Original Research Article

IMMUNO-PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF OCIMUM GRATISSIMUM LEAF IN CYCLOPHOSPHAMIDE-INDUCED MYELOTOXICITY IN WISTAR RAT.

ABSTRACT

The bone marrow is the manufacturing center of blood cells; the suppression of its activity causes deficiency of blood cells. This condition can rapidly lead to life-threatening infection. *Ocimum gratissimum* plays a vital role in preventing some disease conditions, but its hematological effects on the bone marrow have not been documented much. This study is designed to investigate the immuno-protective activity of the aqueous leaf extract of *ocimum gratissimum* in cyclophosphamide-induced myelotoxicity. Twenty-four Wistar rats aged two to three months, weighing 170-200 g were used for the study. The rats were divided into six groups of four rats each, labeled A to F. The rats in the control group (group A) were given feed and water. Group B was administered with 3 mg/kg of cyclophosphamide intraperitoneally daily for seven days to induce myelotoxicity. Groups C and D were treated daily with 100 mg/kg and 200 mg/kg of the extract for fourteen (14) days respectively. Groups E and F were treated with the extract for seven (7) days and then induced and treated with myelotoxicity and extract simultaneously for the next seven (7) days. On the 15th day, blood samples (3.0 ml) were collected from each rat through the retro-orbital plexus. Also the bone marrow was harvested and analyzed using standard histological procedure. Data were analyzed with Pearson's correlation test and multivariate analysis of variance using Statistical Package for Social Sciences (SPSS) version 21 and results were expressed as mean ± SD. Myelotoxicity was achieved in Group B rats. Group E and F rats showed a significant increase in hemoglobin (Hb), hematocrit (Hct), and total white blood cell count (TWBC) compared with Group B. The Group C and D rats revealed a significant increase in Hb, Hct and TWBC when compared with group A. Aqueous leaf extract of *Ocimum gratissimum* may possess immuno-protective properties when orally administered in cyclophosphamide-induced bone marrow suppression.

Keywords; *Ocimum gratissimum*, cyclophosphamide, hematological parameters, leaf extract, myelosuppression.

INTRODUCTION

Cancer and its treatment have over the years posed great difficulties in the field of medicine. Some of the treatment options like chemotherapy have discouraging side effects which on its own pose a threat to the life of the oncology patient. Myelosuppression and liver injury is the most common dose-limiting side effect of chemotherapy [1]. The bone marrow is the spongy tissue in the center of the bones. It is where red blood cells (RBCs), white blood cells (WBCs) and platelets are made. With bone marrow suppression, the bone marrow doesn't make normal numbers of blood cells. It leads to lower levels of one or more types of blood cells. Bone marrow suppression is also called myelosuppression.

The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [2]. Because of these advantages, the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. According to a survey (1993) of World Health Organization (WHO), the practitioners of the traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh [3].

Herbal medications are used widely in developing countries for the treatment of various diseases and ailments. This is because they are seen as alternatives to orthodox medicines in terms of costs and perceived side effects [4]. One of the main problems found in the field of medicinal plants is the lack of pharmacological, toxicological and clinical evidence. In many cases, this practice is commonly associated with their traditional use. Only a small fraction of thousands of medicinal plants used in the world have been rigorously tested in controlled studies, therefore the evidence of toxicity risks and verification of efficacy is even lower [5].

Ocimum gratissimum, also known as **clove basil**, **African basil**, and in Hawaii a **wild basil**, is a species of *Ocimum*. It is native to Africa, Madagascar, southern Asia, and the Bismarck Ar chipelago, and naturalized in Polynesia, Hawaii, Mexic, Panama, West Indies, Brazil, and Bolivia. *Ocimum gratissimum* is one among the leafy vegetables consumed by Nigerians. It is usually seen as a small shrub with many branches and simple oval leaves. The leaves are used as food additives for their believed nutritive and medicinal values [6]. It also adds flavor or aroma to the food. It is used mainly as a spice [7][8]. It is a medicinal plant which can be used for therapeutic purposes or which are precursors for the synthesis of curative drugs [9]. It can be used as flavoring agent in a pharmaceutical product. The *Ocimum gratissimum* oil could be used to mask the bitter taste of most drugs, particularly the anti-malaria tablets [10]. Although much has been documented on the medicinal properties of this plant, this work, however, is designed to evaluate the myelo-protective property of the extract of this plant in order to know the best extract to use in the treatment or amelioration of myelotoxicity in folk medicine.

