

Hypoglycemic Activity of Irradiated Banaba (*Lagerstroemia speciosa* Linn.) Leaves

¹Custer C. Deocaris, ²Ranelle R. Aguinaldo, ²Josephine L. dela Ysla,
²Amelia S. Asencion and ³Elmer-Rico E. Mojica

¹Gene Function Research Center, National Institute of Advanced Industrial Science and Technology (AIST),
1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan.

²Department of Biology, College of Arts and Sciences, University of the Philippines Manila,
Manila, Philippines

³Institute of Chemistry, University of the Philippines Los Baños, College, Laguna, Philippines.

Abstract: Hypoglycemic activities of irradiated and non-irradiated banaba (*Lagerstroemia speciosa* Linn.) extracts were tested on alloxan-treated diabetic mice. The effects of 25% nBLE (non-irradiated BLE), 50% nBLE, 25% iBLE and 50% iBLE were comparable to the hypoglycemic effect of insulin (reference). Time-course monitoring indicated that blood glucose levels started to decline within 1.5 hours post-administration of BLE.

Key words: Hypoglycemic, activity banaba, irradiation

INTRODUCTION

Banaba (*Lagerstroemia speciosa* L.), a deciduous herbal tree found in the Philippines, has been extensively studied for its different biological activities particularly its potency in curing diabetes. The tea from its leaves is being used as a beverage, as well as, for alleviating symptoms of diabetes mellitus^[14]. Its hypoglycemic effect has been elegantly demonstrated in rabbit^[5] and mouse models^[11]. Phase II/III clinical trials in the application of banaba leaf decoctions to manage diabetes mellitus show promising data^[6,9].

Originally, because of its hypoglycemic activity, banaba leaf is thought to contain an “insulin-like principle”, a type of plant-derived peptide hormone^[5]. Further characterization of the extract reveal, otherwise, a triterpenoid compound, known as corosolic acid, which accelerates and stimulates glucose transport into cells^[13]. Also present in the banaba leaf are three active ellagitannins: lagerstroemin, flosin B and reginin A, which were recently isolated and found to increase glucose uptake in rat adipocytes^[7].

The use of banaba decoction is still the most efficacious and practical approach for “ethnopharmacologically” managing diabetes. Synthesis of its active principle, corosolic acid, and much so, its isolation,

have been very complex and costly a industrial process. The Philippine government has made previous attempts at exporting air-dried banaba leaves to Japan to generate revenues from this endemic medicinal plant, however, attempts have not been successful with our failure to meet stringent export standards, i.e. the SAL of 10⁻⁶. It is in this light that we investigate the possibility of irradiating banaba leaves to reduce bioburden. It is common knowledge that radiation can be used to decontaminate or reduce microbial burden in food and medical products^[1,15], and in this preliminary research, we posed the question: Will radiation-treatment of dried banaba leaves at 10 kGy (a ceiling radiation dose set by WHO on food stuff) have any effect on the hypoglycemic activity of banaba? We used alloxan-induced diabetic mouse model to show that banaba leaf does tolerate radiation treatment without compromising its anti-diabetic property.

MATERIALS AND METHODS

Sample Extraction: Fresh leaves (15-20 cm) were collected from banaba tree in the University of the Philippines, Diliman campus and were air-dried. A portion of the leaf sample was cut into strips and irradiated at 10 kGy in the multi-purpose Co-60 facility of PNRI. Banaba leaf extracts (BLE) were extracted from 100 g of samples (irradiated and non-irradiated leaves) in 300 ml 80% ethanol.

Corresponding Author: Elmer-Rico E. Mojica, Institute of Chemistry, University of the Philippines Los Baños, College, Laguna, Philippines.

Hypoglycemic Assay: Fifty (50) two month-old, male mice weighing from 16-24 grams were used in the experiment. Following a completely randomized design, four mice were assigned per treatment and control. The mice were allowed to fast prior to alloxan treatment.

Specific concentrations of aqueous solution of alloxan (Sigma Chemicals) were calculated based on the weights of the mice to induce diabetes. The prepared solutions (100% BLE, 75% BLE + 25 % insulin, 50% BLE + 50 % insulin and 25% BLE + 75% insulin) were introduced subcutaneously into the mice 24 hours after alloxan administration to the designated mice at a dosage of one unit as calibrated by a tuberculin syringe. A positive control of 100 % insulin (100 iu/mL) (Novo Nordisk) was used while the negative control used was the balanced salt solution (vehicle).

