inhibition on pancreatic lipase[10]. Alkaloids such as Broussonetinine A and B has been known to have potent α-glucosidase activity which prevents the metabolism and subsequent absorption of glucose[9]. Tannins such as epigallocatechin-3-gallate from black tea has been known to have pancreatic lipase activity[15]. High dose of squalene has been reported to have prevented the increase of body

fat, blood pressure, blood levels of leptin, glucose, cholesterol, and triglycerides[16]. Phytosterols have a chemical structure similar to cholesterols which have been reported to decrease cholesterol absorption and plasma Low Density Lipoprotein (LDL) values[17]. This is the first study done on the anti-obesity property of *B. luzonicus* leaves and it have shown great potential which can be further studied and develop.

EXPERIMENTAL SECTION

5.1 Collection and Authentication of *Broussonetia luzonicus* leaves

One thousand and three hundred grams (1300g) of fresh leaves was collected at Santa Fe, Nueva Vizcaya. The plant specimen was authenticated by Manuel D. Ching, a botanist from the Bureau of Plant Industry (BPI). The specimen was deposited with the document number (PLT-ID-CRD-256-15) as certification for plant authentication.

5.2 Extraction of the crude methanolic extract of *Broussonetia luzonicus* leaves

The leaves were air-dried and ground using a blender. The ground leaves were extracted by percolation using methanol as the solvent. Exhaustive extraction was used to obtain more extracts from the leaves. After collecting the extracts, it was concentrated under reduced pressure using a rotary evaporator.

5.3 Preparation of the test animals

Twenty-five (25) male albino Swiss mice 4-6 weeks of age were purchased from the Department of Science and Technology (DOST) animal house and housed at DOST's standard and testing division where environmental conditions are controlled. The mice were given laboratory pellets and water *ad libitum,*12 hours light/dark cycle, wood shavings were used as bedding, and the cages were cleaned every day. The mice were acclimatized for 7 days prior to any experimentation.

5.4 Induction of obesity

The mice were given high caloric diet which was regular laboratory pellets supplemented with pork rinds. The weights were measured every 7 days for 35 days. Baseline weights were also collected to compare the difference in weight over the period of experimentation.

5.5 Determination of the anti-obesity activity

Twenty-five (25) albino Swiss mice were divided into 5 groups with 5 mice each: negative control, positive control, 1000mg/kg BW, 1500 mg/kg BW, and 2000 mg/kg BW of the methanolic extract of *Broussonetia luzonicus* leaves. The negative control was given laboratory pellets with pork rinds without any treatment, the positive control was given high caloric diet with 22 mg/kg BW based on the study of Adnyana et al.[18]. The three test groups were given high caloric diet with different doses of the extract 1000 mg/kg BW, 1500 mg/kg BW, and 2000 mg/kg BW of the crude methanolic extract of *B. luzonicus* leaves respectively. The doses were administered using an oral gavage and they are given daily before meals. Weights were measured every 7 days for 28 days.

5.6 Histopathology

On the 28th day, the mice were sacrificed by carbon monoxide method and epididymal fats were collected. Samples of epididymal adipose tissue were fixed with 10% buffered formalin and embedded in slides. Standard sections of 5 μm were cut and stained with hematoxylin and eosin (H&E). The adipose tissue stained sections were viewed via electronic microscope and photos were captured by the computer directed from the microscope.

5.7 Quantification of adipocytes

The adipocyte area was quantified by using Image J v 1.49 and viewed under HPO measuring adipocyte by area of randomly selected 16 adipocytes based on the study of Osman[19]. The area of the adipocytes was calculated using the formula: Area = π * A * B where A is the radius of the height and B is the radius of the width.

Quantification of Adipocytes

Based from the histopathological samples obtained from the epididymalfats of mice, figure 2A shows the adipocytes of the negative control which indicates obese adipocytes. Figure 2B shows the adipocyte of the positive control (orlistat) which indicates the reduction of size compared to that of figure 2A. It also shows that the shrinkage as well as the destruction of the adipocytes. Figure 2C shows that the adipocyte of the mice administered with 1000 mg/kg BW of the methanolic crude extract of *Broussonetia luzonicus* leaves indicates a relative reduction of size of the adipocytes. Figure 2D shows that the adipocyte of the mice administered with 1500 mg/kg BW of the methanolic

crude extract of *B. luzonicus* leaves indicates that there is a destruction of the cell wall of the adipocytes which affects the integrity of the cell. This destruction can cause leaking of the contents of cytoplasm which causes apoptosis of the adipocytes. Figure 2E shows that the adipocyte of the mice administered with 2000 mg/kg BW of the crude methanolic extract of *B. luzonicus* leaves indicates severe shrinkage of adipocytes as well as destruction of cell wall.

