THE ANTI-OBESETY EFFECTS OF THE AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF BLUMEA BALSAMIFERA ON DIET-INDUCED OBESE SPRAGUE-DAWLEY RATS

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ABSTRACT

The present study aims to evaluate the effectiveness of aqueous and ethanolic leaf extracts of Blumea balsamifera in reducing obesity on diet-induced obese Sprague-Dawley rats. Aqueous and ethanolic leaf extracts were obtained by maceration and percolation, respectively, of air-dried, ground leaves. The test animals were given a high fat diet (HFD) for 21 days, except for one negative control group fed with a standard diet (SD). The Blumea balsamifera extracts were given at doses of 300 mg/Kg and 600 mg/Kg for BBAE and BBEE groups and the positive control group, Orlistat, was given at 21.6 mg/Kg dose. After 24 days of treatment, the statistical difference of parameters such as Lee’s index and lipid profile of each group before and after the treatment period were determined separately using Tukey’s test of two-way Analysis of Variance (ANOVA). The statistical results showed that the 600mg/kg dose of BBAE and BBEE had greatly lowered the Lee’s index among the other doses while the 300 mg/Kg dose BBEE, 600 mg/Kg BBAE and 300 mg/kg BBAE lowered the total cholesterol level, LDL level and VLDL and total triglyceride level respectively. The extracts, however, lowered the HDL level which was also exhibited by the standard drug, Orlistat.

KEYWORDS: Adipocyte, adipogenesis, Blumea balsamifera, Lee’s index, obesity, sambong.
1. INTRODUCTION

Obesity predisposes a person to chronic diseases that leads to death. In the Philippines, 3 out of 10 adults aged 20 years and above are either overweight or obese. The factors contributing to obesity include unhealthy eating habits and a sedentary lifestyle. It has also been evaluated in a study that the prevalence rate of obesity is in proportion to the increasing urbanity. People with obesity double their risk of dying from coronary artery disease and triple their chance of dying from type 2 diabetes mellitus. Likewise, obesity elevates blood pressure and cholesterol levels.

Drug therapy and surgery are the current interventions of treating obesity. Most anti-obesity drugs available in the market are appetite suppressants that act in the central nervous system to inhibit energy intake. Some drugs increase metabolic rate or affect metabolism through peripheral action, act on the gastrointestinal tract or alter the overall metabolic syndrome. The use of synthetic anti-obesity drugs such as Orlistat, Phentermine and Lorcaserin has been associated with nausea, vomiting, constipation, stomach pain, diarrhea and other gastrointestinal disturbances. Bariatric surgery is performed for extreme obesity where drug therapy is insufficient. It causes immediate significant weight loss, but it is costly and can lead to complications. The undesirable effects and complications of these interventions show the need of finding a safer and alternative therapy that may be derived from herbal sources.

*Blumea balsamifera*, or commonly known as sambong, has been studied extensively for its pharmacologic utilities. It is classified under the Philippine National Drug Formulary (2012) as a diuretic agent for the treatment of kidney stones. Researches also show that the leaf extracts of *Blumea balsamifera* can reduce blood pressure and inhibit sympathetic nervous system. An *in vivo* assessment of the antidiabetic and antioxidant activities of *Blumea balsamifera* proved that its leaf extract can cause significant reduction in blood glucose level and alteration in elevated lipid profile level.

A study conducted by Kubota et al. (2009) using 3T3-L1 mouse cells showed that *Blumea balsamifera* leaf extracts can decrease the activity of glycerol-3-phosphate dehydrogenase (GDPH) resulting to the reduction in the rate of the adipogenesis which can be valuable in the treatment of obesity.
Blumea balsamifera leaves have been formulated by the National Integrated Research Program on Medicinal Plants (NIRPROMP) and the DOH’s Philippine Institute of Traditional and Alternative Health Care as diuretic tablets. There are also commercially-available tea leaves claimed to treat kidney and gall bladder stones. However, their potential use as an anti-obesity agent has not been fully explored.

This research aimed to investigate the in vivo anti-obesity effect of the aqueous and ethanolic leaf extracts of Blumea balsamifera on diet-induced obese Sprague-Dawley rats.

2. MATERIALS AND METHODS

A. Plant Collection and Extraction

The Blumea balsamifera leaves were collected from the Science City of Munoz, Nueva Ecija, Philippines. The leaves were air dried and ground for the preparation of the two extracts. The ethanolic extract was prepared by percolation method with 80% ethanol (1:8 w/v) while the aqueous extract was prepared by maceration and decoction in distilled water (1:15 w/v) for an hour and was lyophilized. The yields for both extracts were 12.7% and 3.6% respectively.

