**Type of article:** Original research paper

**Title:**

PROPHYLAXIS POTENTIAL OF MUCUNA FLAGELLIPES AGAINST CYCLOPHOSPHAMIDE-INDUCED BONE MARROW SUPPRESSION

**Running title:** Mucuna flagellipes ameliorates myelosuppression

**This paper is original and has not been presented in any conference nor submitted to any other journal for the purpose of publication**

## ABSTRACT

The use of cyclophosphamide in the treatment of malignancies is associated with bone marrow suppression, with its attendant decrease in blood parameters leading to leukopenia, anaemia, thromobcytopenia and other abnormalities. These side effects limit the use of cyclophosphamide, as ferrous sulphate has not been able to solve this problem. Many clinicians result to blood transfusion in order to boost the blood parameters to a level that will withstand cyclophosphamide use. Twenty Wistar rats were used for this experiment. The rats were divided into 5 groups, each group had 4 rats. Group 1 was control; groups 2-5 were treated with 100mg/Kg of cyclophosphamide; groups 3 and 4 also received 250mg/Kg and 500mg/Kg of extract, respectively, while group 5 received 400mg/Kg of ferrous sulphate. There was significant decrease ($P<0.05$) in the packed cell volume and white blood cell count in group 2 when compared with group 1. Groups 3 and 4 showed statistically significant increase in the packed cell volume ($P=0.003$) and white blood cell count ($P=0.015$). There was also increase in the number of proliferating cells in the bone marrow of groups 3 and 4. Mucuna flagellipes leaf extract increases packed cell volume and white blood cell count and number of proliferating cells in the bone marrow in the presence of cyclophosphamide. Mucuna flagellipes leaf extract can be used in clinical practice to cushion the bone marrow suppression effect, leukopenia and anaemia, that follows the use of cyclophosphamide in treatment of malignancies.

**KEYWORDS:** Cyclophosphamide, Mucuna flagellipes, Myelosuppression, Anaemia

## INTRODUCTION

Bone marrow suppression is one of the common side effects of use of chemotherapeutic agents. This is usually anticipated when these chemotherapeutic agents are used in the treatment of malignancies (Carey, 2003). Bone marrow suppression usually affects the production of precursors of leucocytes, erythrocytes and platelets, with attendant leucopenia, anaemia and thrombocytopenia, respectively, thereby predisposing the patients to increased susceptibility to infection, weakness and bleeding. These life-threatening events of chemotherapeutic agents seriously affects the use of these agents in cancer therapies, as patients may die of complication of the treatment instead of the disease (Carey, 2003).

Cyclophosphamide is among the commonly used chemotherapeutic agents. It can be administered singly or in combination with other therapeutic agents. And it is given intravenously. In experimental animals, it can be given orally, as gavage, subcutaneously, or through the intravenous route with a wide range of dosing regimens and usage protocols (Hubbard and Fidanze, 2007). Cyclophosphamide finds its use in the treatment of different forms of malignancies such as lymphomas, leukaemias, breast cancer, multiple myeloma, ovarian cancers, retinoblastoma, neuroblastoma, mycosis fungoides (Hubbard and Fidanze, 2007). Cyclophosphamide is excreted in the urine and may cause haemorrhagic cystitis (Reynard et al., 2013). Several other side effects that can be seen with the use of cyclophosphamide include nausea, vomiting, alopecia (Hubbard and Fidanze, 2007). Cyclophosphamide and other chemotherapeutic agents may not be given if the white blood cell count, platelet count and the haemoglobin concentration or packed cell volume are below the optimal value (Reynard et al., 2013).

There are several options that are available to help build up and improve the blood parameters prior to the administration of these chemotherapeutic agents. These options include use of

haematinics such as Ferrous sulphate, Vitamin C and Vitamin E; and at the extreme, blood transfusion. Blood transfusion could be whole blood or blood fractions such as platelet concentrate, granulocyte colony stimulating factor and red cell concentrate. In severe cases of anaemia, where these agents are needed urgently, these agents may not be able to get the blood level to optimal level. They are also usually not readily available in resource poor societies and centres (Liumbruno et al., 2009). *Mucuna flagellipes* leaf have been used traditionally for treatment of anaemia (Ihedioha and Okoye, 2011).

## MATERIALS AND METHOD

### Collection of plant material

Fresh leaves of Mucuna flagellipes were obtained from Udi, Enugu, Nigeria. The leaf was authenticated by the department of botany, Enugu State University of Science and Technology, Enugu, Nigeria.

### Preparation of extract

The stalks of the leaves were removed, then the leaves washed with distilled water and subsequently air-dried under shade at room temperature. Then pulverized to fine powdered form using mortar and pestle. The powdered material was then sieved using sieve with little pores to remove the ungrounded fibres as previously described by Auwal et al. (2018). One thousand grams of the powdered leaves was extracted exhaustively, each time. The aqueous extract was filtered using Whatman No. 2 filter paper. And then concentrated with rotary evaporator at 40°C and stored in refrigerator at 4°C until use as previously described by Alabi et al. (2012).

