

Effect of selected plants on Haematological parameters of DMBA-induced breast cancer of albino rats

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Abstract

The present study was aimed to investigate the effect of selected plants on haematological parameters of DMBA induced breast cancer of albino rats. Sixty-three female albino rats were used for the study and they were grouped into nine groups of seven animals each. Group I (control) was fed with normal feed. Groups II to IX, were orally administered 20mg DMBA/kg to induce tumour. Group II was untreated; Group III was treated with tamoxifen(6.6mg/kg). Groups IV- IX were treated with 500mg/kg *Sorghum vulgare*, 1000mg/kg *Sorghum vulgare*, 500mg/kg *Eremomastax polysperma*; 1000mg/kg *Eremomastax polysperma*, 500mg/kg *Brillantaisia owariensis* and 1000mg/kg *Brillantaisia owariensis* respectively. Treatment with the different aqueous concentrate demonstrated significant increase ($p \leq 0.05$) of haemoglobin, and packed cell volume levels of the treated groups when compared to the DMBA group. There was a decrease in Neutrophil, N/L, P/L and platelets level in the treated groups when compared to the DMBA group (though not significant, $p \leq 0.05$). The present study reveals that the plants might be able to reduce anaemia posed as a result of breast cancer.

Keywords: Breast Cancer; Haematological Parameters; DMBA

Introduction

Therapeutic plants are various plants used in herbal-treatment and have curative actions [1]. The plant that contains certain bioactive components has been confirmed to have anticancer activities [2]. *Sorghum vulgare* is of the Poaceae (grass) family and have been known to contain pharmacological compounds [3]. *Sorghum vulgare* possess antioxidant, anti-inflammatory, anti-proliferative (on cancer cells) activities, chemoprotective, haematopoietic and hepatoprotective properties [4]. Another group of annotation is the Acanthaceae group of which *Eremomastax polysperma* and *Brillantaisia owariensis*, stake in the family. *E.*

polysperma have been revealed to have anti-anaemic and antidiabetic effects [5], while *B. owariensis* have antimicrobial effect [6]. These plants have been demonstrated to possess flavonoids, saponins, kaempferol and high concentrate of ribalinidine (an alkaloid) [7].

Mammary cancer is a disease condition where normal cell control mechanism that manages the survival of the cell, its proliferation and its differentiation is lost. In women around the globe, it is the paramount kind of cancer [8]. Breast cancer can begin in different areas of the breast – the ducts, the lobules, or in some cases, the tissue in between. There are several diverse types of

breast malignancy, with different stages (spread), aggressiveness, and genetic makeup [9]. Regardless of the numerous approaches in the treatment of breast cancer such as surgery, chemotherapy and radiation, metastatic disease still remains a great clinical challenge [10].

The assessment of haematological parameters can be a pinpointer of adverse effects of foreign compounds on the blood constituents of an animal [11]. Anaemia is a common disease faced in cancer patients (such as cervical, ovarian, and endometrial cancer) and, as a result, anaemic patients suffer from shortness of breath, fatigue, and shrunk energy. Haemoglobin (Hb) and packed cell volume (PCV) are indirectly connected with raised risk of cardiac failure in cancer patients [12]. Furthermore, systemic inflammatory response has been identified to affect survival in a number of malignancies, of which white blood cells are key mediators in this response. In addition, angiogenesis a crucial step for tumour growth, progression and metastasis, requires the involvement of platelets [13,10]. The present study is aimed to investigate the effect of selected plants on haematological indices of DMBA induced breast cancer of albino rats.

Methodology

Collection of Plant sample

The plants; *Sorghum vulgare* leaf sheath was bought in mile 3 market while *Eremomastax polysperma* and *Brillantaisia owariensis* were gotten from a farm at Rumokoro (Lat 4.88999; long 6.96922) all in Port Harcourt, Nigeria. The plants were identified with voucher numbers: UPH/V/1325 (*Brillantaisia owariensis*), UPH/V/1326 (*Sorghum vulgare*, synonyms *Sorghum bicolor*) and UPH/V/1346 (*Eremomastax polysperma*). They were dried and ground into fine powder with a blender and stored in an air tight container.

Preparation of aqueous extract of the plants

The plants were ground and the powder was macerated in distilled water for 12hrs (1kg/l). The macerate was filtered using Whatman filter paper (No 3), and the filtrate was concentrated using a rotary evaporator (60°C) to obtain concentrated crude extract. The extract was stored in a freezer until further use.

