INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 2, p. 230-239, 2022

RESEARCH PAPER OPEN ACCESS

Blood Differential Count effects of *Diplazium polypodioides* Blume, *Diplazium maximum (D.Don)* C. Chris. and *Stenochlaena palustris* edible fiddlehead ferns from Northern Luzon Philippines on ICR mice (*Mus musculus)*

Pablo M. Afidchao Jr.[1*], Michael B. Ples[2], Esperanza Agoo[2]

[1]*College of Medicine, Cagayan State University, Carig, Tuguegarao City, Cagayan, Philippines*

[2]*Department of Biology, College of Science, De La Salle University Manila, Philippines*

Key words: Fiddleheads, Differential count, Edible ferns, Complete blood count (CBC).

http://dx.doi.org/10.12692/ijb/21.2.230-239 Article published on August 20, 2022

Abstract

Diplazium polypodiodes, Diplazium maximum locally called sarabat 1 and 2 respectively and *Stenochlaena palustris* or red-fern fiddlehead extracts were evaluated on blood cells of ICR mice. Thirty-two (32) twelve-week-old mice were divided into eight treatments with four per treatment along with positive and negative controls. 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 red-fern. 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 elevated in the positive control but not significantly different with fern extracts with a *p*-value of 0.119 and 0.208, respectively. White blood cells indicated the negative control and low dose fern extract with mean values of 11.75 and 13.07, respectively, were slightly higher but not significantly different with a *p*-value of 0.185. Effects on neutrophil and absolute lymphocyte counts follow the same trend with a *p*-value of 0.249 and 0.119, respectively. Platelet seems elevated in low dose s*arabat* 1 and 2 as well as low dose red fern but not significant with a 0.370 *p*-value. Comparison of pre and post-test treatments with extracts of *Sarabat* 1, 2 and red fern on blood counts at two dosages showed that **a**mong the parameters tested, only WBC showed a highly significant (0.002) variation at post-treatment which seemed to indicate WBC stimulation which alerts us to caution, while the rest of the differentials are within safe reference parameter ranges.

* **Corresponding Author:** Pablo M. Afidchao Jr.✉ docafidchao@gmail.com

Introduction

Medicinal ferns are not common in comparison to angiosperms in terms of diverse uses in traditional medicine (Corrêa *et al.*, 2015). 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 marketplaces of Nueva Vizcaya and adjoining provinces. Sarabat 2 fiddleheads are larger, succulent and dark green, Sarabat 1 is smaller dark green covered in whitish scales and tiny thorns. 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 marketplaces probably due to their limited distribution and seasonal occurrence.

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 and 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 especially Dryopteridaceae, Osmundaceae, and Woodsiaceae, exhibit powerful antioxidant activities with some demonstrating acetyl cholinesterase activity and antidiabetic activity (Hort *et al*, 2008, Chai *et al.*, 2012, Cao *et al.*, 2015). And some crude extracts obtained from ferns showed powerful antioxidant and free radical scavenging activities more than vitamin C (Chen *et al.*, 2007; Ding *et al.*, 2008; Hort *et al.*, 2008; Lee *et al.*, 2011,Shin and Lee, 2012;), 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.*, 2013,2015).

It is against these backgrounds that further studies need to be undertaken to illuminate the benefits and safety and toxicity of the edible vegetable ferns, especially the *Pako* family. Apart from the fact that there are very limited animal studies on the medicinal uses of ferns in the country. These ferns can be consumed regularly as indigenous vegetables depending on their abundance or seasonal occurrence, while the safety and toxicity of these fiddlehead fern vegetables have not been thoroughly evaluated. Meanwhile, the reported presence of beneficial and toxic phytochemical constituents in edible ferns prompted us to evaluate its physiologic and hematologic effects. In particular, the reported hemolytic and immunosuppressant effects of the edible Diplazium genera of ferns warrant further study .This study aims to establish the hematological profile on regular consumption of these edible ferns on ICR mice models due to its regular consumption as vegetables and identify potential ill effects on blood cells for public health precautions.

