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HEMATOLOGICAL EFFECTS OF *SARABAT* (*Diplazium sp.*) AND *BARANGBANG* (*Stenochlaena palustris*) EDIBLE FIDDLEHEAD FERNS FROM NORTHERN LUZON PHILIPPINES ON ICR MICE (*Mus musculus*)

Pablo Afidchao[1*], Michael B. Ples [2] and Esperanza Maribel Agoo[3]
[1,2,3] *De La Salle University Manila*
**Corresponding Author: pablo_afidchao@dlsu.edu.ph*

Abstract: The reported immunesupression and hemolytic effect of some edible ferns provided us basis to evaluate effects of local edible ferns namely *Sarabat (Diplazium polypodiodes, Diplazium sp.*) and *barangbang* (*Stenochlaena palustris*) fiddleheads on blood cells of ICR mice. Thirty-two (32) twelve-week old mice were divided into eight treatments with four per treatment. Mice were fed daily for four weeks with regular food, water plus extracts in low dose (40mg/kg) and high dose (80mg/kg) of *sarabat1, sarabat2*, and *barangbang*. Negative control was given food and water only while positive control received food, water plus vitamins with iron. Blood was extracted by tail tipping on Day 0, 14, 28 and complete blood count (CBC) with platelets was analyzed. Hematocrit and red blood cells (RBC) was noted to be high in the positive control but not significantly different with fern extract treatment groups with a p value of 0.119 and 0.208 respectively. Effects on white blood cells showed the negative control and low dose fern extract with mean values of 11.75 and 13.07 respectively were slightly higher compared to other treatments but not significantly different with p value of 0.185. Effects on neutrophil and absolute lymphocyte counts follow the same trend with p value of 0.249 and 0.119 respectively. Platelet seem elevated in low dose s*arabat* 1 and 2 as well as low dose red fern but not significantly different with 0.370 p value. Comparison of pre- and post-test treatments with extracts of *Sarabat* 1, 2 and red fern on RBC at two dosages showed that **a**mong the parameters tested only WBC showed highly significant variation at post treatment with p value of 0.002. *Sarabat.* and *barangbang* fiddleheads have no hematologic issues in maintaining blood reference ranges as manifested by no statistical significant differences with the control treatment and slight increase final body weight.

Key Words: fiddlehead ferns; hematologic; differential count; complete blood count; edible ferns

1. INTRODUCTION

Medicinal ferns are not common in comparison to angiosperms in terms of diverse uses in traditional medicine (Corrêa *et al.*, 2015) yet, different fern species throughout the world are presently utilized to treat various ailments, mostly in developing countries, where herbal products still occupy a significant place in primary healthcare for cultural and economic reasons (Ho *et al.*, 2011)

Sarabat is the fiddlehead shoot and edible stalk of 2 vegetable fern species occasionally sold in the market places of Nueva Vizcaya and adjoining provinces. One of the species is larger, succulent and dark green with brownish hairy covering with a white line running with stalk to fiddlehead, The other lacks the white line and slender. Unlike its common □pako□ fern genus (*Diplazium esculentum*) which is fairly widely distributed and seen in marketplaces, these big fiddlehead ferns are not readily available in most market places probably due to its limited distribution.

De Long *et al.* (2011) in their study of fiddlehead ferns in Canada showed that the edible ostrich fern (*Matteuccia struthiopteris)* fiddlehead