MATERIAL AND METHODS

Plant Material Collection

Fresh plant leaves of *Ocimum gratissimum* was harvested from a garden in the University of Nigeria, Enugu Campus. The plant was authenticated by a taxonomist at the Department of Botany, University of Nigeria, Nsukka.

Plant Extraction and Phytochemical Analysis

Fresh leaves *of ocimum gratissimum* were washed in fresh water and air dried in the laboratory at room temperature. The air dried leaves were milled into fine powder in an electric blender and the fine powder soaked in 2 litres of distilled water for 24 hours, filtered with whatman No.1 filter paper (150 mm), and evaporated with water bath / rotary evaporator at 50^{o}C to crude extract of *ocimum gratissimum*. Crude extract residue was weighed and dissolved in distilled water for use on each day of our experiment [11]. 10 g of crude extracted was dissolved in 100 ml of normal saline to get the stock solution of extract used for daily administration.

Phytochemical screening was carried out to qualitatively assess the secondary metabolites in the plant leave extract using the standard laboratory procedures of precipitation and coloration reaction as described by Sofowara[6] to identify the secondary metabolites present.

Animal Procurement

Twenty-four (24) male (170g-200g) adult Wistar rats were used for the experiment. After being purchased from the animal house of College of Medicine, University of Nigeria, Enugu Campus, they were housed in aluminum cages, placed in a well-ventilated house with optimum condition (temperature 30^{o}C photoperiod; 12hours natural light and 12hours dark; humidity is 40-50%). The animals were fed growers mash manufactured by Top Feed Nigeria Limited and allowed water *ad libiitum.* They were allowed acclimatization period of 2 weeks and throughout the experimental period; the animals were handled according to the guidelines for animal research in National Institute of Health guidelines for care and use of laboratory animals. The study was carried out in accordance with the principles of laboratory animal care and standard experimental procedure.

Ethical Approval

Ethical clearance was sought for and approved by the Research and Ethical Clearance committee, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUCOM/FBMS/ETR/2022/013)

TABLE 1: EXPERIMENTAL DESIGN AND ADMINISTRATION

EXPERIMENTAL GROUPS	RATS	ADMINISTRATION	TREATMENT
GROUP A (-ve control)	4	CONTROL	Distilled water and food pellet were only given for 14 days.
GROUP B	4	Cyclophosphamide (3mg/kg)	3mg/kg body weight of cyclophosphamide was given without treatment per day for 14 days.
GROUP C (Extract LD)	4	OC (100mg/kg	100mg/kg of the extract was only given daily for 14 days.
GROUP D (Extract HD)	4	OC (200mg/kg)	200mg/kg of the extract was only given daily for 14 days.
GROUP E (Protective)	4	Cyclophosphamide 3mg/kg+OC 100mg/kg	100mg/kg of extract was given per day for 7 days before myelotoxicity was induced through the administration of 3mg/kg of cyclophosphamide per day for the next seven days.
GROUP F (Protective)	4	Cyclophosphamide 3mg/kg + OC 200mg/kg	200mg/kg of extract was given per day for 7 days before myelotoxicity was induced through the administration of 3mg/kg of cyclophosphamide per day for the next seven days.

***OC = Ocimum gratissimum.**
HD = High dose; LD = Low dose

Body Weight Measurement and Animal Sacrifice

Measurement of the body weight of all the animals in each group

The body weights of all the animals in each group were measured and recorded on the first and last days of study before sacrifice. The measurements were done using a digital electronic scale and recorded to the nearest gram. At the end of the 14 days treatment period, the animals were sacrificed with anesthetic agent called thiopental at dose of 50mg/kg body weight which was injected intraperitoneally 24 hours post treatment.

Blood Analysis

On the 15th day, Blood samples were collected from each of the groups with the aide of capillary tubes through the medial optical vessels for evaluation of blood parameters (complete blood count). Complete blood count was performed with an automated hematology system (Sysmex KX-2N hematology analyzer, Sysmex Incorporation Kobe, Japan).