Material and methods

Procurement of plant samples

"Sarabat" or Fiddlehead Fern Shoots and edible stalks of two kinds along with red fern were 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 fiddlehead shoots were used in the study. Sample plant specimens were collected and brought to DLSU Biology Department and with the help of Forester Rey Callado of the National Museum, identified and authenticated the fern vegetables. Voucher specimens for Sarabat 1 and 2 were subsequently deposited in the DLSU Herbarium under Voucher specimen no. 5602 and 5603.

Test animals and set-up

The experimental set-up followed the protocol as adopted by Montejo *et al.* (2015) in the Hematological Effects of *Ipomea batatas* (camote) and *Phyllantus niruri* from the Philippines in ICR Mice (*Mus musculus*) with slight modifications. A total of 32 12-week-old (approximately 25-35g) ICR mice (*Mus musculus*) of either sex were 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 were observed and the experiment was approved by the Institutional Animal Care and Use Committee of the same institution.

Preparation of plant treatments

Three kinds of fiddlehead fern shoots were dried and ground. These were subjected to water extraction using standard procedures. The crude extracts were then brought for lyophilisation 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 mouse. 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's 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 were 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 $P<0.05$.

Results

Body weight

The mice's weight was recorded at the start of the experiment and served as the basis for comparison of recorded body weight at 28-day post-treatment (Table 4). The result of pre- and post-treatment body weight revealed statistical differences at a 0.001 level of significance (Table 4). 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 (Fig. 1 and 2). Further analysis employing Bonferroni test shows significant differences in body weights esp. Group 7 (low dose Red fern) has a statistically high mean body weight (40.52g) as compared to other treatments.

Table 1. 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 (SAR 1 LD)	47.80 ± 8.49
Treatment C (SAR 1 HD)	60.10 ± 1.04
Treatment D (SAR 2 LD)	47.90 ± 18.00
Treatment E (SAR2HD)	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

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 a *p*-value of 0.119.

Effects on RBC count

The effect of the different experimental treatments on RBC count at high and low doses is shown in Tables 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.

Table 2. 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 (SAR 1 LD)	59.05 ± 3.18
Treatment C (SAR 1 HD)	57.00 ± 3.34
Treatment D (SAR 2 LD)	54.47 ± 1.89
Treatment E (SAR2HD)	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

Effects on WBC cell count

The effects of the various experimental treatments on White Blood Cell Count (WBC) are shown in Table 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 decreases may point to the inflammatory induction potential,immunostimulant or immunosuppressive effects of the fern extracts or extraneous variables like stress in handling as well as the health status of mice specimens. On the one hand, the HD *sarabat* indicating a drop in WBC may lead to immunosuppression consistent with the findings of Roy *et al.* (2013), although not statistically different between and among each other at *p*-value of 0.185.

Table 3. Mean values of WBC count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead fern 2 and red fern.

Treatment	Mean ± sd
Treatment A (negative control)	11.75 ± 4.00
Treatment B (SAR 1 LD)	11.10 ± 1.98
Treatment C (SAR 1 HD)	7.97 ± 0.50
Treatment D (SAR 2 LD)	9.13 ± 4.65
Treatment E (SAR2HD)	7.70 ± 0.00
Treatment F (positive control)	8.63 ± 0.49
Treatment G (Red Fern LD)	6.60 ± 0.99
Treatment H (Red Fern HD)	13.07 ± 0.93

Effects on Neutrophil count

The neutrophil counts in the various treatments are not statistically significantly different from each other, as shown in Fig.3 (top). Although these mean values fall within the reference range, both positive and negative controls, as well as Treatment G, recorded slightly lower mean counts.

Effects on absolute lymphocyte count

Fig. 4 (bottom) 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.

Table 4. Comparison of mean body weight of mice at pre- and post-treatments.

Comparison of Body before and after trearments		Mean ± sd	t	df	Sig. (2-tailed)
Pair 1	$WT_1 - WT_2$	-7.08 ± 4.91	-7.899	29	0.000

Effects on absolute monocyte count

Effects on Absolute monocyte count are noted in Fig 5 (top). Results of mean counts show that the mean values are not significantly different and except for Treatment H which falls within the normal reference parameters of a differential for monocytes.

