

Effect of *Eremomastax polysperma* on some Tumour Markers of 7,12-Dimethylbenz(A) Anthracene Induced Mammary Carcinogenesis in Female Rats

Udiomine Brantley Akuru, Ndidi Nwachoko and Ekene Marcus Agbaraku

Department of Biochemistry, Rivers State University, Oroworukwo P.M.B. 5080, Port Harcourt, Nigeria

ABSTRACT

Background and Objective: Breast cancer is one of the leading causes of death in women in the world. Several medicinal plants have been studied for their anti-proliferative effect on breast cancer. This study investigated the protective properties of *Eremomastax polysperma* on some tumour markers of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female rats.

Materials and Methods: Twenty-eight female rats were randomly divided into 4 groups of 7 rats each including control, carcinogenic (DMBA induced rats only), Tamoxifen treated group and *Eremomastax polysperma* treated group. The female rats were induced orally with a single dose of DMBA (20 mg kg⁻¹) before treatment with *Eremomastax polysperma* (500 mg kg⁻¹) and Tamoxifen (6.6 mg kg⁻¹). At the end of 8 weeks of the study, blood samples were collected for the determination of tumour markers using the Enzyme-Linked Immune-Sorbent Assay (ELISA) method and the breast tissues were collected from each group for histological examination. Data were compared statistically between groups using ANOVA.

Results: From the result, carcinoembryonic antigen, estrogen receptor, Human C-Reactive Protein (hCRP) and human epidermal growth factor receptor (HER) levels were significantly ($p \leq 0.05$) increased in the carcinogenic group when compared to the other groups (both the extract and the drug). Histological studies of the treated group showed characteristic mammary histomorphology with typical lobular organization and few expressions of pyknotic cell mass when compared to the carcinogenic group which showed hyperdense lobular intercalation with numerous pyknotic cell mass, suggesting pathological alteration. **Conclusion:** The results suggest that the plant could attenuate mammary carcinoma by reducing the concentration of these tumour markers and also the growth of the tumour. The reduced cell growth or tumour formation suggests that *Eremomastax polysperma* extract may reduce the effect of mammary carcinogenesis.

KEYWORDS

Eremomastax polysperma, tumour markers, 7, 12-dimethylbenz(a)anthracene-induced, mammary carcinogenesis

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INTRODUCTION

Mammary carcinoma is the most malignant neoplasm in the female population¹. Early diagnosis of mammary carcinoma is crucial for effective treatment and positive prognosis². Although there are several treatments for breast carcinoma which include chemotherapy, surgery, hormone replacement therapy and radiation therapy³, they are quite expensive and might have some level of side effects. The use of herbs in the treatment of people with mammary carcinoma is considered a natural alternative, because some plants may have properties that naturally can treat mammary carcinoma³.

Medicinal plants are natural plant materials used in the treatment of diseases⁴. Natural plant products have shown promising results as anti-tumour and anti-cancer agents. This is due to the presence of constituents of natural products such as flavonoids, alkaloids, terpene and coumarins which have antioxidant and anti-inflammatory properties⁵.

Eremomastax polysperma is of the Acanthaceae family and has anti-anaemic and antidiabetic properties⁶. *Eremomastax polysperma* has shown significant benefit in the use of its extract in the management of female fertility⁷. Thus, this research was carried out to determine the protective properties of *Eremomastax polysperma* on some tumour markers of 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis in female rats.

MATERIALS AND METHODS

Study area: The study was done from April to June, 2019 in the Department of Chemistry, Rivers State University, Port Harcourt, Nigeria.