tissue had an unusual fatty acid composition including γ-linolenic, dihomo-γ-linolenic, arachidonic and eicosapentanoeic acids. It contains antioxidant compounds such as ascorbic acid, a- and γ-tocopherol and a- and ß-carotene. Other edible vegetable ferns in southeast Asia as listed by de Winter and Amoroso (2003) include the green fern *Diplazium esculentum* (Retz), red fern *Stenochlaena palustris , Achrosticum aureum* L.*, Angiopteris evecta* (G. Forst), *Blechnun orientale* L., *Cyathea contaminans* (Wall.ex.Hook) *Nephprolepis hirsutula* (G. Forst), *Pleocnemia irregularis* (C. Presl), *Pteris ensiformis*. Of these *Diplazium esculentum* was found to be the most palatable. *Stenochlaena palustris* (Chai *et al*., 2015a) and *Diplazium esculentum* were demonstrated to have alpha glucosidase inhibition hence have antidiabetic activities (Chai *et al*., 2015b) while Tongco *et al.* (2014) studied the nutritional and phytochemical constituents of this common pako (*Diplazium esculentum*) fern vegetable. Many ferns studied specially, Dryopteridaceae, Osmundaceae, Woodsiaceae exhibit powerful antioxidant activities with some demonstrating acetylcholinesterase activity and antidiabetic activity (Cao *et al*., 2015; Chai *et al*., 2012; Hort, 2008)]. And some crude extracts obtained from ferns showed powerful antioxidant and free radical scavenging activities more than vitamin C (Shin and Lee, 2012; Chen *et al*., 2007; Ding *et al*., 2008; Hort *et al*., 2008; Lee *et al*., 2011), and certainly beneficial for many diverse chronic medical conditions. Also notable are some research studies pointing to carcinogenic activities and toxicities related to some fern species (Wilson et al., 2008; Bringuier *et al*., 1995) and *Diplazium esculentum* or □pako□ as immunosuppressant and hemolytic (Roy et al., 2013).

It is against these background that further studies need to be undertaken to illuminate the benefits and safety or otherwise of the edible vegetable ferns especially the *Pako* family. Apart from the fact that there are very limited animal studies on medicinal uses of ferns in the country. The folkloric claims in regard to the fiddlehead fern or □Sarabat□ as indicated for body weakness and cure for ulcers, diabetes need further investigation. Meanwhile the reported presence of beneficial and toxic phytochemical constituents in edible ferns prompted us to evaluate it physiologic and hematologic effects. In particular, the reported positive nutritional effects as well as reported hemolytic and inflammatory effects of the Diplazium genera of ferns warrant further study.

Objectives of the Study

The study evaluated the hematologic effects of □*Sarabat*□ fern shoots and *Barangbang* or red fern as a functional food with possible nutritive and pharmacologic effects in blood health maintenance.

Specifically,

1. The hematologic effects of the 3 species of edible fiddlehead ferns in Nueva Vizcaya labeled as Sarabat 1 and Sarabat 2 and Barangbang or red fern was evaluated;
2. Minimum dosage of the Sarabat noted to maintain normal blood reference range in albino mice was noted
3. Pre- and post-treatment body weight in mice as a determinant of mice health status was compared.

2. METHODOLOGY

Procurement of Plant samples

□Sarabat□ or Fiddlehead Fern Shoots and edible stalks of two species was purchased in Bayombong and Solano public markets in Nueva Vizcaya which were obtained from Quezon, Kasibu or Ambaguio municipalities in Nueva Vizcaya. Only fresh stalks with unfurled fiddleheads shoots was used in the study.

Test animals and set-up

The experimental set-up followed the protocol as adopted by Montejo et.al. in Hematological Effects of *Ipomea batatas* (camote) and *Phyllantus niruri* from Philippines in ICR Mice (*Mus musculus*) with slight modifications (Montejo *et al.,* 2015). A total of 32 12-week old (approximately 25-35g) ICR mice (*Mus musculus*) of either sex was obtained from the Phil Institute of Traditional and Alternative Health Care (PITAHC), a research institute under the Department of Health. These were kept in separate, standard-sized cages in the animal house of the same institution. All cages were sanitized and bedded with autoclaved paddy husk. Proper handling and maintenance of the mice was observed and the experiment was approved by the Institutional Animal Care and Use Committee of the same institution.

Preparation of plant treatments

Three kinds fiddlehead fern shoots were dried and grinded. These were subjected to water extraction using standard procedures. The crude extracts were then brought for lyophilization at the Department of Chemistry, DLSU. The lyophilized extracts were stored in clean and air- tight containers.