Histological Study

The histological analyses of the tissues were done according to the method of Drury [12]. The stained tissues were micrographed and interpreted by a pathologist at the University of Nigeria, Nsukka.

Statistical Analysis

Results of haematological analysis (Total blood count) were analyzed using SPSS (version 21), expressed as Mean ±Standard Error of Mean (SEM). Statistical difference in mean between groups were analyzed using one way ANOVA (Analysis of variance). P-value less than 0.05 were considered as statistically significant.

RESULTS

Result of Phytochemical Analysis

The phytochemical analysis of the aqueous leave extract of *Ocimum gratissimum* revealed the presence of tannins, steroids, terpenoids, flavonoids and cardiac glycosides. Anthraquinones was also detected in the aqueous extract while steroids were absent (Table 2).

Table 2: Phytochemicals in aqueous leaf extract of *Ocimum gratissimum*.

S/No	Parameter

1	Alkaloids	**+++**
2	Saponins	**++**
3	Tannins	**+**
4	Phlobatannins	**+**
5	Anthraquinones	**+**
6	Steroids	**-**
7	Terpenoids	**+**
8	Flavonoids	**+++**
9	Cardiac glycosides	
	With steroidal ring	**++**
	With deoxy-sugar	**++**
10	Essential oil	**+++**

+++ = Present in high concentration
++ = present in moderate concentration
+ = slightly or sparingly present
- = Absent

Result of the Effect of Aqueous Leaf Extract of *Ocimum Gratissimum* on Body Weight of the Wistar Rats

Table 3: Effect of *Ocimum Gratissimum* extracts on the body weight of the Wistar rats.

Groups	Initial Weight	Final Weight	% change in weight
A (normal control)	293.3±19.4	306.4±18.8	4.5%
B (CYC control)	237.0±8.4	218.9±42.8[a]	-7.6%
C (100mg kg^{-1} extract)	211.6±4.4	216.2±26.8[b]	4.6%
D (200mg kg^{-1} extract)	163.3±4.0	197.8±9.2	21.1%
E (Protective I)	188.1±3.9	207.1±26.1[ab]	10.1%
F (Protective II)	168.6±6.8	183.5±18.6[ab]	8.8%

[a]$P < 0.05$ (statistically significant compared with control), [b]$P < 0.05$ (statistically significant compared with myelosuppressed rats).

Result of the Effect of *Ocimum Gratissimum* on the Haematological Parameters of the Treated Rats

Bone marrow suppression occurred in the group B rats that were treated with only cyclophosphamide. When compared to group A (negative control) there was no significant ($p>0.05$) increase in PCV, TWBC, RBC and Hb in group C and D rats which received only 100 mg/kg and 200 mg/kg of the extract respectively. However, there was a significant increase ($p<0.05$) in the above mentioned blood parameters of the group C and D rats when compared to the group B that was treated with only cyclophosphamide. The increase was moderately dose dependent.

The administration of both the extract and cyclophosphamide brought about a protective activity. This was evident in the group E and F rats which had no significant decrease ($p>0.05$) in HCT, Hb, RBC and TWBC when compared to the control group. The group E and F rats received 100mg/kg and 200mg/kg extract respectively.

The mean differences in RBC, TWBC, HCT, Hb and platelet of group E were slightly lower when compared to the normal control group. Meanwhile, the HCT, Hb, RBC, TWBC and platelet amount are significantly higher ($p<0.05$) than that of the cyclophosphamide group.

Table 4: Effect of aqueous leave extract of *ocimum gratissimum* on some hematological indices of cyclophosphamide treated rats.

Groups	PCV (%)	WBC ($\times 10^9 L^{-1}$)	RBC ($\times 10^9 L^{-1}$)	Hb (gdL^{-1})
A (normal control)	49.0±2.65	6666.7±231.0	245.0±5.00	17.7±0.91
B (CYC control)	23.3±3.50	4000.0±400.0[a]	216.7±15.3[a]	14.7±2.88[a]
C (100mg kg^{-1} OG)	45.0±1.00[b]	7333.3±1222.0[b]	236.7±25.2[b]	15.3±0.37[b]
D (200mg kg^{-1} OG)	51.7±6.51[b]	8533.3±2023.2[b]	236.7±15.3[b]	17.6±2.21[b]
E (Protective I)	35.7±6.14[b]	5400.0±229.2[ab]	235.0±8.67[ab]	18.9±2.76[b]
F (Protective II)	39.0±7.14	6266.7±1205.5	246.7±15.3[b]	15.0±2.70

[a]$P < 0.05$ (statistically significant compared with control), [b]$P < 0.05$ (statistically significant compared with myelosuppressed rats).
OG = Ocimum Gratissimum
CYC = Cyclophosphamide

Table 4: Effect of aqueous leave extract of *ocimum gratissimum* on some hematological indices of cyclophosphamide treated rats continued.