Experimental animals

Sixty-three albino rats were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. The animals were distributed randomly into nine groups of seven animals each. They were housed and fed *ad libitum* with water and growers mash and was allowed for a week for acclimatization. The experiment

was conducted for a period of 14weeks and the animals were treated as follows:

- I. Control: normal rats fed with normal rat feed
- II. DMBA: Carcinogenic control, animals given 20mg/kg of 7,12-dimethyl benz(a)anthracene
- III. DMBA STD: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with a standard drug (tamoxifen 6.6mg)/kg
- IV. DMBA SVL500: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with 500mg/kg of *Sorghum vulgare* leaf sheath.
- V. DMBA SVL1000: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with 1000mg/kg of *Sorghum vulgare* leaf sheath.
- VI. DMBA E.P.500: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with 500mg/kg of *Eremomastax polysperma*
- VII. DMBA E.P.1000: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with 1000mg/kg of *Eremomastax polysperma*
- VIII. DMBA B.W. 500: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with 500mg/kg of *Brillantaisia owariensis*
- IX. DMBA B. W. 1000: animals given 20mg of 7,12-dimethyl benz(a)anthracene and treated with 1000mg/kg of *Brillantaisia owariensis*

Induction of cancer

Mammary gland tumours were induced by a single dose of 20mg of 7,12-dimethylbenz(a)anthracene (DMBA) diluted in soy oil (1 mL) given intragastrically by gavage. Physical examinations were performed monthly. Each rats' mammary gland was checked by inspection, touching, and palpation. At the 8th week after DMBA administration, biopsy test was done to ascertain that the tumours formed are cancerous [14]. The Mammary tumours was cut and representative fragments of the tumours was fixed in 10% formaldehyde and paraffin embedded. The blocks were sectioned every 5 mm, and slides was prepared with haematoxylin-eosin stain and examined using light microscopy [15,14].

After the induction of cancer (and left for 8weeks before treating with the extracts), the animals were treated for the next six weeks with aqueous extract of different plants and the standard drug. The first group was the control; group II received 20mg DMBA /Kg to induce cancer; Group III received 20mg DMBA/kg and treated with tamoxifen (6.6mg/kg). Groups IV, V, VI, VII, VIII and IX received 20mg DMBA and given dissimilar concentration of aqueous extract of the various plants.

The administration of all drugs was orally done for duration of 6 weeks. At the expiration of the study, the rats were weighed, fasted overnight and sedated by exposure to chloroform. They were sacrificed and their blood samples and tissue was collected for haematological analysis.

Haematological Analysis

Red blood cell was determined and the red cells counted in the small squares and read as the number of cells per litre using x 10 objective lens. Mean cell haemoglobin concentration (MCHC) was calculated as the ratio of Hb/PCV; Mean cell volume (MCV) as PCV/RBC and Mean cell haemoglobin (MCH) as Hb/RBC. Packed cell volume (PCV) was read after centrifuging the blood sample using the micro-haematocrit reader. White blood cell was determined and the cells were counted microscopically using an improved Neubauer counting chamber and the number of WBC per litre of blood was calculated. Platelets were tested utilizing an enhanced neubauer ruled count chamber and the quantity of platelets per litre of blood was numbered.

Statistical Analysis

Data-information was taken as mean \pm SEM, and subjected to One-way Analysis of Variance (ANOVA) and T-test utilizing Statistical programming SPSS. A level of $p \leq 0.05$ was seen as statistically significant.

Results

The results of the erythropoietic indices of DMBA treated albino rats, given aqueous concentrate of the different plants are shown on Table 1.

From Table 1 the PCV level and haemoglobin concentration of all treated groups were fundamentally huge ($p \leq 0.05$) than the control. While when contrasted to DMBA untreated group, they were higher, though not significantly significant ($p \leq 0.05$).

Table 2 demonstrates the red cell indices of DMBA treated albino rats, given aqueous concentrate of the different plants. There was no significant distinction

($p \leq 0.05$) in all the groups for MCHC, MCV and MCH concentrations.