### Procurement of rats

Twenty male Wistar rats with average weight of 150g were procured from animal house of Department of Anatomy, Enugu State University of Science and Technology. The rats were handled carefully according to guideline of the Committee for the purpose of control and supervision of experiments on Animals, India. The rats were grouped into 5. Each group had 4 rats and was placed in separate clean cages in the animal house of Department of Anatomy, Enugu State University of Science and Technology. They had two (2) weeks to acclimatize. During this time, they had free access to rat chow and water.

**Administration of extract**

Group 1 served as negative control and was given 1ml Normal saline, orally, throughout the experiment.

Group 2 was given subcutaneous cyclophosphamide 100mg/Kg from day 12-14 of the experiment.

Group 3 was given 250mg/Kg of extract, orally, from day 1-14 of the experiment.

Group 4 was given 500mg/Kg of extract, orally, from day 1-14 of the experiment.

Group 5 was positive control and was given Ferrous sulphate 400mg/Kg from day 1-14 of the experiment.

At the end of the experiment (day 15), the rats were anaesthetized by inhalation of chloroform. Blood samples were then collected from the ophthalmic vein through the medial canthus of the rats. The rats were then sacrificed and the bone marrow aspirated and slide smear made immediately and fixed before further analysis.

**RESULTS**

There was significant reduction in the number of white blood cells and packed cell volume following treatment with cyclophosphamide, as shown in table 1 (group 2). However, there was no marked reduction in the number of white blood cell count and packed cell volume in the groups that received the *Mucuna flagellipes* leaf extract before treatment with cyclophosphamide (groups 3 and 4). This is evidenced by the statistically significant difference in the packed cell volume (P=0.003) and white blood cell count (P=0.015) in groups treated with the extract when compared with those that did not receive the extract (groups 2). There was significant reduction in the packed cell volume and white blood cells in group 5. So, no statistically significant difference between group 5 and group 2.

The histology showed near absence of proliferating cells in the group treated with cyclophosphamide in the absence of the extract (Figure 2). There was no marked reduction in number of cells in the group that received 500mg/Kg (Figure 4) when compared to control (Figure 1). The group treated with fesolate had more reduction in number of cells (Figure 5) when compared with those treated with 250mg/Kg of extract (Figure 3) and 500mg/Kg (Figure 4).

**DISCUSSION**

This study buttressed the fact that cyclophosphamide causes bone marrow suppression (Lee and Lim, 2013). This is as evidenced by the reduction in the white blood cell count and packed cell volume of the experimental animals. Ihedioha and Okoye (2011) also got similar result in their experiments. The decreases in the packed cell volume and the white blood cell count were statistically significant in groups 2 and 5. The histology of the bone marrow also showed reduction in the number of proliferating cells in the groups treated with cyclophosphamide only.

The leaf extract of *Mucuna flagellipes* prevented excessive suppression of the bone marrow by the administered cyclophosphamide. This is evident in the number of proliferating cells seen in the

histology of the groups treated with graded doses of *Mucuna flaggellipes* and cyclophosphamide (groups 3 and 4). The proliferating cells in the group treated with 500mg/Kg of leaf extract of *Mucuna flagellipes* and cyclophosphamide exhibited a greater number of proliferating cells more than that of the group treated with 250mg/Kg of the extract. Though the number of this proliferating cells were not up to that of the control. This could be due to the dose of the extract used in the experiment. A higher dose may have maintained these parameters at a level that is close to the control.

There was also no statistically significant reduction in the packed cell volume and the white blood cell count in the groups treated with leaf extract of *Mucuna flagellipes* and cyclophosphamide when compared to the group treated with cyclophosphamide alone. The group that received ferrous sulphate and cyclophosphamide had statistically significant reduction in the packed cell volume and white blood cell count when compared to control and Groups 3 and 4.

## CONCLUSION

*Mucuna flagellipes* leaf extract minimizes the deterioration in the haematological parameters, such as packed cell volume and white blood cell count in the setting of cyclophosphamide administration. It also prevents destruction of proliferating cells in the bone marrow. These effects were better than those seen with ferrous sulphate. This means that *Mucuna flagellipes* leaf extract has a better prophylaxis ability than ferrous sulphate against cyclophosphamide-induced bone marrow suppression at the dose used. So, *Mucuna flagellipes* leaf extract can be used in clinical practice where anaemia and leucopenia are some of the limiting factors in the effective use of cyclophosphamide in treatment of different organ malignancies.