Groups	MCV (fl)	MCH (pg)	MCHC (gdL^{-1})	PLATELETS ($\times 10^9 L^{-1}$)
A (normal control)	0.20±0.01	0.68±0.04	0.28±0.02	243.3±5.77
B (CYC control)	0.20±0.05	0.69±0.18	0.37±0.23	150.0±20.0
C (100mg kg^{-1} OG)	0.19±0.02	0.65±0.08	0.26±0.01	220.0±20.0
D (200mg kg^{-1} OG)	0.22±0.03	0.75±0.12	0.26±0.02	240.0±17.3
E (Protective I)	0.22±0.03	0.74±0.09	0.31±0.02	230.0±30.0[b]
F (Protective II)	0.18±0.04	0.61±0.14	0.30±0.01[b]	240.0±10.0[b]

[a]$P < 0.05$ (statistically significant compared with control), [b]$P < 0.05$ (statistically significant compared with myelosuppressed rats).
OG = Ocimum Gratissimum
CYC = Cyclophosphamide

Effect of Aqueous Leave Extract of *Ocimum Gratissimum* on Histo-Architecture of Cyclophosphamide treated Wistar Rats.

The bone marrow micrograph of the Wistar rats in each of the groups were examined and the following were observed:

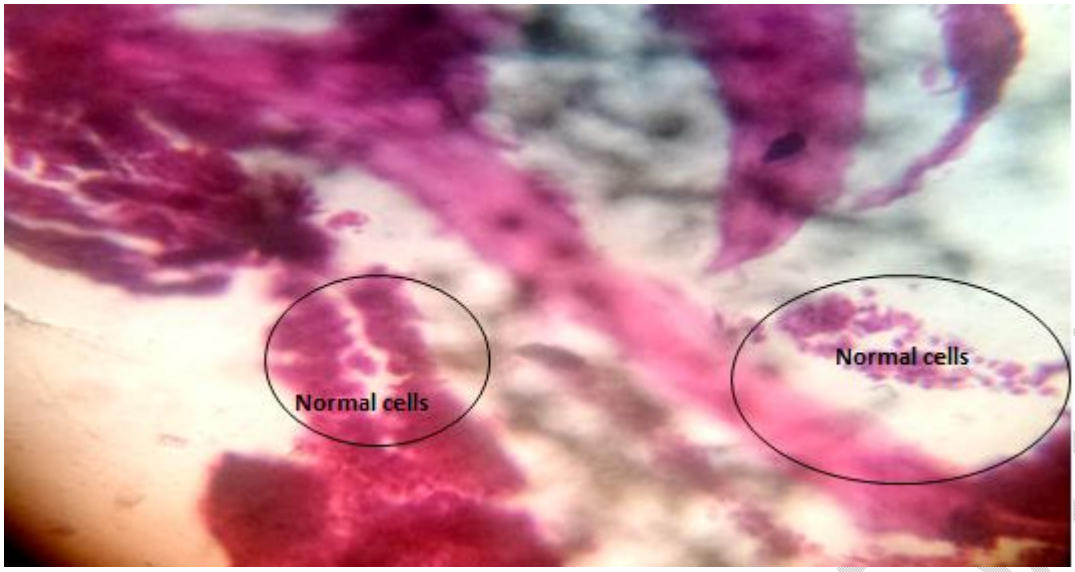

Plate 1: Photomicrograph of bone marrow of Wistar rats in group (A) showing clustered normal cells (circles) with clearly visible connective tissues. H&E. mag. 400X.