The Result of White blood cells of DMBA treated albino rats, given aqueous concentrate of the different plants is appeared on Table 3. From Table 3, the WBC concentration of DMBA BW500 and DMBASVL1000 was altogether increased ($p \leq 0.05$) when differentiated to the control and DMBA un-treated groups. Other groups demonstrated no Essential distinction ($p \leq 0.05$) with the control and DMBA groups. For Neutrophil and Eosinophil level, there was no significant contrast ($p \leq 0.05$) in every-one of the groups. For L level, DMBASTD (73.33 \pm 3.37%), DMBASVL1000 (75.00 \pm 2.88%), DMBA BW500 (74.00 \pm 1.00%) had fundamentally higher values ($p \leq 0.05$) when differentiated to the DMBA-untreated groups while other groups demonstrated no significant contrast ($p \leq 0.05$). Whereas that of Monocyte count, DMBA BW500 group had an essentially diminished value ($p \leq 0.05$) when differentiated to the control and the DMBA untreated group.

Table 4 demonstrates the Blood platelets concentration of DMBA treated albino rats, given aqueous concentrate of the different plants (*S. vulgare*, *E. polysperma* and *B. owariensis*). There was noteworthy increase ($p \leq 0.05$) of the DMBA group (346.66 \pm 28.40 $\times 10^9$) when contrasted to the control (253.33 \pm 14.52 $\times 10^9$) and DMBASTD (233.33 \pm 8.81 $\times 10^9$) groups. The other groups showed no noteworthy difference ($p \leq 0.05$), although had lower values than the DMBA group.

Table 5 demonstrates the Neutrophil-lymphocyte proportion and platelet - lymphocyte proportion of DMBA administered albino rats, given aqueous concentrate of the different plants. There was a reduction (though not noteworthy, $p \leq 0.05$) of Neutrophil-lymphocyte ratio (N/L) of DMBASTD (0.37 \pm 0.02), DMBASVL1000 (0.30 \pm 0.03) and DMBABW500 (0.35 \pm 0.01) and other groups when contrasted to the DMBA-untreated group (0.46 \pm 0.01) except DMBA E.P.1000 which had similar values with the DMBA-untreated group. For the platelet-lymphocyte ratio (P/L), it was not significant ($p \leq 0.05$), in all the groups.

Groups	WBC(X 10 ⁹ /L)	N(%)	L(%)	E(%)	M(%)
Control	5.66 \pm 0.16 ^a	27.66 \pm 3.92 ^a	69.00 \pm 5.00 ^a	1.00 \pm 0.88 ^a	2.33 \pm 0.66 ^a
DMBA	5.60 \pm 0.49 ^a	29.66 \pm 1.45 ^a	65.00 \pm 2.08 ^a	1.66 \pm 0.57 ^a	3.66 \pm 0.33 ^b
DMBA STD	6.83 \pm 1.48 ^a	27.66 \pm 1.20 ^a	73.33 \pm 3.37 ^c	0.00 \pm 0.00 ^a	1.00 \pm 1.00 ^a
DMBASVL500	8.43 \pm 2.07 ^a	29.66 \pm 3.92 ^a	67.33 \pm 3.92 ^a	0.33 \pm 0.33 ^a	2.66 \pm 0.33 ^a
DMBASVL1000	10.23 \pm 0.64 ^{ab}	22.66 \pm 1.76 ^a	75.00 \pm 2.88 ^c	1.00 \pm 0.57 ^a	1.33 \pm 0.66 ^b
DMBA E.P 500	6.26 \pm 0.78 ^a	30.00 \pm 2.88 ^a	68.33 \pm 3.33 ^a	0.67 \pm 0.67 ^a	1.00 \pm 1.00 ^b

DMBA E.P1000	7.40±1.40 ^a	33.33±2.40 ^a	64.33±2.33 ^a	0.00±0.00 ^a	2.33±0.33 ^a
DMBA BW500	10.60±0.60 ^{ab}	26.00±0.57 ^a	74.00±0.57 ^c	0.00±0.00 ^a	0.00±0.00 ^{ab}
DMBA BW1000	9.75±2.75 ^a	31.00±0.57 ^a	65.00±0.00 ^a	1.00±1.00 ^a	3.00±0.00 ^a

Table 1: Erythropoietic indices of DMBA treated albino rats, given aqueous extract of the various plants. Values are stated as Mean ± SEM. Values in a column with alike alphabetical superscript do not contrast significantly $p \leq 0.05$.