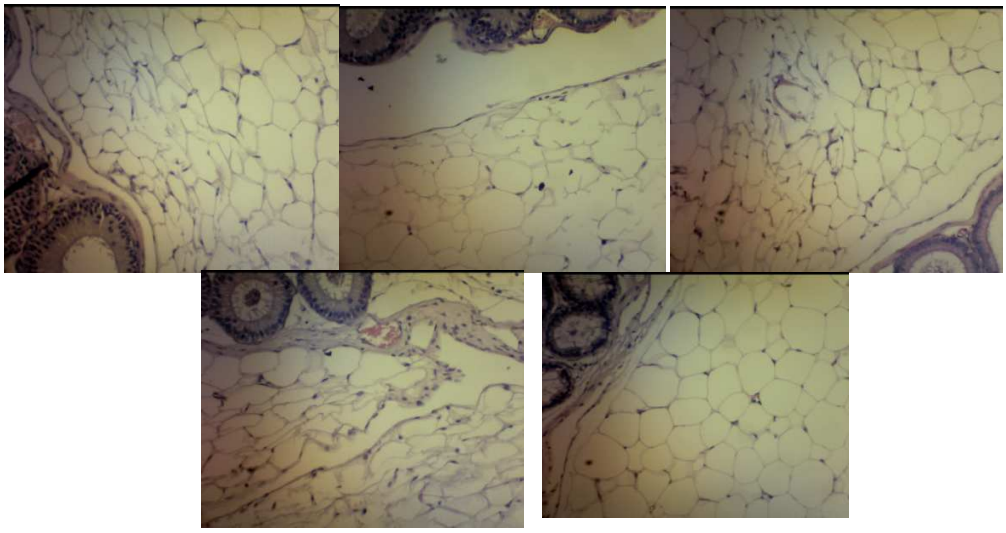
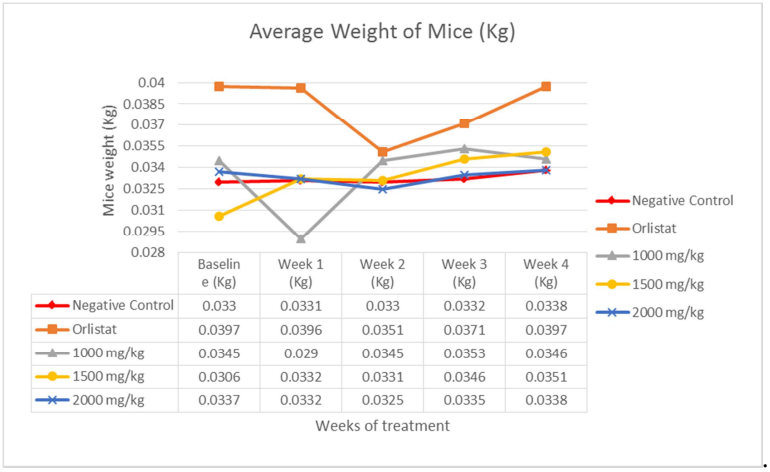


Figure 1A-1E Histopathology of adipocytes: top left: Negative control, top middle: Positive control (Orlistat), top right:1000 mg/kg BW of extract, bottom left: 1500 mg/kg BW of extract, bottom right: 2000 mg/kg BW of extract

To assess the adipocytes objectively, the study utilized image J v. 1.49 to quantify the size of the adipocytes. Sixteen adipocytes were measured per slide randomly[19]. The area of the adipocytes were calculated and the mean per test group were collected.

Figure 2: Weight of mice (Kg) vs. weeks of treatment



5.8 Weight monitoring

Figure 2 The weight of the mice monitored over the 4 weeks of treatment and their average weights in kilograms are shown **Fig 1**. The negative control has been maintained throughout the experimentation period. However orlistat and 1000 mg/kg of the plant extract showed an erratic change in weight as well as in dose 1500 mg/kg and 2000 mg/kg of the plant extract resulted in an increase in body weight. The reason for this irregularity may be because of the young age of the test animals (4-6 weeks). It is also important to mention that metabolism, bone and muscle growth are still active during this period.

