

Anti-Proliferative Properties of Aqueous Extracts of Selected Medicinal Plants on DMBA-Induced Benign Breast Disease in Wistar Rats

¹Udiomine Brantley Akuru and ²Benjamin Achor Amadi

¹Rivers State University, Port Harcourt, Nigeria

²University of Port Harcourt, Port Harcourt, Nigeria

ABSTRACT

Background and Objective: Benign breast diseases such as hyperplasia, sclerosing adenosis, intraductal papillomas and radial scar are non-cancerous tumours on the breast. Although, benign diseases are not life-threatening, some are linked with a higher risk of developing mammary cancer. Hence, a plant-based approach is necessary since some of these herbal plants have been shown to have a positive effect in acting as antioxidants and might thus reduce the burden of mammary cancer to some extent. Biochemical effect of aqueous extract of selected medicinal plants on 7,12-dimethylbenz(a)anthracene-induced benign breast disease in Wistar-rats was investigated. **Materials and Methods:** Wistar rats were used for this study, they were sixty-three in number, all females and weighted 120-250 g. They were assembled into nine different groups of seven animals each. Group I (control) was given normal feed. The other 8 groups were given 20 mg kg⁻¹ DMBA orally to induce benign breast disease, group 2 (was not treated), group 3 (was given 6.6 mg kg⁻¹ of tamoxifen) and groups 4-9 were given various concentrations of the three different plants at 500 and 1000 mg kg⁻¹. **Results:** Treatment with the various concentrate revealed a significant reduction ($p \leq 0.05$) of cancer antigen 15-3, prolactin, estrogen and malondialdehyde, levels when compared to the DMBA group. The glutathione, catalase and superoxide dismutase, levels of the treated groups was significantly higher ($p \leq 0.05$) than the DMBA group. Histological changes on the breast tissue caused by DMBA-induced damage was decreased by the different plant's. **Conclusion:** The present investigation reveals that the plants have properties that can lessen benign breast disease and conceivably breast cancer disease.

KEYWORDS

Benign breast disease, breast cancer, plant's extract, oxidative properties, hormonal prolife, breast histopathology, lipid profile

Copyright © 2022 Udiomine Brantley Akuru and Benjamin Achor Amadi. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

INTRODUCTION

Benign breast disease is all non-malignant conditions of the breast¹. There is an established relationship that benign breast is a risk factor for breast cancer². Breast cancer is a disease characterised by a loss in the normal control mechanism that governs cell survival, proliferation and differentiation of the breast³. The risk of developing breast cancer increases gradually with cell proliferation and atypia to more than



4-fold². Although, there had been technological advancement in the diagnosis and treatment of breast cancer, new strategies are needed to combat this disease⁴.

Breast cancer grows in a hormone-rich environment and this affects its biological features and its clinical behaviour⁵. Estrogen is a sex steroid hormone, that controls several physiological functions in many target tissues extending from regulation of the menstrual cycle and reproduction to modulation of bone density, brain function and cholesterol mobilization⁶. Estrogen plays a cogent role in promoting the proliferation of both normal and neoplastic breast epithelium⁷. Progesterone levels, on the other hand are associated with a lower proliferation rate⁵. Although, progesterone in combination with estrogen pre-treatment can stimulate cell-proliferation of progesterone receptor cells (PR-cells) and hormone receptor cells (HR-cells)⁸. Prolactin affects cellular growth, angiogenesis, proliferation and differentiation of breast tissue. It also affects the initiation and maintenance of milk production after priming of the breast tissue by estrogen and progesterone⁹. Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are glycoproteins hormone that controls crucial steps in human reproduction. They exert these activities through their receptors FSH receptor and LH receptor in the ovaries and testes, where, they control steroidogenesis and gametogenesis. Numerous studies suggest that the FSH receptor and LH receptor are expressed in several cancers, which might indicate that gonadotropins may encourage the development and progression of these diseases⁴.

Oxidative stress is also an important factor in the generation of cancer, they cause gene mutation and have effects on signal transduction and transcription factors. Oxidative stress causes damages to DNA, phospholipids, proteins and carbohydrates on the cell membrane¹⁰.