Effects on platelet count

Fig. 6 (bottom) shows the mean values of platelet counts for the various treatments. The table shows that Treatments G, B and D registered 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.

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 with the exception of WBC and even resulted in 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 study, however, is consistent with the study of Roy *et al.* (2013) which demonstrated the immunostimulant and pro-inflammatory effects of *Diplazium esculentum*

aqueous extract as shown by fluctuations in WBC differential counts. Also, dose-dependent hemolysis can, however, be a high possibility due to hemolytic phytochemical constituents present particularly saponins.

At low consumption levels however, 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).

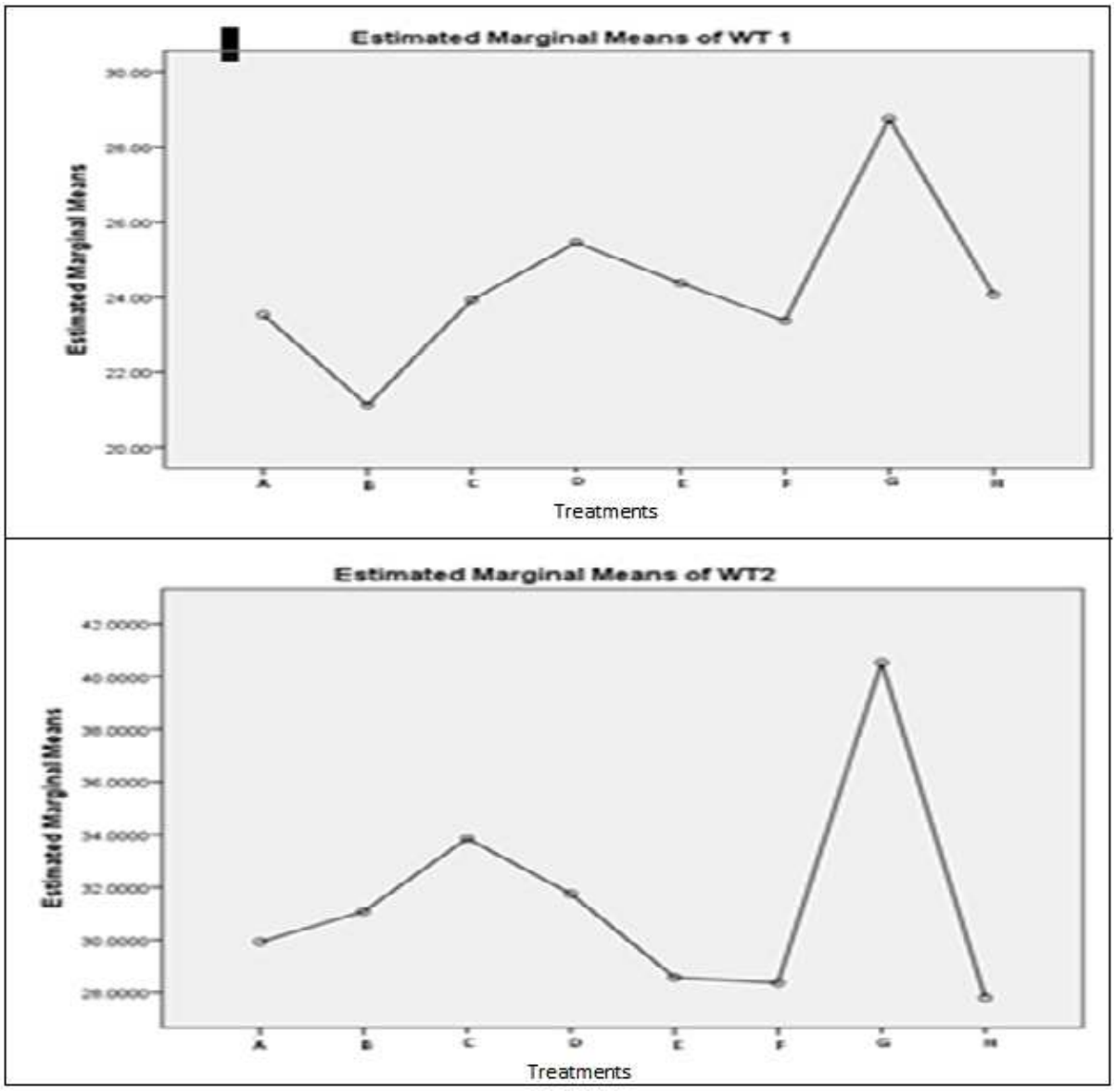

Fig. 1 and 2. Mean body weight of mice at pre-treatment (top) and at 28-day post-treatment (bottom).

Hematocrit or packed cell volume provides the fastest, most accurate estimation of the oxygen-carrying capacity of the blood and yields important information, especially the 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 are no statistically significant differences between and among the treatments. It is notable that the positive

control (Treatment F) due to the direct introduction of iron and vitamin supplementation showed a slightly greater yield for hematocrit as expected, although not very significant with the rest of the treatments. Leukocyte count or WBC is shown in Table 3 of which the Red fern or Treatment H seems 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 increases 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 or the initial stage of immunosuppression, as shown by Roy *et al.* (2013, 2015) in their study of *D. esculentum*.

