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**Selective immune-boosting activity of *Buchholzia coriacea* seed extract on leucopenic Wistar rats**Ilesanmi, A.O.<sup>1</sup>, Akinosun, B.<sup>2</sup>, Akinleye, W.A.<sup>1</sup>, Obisesan, A.J.<sup>1</sup>*Department of Medical Laboratory Science, Babcock University, Ilishan-Remo<sup>1</sup>, Department of Chemical Pathology, University College Hospital, Ibadan<sup>2</sup>**Author for Correspondence: ayodeleilesanmi2002@yahoo.com/+234-***Abstract**

*Buchholzia coriacea* has been acclaimed to have certain medicinal properties locally. The claims have not been backed sufficiently with empirical data. This aim of this study to assess the effects of *Buchholzia coriacea* on certain haematological parameters of leucopenic rats, in particular the cellular component of the rats' immune system. We induced leucopenia in experimental Wistar rats by injecting the animals with the drug, cyclophosphamide. The rats induced for leucopenia were divided into two groups; one group was administered the extract of *B. coriacea* while the other group received no treatment. The third group of rats serving as control was neither induced for leucopenia nor administered with seed extract. We performed total and differential white blood cell counts of the rats at basal level as well as in the post-induction and post-treatment period, in order to determine if any of the diet supplements administered has any effect on these parameters. The group of rats induced with cyclophosphamide without treatment, displayed a significantly reduced mean total white blood cell count (TWBC) ( $8.93 \pm 2.14$  cell/ $\mu$ l) than those treated ( $16.44 \pm 0.54$  cell/ $\mu$ l) ( $t=3.41$ ,  $p<0.05^*$ ). There was no statistically significant difference in the mean TWBC values of rats treated with the ethanolic seed extract ( $8.96 \pm 2.04$  cell/ $\mu$ l) and the untreated ( $8.93 \pm 2.14$  cell/ $\mu$ l) ( $t=0.009$ ,  $p>0.05$ ). Also, there was no statistically significant differences in the mean percentage neutrophil count in rats treated with ethanolic extract of *Buchholzia coriacea* ( $31.33 \pm 3.94\%$ ) as compared to the untreated rats

( $22.33 \pm 3.35\%$ ) ( $t=1.74$ ,  $p>0.05$ ). The effect of the ethanolic extract of *Buchholzia coriacea* was studied on the lymphocyte count of rats, there was reduction in the mean percentage lymphocyte count of rats treated with ethanolic extract of *Buchholzia coriacea* ( $64.00 \pm 4.75\%$ ) compared to the untreated ones ( $73.67 \pm 2.32\%$ ) and the difference however was not statistically significant ( $t=1.74$ ,  $p>0.05$ ). However, in absolute terms, the difference between the treated and the untreated in both the neutrophil and lymphocyte counts was significant ( $p<0.05$ ).

**Keywords:** Leucocytes, immunity, inflammation, herbal extract, leucopenia.

**Introduction**

White blood cells (leukocytes) are blood components that protect the body from infectious agents and combat inflammation. White blood cells play an important role in the immune system by identifying pathogens, damaged cells, cancerous cells, and foreign matter and targeting them for destruction and removal from the body. Inadequate number of white blood cells could lead to inability of the body to contain bacteria or viral infections (Ochei and Kolhatkar, 2008). Low white cell counts, known as leukopenia may be due to acute bacterial or viral infections, or may be induced by certain forms of chemotherapy, radiation therapy, presence of leucocyte antibodies, myelofibrosis and aplastic anaemia. HIV and AIDS in which the immune cells are the target of attack for the virus are also possible cause of diminished number of white cells. Other causes of low white blood cell count include bone

marrow failure, auto-immune diseases like systemic lupus erythematosus, Hodgkin's lymphoma, myelodysplastic syndromes, some types of cancer, typhoid, malaria, tuberculosis, dengue, sepsis, splenomegaly, folate and Vitamin B<sub>12</sub> deficiencies. Many other causes exist, but some of the cases seen in practice are secondary to deficiency of certain minerals like copper and zinc (Nicholson *et al.*, 1995). Some medications can also have an impact on the number and function of white blood cells. Medications which can cause leukopenia include bone marrow suppressants like chloramphenicol, lamotrigine, cyclophosphamide and others like clozapine, an antipsychotic medication with a rare adverse effect that may lead to the total absence of all granulocytes (Nicholson *et al.*, 1995).