The whole, essence of using plants was to see a more natural root of treating benign breast disease and possibly breast cancer since plants have been a reservoir of natural chemicals that provide chemo-protective abilities against cancer¹¹. Hence, the objective of this study was to investigate the biochemical effect of aqueous extract of selected medicinal plants (*Sorghum vulgare* leaf-sheath, *Brillantasia owariensis* and *Eremomastax polysperma* leaves) on 7,12-dimethylbenz(a)anthracene-induced benign breast disease in Wistar-rats.

MATERIALS AND METHODS

Framework of the study: The study was done from June to October, 2017 in the Department of Chemistry, Rivers State University and Department of Biochemistry, University of Port Harcourt, Nigeria.

Preparation of aqueous extract of the plants: The plants were dried and ground into a fine powder, the powder was extracted using a slight modification of Dahiru *et al.*¹².

Experimental animals: Sixty-three albino rats (all female) who weighed 120-250 g, were obtained from the animal house of the Department of Biochemistry, The University of Port Harcourt and they were assembled into nine different groups of seven animals each. They were housed and was allowed for 7 days for adaptation.

Experimental design: The investigation was done for 14 weeks in which n = 7 as presented in Table 1 (showing the groups for experimental design and how the drugs were administered).

Induction of cancer: Induction of breast tumours was done by a lone dose of 20 mg kg⁻¹ of 7,12-dimethylbenz(a)anthracene (DMBA) (sigma-aldrich) diluted in soy oil (1 mL) and was intragastrically given by gavage. Physical examinations were made once a month. Individually rat's breast was examined by observing the breast, touching it and palpating the breast^{13,18}. This was left for 8 weeks afore the administration of the plant's extract.

Table 1: Groups for experimental design

Groups (n = 7)	Treatments
Control	Normal rats fed with normal rat feed
DMBA	Carcinogenic control: Animals induced with tumour using 20 mg kg ⁻¹ of 7,12-dimethyl benz(a) anthracene ¹³
DMBA STD	Animals given 20 mg kg ⁻¹ of 7,12-dimethyl benz(a)anthracene ¹³ and treated with a standard drug (tamoxifen 6.6 mg kg ⁻¹) ¹⁴
DMBA GLC500	Animals were given 20 mg kg ⁻¹ of 7,12-dimethyl benz(a)anthracene ¹³ and treated with 500 mg kg ⁻¹ of <i>Sorghum vulgare</i> leaf sheath ¹⁵
DMBA GLC1000	Animals were given 20 mg kg ⁻¹ of 7,12-dimethyl benz(a)anthracene ¹³ and treated with 1000 mg kg ⁻¹ of <i>Sorghum vulgare</i> leaf sheath ¹⁵
DMBA <i>E. poly</i> 500	Animals given 20 mg kg ⁻¹ of 7,12-dimethyl benz(a)anthracene ¹³ and treated with 500 mg kg ⁻¹ of <i>Eremomastax polysperma</i> leaf ¹⁶
DMBA <i>E. poly</i> 1000	Animals given 20 mg kg ⁻¹ of 7,12-dimethyl benz(a)anthracene ¹³ and treated with 1000 mg kg ⁻¹ of <i>Eremomastax polysperma</i> leaf ¹⁶
DMBA BW500	Animals were given 20 mg kg ⁻¹ of 7,12-dimethyl benz(a)anthracene ¹³ and treated with 500 mg kg ⁻¹ of <i>Brillantaisia owariensis</i> leaf ¹⁷
DMBA BW1000	Animals were given 20 mg of 7,12-dimethyl benz(a)anthracene ¹³ and treated with 1000 mg kg ⁻¹ of <i>Brillantaisia owariensis</i> leaf ¹⁷

Succeeding the initiation of the tumour using DMBA, the animals were orally administered the aqueous extract of the different plants and the standard drug for the next 6 weeks. At the close of the study, the rats were weighed, fasted overnight and anaesthetized by exposure to chloroform. They were sacrificed and their blood samples were collected for biochemical investigation and breast tissue was collected for histological study.

