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Hematological effects of *Ipomoea batatas* (camote) and *Phyllanthus niruri* (sampa-sampalukan) from Philippines in the ICR mice (*Mus musculus*)

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ABSTRACT

Objective: To analyze the hematological effects of administering *Ipomoea batatas* (*I. batatas*) and *Phyllanthus niruri* (*P. niruri*) in the ICR mice.

Methods: Powdered leaves of *I. batatas* and *P. niruri* were fed to mice for 4 weeks. A total of six groups were used to determine the effect of the plants to the complete blood count of the mouse. Group A (blank control) mice were feed with pellets only; Group B (negative control) mice were fed with pellets coated with honey; Group C (low dosage) mice were fed with honey-coated pellets and powdered leaves of *I. batatas* at 10 g/kg body weight of the mouse; Group D (high dosage) mice were fed with honey-coated pellets and powdered leaves of *I. batatas* at 20 g/kg body weight of the mouse; Group E (low dosage) mice were fed with honey-coated pellets and powdered leaves of *P. niruri* at 10 g/kg body weight of the mouse; and Group F (high dosage) mice were fed with honey-coated pellets and powdered leaves of *P. niruri* at 20 g/kg body weight of the mouse. Complete blood count was performed on Days 0, 14 and 28.

Results: It was shown that *I. batatas* can increase the values of hematocrit and hemoglobin on both the low dose and high dose at Day 28 and red blood cells (RBC) on both Days 14 and 28 of testing. On the other hand, *P. niruri* can increase RBC, hematocrit and hemoglobin on Day 28 with only the low dose. There were no significant differences with white blood cell, absolute granulocyte, lymphocyte and monocyte, and platelet counts observed for both plant samples.

Conclusions: *I. batatas* and *P. niruri* have effects on the hematocrit, RBC and hemoglobin levels in mice.

1. Introduction

Ipomoea batatas (L.) Lam. (*I. batatas*) or sweet potato is locally called camote. It is a warm season crop which is extensively cultured in tropical, subtropical and temperate regions[1]. This crop is available all year round and is easily propagated from stem cuttings[2]. Specifically, the leaves has been shown to have nutritive and anti-nutritive effects[3]. The leaves contain cyanide, tannins, oxalate and phytic acid as antinutrients and a couple of minerals (calcium, magnesium, iron, zinc, potassium, manganese, phosphorus, copper and sodium) and vitamins (vitamin A, B6, B12, C and D)[4,5]. A study showed that the purple leaves of camote contain 6 g of phenolics and 21.5 μg of β-carotene per 100 g. It also has been hypothesized to protect the human body from oxidative stress which is associated with many diseases[6].

Phyllanthus niruri L. (*P. niruri*) is locally called sampa-sampalukan. It is a common roadside and garden weed which is

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found in the tropics[7]. It is a branching herb with small oblong leaves and fruits in its branches. This plant, however, is usually ignored because it is just regarded as a weed in vacant lots and roadsides. Phytochemical analysis of the leaf reported that it consists of multiple compounds which include alkaloids, saponins, tannins, oxalate, flavonoids, glycosides, lignins, terpenoids, polyphenols and coumarins[8,9]. Mineral constituents include lead, phosphorus, magnesium, copper, calcium, iron, nitrogen, zinc, selenium, sodium and potassium[10].

Vast sudies have been made on the effects of *I. batatas* such as anti-mutagenic[1], anti-diabetic[11], antibacterial and antifungal[12], anti-inflammatory[13], antioxidant[1], wound healing[14], antiulcer[15], cardiovascular[16], hepatoprotective[17], immunomodulatory and anticancer activities[13]. On the other hand, *P. niruri* have been shown to have hepatoprotective effect[18], inhibiting HIV replication[19], lipid lowering activity[20], anti-diabetic activity[21], anti-malarial activity[22], anti-spasmodic activity[23], analgesic activity[24], antioxidant activity[9] and inhibiting chromosomal aberrations[7].