Inflammation is a protective response of the body to infection or injury. When infection or injury affects a body tissue, an inflammatory response is triggered to eliminate the pathogens or begin the healing process. Inflammation is usually characterized by pain, elevated temperature, redness, and swelling around the lesion. The early stages of inflammation, irrespective of the causal mechanism - whether by infection or injury, involve changes of the volume (and blood flow rate as a result) and permeability of the blood vessel in the region of inflammation. Recruitment of leucocytes from the blood stream to the site of inflammation, facilitated by the changed permeability of the vessel wall, aids the destruction of the pathogens, with the leucocytes eliminating them by phagocytosis (Hwang *et al.*, 2010). In the process of fighting infections, some leucocytes are lost in battle, thus sometimes resulting in leukopenia. Leukopenia may be treated by stimulating the bone marrow, so that additional white blood cells can be produced. However, where leukopenia is caused by nutritional deficiency, multivitamin supplement which contain copper and zinc may be administered to ensure that the key nutrients required to produce healthy blood cells are made available.

In recent times, attention is being paid to the use of our local herbs in the treatment of many common ailments and some refractive diseases.

In addition, herbal remedies are now seen as viable natural alternatives to the use of synthetic medications, perceived as being incompatible with nature's pharmaco-physiology. In this regard, the seed of *Buchholzia coriaceae*, a local herb touted for its multiple healing powers, is considered a good candidate for empirical evaluation. *Buchholzia coriaceae* belongs to the family Cappariaceae and is widely distributed in several tropical countries. It is a forest tree with large, glossy, leathery leaves and conspicuous cream-white flowers in racemes at the end of the branches. The seed produced by the tree are edible and taste peppery. It has been used locally for decades to ameliorate a variety of illnesses and since it has been used continually over many generations and among several people groups, it is hypothesized that the kola seed actually has an effect against some illnesses. Due to the several documented beneficial effects of the extract of seeds of *B. coriacea*, it has earned the name *wonderful kola* in folkloric use (Adisa *et al.*, 2011; Mbata *et al.*, 2009). The leaves and stem bark of *Buchholzia* in various formulations, decoctions and concoctions have anti-inflammatory effect on the body, in addition to its antimicrobial and cytotoxicity effects on microorganisms (Ajaiyeoba *et al.*, 2003; Ayoola *et al.*, 2011 Okoli, *et al.*, 2010). *In light of the several touted healing activities of the seed of Buchholzia coriaceae, it was thought proper to conduct more investigations on its healing properties. An important segment of its healing property considered for evaluation is the potential for cellular immune-boosting activity. Infectious diseases are rampant in many tropical countries and consequently, a natural immune boosting agent is desirable. In addition, the white blood cell count value of Africans is comparatively low to those of Caucasians (Reich et al., 2009; Schoenfeld et al., 2009) and ways to mitigate further lowering of the leucocyte count by organic diet supplementation should be explored.*

*The aim of this study was to investigate the cellular immune-boosting effect of Buchholzia coriaceae on leucopenia-induced Wistar rats fed the seed extract of Buchholzia coriaceae, with a view to seeing if the white blood cells would be quickly replenished.*

## Materials and Method

### Seed extract processing

The fresh 'wonderful' kolanuts of *B. coriaceae* were obtained from Sagamu main market and verified at the Botany Department, University of Ibadan, Ibadan. They were cleaned by washing thoroughly with distilled water to remove adhering particles. The kolanuts were then diced to facilitate drying at 50°C using an incubator. The dried kolanuts were pulverized using a grinding mill. The powder was stored in polyethylene bags to prevent moisture absorption and contamination. Exactly 200g of the powdered seeds was soaked in 1litre of 70% ethanol for 72 hours. Afterwards, the mixture was then stirred vigorously and sieved using Whatman No1 filter paper to obtain the filtrate. The filtrate was evaporated to dryness to obtain the ethanol extract, using a rotary evaporator.

### Experimental design

A total number of twenty-seven rats of an approximate average weight of 200g, obtained from Babcock University's farm house, were used for the experiment. They were distributed into three groups (A, B and C) with a population of 9 rats in each group. Group A represents the group of healthy rats without induction of leukopenia or treatment. Group B represents the group of rats induced for leukopenia without treatment. Group C represents the group of rats induced with leukopenia and treated with the herbal extract. Cyclophosphamide was the agent used for inducing leukopenia in the rats and the dosage used was 150mg, single-dose. This agent was administered intramuscularly.