## TABLES AND FIGURES

**Table 1: Effect of Mucuna flagellipes on packed cell volume (PCV) and white blood cell count (WBC)**

| Groups | PCV (%) | WBC (x$10^9$/L) | Neutrophil (%) |
|---|---|---|---|
| **1** | 46.51 ± 0.71 | 7.74 ± 2.12 | 57.50±2.11 |
| **2** | 20.50 ± 0.70* | 4.20 ± 0.01* | 50.50±0.01 |
| **3** | 30.01 ± 7.07 ** | 6.25 ± 1.76 | 43.50±13.44 |
| **4** | 31.50 ± 0.70** | 7.45 ± 1.41* | 47.00 ±1.41 |
| **5** | 24.50 ± 0.71 ** | 4.10 ± 0.00^* | 34.01 ±5.66 |
| | **P=0.0032** | **P=0.0150** | **P=0.1037** |

Values were expressed as Mean± SD . where *P<0.05 showed a significant difference compared to group 1 and **P<0.05 showed a significant difference compared to groups 2, and ^* showed significant difference compared to 2,3,4, while values without superscript shows no significant difference (P>0.05) using One– way anova with Tukey HSD test for multiple comparison.

## Histology of the bone marrow aspirate

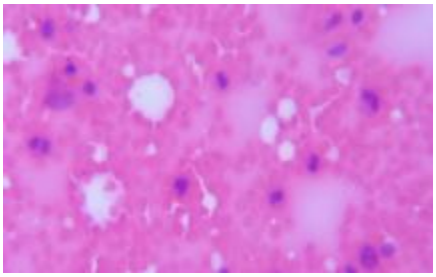

Figure 1: Histology of bone marrow of group 1.

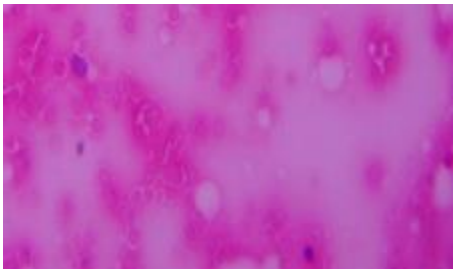

Figure 2: Histology of bone marrow of group 2.

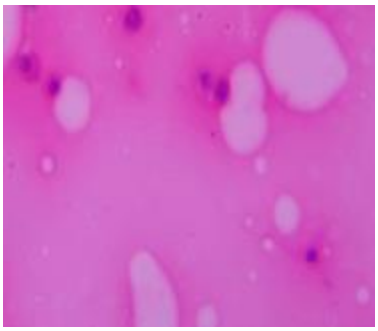

Figure 3: Histology of bone marrow of Group 3.

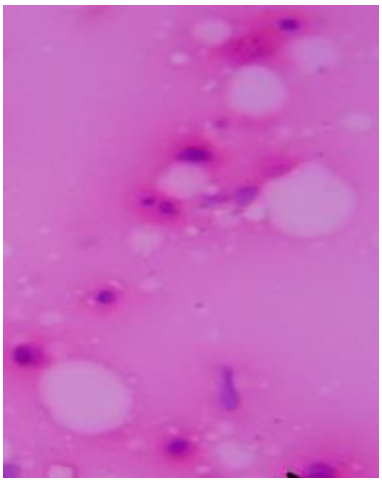

Figure 4: Histology of bone marrow of group 4.

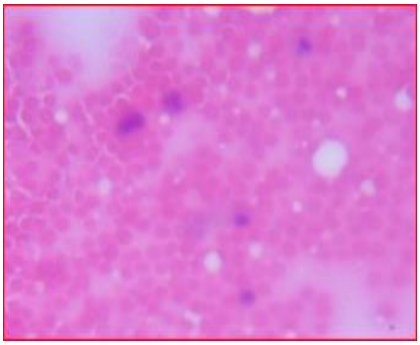

Figure 5: Histology of bone marrow of group 5.

**REFERENCES**

1. Alabi OA, Haruna MT, Anokwuru CP, Jegede T, Abia H, Okegbe VU, Babatunde EE. (2012). Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of Carica papaya on clinical bacterial and fiungal isolates. Advances in Applied Science Research. 3(5): 3107-3114.
2. Auwal U, Olanitola SO, Lawan FD, Muhammad A. (2018). Antibacterial Activity of Fractionated Extracts of Carica papaya Leaves and Stem Bark against Clinical Isolates of Methicillin Resistant Staphylococcus aureus (MRSA). Modern application in pharmacy and pharmacology. 1(5): 520-525.
3. Carey PJ. (2003). Drug-induced myelosuppression: diagnosis and management. Drug Saf. 26(10):691-706.
4. Hubbard RD, Fidanze S. (2007). Therapeutic areas II: cancer, infectious diseases, inflammation and immunology and dermatology. in Comprehensive Medicinal Chemistry II
5. Ihedioha JN, Okoye COB. (2011). Nutritional evaluation of Mucuna flagellipes leaves: an underutilized legume in Eastern Nigeria. American Journal of Plant Nutrition and Fertilization Technology. 1(1): 55-63
6. Lee J, Lim KT. (2013). Protection against cyclophosphamide-induced myelosuppression by ZPDC glycoprotein (24 kDa). Immunol Invest. 42(1):61-80.
7. Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G. (2009). Recommendations for the transfusion of red blood cells. Blood transfusion. 7(1). 49-64.

8. Reynard J, Brewster S, Biers S. (2013). Prostate cancer: management of advanced disease-castrate-resistant prostate cancer. Oxford handbook of urology. 3ed. Oxford university press. 358-363.