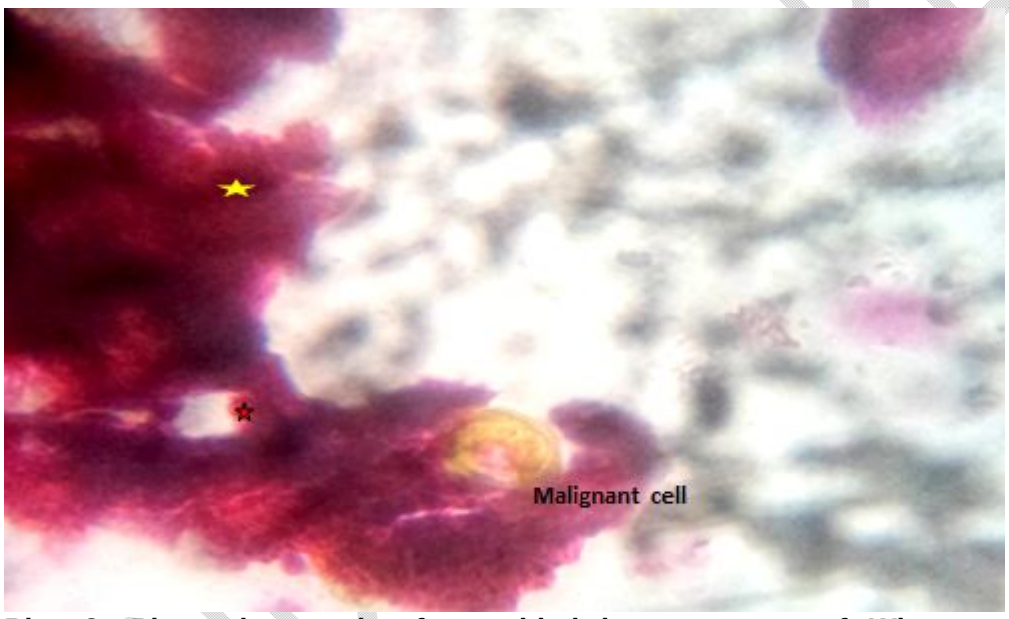

Plate 2: Photomicrograph of atrophied bone marrow of Wistar rats in group (B) showing myelodysplastic syndrome (yellow star) together with clear appearance of tumor cell (red star) (lymphoid progenator cells) which usually has yellow appearance. H&e. Mag. 400x.

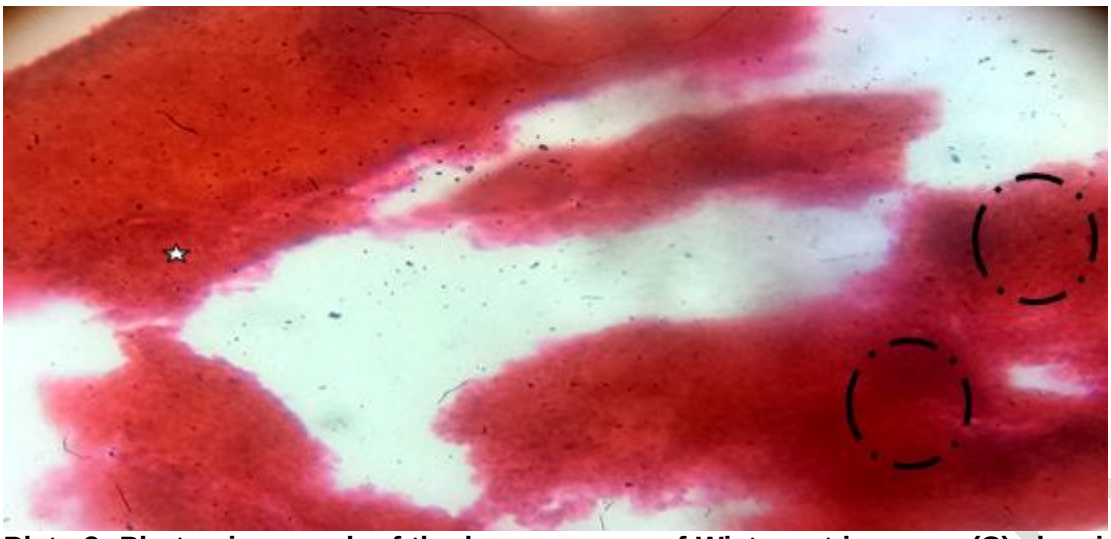

Plate 3: Photomicrograph of the bone marrow of Wistar rat in group (C) showing normal clustered cells with the presence of megakaryocytes and hematopoietic chords. H&E. mag 400X.