### Animals

Male and female adult albino rats of average weight of 200g that have been quarantined for a week were obtained from the breed of the animal house of the Physiology Department, Babcock University. They were kept in cages under standard environmental conditions and fed with commercial pellets and tap water. They were acclimatized for 3 weeks on normal diet of pelletized mouse dice, with water at room temperature. The animals were then deprived of food but only provided with tap water 24 hours prior to experimentation. Thereafter, the group of animals designed for treatment were

administered with the seed extract and subsequently fed with the animal pellets according to the experimental design outlined above. The study was performed with the approval of the Babcock University Ethical Committee. The use of animals in this experiment conforms with the ARRIVE Guidelines.

### Sample collection

Blood sample collection was through the retro-orbital sinus and about 1ml of blood was collected from the rats into EDTA bottles.

### Sample Analysis

#### Total white blood cell count (manual method)

Whole blood was diluted appropriately, using a diluent (Turk's solution) which haemolyses red cells, leaving all the nucleated cells intact. The number of white cells in a known volume and known dilution were counted using a counting chamber. Briefly, 0.02ml of well-mixed EDTA anticoagulated blood was added to 0.38ml of the diluent in a tube. After mixing and allowing settling for a few minutes, the mixture was charged to a Neubauer blood cell counting chamber. The cells in the four large corner squares of a Neubauer chamber were then counted (Ochei and Kolhatkar, 2008).

### Differential count

A thin blood film was made from the anticoagulated blood, allowed to dry, and stained with Leishman stain for 12 minutes. A drop of immersion oil was added to the blood film and viewed under the light microscope using the x100 objective. The different white blood cell types were then counted in percentage ratio.

### Results

The number of the rat samples analyzed was twenty-seven (27). The summary of data obtained after analysis is displayed in form of graphs. A p-value of  $\leq 0.05$  is considered statistically significant. The results for the baseline differential white blood cells count are: lymphocyte - 67%, neutrophils - 28%, monocyte - 2%, eosinophil - 2% and basophil - 1% for the control group of the experimental rats.

The effect of cyclophosphamide on the Total white blood cell count (TWBC) of rats was assessed. A comparison of mean TWBCs between rats not treated with cyclophosphamide ( $16.44 \pm 0.54$  cell/ $\mu$ l) and those treated ( $8.93 \pm 2.14$  cell/ $\mu$ l) showed a significant difference in which those treated with cyclophosphamide displayed a significantly reduced mean TWBC than those not treated ( $t=3.41, p<0.05$ ).

In figure 1, the effect of ethanolic extract of *Buchholzia coriacea* supplementation was observed on the TWBC count of rats; Group A (the control sample population) demonstrated a normal stable count initially but began to decline mildly from day 8. On its part, Group B (the group of rats induced with cyclophosphamide without further treatment) showed an initial pattern of increase but which declined sharply from day 8 post-induction.