However, there is little research on its effect on the hematology of normal ICR mice. Therefore, this study aims to analyze the effects of camote and sampa-sampalukan on the hematology of the mice, specifically hematocrit, red blood cell (RBC) count, hemoglobin, white blood cell (WBC) count, granulocyte count, lymphocyte count, monocyte count and platelet count. If proven to be effective, these plants may be used as an alternative for high-priced drugs which is currently being used for treatment of patients who have problems with blood.

2. Materials and methods

2.1. Procurement of plant samples

I. batatas leaves were purchased from vegetable vendors in Pablo Ocampo Street, Malate, Manila, Philippines. Only those with purple and undamaged leaves were considered for use.

P. niruri leaves were procured from the Bureau of Plant Industry, Department of Agriculture, Philippines. Only those with green and undamaged leaves were considered for use.

2.2. Test animals and setup

A total of 24 12-week old (approximately 30-40 g) ICR mice (*Mus musculus*) of either sex were obtained from the Bureau of Animal Industry, Department of Agriculture, Philippines. These were kept in separate, standard-sized cages in the animal house of De La Salle University (DLSU). All cages were sanitized and bedded with autoclaved paddy husk. The plates and bottles were washed, sanitized and dried twice a week. Prior to experimentation, mice were acclimatized for one week to adjust to a 12 h light, 12 h dark cycle at 28-30 ˚C. Proper handling and maintenance of the mice were observed and the experimental use was approved by the Institutional Animal Care and Use Committee of DLSU.

2.3. Preparation of plant treatments

The purple camote and sampa-sampalukan leaves were lyophilized at the Department of Chemistry, DLSU. The dried samples were then grinded and stored in clean and air-tight containers. A mixture of 25 g of pellets were coated with 2.5 mL honey and were administered corresponding to the dose of treatment for each experimental group.

2.4. Experimental procedures

The animals were randomly assigned into six Groups-A, B, C, D, E, F with 4 mice each. The diet and treatment given to each group were as follows: Group A (blank control) mice were feed with pellets only; Group B (negative control) mice were fed with pellets coated with honey; Group C (low dosage) mice were fed with honey-coated pellets and powdered leaves of *I. batatas* at 10 g/kg body weight of the mouse; Group D (high dosage) mice were fed with honey-coated pellets and powdered leaves of *I. batatas* at 20 g/kg body weight of the mouse; Group E (low dosage) mice were fed with honey-coated pellets and powdered leaves of *P. niruri* at 10 g/kg body weight of the mouse; and Group F (high dosage) mice were fed with honey-coated pellets and powdered leaves of *P. niruri* at 20 g/kg body weight of the mouse.

All animals were weighed prior to administration of treatment and fed 10% w/w mice feeds. It was ensured that the food pellets together with the treatment were consumed in one day. The experimental procedure lasted for 4 weeks with blood collected at Day 0, Day 14 and Day 28 of treeatment.

2.5. Blood analysis

Blood was collected by tail tipping at Day 0, Day 14 and Day 28 from 6:00 am to 7:00 am to prevent variations for analysis and placed in ethylene diamine tetraacetic acid tubes for analysis[25]. Blood was analyzed using Mythic 18 Vet (Orphee SA, Switzerland).

2.6. Statistical analysis

The data on blood parameters that expressed as means were subjected to multivariate analysis of variance. Means were compared with Tukey's test using SPSS version 22 to determine significant differences among treatment groups. The level of significance in all parameters used was $P<0.05$.

3. Results

3.1. Hematocrit

Table 1 shows the mice which were treated with high dose of camote had a significant difference from the negative and blank control only after 4 weeks of treatment administration. There were no significant differences from all treatment groups from the baseline and at Day 14 of blood testing.

Table 1

Effects of camote and sampa-sampalukan on hematocrit (%).

Treatment groups	Day 0	Day 14	Day 28
Group A	29.88±4.48	30.35±2.68	30.90±2.93[a]
Group B	29.38±7.31	30.28±4.93	30.75±4.33[a]
Group C	27.73±5.47	31.58±5.69	35.48±6.15[a,b]
Group D	31.00±5.19	37.75±5.63	42.20±5.27[b]
Group E	30.76±5.32	30.73±4.27	30.80±3.40[a]
Group F	31.68±5.02	31.53±3.63	31.99±1.73[a]

[a,b] Values with the same letter within columns are not significantly different from each other at $P<0.05$.