5.9 Statistical Analysis

The size of the adipocytes were statistically analyzed by One-way ANOVA and Scheffe as the post-hoc analysis using SPSS v. 22 statistical software. Results were considered significant at p-value lesser than 0.05.

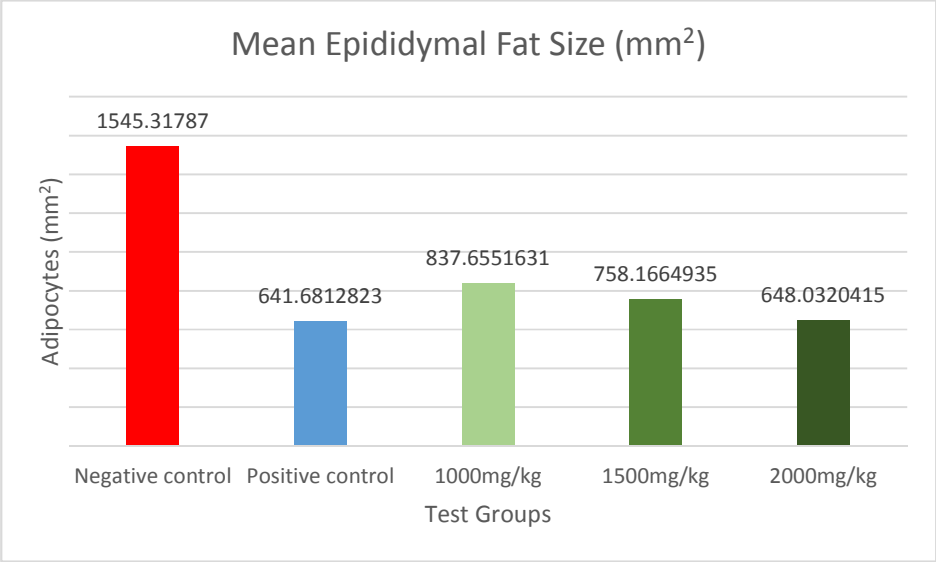


Figure 3: Mean Size of Adipocytes (mm^2) among the Test Groups

As shown in **Figure 3**, the mean size negative control which is the high caloric diet group without treatment was larger compared to the positive control, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg crude methanolic extract of *B. luzonicus* leaves. The treatment group have was also comparable to the effects of the positive control group. This also shows the trend of the doses, the higher the dose given, the greater the reduction in the adipocyte sizes. Statistical analysis with One-way ANOVA shows that there is a significant difference between all the groups (p=0.002). However, post-hoc analysis using Scheffe indicates that there is no significant difference between the positive control and the 3 different dose groups 1000 mg/.kg (p=0.941), 1500 mg/kg (p=0.989), and 2000 mg/kg (p=1.000). Therefore this study reports that the anti-obesity effect of *B. luzonicus* leaves is comparable to the anti-obesity effect of Orlistat which is currently available in the market.

RESULTS AND DISCUSSION

Based on the data obtained from the study, the result from the weight monitoring produced irregular readings which may be due to young age of the mice. However, based on the size of the adipocytes, there is a marked reduction in the size of the cell compared to the non-treatment group. The activity of the different doses of the crude extract was also comparable to that of the positive control which is Orlistat. This evidence can support the anti-obesity activity of the crude methanolic extract of *Broussonetia luzonicus* leaves.

CONCLUSION

Broussonetia luzonicus is an endemic tree found in the Philippines and the leaves and flowers are commonly eaten as vegetables. Some of the secondary metabolites present can be related to its anti-obesity activity. This study have proven the anti-obesity activity of the plant was comparable to Orlistat which is the most used agent in the market.

Recommendation

However, further studies should be made on the acute and chronic oral toxicity of the extracts of *Broussonetia luzonicus* leaves, complete identification of the secondary metabolites present, optimization of the propagation of *B. luzonicus* which can increase the yield of active secondary metabolites, and standardization of the amount of secondary metabolites and dose for its therapeutic activity.

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