B. In vivo Anti-Obesity Evaluation

The study was conducted at University of Santo Tomas using the Institutional Animal Care & Use Committee (IACUC) approved test protocol. The study was conducted to 21 adult male Sprague-Dawley rats which were divided into 7 groups with 3 rats each after one week of acclimatization. During the 21-day induction of obesity, all groups received a high fat diet (HFD) except for one control group which was given a standard diet (SD). The body weights were recorded weekly for Lee’s index. After 20% increase in the body weights of all the rats, the 24 days administration of the treatment was done. The blood samples were collected after a 9-hour fast in the post-induction and post-treatment periods for the determination of the following parameters: triglyceride (TG) level, total cholesterol (TC) level, high-density lipoprotein (HDL) cholesterol level and low-density lipoprotein (LDL) cholesterol level. The groups and their respective diets are shown in table 1.

The statistical difference in Lee’s index and serum parameters over time within and between groups were determined separately by two-way ANOVA.
Table 1: Group Assignments of Test Animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
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<tbody>
<tr>
<td>Negative Control Group A</td>
<td>SD</td>
</tr>
<tr>
<td>Negative Control Group B</td>
<td>HFD</td>
</tr>
<tr>
<td>Positive Control Group with Orlistat</td>
<td>HFD</td>
</tr>
<tr>
<td>BBEE Group 300mg/kg BW</td>
<td>HFD</td>
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<tr>
<td>BBEE Group 600mg/kg BW</td>
<td>HFD</td>
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<tr>
<td>BBAE Group 300mg/kg BW</td>
<td>HFD</td>
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<tr>
<td>BBAE Group 600mg/kg BW</td>
<td>HFD</td>
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</tbody>
</table>

Legend: BBEE: *Blumea balsamifera* ethanolic extract; BBAE: *Blumea balsamifera* aqueous extract.

3. RESULTS AND DISCUSSION

A. Assessment of Obesity of the Sprague-Dawley Rats

There was a steady increase in the mean body weight of the HFD and SD group as compared to the treatment groups and the positive control group, Orlistat (shown in Fig. 1). The latter two showed a decrease in their mean body weight at week 7 which was within the treatment period. The weight and nasoanal length were also recorded weekly for the computation of the Lee’s index shown in Fig. 5. In the pre-induction period, the treatment groups namely 300 mg/Kg BBAE, 600 mg/Kg BBAE, 300 mg/Kg BBEE and 600 mg/Kg BBEE and the group treated with Orlistat had a significant increase in their mean Lee’s Index at the end of the induction period. It implies that the high fat diet used was effective in inducing obesity to the animal models.

The Lee’s index of the test animals was used to assess the effect of *Blumea balsamifera* leaf extracts in reducing the percent mean body fat of the treatment groups. The mean Lee’s index of the negative control groups, HFD and SD were used as the baseline values since there are no available baseline values for the interpretation of Lee’s index.

Based on the data obtained, the mean Lee’s index of the treated groups showed no significant reduction (p >0.05) in the post treatment period. Despite the statistical result, there was a notable decrease in the mean Lee’s index of the treated groups, as illustrated in Fig. 2, in which 600 mg/Kg BBAE and 600 mg/kg BBEE decreased the most.
B. Analysis of Serum Parameters

The total cholesterol level represents the total amount of cholesterol found in the systemic circulation which also comprise of the LDL and HDL. In Fig. 3, the mean total cholesterol level of the SD group was nearly maintained while the HFD group had increased continuously in the post treatment period. Conversely, the mean total cholesterol level of the treatment groups and the Orlistat group had an observable decrease. However, all of the treated groups, except 600mg/Kg BBAE, showed no significant deviation (p >0.05) in the difference of their mean total cholesterol level when compared to the SD group. In addition, the mean total cholesterol level of 300 mg/Kg BBEE group lowered notably among all the other treatment groups and it was comparable to the result exemplified by the Orlistat group.
Figure. 3 Effects of *Blumea balsamifera* leaf extracts administration on Mean Total Cholesterol level.

In contrast to the beneficial result on the lowered mean total cholesterol level of the treatment groups, their mean HDL level had apparently decreased whereas the mean HDL level of the HFD and SD groups retained as shown in Fig. 4. Since HDL represents the good cholesterol, it is ideal to have a high level of HDL found in the blood. Despite this, the effect elicited by the *Blumea balsamifera* extracts was also exemplified by the standard drug, Orlistat and only the 600 mg/Kg BBEE group significantly decreased (p <0.05) its mean HDL level. The remaining treatment groups namely 300 mg/Kg BBAE, 600 mg/Kg BBAE and 300 mg/Kg BBEE and the positive control group, Orlistat, showed no significant decrease in their mean HDL level (p >0.05) statistically in the post treatment period.

