

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

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### 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 leucopenia, anaemia, thrombocytopenia 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. This study aims at exploring the prophylaxis potential of *Mucuna flagellipes* leaf extract against cyclophosphamide-induced bone marrow suppression. 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 affect 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* leaves are good sources of plant crude fibre and minerals especially calcium, magnesium, iron and potassium with moderate levels of phosphorus, sodium, phytate, nitrate, cadmium and lead (Ihedioha and Okoye, 2011). Rural dwellers have overtime used it traditionally for treatment of anaemia with huge success and high iron content in *M. flagellipes* tends to support their claims (Ihedioha and Okoye, 2011).

Therefore, natural compounds such as *Mucuna flagellipes*, having high percentage of iron could help in boosting blood level in anaemic conditions and could be used as alternative treatments to help build up and improve the blood parameters prior to the administration of chemotherapeutic agents. To the best of our knowledge, the effect of leaf extract of *Mucuna flagellipes* on the bone marrow and blood parameters of rats treated with cyclophosphamide has not been studied. Hence, the purpose of this study was to investigate the prophylaxis potential of *Mucuna flagellipes* leaf extract against cyclophosphamide-induced bone marrow suppression.

## 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 retro-orbital vein of the rats as described by Parasuraman et al (2015) for haematological analysis. The rats were then sacrificed and the bone marrow aspirated and slide smear made immediately and fixed before histological analysis using haematoxylin and Eosin stain

The haematological parameters were obtained for each blood sample using ERMA, PCE-210, automated haematology Analyzer (made in Japan).

## 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

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

Groups	PCV (%)	WBC (x10 <sup>9</sup> /L)	Neutrophil (%)	Lymphocytes (%)	Basophils	Eosinophil
1	46.51 ± 0.71	7.74 ± 2.12	57.50±2.11	42.50±2.12	Nil	Nil
2	20.50 ± 0.70*	4.20 ± 0.01*	50.50±0.01	48.50±0.71	Nil	Nil
3	30.01 ± 7.07 **	6.25 ± 1.76	43.50±13.44	56.50±13.44	Nil	Nil
4	31.50 ± 0.70**	7.45 ± 1.41*	47.00 ±1.41	62.50±0.71	Nil	Nil
5	24.50 ± 0.71 **	4.10 ± 0.00^*	34.01 ±5.66	43.50±2.12	Nil	Nil

$P=0.0032$   $P=0.0150$   $P=0.1037$   $P=0.00984$

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.

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 as depicted in table 1 (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 (groups 3 and 4), when compared with those that did not receive the extract (groups 2) as shown in table 1. There was significant reduction in the packed cell volume and white blood cells in those treated with Fesolate (group 5) when compared with control (group 1). Also, no statistically significant difference between group 5 (treated with Fesolate) and group 2 (treated with cyclophosphamide) as shown in table 1.

The histology showed near absence of proliferating cells in the group treated with cyclophosphamide in the absence of the extract (group 2) as shown in Figure 2.

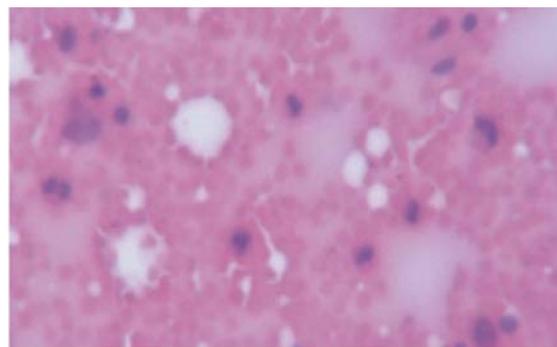


Figure 1: Histology of bone marrow of control (group 1).

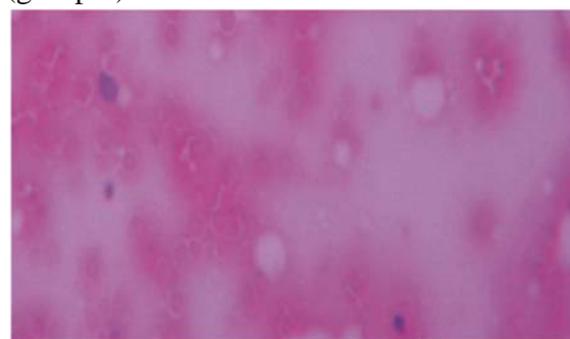


Figure 2: Histology of bone marrow of group treated with cyclophosphamide (group 2)

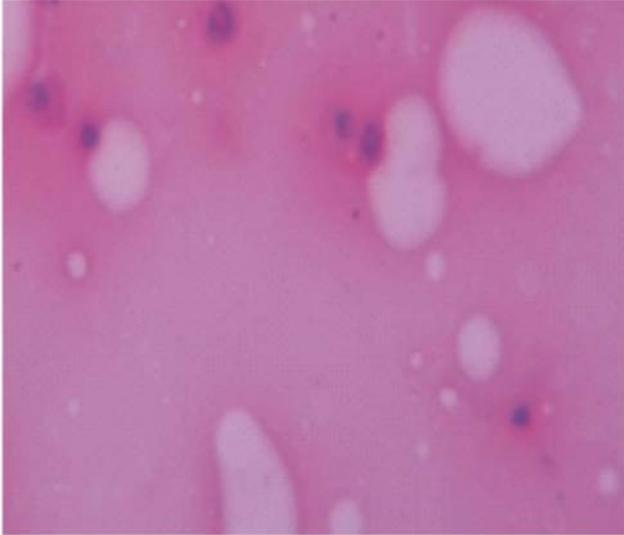


Figure 3: Histology of bone marrow of group treated with 250mg/Kg of *M. flagellipes* leaf extract (group 3).