Plate 4: Photomicrograph of the bone marrow of Wistar rat in group (D) showing normal clustered cells with the presence of megakaryocytes and hematopoietic chords. H&E. mag. 400X.

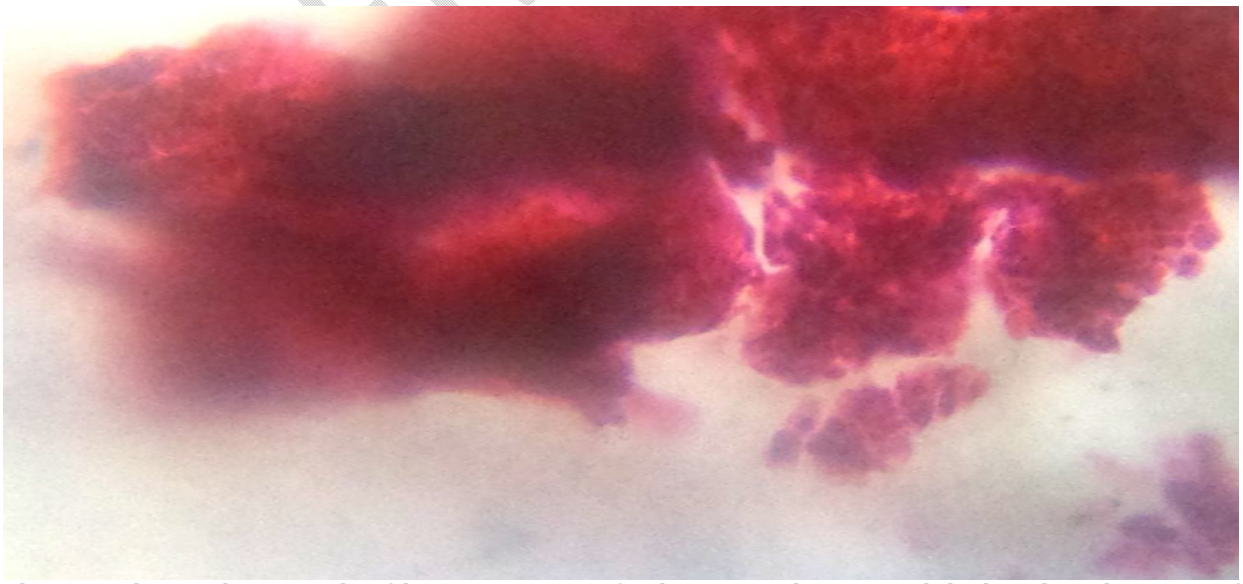

Plate 5: Photomicrograph of bone marrow of Wistar rats in group (E) showing damage of the blood forming cells in the bone marrow and this condition can be referred to as myelodysplastic syndrome mainly of secondary origin. H&E. mag. 400X.