Collection and preparation of sample: *Eremomastax polysperma* was gotten from a farm at Rumokoro, Port Harcourt, Nigeria. The plant was identified with voucher number: UPH/V/1346 (*Eremomastax polysperma*). The plant was washed, sun-dried and ground into fine powder. The aqueous extract of the plant was obtained using the hot water extraction method as described by Yadav and Agarwala⁸. Finely dried powder of *Eremomastax polysperma* (5 g) was put in a beaker and 200 mL of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 40°C for 20 min and allowed to cool. The mixture was then filtered using filter paper. An aliquot of the filtrate was concentrated to paste to determine the concentration of the sample in mg mL⁻¹ and this was used to extrapolate the concentration for the remaining aqueous extract. The aqueous extract was then kept in a refrigerator for further use.

Experimental animals: Twenty-eight female rats were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt and distributed into four groups of seven animals each. They were housed and fed *ad libitum* with water and growers' mash and left for a week for acclimatization.

Experimental design: This study was carried out for eight weeks. The animals were divided into four groups. Group I serve as a control (animals were given normal feed and water), group II was the Carcinogenic control and the animals were administered 20 mg kg⁻¹ of 7,12-dimethyl benz(a)anthracene to induce tumour, group III (animals were given 20 mg kg⁻¹ of 7,12-dimethyl benz(a)anthracene and given a standard drug (Tamoxifen 6.6 mg kg⁻¹) while in group IV, animals were administered 20 mg kg⁻¹ of 7,12-dimethyl benz(a)anthracene and treated with *Eremomastax polysperma* (500 mg kg⁻¹).

Induction of tumour: Mammary tumour was induced by a freshly prepared single dose of 20 mg kg⁻¹ of 7,12-dimethylbenz(a)anthracene (DMBA) (Sigma Aldrich) diluted in soy oil (1 mL) and was given intragastrically by gavage method as described by Barros *et al.*⁹. After 4 weeks of induction, the animals were treated orally for the next 4 weeks with the aqueous extract of the plant and Tamoxifen. At the close of the study, the animals were fasted overnight, weighed and anaesthetized by exposure to chloroform. They were sacrificed and the blood samples were collected for biochemical analysis while the breast tissue was collected for histopathology.

Determination of tumour markers: Rats' Carcinoembryonic Antigen (CEA), estrogen receptor, Human C-Reactive Protein (hCRP) and Human Epidermal Growth Factor Receptor (HER) levels were determined using ELISA kits.

Histological determination: Breast tissue from the control and experimental groups was taken from the rats for histological examination. The mammary gland tissues were fixed in 10% formal saline and then embedded in infiltrating medium. This was cooled at room temperature and then dehydrated in alcohol. The tissues were prepared to form trimmed blocks and were sectioned at 7 microns to obtain tissue ribbon for staining and subsequently stained with haematoxylin/eosin. This was differentiated by dipping in 1% alcohol for 5 min. The slides were then dehydrated and cleared using alcohol and xylene, respectively. The slides were mounted in DPX (Dibutylphthalate Polystyrene Xylene) and captured microscopically at $\times 400$ magnification.

Statistical analysis: Statistical analysis of data was represented as Mean \pm SEM and subjected to One-way Analysis of Variance (ANOVA) using statistical software SPSS. A level of $p \leq 0.05$ was considered statistically significant.

RESULTS

The weight of DMBA-treated animals is shown in Table 1. There were variations between the groups in the various weeks but in the 8th week, the carcinogenic group (204.28 ± 8.12^b) had a significant increase in body weight of the animals when compared to other groups.

The effect of *Eremomastax polysperma* on some tumour markers of 7, 12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female rats is shown in Table 2. There was a significant increase ($p \leq 0.05$) in CEA level in the rats that received DMBA only (carcinogenic group) ($3.61 \pm 0.60 \text{ ng mL}^{-1}$) when compared to other groups. The ER, hCRP and HER levels of the carcinogenic group was also significantly higher than the other groups ($331.34 \pm 19.39 \text{ pg mL}^{-1}$, $13.41 \pm 1.77 \text{ ng mL}^{-1}$ and $12.76 \pm 1.75 \text{ ng mL}^{-1}$ for ER, hCRP and HER, respectively).