Blood glucose levels of the mice were monitored before the induction of diabetes (original glucose level), 24 hours after the induction of diabetes (diabetic blood glucose level) and every hour after the introduction of the experimental treatments. This was done by obtaining blood sample from the tail of the mice by venipuncture method and then by observing the color reaction of the blood sample on the haemoglucotest strips.

Statistical Analysis An analysis of variance (ANOVA) was carried out to determine the effects of radiation treatment and concentration of BLB on blood glucose at $p < 0.05$ level. Least significant difference (LSD) was determined to detect difference between mean glucose levels of alloxan-treated mice injected with banaba leaf extract with insulin.

RESULTS AND DISCUSSIONS

The hypoglycemic activity of BLE, both irradiated and non-irradiated, are shown in Figures 1 and 2. This trend was determined from changes in blood glucose level for 4 hours. After 1 hour following injection, treatments containing 25-50% BLE with 75-50% insulin showed rapid lowering of blood glucose levels for both irradiated and non-irradiated banaba leaves. Mice receiving 75-100% BLE with 0-25% insulin failed to elicit a hypoglycemic trend and behaved similarly with that of the vehicle. This concentration effect of BLE supplementation is confirmed with ANOVA.

A comparison of the normalized mean % reduction of glucose level is shown on Figure 3. In the irradiated banaba leaf extract (iBLE), 25% iBLE + 75% insulin was most effective (with 57.69% reduction in the blood glucose level), followed by 50% iBLE + 50% insulin, 75% iBLE + 25% insulin and 100% iBLE. The same trend was

also observed for non-irradiated banaba leaf extract (nBLE). Analysis of variance between treatments, however, suggests that radiation has also significantly altered the hypoglycemic property of banaba leaf extract. Test of means by LSD showed that BLE exhibited higher hypoglycemic potentials after radiation treatment

As expected, the greater the amount of insulin in the treatment, the greater the effectiveness in lowering blood glucose level in alloxan-treated mice. Pure BLE have minimal effects on the blood glucose level, but when combined with insulin, the activity is synergistically enhanced.

Corosolic acid (Figure 4) is a triterpenoid compound that constitutes 1% of the total content of banaba leaves. This was found to absorb glucose into cells promptly as insulin does based on data derived from Ehrlich ascites tumor cells^[13], to alleviate insulin-dependent type of diabetes (type II). Purified 1% corosolic acid, simulating that of the banaba leaf, given to Type II diabetics was found to work fairly well^[10].

Gamma radiation of banaba leaves could have lead to effective extraction of either of corosolic acid, the tannins, or both. This contention is based on published data demonstrating improved lipid extraction efficiency in dried caraway and caradom seeds exposed to different doses of radiation^[8,2]. Although it is commonly shown that radiation destroys important biomolecules to a certain degree, radiation effects can also bring about improved chemical stability in some natural products. For example, β -carotene irradiated in aqueous solvents form a more stable molecular orientation^[16]. At a certain range, irradiation of fresh fruits increased antioxidant activity of apple juice^[3], but it has no effect on antioxidant properties of anise, caraway, cumin and fennel essential oils extracted^[4]. Considering these possible radiation effects, irradiation of banaba leaf may facilitate unlocking active components from its complexed natural state and allow for more efficient extraction and increased in observed hypoglycemic activity.

In this study, irradiated banaba leaf extract mixed with insulin was found to have a higher hypoglycemic activity in comparison with the mixtures of nBLE and insulin. The activities of the resultant mixture were found comparable with pure insulin. Potentially, this may lessen the financial costs of managing diabetes by abating heavy dependence on recombinant insulin. Our results, however, seemed to differ from a recent pharmacologic report that BLE failed to exhibit synergistic nor additive effects with insulin in 3T3-L1 adipocytes based on glucose uptake