The diet and treatment given to each group are as follows: Treatment A-negative control (mice are fed with pellets only); Treatment B- low dose Sarabat 1 (mice are fed with pellets plus 40mg Sarabat 1); Treatment C-high dose Sarabat 1 (mice are fed with pellets plus 80mg Sarabat 1); Treatment D- low dose Sarabat 2 (mice are fed with pellets plus 40mg Sarabat 2); Treatment E- high dose Sarabat 2 (mice are fed with pellets plus 80mg Sarabat 2); and Treatment F-positive control (mice were fed with pellets and vitamins with iron supplement); Treatment G-low dose Red fern (mice are fed with pellets plus 40mg Red fern) and; Treatment H- high dose Red fern (mice are fed with pellets plus 80mg Red fern).

All animals were weighed prior to administration of treatment extracts and fed mice feeds. Administration of the extracts was done by oral gavage individually to ensure the correct dose per mice. The procedure lasted for 4 weeks with blood collected at Day 0, Day 14 and Day 28 of treatments. The animals were again weighed on Day 28 at the completion of the study to determine weight gain or loss as a determinant of the mice health status.

Blood analysis

Blood was collected by tail tipping at Day 0, Day 14 and Day 28 from 8:00 am to 9:00 am to prevent variations for analysis and placed in ethylene diamine tetraacetic acid violet microtubes and immediately brought and analyzed at the Regional Central Laboratory Integrated Laboratory Division of the Department of Agriculture in Tuguegarao City using Auto-Hematology Analyzer Model KT 6180 s2015.

Statistical analysis

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

3. RESULTS AND DISCUSSION

Effect of Fiddlehead Ferns on percentage hematocrit or pack cell volume (%PCV)

The effect of the different plant treatments at varying dosages is reflected in Table 1. Treatment G (red fern) and Treatment F or positive control manifested the highest mean percentage values for hematocrit. However, this is not statistically different from other treatments used in the study as shown in Table 1 with p-value of 0.119.

Table1. Mean values of PCV count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

Treatment	mean sd
Treatment A (negative control)	59.82 ± 4.94
Treatment B (SAR1 LD)	47.80 ± 8.49
Treatment C (SAR1 HD)	60.10 ± 1.04
Treatment D (SAR2 LD)	47.90 ±18.00
Treatment E (SAR2 HD)	55.20 ± 0.00
Treatment F (positive control)	66.13 ± 4.60
Treatment G (Red Fern LD)	64.40 ± 3.82
Treatment H (Red Fern HD)	43.30 ± 13.55

Effects on RBC count

The effect of the different experimental treatments on RBC count at high and low doses is shown in Table 3 and Figure 2. Among the various fern treatments, Treatments A, B, C and F (positive control) recorded higher mean RBC counts over other treatments yet this is not statistically different with the various treatments with a p value of 0.208.

Table2. Mean values of RBC count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

Treatment	mean Sd
Treatment A (negative control)	59.80 ± 4.62
Treatment B (SAR1 LD)	59.05 ± 3.18
Treatment C (SAR1 HD)	57.00 ± 3.34
Treatment D (SAR2 LD)	54.47 ± 1.89
Treatment E (SAR2 HD)	49.90 ± 0.00
Treatment F (positive control)	58.73 ± 3.36
Treatment G (Red Fern LD)	55.60 ± 2.12
Treatment H (Red Fern HD)	53.67 ± 4.61

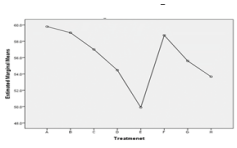

Fig. 2. Mean values of RBC counts

Effects on WBC cell count

The effects of the various experimental treatments on White Blood Cell Count (WBC) is shown in Table 3 and Figure 3. Decreased mean WBC can be seen with Treatments C and E both high dose Sarabat and slight elevations are noted with Treatments A, B and H. Such elevations or decrease may point to the inflammatory induction potential of the fern extracts or extraneous variables like stress in handling as well as health status of mice

specimens. Although not statistically different between and among each other at p-value of 0.185.