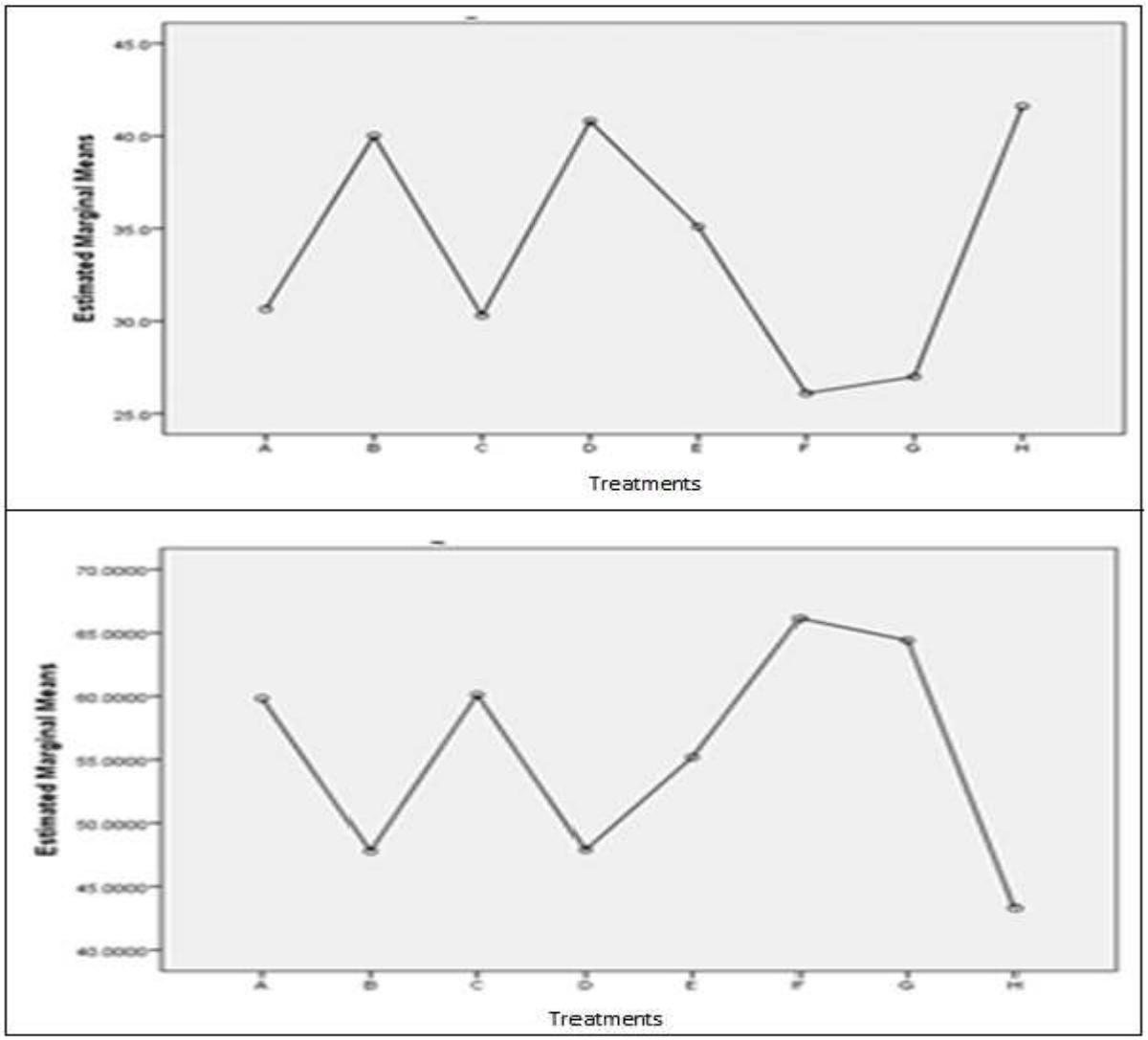

Fig. 3 and 4. Mean values of neutrophil (top) and absolute lymphocyte (bottom) count from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

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. 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 cells was not noted which is expected in these saponin-containing fern vegetables and their physiologic recycling seems normal with the normal weight gains noted. The presence of such antioxidant and nutritive components in both Diplazium species (Sarabat) is also very likely and needs further elucidation. Notable also is that in the differential count, no neutrophilic or lymphocytic predominance was recorded, indicating that neither acute nor chronic pathophysiologic processes are going on in the mice specimens in the span of a 28-day feeding period.