Groups	PCV(%)	HB(g/L)	RBC($\times 10^{12}/L$)
Control	30.00±0.00 ^a	10.00±0.00 ^a	4.03±0.03 ^a
DMBA	34.00±1.15 ^a	11.33±0.37 ^a	4.50±0.25 ^a
DMBA STD	35.33±2.33 ^c	11.76±0.76 ^c	4.90±0.65 ^a
DMBASVL500	36.33±1.33 ^c	12.13±0.43 ^c	5.06±0.47 ^a
DMBASVL1000	35.00±2.88 ^c	11.66±0.95 ^c	4.93±0.69 ^a
DMBA E.P 500	35.33±1.85 ^c	11.76±0.62 ^c	5.06±0.47 ^a
DMBA E.P1000	36.66±1.76 ^c	12.20±0.58 ^c	5.33±0.60 ^a
DMBA BW500	37.00±0.07 ^c	12.38±0.20 ^c	5.33±0.08 ^a
DMBA BW1000	36.00±0.00 ^c	12.00±0.00 ^c	5.03±0.03 ^a

Table 2: Red cell indices of DMBA treated albino rats, given aqueous extract of the various plants. Values are stated as Mean ± SEM. Values in a column with alike alphabetical superscript do not contrast significantly $p \leq 0.05$.

Groups	MCHC(g/l)	MCV(μ L)	MCH(pg)
Control	3.00±0.00 ^a	7.57±0.20 ^a	2.48±0.02 ^a
DMBA	3.00±0.01 ^a	7.32±0.44 ^a	2.53±0.07 ^a
DMBA STD	3.00±0.00 ^a	7.24±0.38 ^a	2.44±0.15 ^a
DMBASVL500	2.99±0.00 ^a	7.19±0.45 ^a	2.42±0.13 ^a
DMBASVL1000	3.00±0.01 ^a	7.02±0.26 ^a	2.38±0.16 ^a
DMBAE.P500	3.00±0.00 ^a	6.97±0.42 ^a	2.33±0.08 ^a
DMBA E.P1000	3.01±0.00 ^a	6.93±0.02 ^a	2.32±0.14 ^a
DMBA BW500	2.99±0.01 ^a	7.20±0.09 ^a	2.32±0.01 ^a
DMBA BW1000	3.00±0.00 ^a	7.15±0.09 ^a	2.38±0.01 ^a

Table 3: White blood cell indices of DMBA treated albino rats, given aqueous extract of the various plants. Values are stated as Mean ± SEM. Values in a column with alike alphabetical superscript do not contrast significantly $p \leq 0.05$.

Groups	PL ($\times 10^9/L$)
Control	253.33±14.52 ^a
DMBA	346.66±28.40 ^b
DMBA STD	233.33±8.81 ^a
DMBASVL500	301.66±7.26 ^b
DMBASVL1000	313.33±23.33 ^b
DMBA E.P 500	296.66±43.70 ^b
DMBA E.P1000	300.00±40.41 ^b
DMBA BW.500	325.00±45.00 ^b
DMBA BW1000	335.00±45.00 ^b

Table 4: Blood platelets concentration of DMBA treated albino rats, given aqueous extract of the various plants. Values are stated as Mean ± SEM. Values in a column with alike alphabetical superscript do not contrast significantly $p \leq 0.05$.

Group	N/L	P/L
Control	0.40±0.08 ^a	3.96±0.47 ^a
DMBA	0.46±0.03 ^a	5.05±0.53 ^b
DMBASTD	0.37±0.02 ^c	3.43±0.29 ^a
DMBASVL500	0.44±0.08 ^a	4.52±0.31 ^a
DMBASVL1000	0.30±0.03 ^c	4.17±0.25 ^a
DMBA E.P.500	0.44±0.01 ^a	4.32±0.63 ^a
DMBA E.P.1000	0.52±0.05 ^a	4.73±0.59 ^a
DMBA BW500	0.35±0.01 ^c	4.41±0.32 ^a
DMBA BW1000	0.47±0.01 ^a	5.20±0.40 ^b

Table 5: Neutrophil-lymphocyte ratio and platelet –lymphocyte ratio of DMBA treated albino rats, given aqueous extract of the various plants.

Values are stated as Mean ± SEM. Values in a column with alike alphabetical superscript do not contrast significantly $p \leq 0.05$.