Table 1: Weight of DMBA-treated animals (g)

Groups	Control	Carcinogenic	Tamoxifen	<i>E. polysperma</i>
Week 1	77.50 \pm 8.81 ^a	165.71 \pm 7.82 ^b	162.85 \pm 6.06 ^b	165.71 \pm 7.82 ^b
Week 2	90.00 \pm 13.09 ^a	175.71 \pm 9.47 ^b	165.71 \pm 7.82 ^b	180.00 \pm 8.45 ^b
Difference in body weight between week 2 and 1	12.50	10.00	2.85	14.28
Week 3	110.00 \pm 12.53 ^a	165.71 \pm 7.82 ^b	158.57 \pm 5.53 ^b	182.85 \pm 8.92 ^b
Difference in body weight between week 3 and 2	20.00	-10.00	-7.14	2.85
Week 4	116.25 \pm 9.43 ^a	162.85 \pm 6.06 ^b	177.14 \pm 7.78 ^b	187.14 \pm 7.14 ^{ab}
Difference in body weight between week 4 and 3	6.25	-2.85	18.57	4.28
Week 5	123.75 \pm 9.98 ^{bc}	172.85 \pm 8.65 ^{ac}	198.57 \pm 9.36 ^{ab}	184.28 \pm 6.85 ^b
Difference in body weight between week 5 and 4	7.50	10.00	21.24	-2.85
Week 6	143.75 \pm 12.66 ^a	177.14 \pm 7.78 ^b	194.28 \pm 11.51 ^b	184.28 \pm 6.85 ^b
Difference in body weight between week 6 and 5	20.00	4.28	-4.28	0.00
Week 7	163.75 \pm 11.79 ^a	170.00 \pm 7.55 ^b	197.14 \pm 5.21 ^{ab}	184.28 \pm 6.85 ^b
Difference in body weight between week 7 and 6	20.00	-7.14	2.85	0.00
Week 8	176.25 \pm 8.22 ^a	204.28 \pm 8.12 ^b	182.85 \pm 8.92 ^a	185.71 \pm 9.22 ^a
Difference in body weight between week 8 and 7	12.50	34.28 [*]	-14.28	1.42

Table 2: Effect of *Eremomastax polysperma* on some tumour and inflammatory markers of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female rats

Groups	Control	Carcinogenic	Tamoxifen	<i>E. polysperma</i>
Carcinoembryonic antigen (CEA) (ng mL ⁻¹)	0.68 \pm 0.26 ^a	3.61 \pm 0.60 ^b	1.08 \pm 0.33 ^a	0.47 \pm 0.26 ^a
Estrogen receptor (ER) (pg mL ⁻¹)	184.84 \pm 30.24 ^a	331.34 \pm 19.39 ^b	134.45 \pm 34.33 ^a	175.27 \pm 51.55 ^a
Human C-reactive protein (hCRP) (ng mL ⁻¹)	3.70 \pm 1.62 ^a	13.41 \pm 1.77 ^b	2.78 \pm 0.49 ^a	3.85 \pm 1.159 ^a
Human epidermal growth factor receptor (HER) (ng mL ⁻¹)	5.16 \pm 1.13 ^a	12.76 \pm 1.75 ^b	6.86 \pm 2.30 ^a	4.53 \pm 2.57 ^a

Histopathology of the breast tissue: Figure 1-4 shows the histopathology of the breast tissue of the animals induced with tumours using DMBA and treated with the aqueous extract of *Eremomastax polysperma*. Figure 1 is the control and it shows characteristic mammary histomorphology with typical lobular organization and no apparent histopathology. Figure 2 is the carcinogenic group which was induced with tumour and not treated. It showed hyperdense lobular intercalation with numerous pyknotic cell masses suggesting pathological alteration. Figure 3 is the Tamoxifen group, in which the animals were induced with tumours and treated with Tamoxifen. Its histology showed characteristic mammary histomorphology with the typical lobular organization with few expressions of pyknotic cell mass. Figure 4 is the ETS group, where the animals were induced with tumours and treated with 500 mg kg⁻¹ of aqueous extract of *Eremomastax polysperma*. Its histology showed characteristic mammary histomorphology with the typical lobular organization with few expressions of pyknotic cell mass when compared with the carcinogenic group.

