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Anti-hyperglycemic effects of aqueous *Lenzites betulina* extracts from the Philippines on the blood glucose levels of the ICR mice (*Mus musculus*)

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ABSTRACT

Objective: To examine the anti-hyperglycemic effects of aqueous *Lenzites betulina* (*L. betulina*) extracts on normoglycemic glucose-loaded mice.

Methods: Different doses of aqueous extract from *L. betulina* were administered to 45 ICR mice (*Mus musculus*) to determine whether there was an effect of *L. betulina* extracts on the blood glucose level of the ICR mice. Aqueous extracts of *L. betulina* were orally gavaged to mice using oral glucose tolerance test. A total of five groups were used to determine the effect of the fungi on blood glucose of the mice. Group A (positive control) was given 16.7 μg/kg glimepiride; Group B (negative control) was given distilled water; Group C (low dosage) was given 200 mg/kg aqueous extract; Group D (mid dosage) was given 400 mg/kg aqueous extract and Group E (high dosage) was given 800 mg/kg aqueous extract. Baseline blood glucose value was firstly acquired before induction of hyperglycemia through D-glucose, after which another check on blood glucose was made after 0.5 h. Immediately, after the acquisition of hyperglycemic blood glucose level, the individual administration of treatments were done. After that, three blood collections were done spanning 3 h with 1 h interval.

Results: The low dose (200 mg/kg) and the mid dose (400 mg/kg) of *L. betulina* extracts were significantly different ($P < 0.05$) from their respective baseline values throughout the whole experiment with the latter surpassing its baseline value during the 3rd hour. On the other hand, the high dose (800 mg/kg) during the 1st hour after administration was not significantly different ($P > 0.05$) from its corresponding baseline value, acting faster than the positive control (glimepiride), which only became significantly different ($P < 0.05$) at the 2nd hour.

Conclusions: Aqueous *L. betulina* extract is able to produce hypoglycemic effects on the mice with all doses, which are able to normalize blood glucose levels at varying times.

1. Introduction

Various species of *Lenzites* sp. are not only widespread around Asia, but also well distributed around the world. The species *Lenzites betulina* var. *flaccida* can be found in almost all parts of the world, while some species, such as *Lenzites vespacea*, are only found in Asia, Africa and Oceania. Other species like the *Lenzites heteromorpha* is native to Europe [1]. In Philippines, multiple species of *Lenzites* are identified to be present such as *Lenzites repanda* which can be found in the islands of Bataan, and *Lenzites acuta* which can be found in Cagayan Province [2].

Lenzites spp. have no value as food since it has an extremely tough exterior [3]. There have been different studies on the various medicinal uses of *Lenzites* spp. as scavengers of free radicals, antimicrobials, anti-oxidant, anti-viral and immunosuppressant

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All experimental procedures involving animals were conducted in accordance to PALAS Code of Practice for the Care and Use of Laboratory Animals in the Philippines and approved by the Institutional Animal Care and Use Committee of De La Salle University.

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[4–8]. Phytochemical analysis of compounds present in *Lenzites* spp. using three different solvents (ethanol, water and petroleum ether) was performed. The results showed that phenolic and steroids were present in the ethanol extract; flavonoids, tannins and steroids were present in the petroleum ether and only saponins were present in the aqueous extract [5]. However, there has been no study yet on the hypoglycemic effects of crude aqueous extract of *Lenzites betulina* (*L. betulina*).

The medicinal herbs have been used increasingly with greater advocacy for complementary and alternative medicine. Since then, there is a need for scientific-based research to find out the exact effects of different herbs and plants within the body. In the Philippines alone, 57.3% of the population is making use of herbal medicine to either control or prevent diseases [9,10]. Although herbal medicine has clinical substantiation for several years, mechanisms of how they provide medicinal effects are not well described. Since majority of the Philippine population is classified under the middle class, not all people can afford prescription medicine for different diseases and the use of *L. betulina* as a control of diabetes can serve as a cheap and accessible alternative prescription medication. Also, the value of *L. betulina* would increase especially in the medical sector and could possibly pave the way for the discovery and development of alternative medicine for the treatment of diabetes. Hence, the present study is aimed to investigate the hypoglycemic effects of crude aqueous extract of *L. betulina*.