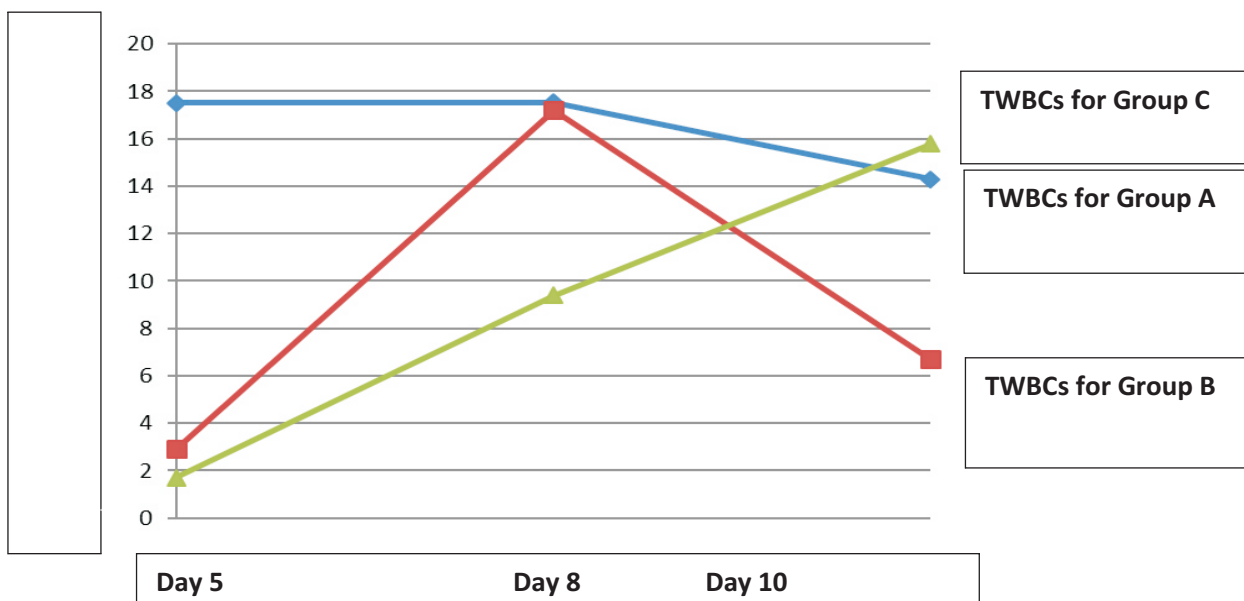
Also, the effect of ethanolic extract of *Buchholzia coriacea* diet on the TWBCs of rats was assessed. The rats in group C (induced with cyclophosphamide and given the seed extract) showed a continuously increasing WBC count pattern. Although there is a difference in the mean TWBC values of rats treated with the seed extract ( $8.96 \pm 2.04$  cell/ $\mu$ l) and the untreated ( $8.93 \pm 2.14$  cell/ $\mu$ l). The difference was however not significant ( $t=0.009, p>0.05$ ).

Similarly, the effect of the ethanolic seed extract

of *Buchholzia coriacea* was studied on the neutrophil count of the rats. While the mean neutrophil count of rats was higher in rats treated with ethanolic extract of *Buchholzia coriacea* ( $31.33 \pm 3.94\%$ ) compared to the untreated ones ( $22.33 \pm 3.35\%$ ), the difference however was not statistically significant ( $t=1.74, p>0.05$ ).

Figure 2 demonstrates the effect of ethanolic extract of *Buchholzia coriacea* on the percentage neutrophil count of rats. While in group A there was no increase, there was a steady increase in the percentage neutrophil count in group C; but in group B there was a significant reduction in the percentage neutrophil count.

The effect of the ethanolic extract of *Buchholzia coriacea* was studied on the lymphocyte count of rats, there was reduction in the mean percentage lymphocyte count of rats treated with ethanolic extract of *Buchholzia coriacea* ( $64.00 \pm 4.75\%$ ) compared to the untreated ones ( $73.67 \pm 2.32\%$ ) and the difference however was not statistically significant ( $t=1.74, p>0.05$ ). In figure 3, the effect of ethanolic extract of *Buchholzia coriacea* on the lymphocyte count of rats is shown. While in group A, there was a stable count pattern, Group B showed an increase, but in group C there was a reduction in the percentage lymphocyte count. However, in absolute terms, the difference between the treated and the untreated in both the neutrophil and lymphocyte counts was significant ( $p<0.05$ ).