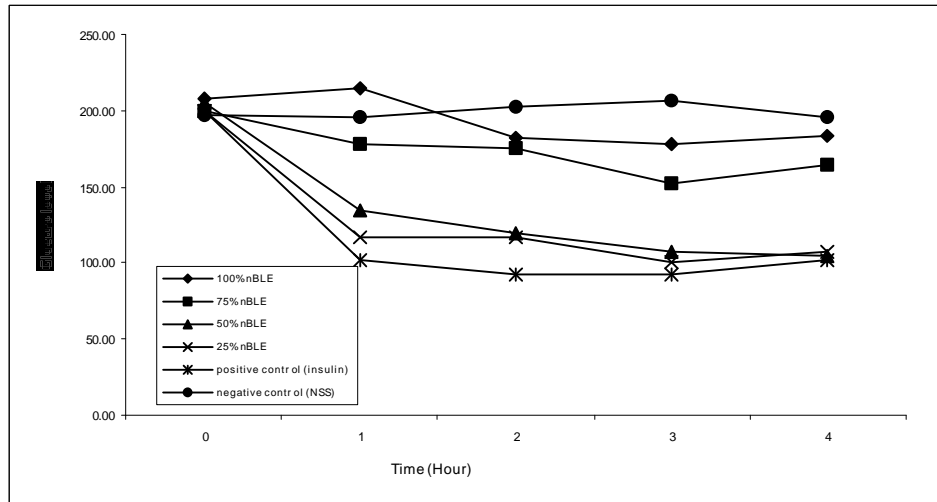


Figure 1: Glucose level of non-irradiated banaba leaf extracts.

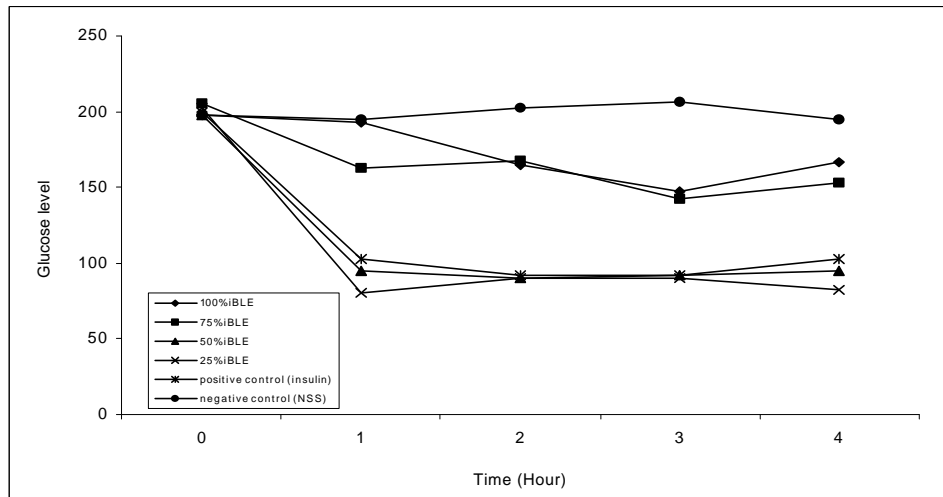


Figure 2: Glucose level of irradiated banaba leaf extracts.

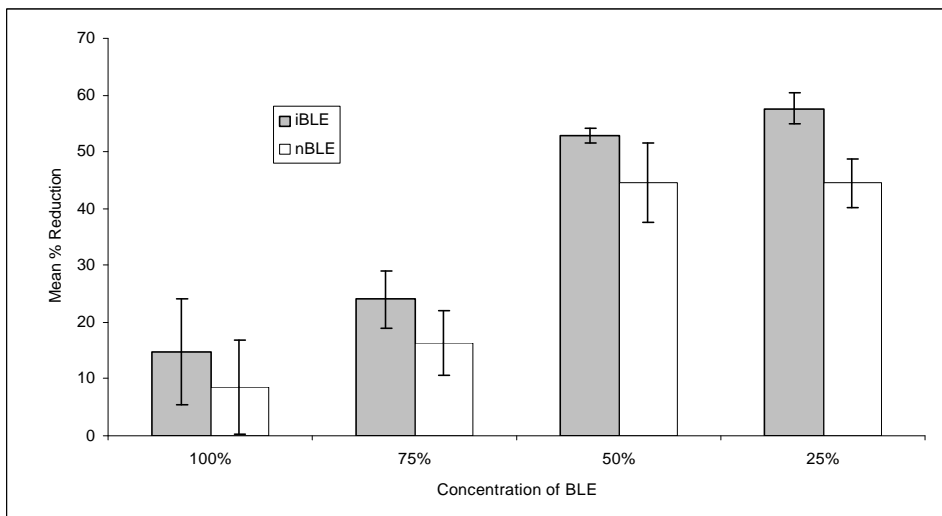


Figure 3: Percent mean reduction of different treatments of banaba leaf extracts

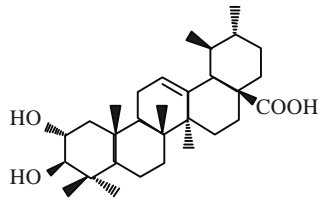


Figure 4: Chemical structure of corosolic acid

(Liu *et al*, 2001). However, such a discrepancy could be explained from the obvious differences in the model systems.

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