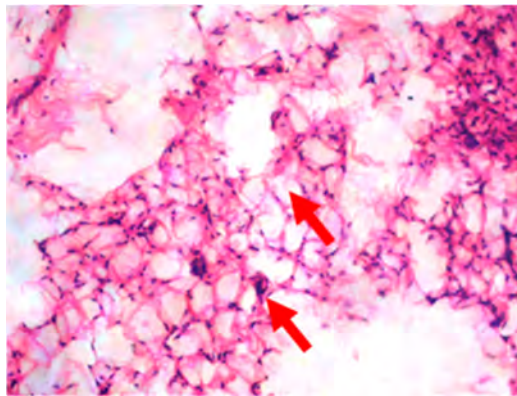


Fig. 1: Control group

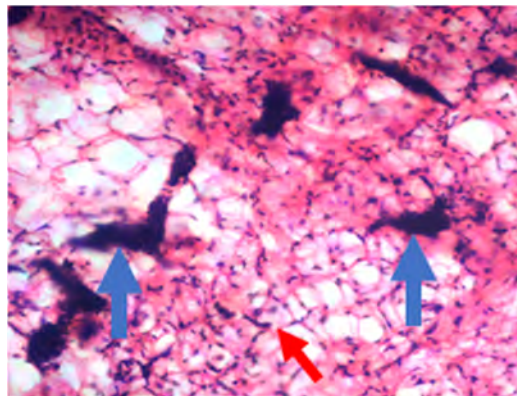


Fig. 2: Carcinogenic group

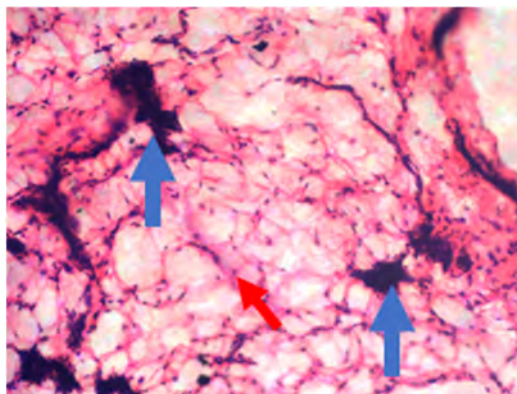


Fig. 3: Tamoxifen group

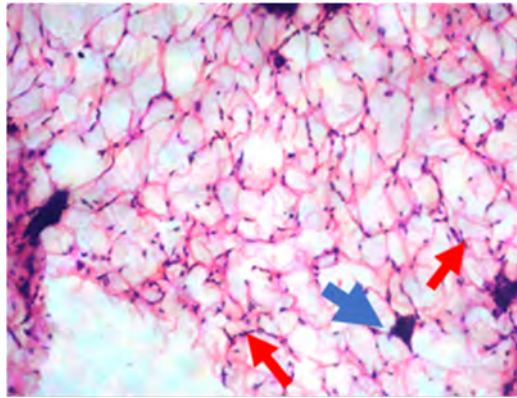


Fig. 4: ETS Group

DISCUSSION

Table 1 showed the result of the observed weekly weight measurement of the animals induced with tumours using DMBA. This is because obesity has an increased risk of developing several cancers including breast cancer¹⁰, especially in women who have been through menopause due to fatty acids which can provide estrogen even if the ovaries had stopped producing the female hormones³ and thus lead to accelerated tumour formation¹¹. There was a significant reduction and variations in the weight of animals that received *E. polysperma* (500 mg kg⁻¹) and the standard drug (Tamoxifen) when compared to the carcinogenic group and the control. Animals that received DMBA only (carcinogenic group) showed a significant increase in weight in the 8th week, which might be a result of the presence of the tumour formed.