2. Materials and methods

2.1. Fungi collection and processing

L. betulina was obtained from tree barks in Quezon, Palawan mostly in sites where kaingin was practiced. Bolos and knives were utilized for the removal of the mushroom from its attachment to the tree. It was authenticated by Edwin R. Tadiosa, Museum Researcher II, Botany Division at the National Museum of the Philippines.

Air-drying was done to prevent deterioration of the sample, after which the samples were then soaked in methanol for 24 h twice for the removal of low-molecular-weight compounds. The residues were extracted with distilled water at 100 °C for 6 h, evaporated to a small volume and subjected to lyophilization in a vacuum [11]. The mushroom sample was dissolved in distilled water to obtain a final concentration of 100 mg/mL.

2.2. Animal procurement and housing

A total of 45 10-week-old female mice of the ICR strain were obtained from the Research Institute for Tropical Medicine in Alabang, Muntinlupa, Philippines. They were housed individually in standard-sized cages in the animal house of De La Salle University. All cages were cleaned twice a week and bedded with autoclaved soft wood shavings. Feeders and water bottles were cleaned and dried twice a week. Prior to the experiment, all mice were acclimatized for a period of one week to adjust to a 12 h-light: 12 h-dark cycle at (24 ± 2) °C and relative humidity of 55% ± 10%. During the entire acclimatization and experimental period, food pellets and distilled water were supplied *ad libitum*. Proper handling and maintenance of the mice were observed and the Institutional Animal Care and Use Committee of De La Salle University approved the experimental protocol used.

2.3. Experimental procedures

Baseline blood glucose values were obtained through tail-tip nicking using an EasyTouch® GCU and immediately after, D-glucose solution (5 mg/kg body weight) was administered to all the mice to induce hyperglycemia. About 30 min after the induction of glucose, blood testing was performed to confirm hyperglycemia.

The mice were divided into 5 groups with 6 mice each. All treatments (glimepiride and *L. betulina* extracts) were diluted in distilled water. Then, Group A (positive control) was given glimepiride (16.7 μg/kg body weight); Group B (negative control) was given 0.2 mL of distilled water; Group C–E was given *L. betulina* at different concentrations (low dose: 200 mg/kg body weight; mid dose: 400 mg/kg body weight; high dose: 800 mg/kg body weight). Three more blood collections at 1st, 2nd and 3rd hour post-glucose administration were performed.

2.4. Statistical analysis

The differences in blood glucose levels (mean ± SD) were analyzed using repeated measures ANOVA and multivariate ANOVA and means were compared using Tukey's test and SPSS version 22 to determine significant differences among the treatment groups at $P < 0.05$.

3. Results

A total of 45 ICR mice were administered with three different doses of *L. betulina* extract (200 mg/kg, 400 mg/kg, 800 mg/kg), and glimepiride was given in the positive control (16.7 μg/kg) and distilled water was given in the negative control. A total of 5 blood collections were done to quantify blood glucose levels (mg/dL) during 3 h (Table 1).

Prior to the induction of hyperglycemia to all mice, *post hoc* tests showed that there were no significant differences between the baseline levels of all groups. This was supported by the fact that all groups were subjected to the same conditions. The values obtained were also within the limit of the normal blood glucose level (160 mg/dL) [12]. This indicated that no hyperglycemic activity was present before the administration of any substance to the mice (Table 1).

After 30 min, the blood glucose values were again compared after the induction of hyperglycemia to all mice. While all groups exhibited the desired hyperglycemic condition and no significant differences were observed across all groups (Table 1).

During the 1st hour, the blood glucose level of the negative control was significantly higher from all the other groups surpassing the hypoglycemic effect of glimepiride. This indicated that the mushroom extract exhibited anti-hyperglycemic activity. Also, within the treated groups, the values from the high dose group were proved to be significantly lower than those from the low and mid dose groups, including the positive control (Table 1). This indicated that the mushroom extract at high levels provided hypoglycemic activity along with the natural ability of the mice to retain its normal blood glucose level.

After 2 h of baseline testing, continuous decrease in blood glucose levels for all groups was observed. This time, the high dose and the positive control groups were not significantly different from each other and glimepiride had the same effect with the high dose of the *L. betulina* extract. Both mid dose and