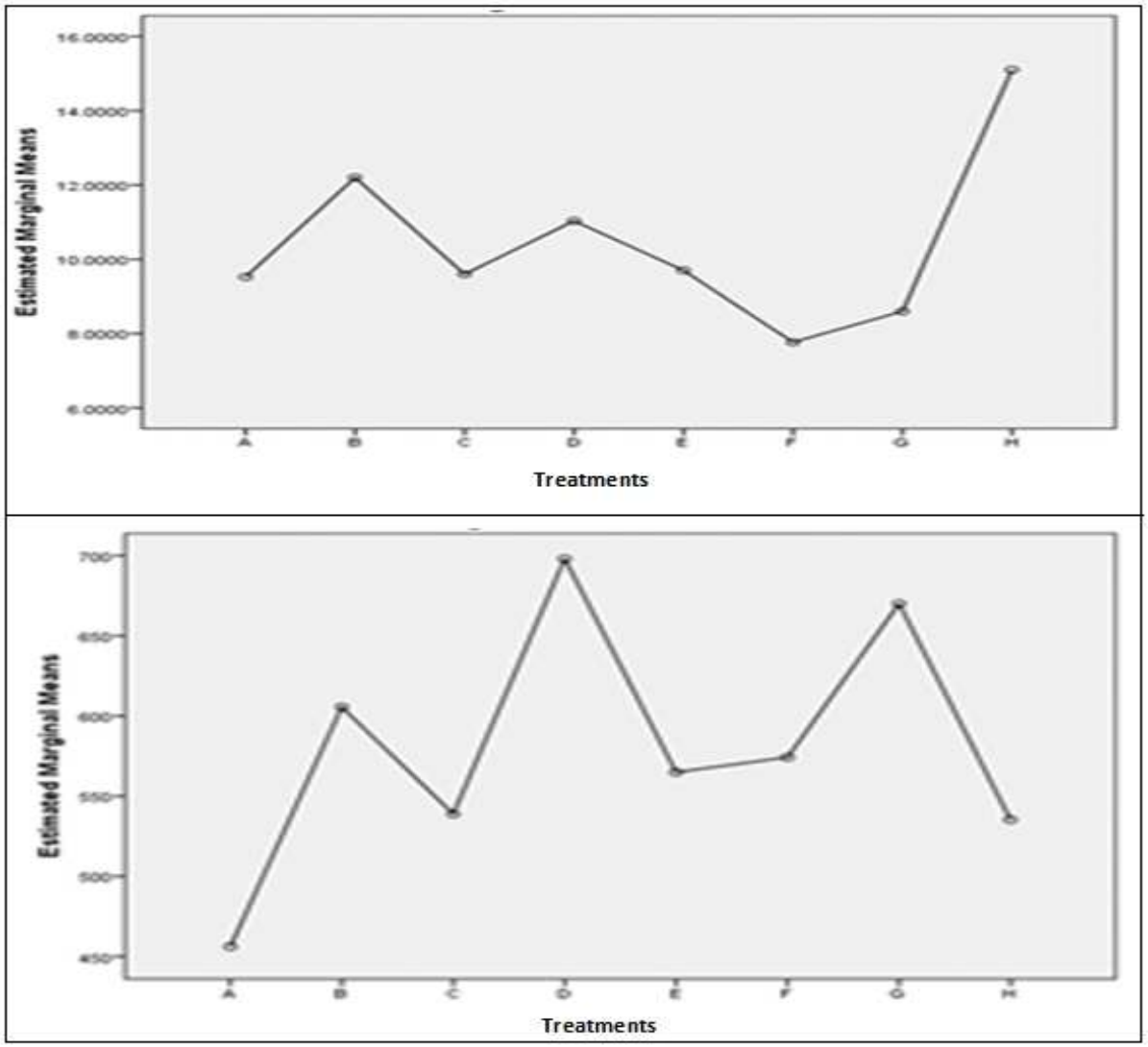

Fig. 5 and 6. Mean values of absolute monocyte count Fig.5 (top) and Fig.6 platelet count (bottom) from mice blood treated with different dosages of fiddlehead fern1, fiddlehead 2 and red fern.

In terms of dosing, no significant differences have been noted in 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.

Conclusion

In general, no significant hematologic issues of the fern extracts on the blood count parameters were noted as they fall within normal reference values as reflected in the serial complete blood count in this

study. In contrast, some fluctuations of some blood parameters, particularly WBC, have been noted which may indicate that the fern extracts may elicit WBC activity which may impact on immune response. This points to caution with overconsumption. Changes may also 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 seems to be a physiologic response to the trauma of tail tipping where clotting pathways are activated including platelet aggregation, Elevation of WBC may point to immune stimulation and likewise, the decrease in WBC at a high dose may be indicative of toxicity and may warrant longer serial observations. Overall, the study demonstrated the nutritive hematopoietic potential of the test fern extracts which correlate with their nutritive