Orlistat is an anti-obesity agent that controls the level of bad cholesterol, LDL, but it does not imply that it can simultaneously increase the level of the good cholesterol. In this case, the results of the *Blumea balsamifera* extracts in the mean HDL level could be associated with the activity of Orlistat.

As mentioned above, LDL represents the bad cholesterol and this is one of the risk factors associated with heart diseases. Therefore, it is important to monitor the amount of LDL present in the blood. In Fig. 5, there was an observable decrease in the mean LDL level of all test groups in the post treatment period. However, the reduction in the mean LDL level of the 600mg/Kg BBAE and 300mg/Kg BBEE groups were significantly larger (p <0.05) compared to the other treatment groups.
Another serum parameter assessed in the experiment was the mean VLDL level. In Fig. 6, there was no significant reduction (p > 0.05) in the mean VLDL level of the HFD and SD groups as well as that of the treated groups in the post treatment period. The reason for the narrow lowering of the mean VLDL level is the probable inhibition in the activity of the enzyme Lecithin-cholesterol acyltransferase (LCAT) which catalyzes the conversion of VLDL to LDL in the bloodstream. In a study by Bielicki et al. (1996), minimally oxidized LDL can inhibit the activity of LCAT in converting VLDL to LDL. In previous studies, *Blumea balsamifera* extract proved to exhibit an antioxidative property. The observable decrease in the mean LDL level of the treated groups, specifically in the 300 mg/Kg BBAE group, had affected the lowering of the mean VLDL level in the post treatment period.

Triglyceride is another type of fat that increases the risk of developing cardiac diseases. In the evaluation of the effect elicited by the leaf extracts in the mean triglyceride level of the treatment groups, there was a varying decrease in the result of their mean Triglyceride level. Nevertheless, the 300mg/Kg BBAE group had significantly decreased (p <0.05) compared to the other groups. The narrow decrease in the mean triglyceride level of the residual groups could be attributed to the fact that triglyceride is transported by the lipoprotein, VLDL, to the peripheral tissues together with cholesterol. With the narrow decrease in the VLDL level, the triglyceride level was consequently reduced minimally.
Figure. 5 Effects of *Blumea balsamifera* leaf extracts administration on Mean LDL level.

Figure. 6 Effects of *Blumea balsamifera* leaf extracts administration on Mean VLDL level.

Figure. 7 Effects of *Blumea balsamifera* leaf extracts administration on Mean Total Triglyceride level.
4. CONCLUSION

The effect of *Blumea balsamifera* in lowering percent body fat, specifically the 600 mg/kg doses of BBAE and BBEE, had showed a promising result within a span of almost 4 weeks of administration. The findings also confirmed the potential of *Blumea balsamifera* leaf extract in managing the total cholesterol level, LDL level, VLDL level and total triglyceride level despite its negative effect in the HDL level. The doses of the *Blumea balsamifera* extracts that showed a promising effect on the serum parameters are as follows: 300 mg/Kg dose BBEE, 600 mg/Kg BBAE and 300 mg/kg BBAE lowered the total cholesterol level, LDL level and VLDL and total triglyceride level respectively.

It is important to note a high level of blood cholesterol does not imply that an individual is automatically obese. The results on the effect of *Blumea balsamifera* in the lipid profile of the test animals provide a broad overview of its scope and mechanism of action.

Since obesity is largely manifested by the percent body fat, the main parameter used to evaluate the anti-obesity effect of *Blumea balsamifera* was the decrease in the body weights of the test animals relative to their nasoanal length which was presented in the study as the Lee’s index.

Further studies in the assessment of the anti-obesity effect of *Blumea balsamifera* is considered necessary specifically in the identification of the active constituent present in the leaf extract that contributed to the management obesity. Alongside with these, studies should also include the determination of the optimal dose of *Blumea balsamifera* as an anti-obesity agent. The future researches could also include groups of rats which are subjected to diet change such as lowered fat and carbohydrate intake while undergoing treatment to fully understand the scope and limitations of the anti-obesity effect of *Blumea balsamifera*. A necropsy analysis should also be taken into consideration for the post treatment analysis of the visceral internal organs for any changes which can occur in the course of the study.

This thesis and the succeeding researches will pave the way toward clinical studies that will use *Blumea balsamifera* as an economical, safe and effective option for the care and treatment of metabolic disease, alongside lifestyle changes such as a healthy balanced diet and exercise.
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REFERENCES