3.2. RBC count

Table 2 shows the effects of camote and sampa-sampalukan on RBC count. It can be seen that at Day 14, the high dose of camote tops had a signficant difference against the control groups. This difference was most evident on Day 28 when the high dose of camote tops was significantly different from the sampa-sampulacan doses as well.

Table 2

Effects of camote and sampa-sampalukan on RBC count (10^6/μL).

Treatment groups	Day 0	Day 14	Day 28
Group A	5.46±1.06	5.35±0.56[a]	5.33±0.93[a]
Group B	5.18±0.98	5.21±0.76[a]	5.28±0.80[a]
Group C	5.34±0.75	6.01±1.07[a,b]	7.06±0.95[b,c]
Group D	5.45±0.44	6.95±0.41[b]	7.81±0.39[c]
Group E	5.43±0.91	5.58±0.29[a,b]	5.48±0.46[a,b]
Group F	5.30±1.11	5.41±0.70[a]	5.33±0.50[a]

[a,b,c] Values with the same letter within columns are not significantly different from each other at $P<0.05$.

3.3. Hemoglobin

Table 3 shows the effects of camote and sampa-sampalukan on hemoglobin counts. It can be seen that there was a significant difference observed on Day 28 with the high dose of camote tops being different from all groups.

Table 3

Effects of camote and sampa-sampalukan on hemoglobin (g/dL).

Treatment groups	Day 0	Day 14	Day 28
Group A	10.74±0.94	10.83±1.26	11.02±0.66[a,b]
Group B	9.83±1.28	9.75±1.28	10.38±0.89[a,b]
Group C	9.75±1.20	11.00±1.69	12.32±1.80[b,c]
Group D	10.22±1.18	12.23±1.53	14.25±1.12[c]
Group E	9.93±1.41	10.03±1.05	9.90±0.85[a]
Group F	9.20±1.16	9.38±1.28	9.63±0.46[a]

[a,b,c] Values with the same letter within columns are not significantly different from each other at $P<0.05$.

3.4. WBC cell count

Table 4 shows the effects of camote and sampa-sampalukan on WBC count. It can be seen that even though there was an increasing trend from all experimental treatment groups from baseline to Day 28, there were no significant differences among treatment groups.

Table 4

Effects of camote and sampa-sampalukan on WBC count (10^3/μL).

Treatment groups	Day 0	Day 14	Day 28
Group A	10.45±0.87	10.28±1.01	10.00±0.39
Group B	9.68±1.77	7.45±5.05	9.88±1.35
Group C	8.98±1.38	9.95±0.72	10.65±0.70
Group D	10.33±1.72	10.88±0.91	11.53±1.17
Group E	10.33±1.18	10.08±0.64	10.55±0.68
Group F	9.15±1.93	10.30±0.47	11.23±0.28

Tables 5, 6, 7 also show that there were no significant differences between the different subtypes of WBC.

Table 5

Effects of camote and sampa-sampalukan on absolute granulocyte count (10^3/μL).

Treatment groups	Day 0	Day 14	Day 28
Group A	9.02±0.46	9.03±0.71	8.67±0.30
Group B	9.13±1.28	8.67±0.96	9.13±0.66
Group C	8.37±0.73	8.75±1.06	9.21±0.88
Group D	8.17±1.69	8.10±0.57	8.65±0.96
Group E	7.73±0.88	9.24±1.55	9.11±0.53
Group F	8.99±1.50	8.75±1.06	8.75±1.33

Table 6

Effects of camote and sampa-sampalukan on absolute lymphocyte count (10^3/μL).

Treatment groups	Day 0	Day 14	Day 28
Group A	0.65±0.19	0.56±0.14	0.58±0.10
Group B	0.51±0.07	0.53±0.09	0.56±0.10
Group C	0.38±0.17	0.34±0.02	0.51±0.14
Group D	0.49±0.28	0.56±0.25	0.52±0.11
Group E	0.43±0.11	0.46±0.07	0.47±0.16
Group F	0.32±0.10	0.39±0.04	0.43±0.05

Table 7

Effects of camote and sampa-sampalukan on absolute monocyte count (10^3/μL).