Table3. Mean values of WBC count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

Treatment	mean Sd
Treatment A (negative control)	11.75 ± 4.00
Treatment B (SAR1 LD)	11.10 ± 1.98
Treatment C (SAR1 HD)	7.97 ± 0.50
Treatment D (SAR2 LD)	9.13 ± 4.65
Treatment E (SAR2 HD)	7.70 ± 0.00
Treatment F (+ Control)	8.43 ± 0.49
Treatment G (Red Fern LD)	6.60 ± 0.99
Treatment H (Red Fern HD)	13.07 ± 0.93

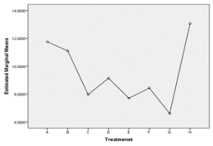

Fig. 3. Mean values of WBC counts

Effects on Neutrophil count

The neutrophil count in the various treatments are not statistically significantly different from each other as shown in Fig.4(left). Although these mean values fall within reference range, both positive and negative controls as well as Treatment G recorded slightly lower mean counts.

Effects on Absolute lymphocyte count

Below Figure4 (right) shows the mean absolute lymphocyte counts of the various treatments. The positive and negative control as well as Treatment G (Red fern) showed a slightly higher lymphocytic count over others and Treatments H, B and D slightly lower yet all are not significantly different.

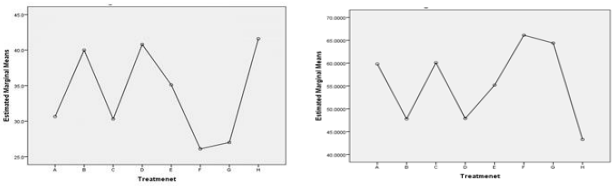

Fig.4. Mean values of neutrophil (left) and absolute lymphocyte (right) count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

Effects on Absolute monocyte count

Effects on Absolute monocyte count are noted in Figure5 (left). Results of mean counts shows that the mean values are not significantly different and except for Treatment H which fall within the normal reference parameters of a differential for monocytes.

Effects on Platelet count

Figure 5 (right) shows the mean values of platelet counts for the various treatments. The table shows that Treatments G, B and D registered the slightly high mean platelet counts over the reference. The mean values for platelet count tend to congregate within the upper limits of the platelet reference range which can be a physiologic reaction to dehydration or the trauma of blood extraction.

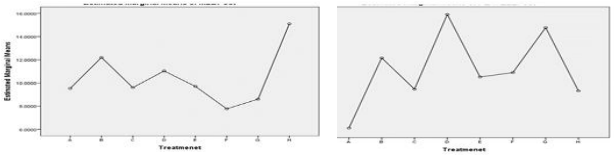

Fig.7. Mean values of absolute monocyte (right) and platelet (left) count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

Body weight

The mice weight was recorded at the start of experiment and served as basis for comparison of recorded body weight at 28-day post-treatment (Table4). Result of pre- and post-treatments body weight revealed statistical differences at 0.001 level of significance (Table4). Univariate analyses on pre and post-treatments indicate an increase in mean body weights with considerable differences in increase of weights among treatment means noted at 28-day post-treatment (Figure 8). Further analysis employing Bonferroni test show significant differences in body weights esp. Group 7 (low dose Red fern) having a statistically high mean body weight (40.52g) as compared to other treatments.

Table4. Comparison of mean body weight of mice at pre- and post-treatments.

Comparison of Body weight before and after treatments		Mean	Std. Deviation	t	df	Sig. (2-tailed)
Pair 1	WT 1 - WT2	-7.0800000	4.9091126	-7.899	29	.000

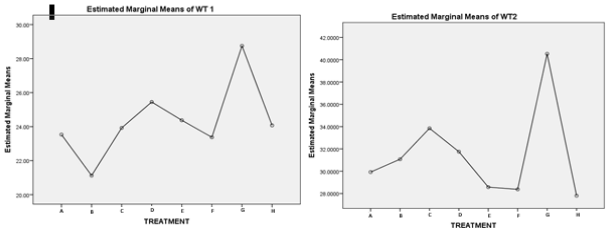

Fig.8. Mean nody weight of mice at pre-treatment (left) and at 28-day post-treatment (right).