Discussion

Complete blood count reflects the response of cellular immunity in a patient with cancer [12]. The Erythropoietic indices of DMBA administered albino rats, given aqueous concentrate of the different plants is appeared on Table 1 & 2. For PCV and Hb concentration, there was significant increase ($p \leq 0.05$) in all the treated groups when contrasted with the control and DMBA group ($p \leq 0.05$). There was additionally increase in the RBC level in all groups when compared with the control and DMBA-untreated group, however not significant ($p \leq 0.05$). The MCHC, MCV and MCH levels demonstrated no striking significance ($p \leq 0.05$) in all of the groups. Red blood cell, haemoglobin and Packed cell volume are markers of anaemia, predicting the increased danger of cancer patients' death due to heart attack [16,11]. Anaemia is observed commonly in cancer patients, which might result from bleeding, nutritional deficiencies, damage of the bone marrow, tumour infiltration and malignancy [17]. The various plants have shown to have anti-anaemic properties; this might be due to the high concentration of iron present in these plants and thus reduce risk of death of patients with cancer from heart failure. This commensurate with the works of Zingue, et al. [18], which demonstrated that ethanolic extract of *Acacia seyal* on DMBA-induced mammary tumours were able to increase MCV, MCHC, MCH, RBC, PCV and haemoglobin concentration when contrasted to the DMBA un-treated animals.

Table 3 demonstrates the white blood cell of DMBA administered albino rats, given the different plants extract. There was a significant increase ($p \leq 0.05$) in the white blood cell level of animals treated with 1000mg/kg of *Sorghum vulgare* leaf sheath and 1000mg/kg *Eremomastax polysperma* when compared with the

control and DMBA-untreated group (Table 3). The DMBA group had the most reduced white blood cell level (however not significant, $p \leq 0.05$). A white blood cells (WBCs) level below normal, pose more at risk for infection [19], whereas a higher WBC counts increases risk of developing invasive breast [13]. There was no significant difference ($p \leq 0.05$) in the neutrophil values in spite of the fact that, DMBASVL1000, DMBABW500 had lower values than the DMBA and DMBASTD groups, indicating the functionality of these extracts, since a high neutrophil count decreases overall survival frequency of patients with cancer of the breast [13]. For the lymphocyte count, DMBASTD, DMBASVL1000 and DMBA BW500 had significantly higher values ($p \leq 0.05$) when compared to the DMBA-untreated group. DMBASVL500 and DMBAE.P.500 had higher values also, when compared to the DMBA-untreated group (though not significantly different, $p \leq 0.05$). Lymphocytes fights cancer, with low levels of it in the blood encourages a relapse and decreased survival rates, while higher lymphocyte count increases the overall survival [16]. These results are in commensurate with those of Chen, et al. [20], which showed that *Puerariae radix* was able to reduce WBC, Neutrophil and lymphocyte levels of DMBA-induced mammary cancer.

Table 4 shows the Blood platelets concentration of DMBA treated albino rats, given aqueous extract of the various plants. There was noteworthy increase ($p \leq 0.05$) in the DMBA-untreated group when compared with the control and DMBASTD group. The various treated group had lesser values than the DMBA-untreated group (however not significant, $p \leq 0.05$). A high platelet count is associated with prognosis of gynaecological cancers [13], since it secretes various growth factors and cytokines that promote angiogenesis; which is a critical step in breast cancer metastasis [21]. The result from the study showed

lower values of platelet count, indicating the usefulness of this extract in reducing blood platelet, and hence angiogenesis. This result is also in line with those of [18], that ethanolic extract of *Acacia seyal* on DMBA induced mammary tumours were able to reduce platelet concentration when compared to the DMBA-untreated group.

For Neutrophil-lymphocyte proportion (N/L), (Table 5) there was a significant decrease ($p \leq 0.05$) in DMBASTD, DMBAVL1000 and DMBA BW.500 when compared with the DMBA-untreated groups while DMBAVL500 and DMBAE.P.500 had lessened values (however not critical, $p \leq 0.05$). This show the concentrates would be utilized to enhance the survival rate of breast malignancy patients, since a lessened N/L expands the general survival rate [13]. For the platelet to lymphocyte proportion (Table 5) there was decrease in all the treated groups when analogized with DMBA-untreated group aside from DMBAE.P1000 group when compared with the control. This outcome proposes that the concentrates could likewise enhance incredibly the survival rate of breast malignancy patients, since a decreased P/L proportion protracts their life expectancy [22,10].

Conclusion

In conclusion, the aqueous extract of *Sorghum vulgare* leaf-sheath, *Eremomastax polysperma* and *Brillantaisia owariensis* leaves have been able to increase red blood cell indices and reduces platelets concentration and stabilize the white blood cell, enabling it to reduce anaemic burden caused by cancer and possibly inflammation.

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