Treatment groups	Day 0	Day 14	Day 28
Group A	0.78±0.23	0.69±0.19	0.75±0.80
Group B	0.76±0.20	0.74±0.19	0.78±0.20
Group C	0.87±0.48	0.95±0.64	1.06±0.43
Group D	0.85±0.49	0.84±0.24	1.07±0.34
Group E	0.79±0.19	0.81±0.16	0.89±0.17
Group F	0.86±0.30	0.78±0.20	0.81±0.14

3.5. Platelet count

Table 8 shows that there were no significant differences between platelet count of the administration of treatment groups and control groups.

Table 8

Effects of camote and sampa-sampalukan on platelet count (10^3/μL).

Treatment groups	Day 0	Day 14	Day 28
Group A	201.75±33.78	217.75±45.22	214.00±40.35
Group B	213.00±35.44	216.75±40.95	218.00±46.08
Group C	204.50±36.10	239.50±36.16	268.75±41.09
Group D	189.75±41.96	268.25±59.43	304.75±58.71
Group E	192.50±38.65	214.50±31.84	235.50±25.37
Group F	214.00±57.65	254.50±50.55	284.00±50.25

4. Discussion

This study showed that there was an increasing trend for the hematocrit, RBC and hemoglobin count in healthy mice after treatment with both camote and sampa-sampalukan. These animals remained healthy throughout the duration of the experiment and no adverse effects especially death of animals were seen during the duration of the treatment.

An increase in RBC count can be attributed to the direct stimulation on hemopoietic tissues such as the liver and bone marrow of both camote and sampa-sampalukan[26]. Camote has been identified to be rich in folic acid, which is needed for the growth and development of RBC[27]. Folic acid, which is evident in the liver, participates in erythropoiesis and plays an important role in DNA synthesis and cellular division[28]. An increase in the RBC count of sampa-sampalukan is similar with the study of Islam *et al.,* where the hemoglobin, RBC and WBC counts were restored to normal after being given to mice with Ehrlich ascites carcinoma[29].

Since hematocrit is a measure of the volume of RBC over the total blood volume. Significant difference in hematocrit in camote is expected since there is a significant difference in the RBC count[30]. This is also similar with a study on the effect of sweet potato extract on hematocrit levels in rabbit[26].

Camote could cause a significant increase in the hemoglobin concentration in mice because it is rich in iron, an important component in the subunits of haemoglobin and vitamin B6, which is needed to produce the heme of the hemoglobin protein because it contains pyridoxine that provides material for the formation of hemoglobin[27].

Though camote and sampa-sampalukan have been found to be abundant in vitamin C, it is still unable to promote an increase in WBC. β-carotene convertes to vitamin A and directly aids the production of WBC[31]. Vitamin C or ascorbic acid contributes to the WBC count by increasing leukocyte enzymes which are needed for the production of competent leukocytes. Insignificant changes in the leukocyte counts may be attributed to the different compounds which act as anti-mutagenic and anti-cancer[1]. In the study of Asare *et al.*, there was a significant reducing activity of *P. niruri*, which specifically acts to suppress bone marrow production[32].

There has been no significant differences among groups with regards to the platelet count. This is opposite to the study on rabbits in which sweet potato extracts were able to increase WBC and platelet count[26].

The findings of this study strongly suggest that the bioactive compounds in both camote tops and sampa-sampalukan leaves can enhance erythropoiesis in animals. The main difference may be attributed to the different concentrations of the main compounds which were needed for erythropoiesis (vitamin B6, B12, C and copper) and hemoglobin production (iron).

This study demonstrates that there is a significant difference on the hematocrit, RBC and hemoglobin levels in mice. There have been no significant effects demonstrated on the WBC count, absolute granulocyte, lymphocyte and monocyte counts, and platelet counts. It can be concluded that the effect of *I. batatas* and *P. niruri* on the different parameters are due to folic acid and iron which is an integral component in the production of RBC and hemoglobin.

Conflict of interest statement

We declare that we have no conflict of interest.

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