Discussion

Blood bathes all the other cells of the body carrying nutrients, oxygen and waste products and is exposed to almost all metabolic processes of these cells, often reflecting any alteration from normal function. Blood is essential in water and electrolyte

balance, temperature control and the functioning of the immune system which is the defense mechanism of the body (Voigt, 2000)

The present study has shown the nutritional effects of 3 edible species of mountain fern fiddlehead aqueous extracts with the general observation that consumption of fern extracts by ICR mice generally maintained the blood cell counts within normal reference parameters and seem not to affect weight gain of mice specimen at the conclusion of the study. Anemia was not induced and the extracts have been generally well tolerated throughout the study. This seem to contrasts with the study of Roy *et al* (2013) which demonstrated the hemolytic effects of *Diplazium esculentum* aqueous extract. Dose dependent hemolysis can however be a high possibility due to hemolytic phytochemical constituents present. Also, maintenance of RBC within normal reference range parameters points to the nutritive hematopoietic enhancing properties of the extracts. This may be due mainly to the presence of vitamins and minerals such as iron, phosphorous and potassium as well as a variety of antioxidant phytochemical constituents such as polyphenols, flavonoids, hydroxycinnamic acid and anthocyanins as demonstrated by Chai *et al* (2012) specifically for *S. palustris* which have powerful antioxidant properties and has also been shown in Pako (*D. esculentum*) studies by Tongco *et al.* (2014).

Hematocrit or packed cell volume provides the fastest, most accurate estimation of the oxygen carrying capacity of the blood and yields important information especially presence of anemia. The purpose of measuring it is to determine the percentage of erythrocytes circulating in the peripheral blood at the time of collection (Voigt, 2000). In the present study, although there is no statistical significant differences between and among the treatments. It is notable that the positive control (Treatment F) due to direct introduction of iron and vitamin supplementation showed a slightly greater yield for hematocrit as expected although insignificant with the rest of the treatments.

Leukocyte count or WBC is shown in Table 5 and Figure 3 of which the Red fern or Treatment H seem to exhibit an elevation followed by Treatment A and Treatment B, negative control and low dose Sarabat 1, respectively. While the elevation falls within the normal reference parameters in general, some components of the differential count indicate leukocytosis. It is important to point out that not all changes in the leukogram result from pathologic or disease processes. Some increase in cell types and total numbers are due to physiologic leukocytosis as occurs in conditions such as, pain, apprehension, digestion, estrus and pregnancy and stress which can be both physiologic and pathologic (Voigt 2000) It could however be indicative of an inflammatory process or the extract itself can induce inflammation as shown by Roy *et al.* (2013) in their study of *D. esculentum.*

Components of the differential counts such as neutrophils, lymphocytes, monocytes show no significant statistical difference between and among the treatments and the values obtained for each treatment also fall within the reference ranges. The antioxidant properties of many fern species have been thoroughly documented by many studies and the antioxidant and reactive oxygen species scavenging activity of almost all of these studied ferns (Chai *et al.,* 2015a; De Long *et al.,* 2011; Lee *et al.,* 2011) point to their potential in overall health maintenance and in the case of red blood cells, maintenance and protection of the integrity of red cell membranes against ROS released as a by-product of metabolism. The rapid breakdown or hemolysis of red cell is prevented and their physiologic recycling is thus assured. The presence of such antioxidant and nutritive components in Sarabat is also very likely and need further elucidation. Notable also is that in the differential count no neutrophilic or lymphocytic predominance was recorded indicating that neither acute or chronic pathophysiologic processes is going on the mice specimens in the span of a 28-day feeding period.

In terms of dosing, no significant differences have been noted on the two dose preparations relative to the various treatments and their effects on the blood count parameters suggesting a need for adjustment in dosing requirements based on toxicity studies.

4. CONCLUSIONS

In general, no hematologic issues of the fern extracts on the blood count parameters were noted but rather maintained normal healthy reference values as reflected in the serial complete blood count in this study. While insignificant fluctuations of some blood parameters have been noted. These changes may be attributable to physiologic adjustments in the internal metabolic and physiologic processes in mice such as adjustments to stress and dehydration, pain

and stress during tail tipping. The observed elevation in platelet counts seem a physiologic response to the trauma of tail tipping where clotting pathways are activated including platelet aggregation , Overall, the study demonstrated the nutritive hematopoietic potential of the test fern extracts which correlate with their reported rich antioxidant property as well as presence of essential elements like iron and B vitamins as shown by the increase hematocrit and RBC values over time and this likewise did not negatively affect weight gain of the mice specimens.

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