constituents as well as the presence of essential elements like iron and B vitamins, as shown by the increased hematocrit and RBC values over time and this is likewise associated with the increase in body weight of the mice specimens. Precaution on longterm overconsumption is however advised pending further toxicological assays.

Acknowledgments

The authors acknowledge with thanks the advice of Dr. Ron Vitor of the DLSU Biology Department, as well as Dr. Abe Bas-ong of PITAHC and the laboratory staff of DA-RO2, Prof. Miladis Afidchao of ISU and Forester Rey Callado of the National Museum.

References

Bringuier PP, Piaton E, Berger N. 1995. Bracken fern-induced bladder tumours in guinea pigs -a model for human neoplasia. American Journal of Pathology; **147(3),** 858–68.PMID 7545876 PMC PubMed Central/ncbi.nlm.nih.gov

Cao J, Zheng Y, Xia X, Wang Q, Xiao J. 2015. Total flavonoid contents, antioxidant potential and acetylcholinesterase inhibition activity of the extracts from 15 ferns in China. Industrial Crops and Products **75,** 135–140. https://doi.org/10.1016/j.indcrop.2014.04.005

Chen YH, Chang FR, Lin YJ, Wang L, Chen JF, Wu YC, Wu MJ. 2007. Identification of phenolic antioxidants from Sword Brake fern (*Pteris ensiformis* Burm.). Food Chemistry **105,** 48–56. https://doi.org/10.1016/j.foodchem.2007.03.055