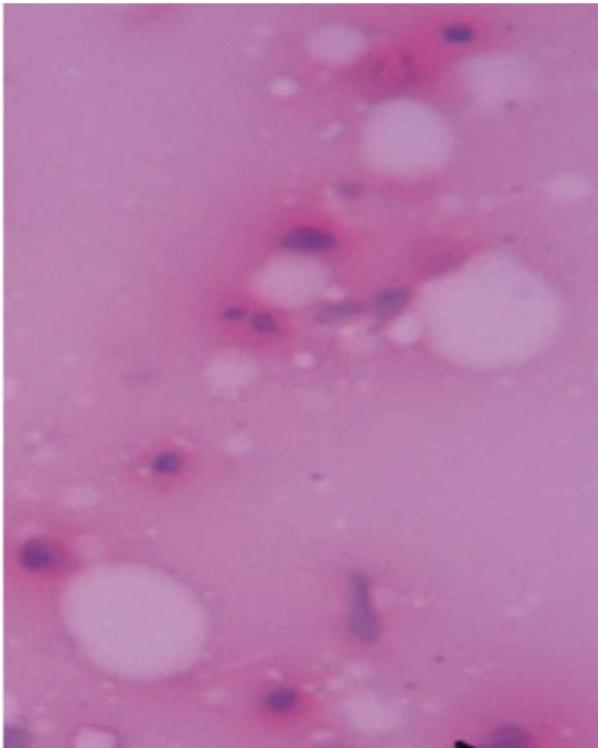


Figure 4: Histology of bone marrow of group treated with 500mg/Kg of *M. flagellipes* leaf extract (group 4).

There was no marked reduction in number of cells in the group that received 500mg/Kg (group 4) as shown in Figure 4 when compared to control (group 1) as depicted in Figure 1.

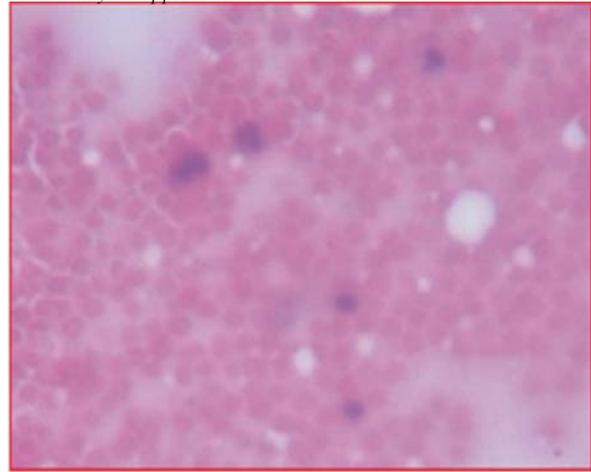


Figure 5: Histology of bone marrow of group treated with 400mg/Kg of Fesolate (group 5).

The group treated with Fesolate (group 5) had more reduction in number of cells as shown in Figure 5 when compared with those treated with 250mg/Kg of extract (group 3) and 500mg/Kg (group 4) as shown in figure 3 and 4, respectively.

## DISCUSSION

Bone marrow suppression usually affects the production of precursors of leucocytes, erythrocytes and platelets, with attendant leucopenia, anaemia and thrombocytopenia, respectively (Carey, 2003). This study showed marked reduction in the white blood cell count and packed cell volume of the experimental animals as shown in Table 1. This was as a result of myelosuppressive effect of cyclophosphamide on haemopoietic cells of the experimental rats. This decrease in the packed cell volume and the white blood cell count were statistically significant in groups 2 and 5 as shown in table 1. The histology of the bone marrow also showed reduction in the number of proliferating cells in the group treated with cyclophosphamide only (group 2) as shown in figure 2. This further buttress the fact that cyclophosphamide causes bone marrow suppression as shown by previous studies (Ihedioha and Okoye, 2011; Lee and Lim, 2013).

*Mucuna flagellipes* plants possess useful phytochemicals of high medicinal value (Sridhar and Bhat, 2007). The seeds constitute a good source of several alkaloids, antioxidants, antitumor and antibacterial

compounds (Sridhar and Bhat, 2007). They are also rich in plant crude fibre and minerals especially calcium, magnesium, iron and potassium with moderate levels of phosphorus, sodium, phytate, nitrate, cadmium and lead. The immature pods and leaves serve as vegetable (Adebowale and Lawal, 2003), contain vitamin C (Aviara et al. 2010) that is very vital for proper functioning of white blood cells and maintenance of the normal reactivity of white blood cells (Lampariello et al. 2012). Cytokines, growth factors, hormones, interactions with stromal cells in the bone marrow and elements, such as iron (Fe), folate and vitamin B12, are involved in control mechanisms of the process. Abnormalities in any of these factors can affect red cell mass, resulting in anaemia or leucopenia (Herbert, 2009).

The results of this study revealed that the extracts boosted blood production thereby preventing 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 flagellipes* and cyclophosphamide (groups 3 and 4). This significant increase in the packed cell volume levels showed that *Mucuna flagellipes* leaf extracts could be used as prophylaxis against cyclophosphamide-induced bone marrow suppression.

The hematological parameters of animal could be influenced by age, breed, nutritional status, current status of the animal and environmental factors (Butler and Waleen, 2006). In this study, the proliferating cells in the group treated with 500mg/Kg (group 4) of leaf extract of *Mucuna flagellipes* and cyclophosphamide exhibited a greater number of proliferating cells more than the group treated with 250mg/Kg of the extract (group 3). Though the number of these proliferating cells was not up to that of the control, it 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 or even higher than that of the control (group 1).

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 (groups 3 and 4) when compared to the group treated with cyclophosphamide alone (group 2). The group that received ferrous sulphate and cyclophosphamide (group 5) 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 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.

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