The study also looked at the effect of *Eremomastax polysperma* on some tumour markers and inflammatory markers of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female rats as shown in Table 2. This is because tumour and inflammatory markers play a role in breast cancer, in that when found in the blood could pose that there might be a disease. The tumour markers studied were estrogen receptor, Human Epidermal Growth Factor Receptor 2 (HER 2) and Carcinoembryonic Antigen (CEA). The HER 2 positive overexpression accounts for about 15-20% of breast cancer¹² and it is linked with more aggressive disease, higher recurrence rate and increased mortality¹³. The HER 2 positives when activated cause epithelial cell growth and differentiation¹⁴. The estrogen receptor is one of the successful serum tumour markers of the breast. Estrogen receptor allows the hormone estrogen to bind to it and cause cellular proliferation, growth and differentiation. Measurement of the levels of ER in breast tumour tissue is useful as a prognostic indicator and in determining the probability of hormone-resistant breast cancer¹⁵. Carcinoembryonic antigen has been elevated in a wide range of malignancies including breast cancer. Reduced serum CEA levels are useful in predicting a positive response to systemic therapy in metastatic breast cancer¹⁶. The CEA levels are associated with metastatic cancer in breast cancer¹⁵. The C-Reactive Protein (CRP) is a commonly used marker for inflammatory status¹⁷. Chronic inflammation is associated with or may increase breast cancer¹⁸. The CRP's relationship with cancer is possibly due to chronic inflammation-associated oxidation damage which could initiate carcinogenesis by causing inactivating mutations in tumour suppressor genes or post-translational modifications in proteins involved in DNA repair or apoptotic control¹⁹.

From the result, the Carcinoembryonic Antigen (CEA), Estrogen Receptor (ER) and Human Epidermal Growth Factor Receptor (HER) concentrations of all the groups were significantly lower than the carcinogenic group. This indicates the extract was able to reduce the level of the tumour markers and thus possibly reduce breast cancer incidence. The Human C-Reactive Protein (hCRP) protein concentration for all the groups was significantly lower than the carcinogenic group, indicating that the extract might be able to reduce inflammation.

Histological study of the breast tissue is shown in Fig. 1-4. Figure 2 shows the carcinogenic group having hyperdense globular intercalation with numerous pyknotic cell mass which suggests a neoplastic transformation indicating a DMBA-induced cell proliferation about 8 weeks after induction with tumour using DMBA. The development of tumours in the breast region in the experimental rats supports the study that animal models have helped demonstrate different classes of chemicals to act as initiators and induce mammary cancer²⁰. The other induced groups which were treated showed few expressions of pyknotic cell mass. The group treated with *E. polysperma* (Fig. 4), showed characteristic mammary histomorphology with typical lobular organization and few expression pyknotic cell mass. The protection of the plant against breast cancer may be achieved as a consequence of its antioxidant and anti-inflammatory properties⁷.

CONCLUSION

The present study showed that the aqueous extract of *Eremomastax polysperma* leaves can be used as an anti-proliferative measure against breast cancer in female albino rats. The histological result revealed the breast tissue of the *Eremomastax polysperma* treated rat has a normal globular organization with few expression of cell mass at the lobules when compared to the untreated group which had a neoplastic transformation. Hence, concluding the extract of the plant might have efficacy for the treatment of breast cancer. The knowledge obtained from the study serves as a resource base and can be scientifically exploited for future research in breast cancer chemoprevention.

SIGNIFICANCE STATEMENT

This study discovered that *Eremomastax polysperma* had some positive effect on some tumour markers (such as CEA, ER and HER) of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female rats that can be beneficial for possible drug exploration. This study will help the researcher to uncover the critical areas of this plant extract on estrogen receptor, carcinoembryonic antigen, human C-reactive protein and human epidermal growth factor receptor and histopathology of the breast tissue. Thus a new theory on this plant used to treat the proliferation of breast tissue may be arrived at.

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