Chai TT, Kwek MT, Ong HC, Wong FC. 2015a. Water fraction of edible medicinal fern *Stenochlaena palustris* is a potent α-glucosidase inhibitor with concurrent antioxidant activity. Food Chemistry **186,** 26-31. https://doi.org./10.1016/j.foodchem.2014.12.099

Chai TT, Yeoh LY, Ismail NM, Ong HC, Manan, FA, Wong FC. 2015. Evaluation of glucosidase inhibitory and cytotoxic potential of five selected edible and medicinal ferns. Tropical Journal of Pharmaceutical Research **14(3),** 449-454. https://doi.org./10.4314/tjpr.v14i3.13

Corrêa R, Santos P, Augusto R, Santiago CP, Medeiros PM, Albuquerque UP. 2015. Journal of Ethnopharmacology **175,** 39-47.

DeLong JM, Hodges DM, Prange RK, Forney CF, Toivenon PMA, Bishop MC, Elliot ML, Jordan MA. 2011. The unique fatty acid and antioxidant composition of ostrich fern (*Matteuccia struthiopteris*) fiddleheads. Canadian Journal of Plant Science **91,** 919-930. https://doi.org/10.4141/cjps2010-042

de Winter WP, Amoroso VB. 2013. Plant resources of Southeast Asia No. **15(2),** Cryptogams; Ferns and Fern allies, Backhuys Publishers Leiden.

Ho R, Teai T, Bianchini JP, Lafont R, Raharivelomanana P. 2011. Ferns: from traditional uses to pharmaceutical development, Chemical Identification of Active Principles. Working with Ferns Issues and Applications.Springer Science and Business Media ISBN1441971629- 2010.

Hort MA, DalBó S. 2008. Antioxidant and hepatoprotective.effects of *Cyathea.phalerata* Mart. (Cyatheaceae). Basic and Clinical Pharmacology and Toxicology **103(1),** 17-24. https://doi.org/10.1111/j.1742-7843.2008.00214.x

Lee H, Shin C, Lim S. 2011. Functional activities of ferns in human health, working with ferns issues and applications. Springer Science and Business Media ISBN1441971629- 2010.

Montejo JF, Mondonedo JAB, Lee MGA, Ples MB, Santos Vitor II RJ. 2015. Hematological effects of *Ipomea batatas* (camote) and *Phyllantus niruri* (Sampasampalukan) from Philippines in ICR Mice (*Mus musculus*). Asian Pacific Journal of Tropical Biomedicine **5(1),** 29-33. https://doi.org/10.1016/S2221-1691(15)30166-0

Roy S, Tamang S, Dey P, Chaudhuri TK. 2013. Assessment of the immunosuppressive and hemolytic activities of an edible fern, *Diplazium esculentum*, Journal Immunopharmacology and Immunotoxicology **35(3).**

Roy S, Tamang S, Dey P, Chaudhuri TK. 2015. Assessment of Th1 and Th2 cytokine modulatory activity of an edible fern,Diplazium esculentum.Food and Agriculture Immunology Taylor and Francis **26(5),** 690-702. https://doi.org./10.1080/09540105.2015.1007449

Shin SL, Lee CH. 2010. Antioxidant effects of the methanol extracts obtained from aerial part and rhizomes of ferns native to Korea. Korean J. Pant Resources **23(1),** 38–46, 1226-3591(pISSN). http://www.kjpr.kr/1988.10.00

Tongco JV, Villaber RAP, Aguda RM, Razal RA. 2014. Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* (Retz.) Sw. from Philippines. Journal of Chemical and Pharmaceutical Research. **6(8),** 238-242.

Voigt Gregg LDVM. 2000. Hematology techniques and concepts for veterinary technicians, Iowa State University Press Blackwell Publishing.

Wilson D, Donaldson LJ, Sepai O. 1998. Should we be frightened of bracken? A review of the evidence. Journal of Epidemiology and Community Health **52,** 812–17. https://doi.org/10.1136/jech.52.12.812