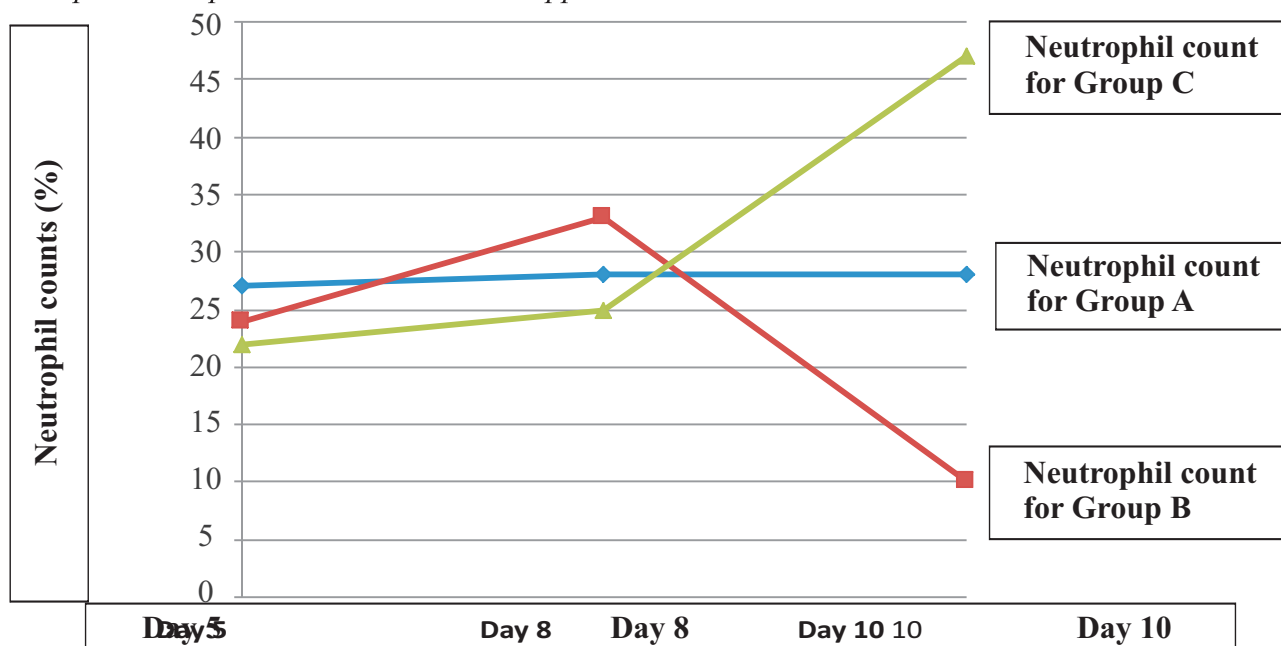
**Figure 1. The effect of ethanolic extract diet of *Buchholzia coriacea* on the total white blood cell count (TWBCs) of rats**

**Key**

Group A: Control (Normal Diet)

Group B: Leukopenia induced + normal diet

Group C: Leukopenia induced + extract-supplemented diet



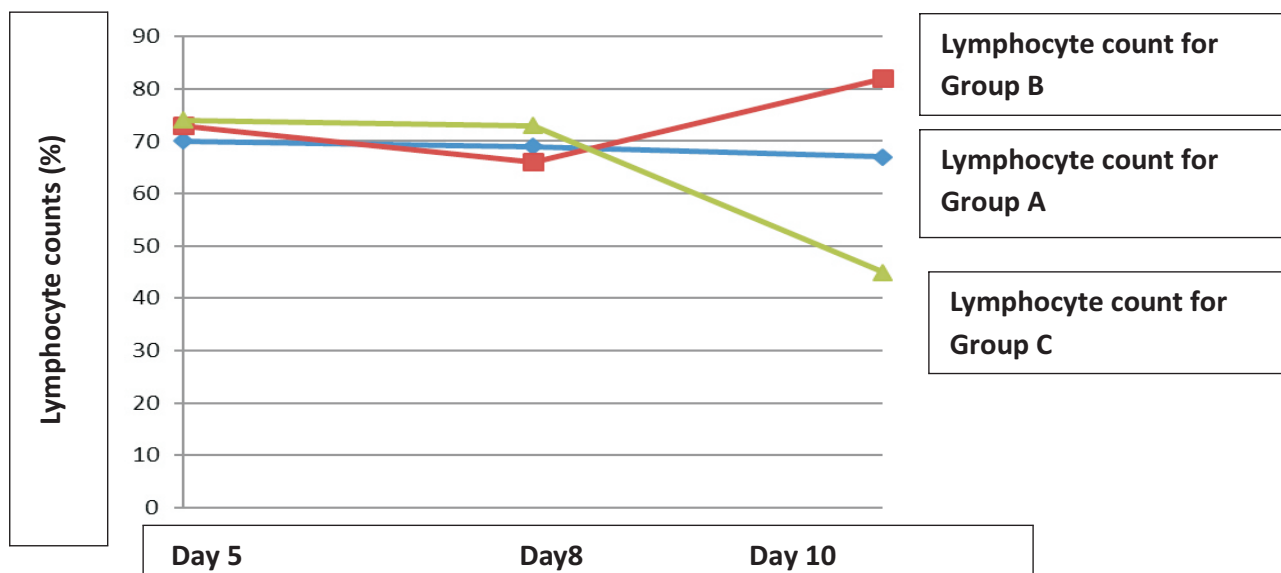
**Figure 2.** The effect of ethanolic extract of *Buchholzia coriacea* on the % neutrophil count of rats

**Key**

Group A: Control (Normal Diet)