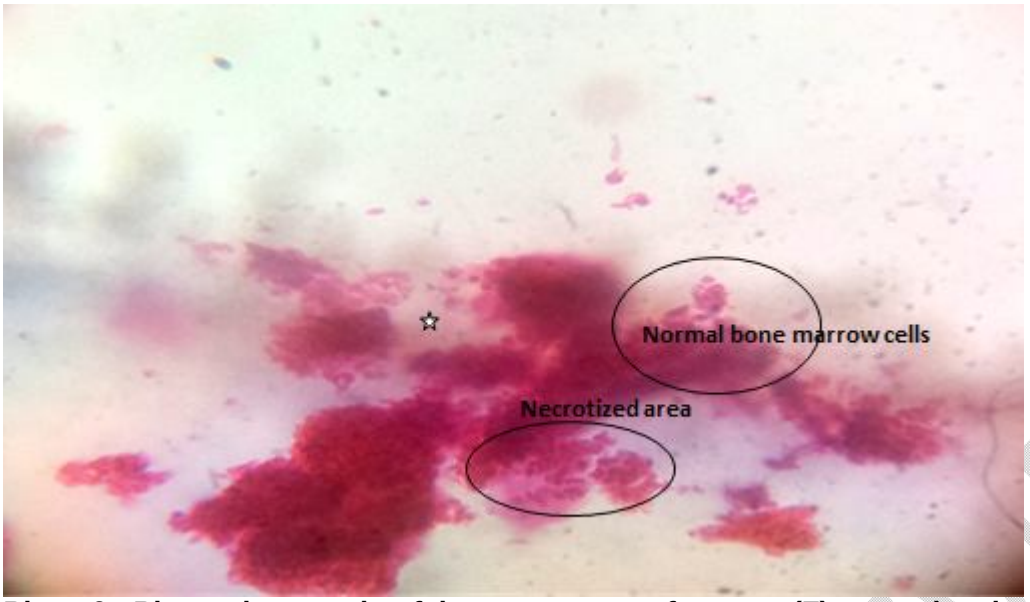

Plate 6: Photomicrograph of bone marrow of group (F) rats showing apparent normocellular (circles) bone marrow with slight loss of some hematopoietic cells which appears quite necrotized (star). H&E. mag. 400X.

DISCUSSION

In this study, the phytochemical analysis of the aqueous leave extract of *Ocimum gratissimum* revealed the presence of tannins, steroids, terpenoids, flavonoids and cardiac glycosides. Anthraquinones was also detected in the aqueous leave extract while steroids were absent (Table 2). Similar result to this was observed by Okoye [13] and Afolabi [14].

de Vasconcelos [15] reported that the phytochemical constituents of a plant are largely affected by the type of soil and environment in which the plant is grown, this could therefore be the reason why in contrast to the result of this study, Akinmoladun [16] reported the presence of steroids.

The high content of flavonoid and alkaloids in the leave extract could be the reason for the protective activity observed in the leave extract. Alkaloids are known to play some metabolic role and control development in living system. They also have a protective role in animals [8]. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anticancer activity [17]. The presence of alkaloid and flavonoid in this study therefore could be the reason for the protective actions of *Ocimum gratissimum*. Also flavonoid could have also been the reason for the reduction in the oxidative cell damage reported in this study. This is in agreement with the studies carried out by Thabrew [18], Halliwell and Gutteridge [19].

Histological results in the Normal control group showed normal cells with clearly visible connective tissues and it is therefore in agreement with the study made by Travlos [20]. The atrophied bone marrow seen in the rats induced with bone marrow suppression is indicative of myelodysplastic syndrome. This is as a result of the myelotoxicity activity of cyclophosphamide and its ability to cause bone marrow lesions, as also recorded by Travlos [20]. The normal clustered cells in the rats that was treated with only extracts implies that aqueous extract of *Ocimum gratissimum* do not have any toxic effect on the bone marrow cells of Wistar rats.

Administering the rats with both the leave extract and cyclophosphamide brought about a protective effect against the cyclophosphamide induced myeolotoxicity on the Wistar rats. This was evident in the treated rats which showed reduced cell death in their histo-architecture. This might be as a result of the presence of alkaloids and flavonoids in the extract. According to Onwueyiagba [17], McMahon [21] and Okoye [13], flavonoids and alkaloids are free radical scavengers, super antioxidants and potent water soluble agents which prevent oxidative cell damage and have strong anticancer activity.

Chemotherapy-induced anemia is one of the most common side effects experienced by cancer patients, occurring in approximately 70-90% of those undergoing treatment for the disease [22]. This is because chemotherapeutic drugs kill rapidly dividing cells in the body including cancer cells and normal cells which include red blood cells at the same time suppress bone marrow ability to produce new ones, resulting in decrease of blood Hb level [11]. In this study, it was observed that gavaging the animals with *Ocimum*

gratissimum extract enhanced the Hb level, platelet count, TWBC, PCV and RBC count which was depleted by cyclophosphamide treatment in the cyclophosphamide control group. The effect of this plant extract on the haematological parameters may be due to free radical scavenging activity or interference with the formation of the active metabolite of cyclophosphamide through the inhibition of cytochrome p-450 enzyme system [11].

CONCLUSION

Aqueous extracts of *Ocimum gratissimum* leaf possess a protective effect against cyclophosphamide induced bone marrow toxicity and consequently justifies the use of this extract as a tonic in traditional medicine.

CONSENT

It is not applicable.

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