Group B: Leukopenia induced + normal diet

Group C: Leukopenia induced + extract-supplemented diet



**Figure 3:** Graph showing the effect of ethanolic extract of *Buchholzia coriacea* on the % lymphocyte count of rats

**Key**

Group A: Control (Normal Diet)

Group B: Leukopenia induced + normal diet

Group C: Leukopenia induced + Extract-supplemented diet

## Discussion

This study was carried out to assess the cellular immune response of *Wistar* rats fed with ethanolic seed extracts of *Buchholzia coriacea*. The parameters assessed include total white blood cell count and the differential white blood cell count for neutrophils and lymphocytes. In this study, we initially induced leukopenia by administering 300 mg/kg of the cyclophosphamide in 150mg/kg divided doses. It was discovered during the course of the study that the dosage used in the research work of Andres *et al.* (2006) for inducing leukopenia in mice in their study (250mg/kg) was too lethal for the species of rats used in our study, resulting in the death of ten of the rats. We therefore resorted to making use of a lower dosage (150mg/kg) in one single dose, to induce the leukopenia in a new set of rats and this gave us an improved performance, as the rats were able to survive.

The results for the baseline differential white blood cells count for the control group of the experimental rats had values that fall within the normal range of the School of Veterinary Medicine, University of Pennsylvania (2002) reference values for laboratory rodents' haematology values. When the data of the total white cell count for both the control group and the test group were compared, there was a significant difference in the mean total white cell count of rats not treated with cyclophosphamide as against those treated with cyclophosphamide ( $p < 0.05$ ). Cyclophosphamide is a well-known immune-suppressive agent (Andres *et al.*, 2006). One of the two groups induced with leukopenia was treated with ethanolic seed extract of *Buchholzia coriacea* diet supplementation (Group C). When the total and the differential count of the white blood cells were carried out for all the groups, it was observed that although there is a difference in the total white blood cell count of rats treated with extract when compared with the untreated group, the difference was however found to be insignificant ( $p > 0.05$ ). This might be due to the short duration of the treatment period as we did not continue the observation beyond day 10 post-induction. From figure 1, it can be seen that the extract-fed group (C) showed a trend of continuous increase in the total white blood cells count as against that of

group B (leukopenia-induced and normal diet). The implication of this finding is that while the seed extract of *B. coriacea* induces a steady increase in the total white blood cell count of these animals, the increase is mild over time. Alternatively, it could be that a significant increase in the total WBC would be a delayed phenomenon. This should be expected since the depressive effect of the cyclophosphamide on the rats' bone marrow may still linger.

The result of the neutrophil count showed that though the mean neutrophil count of rats treated with ethanolic extract of *Buchholzia coriacea* was higher than the untreated ones, the difference was found to be insignificant ( $p > 0.05$ ). We also observed that the group fed with *B. coriacea* extract showed a trend of continuous increase in the mean percentage neutrophil count for the duration of treatment as against that of the group B (leukopenia-induced on normal diet) (Figure 2). This observation also implies that the increased WBC pattern observed in this study is a consequence of the increase in the neutrophil fraction. The neutrophil cell line is the lineage responsible for innate cellular defense in mammals and they play a critical role in the body's defense against bacterial invasion and inflammation. Therefore, the use of the seed extract of *Buchholzia coriacea* is expected to slowly promote this line of defense.

The group of rats fed with the ethanolic extract of *Buchholzia coriacea* showed a reduction in the mean percentage lymphocyte count compared to the untreated ones and the difference was found to be significant ( $p < 0.05$ ). This observation is a *sequela* of the increase in the relative percentage of neutrophils in the rats' blood circulation. The cell line most negatively affected when cyclophosphamide is used is the lymphoid cell line, since the impact of the extract could not rescue the lymphocytes from the deleterious effect of cyclophosphamide.

Generally, from this study, the seed extract of wonder-kola only partially boosts the cellular immune system, working to ameliorate only the neutrophil cell line; and that, slowly too. It is clear that since there was no significant rise in the total WBC count of rats induced with

cyclophosphamide and a mild but insignificant rise in the neutrophil fraction and a concurrent decline in the lymphoid cell percentage, the mild increase in total WBC is essentially due to the improvement in the number of neutrophils. According to Ibrahim and Fagbohun (2012), the seed extract of *Buchholzia coriacea* which contain alkaloids, saponins, glycosides, steroids, tannins, flavonoids, terpenes, and phenols are said to have a boosting effect on the immune system. It appears from this study that one of the immune-boosting activities may be based on its ability to slowly raise blood neutrophil levels.

### Conclusion

We conclude from this study that cyclophosphamide induction of leucopenia in rat model experiments should be customized to get best results. While the granulocyte fraction of the cellular immune components may be rescued by supplementation with the ethanolic seed extract of *Buchholzia coriacea*, it appears the lymphocyte fraction is not positively affected. We therefore suggest that the ethanolic seed extract of *Buchholzia coriacea* may contain some biochemical substances that may slowly boost the leukocyte count and in particular, the neutrophil cell line, thereby raising the innate cellular immunity of rats. An expanded version of this study designed for much longer duration is suggested. It may be that a significant effect of boosting both the total leukocyte count and the neutrophil cell line would be achieved on a long term. In addition, the active ingredient responsible for the immune-boosting activity needs to be identified.

### References

- Adisa, R.A., Choudhary, M.I. and Olorunsogo, O.O. (2011). Hypoglycemic activity of *Buchholzia coriacea* (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. *Experimental and Toxicologic Pathology*; 63: 619-625.
- Ajaiyeoba, E.O., Onocha, P.A., Nwozo, S.O. and Sama, W. (2003). Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia*; 74(7-8): 706-709.
- Andres, F.Z., Beatriz, E.S., Carlos, A.R., Ana, X.Z., Maria, A. and Omar V. (2006). Neutropenia induced in outbred mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. *B M C Infectious Diseases*; 6:55.
- Ayoola, M.D., Gbolade, A.A., Ibitoye, S.F., Moody, J.O. (2011). In vivo anti-inflammatory activity of methanol extracts of *Treculia Africana Decne and Buchholzia coriacea* engl, *Nigerian Journal of Pharmaceutical Sciences*; 10(1): 27-31.
- Hedges, S.B. (2002). The origin and evolution of model organisms. *Nature Reviews of Genetics*; 3 (11): 838–849.
- Hwang M., Berceli S.A. and Tran-Son-Tay R. (2010). Modelling and role of leucocytes in inflammation. In: Garbey, M., Bass, B., Collet, C., Mathelin, M., Tran-Son-Tay, R. (Eds). *Computational Surgery and Dual Training*, Springer, Boston, MA.
- Ibrahim, T.A. and Fagbohun, E.D. (2012). Phytochemical and Nutritive Quality of Dried Seeds of *Buchholzia coriacea*, *Greener Journal of Physical Sciences*; 2 (5): 185-191.
- Mbata, T.L., Ikenebomeh, M.J. and Alaneme, J.C. (2009). Studies on the microbiological, nutrient composition and antinutritional contents of fermented maize flour fortified with bambara groundnut (*Vigna subterranean L.*). *African Journal of Food Science*; 3:165-171.
- Nicholson, R.J., Kelly, K.P. and Grant, I.S (1995). Leucopenia associated with Lamotrigine *British Medical Journal*; 310(6978): 504.
- Ochei, J. and Kolhatkar, A. (2008). *Medical Laboratory Science: theory and practice* (6<sup>th</sup> Edition). Tata McGraw-Hill. New Delhi: 367-376.
- Okoli, B.J., Okere, O.S. and Adeyemo, S.O. (2010). The antiplasmodial activity of *Buchholzia coriacea*. *Journal of Medical and Applied Biosciences*; 2: 21-29.
- Reich, D., Nalls, M.A., Linda-Kao, W.H., Akyibekova, A.T., Patterson, N., Mullikin, J., Hsueh, W.C., Cheng, C.Y., Coresh, J.,

Boerwinkle, E., Li, M., Waliszewska, A., Neubauer, J., Li, R., Leak, T.S., Ekunwe, L., Files, J.C., Hardy, C.L., Zmuda, J.M., Taylor, H.A., Ziv, E., Harris, T.B. and Wilson, J.G. (2009). Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy Antigen receptor for chemokine genes. *PLoS Genetics*; 5 (1): e1000360.

Schoenfeld, Y., Alkan, M.L., Asaly, A., Carmeli, Y., Katz, M. (2009) Benign familial leucopenia and neutropenia in different ethnic groups *European Journal of Haematology* 41 (3): 273-277.

University of Pennsylvania School of Veterinary Medicine (2002) Rodent reference ranges. [www.cal.vet.upenn.edu/project](http://www.cal.vet.upenn.edu/project) (accessed 30/08/2017).

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