Determination of CA 15-3: The CA 15-3 was done and absorbance was read at 450 nm in a microplate reader or ELISA machine¹⁹ [Enzyme Linked Immunosorbent Assay (ELISA) Monobind Inc., Lake Forest CA92630, USA].

Determination of hormonal profile: Estrogen, progesterone, prolactin, luteinizing hormones and follicle-stimulating hormone concentrations were determined using ELISA kits (Monobind Inc., 100 North points drive Lake Forest, 92630 USA).

Determination of oxidative markers: Malondialdehyde (MDA) concentration was determined and its absorbance read at 532 nm in a spectrophotometer (Genesys 10-S, USA). Glutathione (GSH) was analyzed and absorbance was read at 412 nm. Catalase activity was determined at 240 nm. Glutathione S-Transferase (GST) activity was assayed and the absorbance was read at 340 nm. Superoxide Dismutase (SOD) activity was determined and Glutathione Peroxidase (GPx) activity was determined at 412 nm.

Lipid profile: Total cholesterol, high-density lipoproteins and triglyceride were determined using a microplate reader at 540 nm. Low-density lipoprotein was determined and Very-Low-Density Lipoprotein cholesterol (VLDL) was also estimated.

Histological studies: Histopathology of the mammary gland was carried out and the breast tissue was examined using light microscopy^{13,18}.

Statistical analysis: Data were represented as Mean ± SEM and subjected to One-way Analysis of Variance (ANOVA) using Statistical software SPSS.

RESULTS

The result for the weight of DMBA-induced benign breast disease in female Wistar rats is shown in Table 2. There was no significant difference in the animals after 1 month of inducing the animals with tumour using DMBA in all the groups except for DMBA STD (141.67 ± 8.33 g for 1 month after inducement

Table 2: Weight of DMBA-induced benign breast disease in female Wistar rats

Groups	Initial weight	One month after induction with tumour using DMBA	Difference in weight between 1 month after induction with tumour using DMBA and initial weight		Two months after induction with tumour using DMBA	Difference in weight between 2 months after induction with tumour using DMBA		One month and 2 weeks after treatment with aqueous extract of the plants	Difference in weight between 1 month and 2 weeks after treatment with aqueous extract of the plants and 2 months after induction	
			tumour using DMBA	using DMBA and initial weight		with tumour using DMBA	with tumour using DMBA		1 month and 2 weeks after treatment with aqueous extract of the plants	1 month and 2 weeks after treatment with aqueous extract of the plants and 2 months after induction
Control	133.33±5.27 ^{bc}	120.83±4.16 ^a	-12.5±9.68	145.83±7.68 ^a	2.50±9.68*	175.00±9.12 ^a	29.16±9.68*			
DMBA	229.16±7.68 ^b	216.67±5.27 ^{bc}	-12.5±10.70	212.50±8.53 ^{bc}	-4.16±10.70	216.68±8.33 ^b	4.16±9.68			
DMBASTD	220.83±11.93 ^b	141.67±8.33 ^a	-79.16±12.50*	145.83±7.68 ^a	4.16±12.50	150.00±6.45 ^a	4.16±12.50			
DMBA GLC500	204.16±7.68 ^{ab}	220.83±7.68 ^{bc}	16.66±18.44	212.50±16.77 ^{bc}	-8.33±18.44	212.50±16.77 ^b	0.00±18.44			
DMBA GLC1000	183.33±5.27 ^{abc}	158.33±5.27 ^a	-25.00±12.07	158.33±5.27 ^a	0.00±12.07	175.00±14.43 ^a	16.66±12.07			
DMBA E. poly500	150.00±0.00 ^{bc}	145.83±11.93 ^a	-4.16±11.02	154.16±7.68 ^a	8.33±11.02	175.00±6.45 ^a	20.83±11.02			
DMBA E. poly1000	158.33±5.27 ^{bc}	150.00±6.45 ^a	-8.33±9.94	145.83±7.68 ^a	-4.16±9.94	158.33±8.33 ^a	12.50±9.94			
DMBA BW500	150.00±0.00 ^{bc}	183.33±16.67 ^{bc}	33.33±16.29	208.33±5.27 ^{bc}	-12.50±16.29	204.16±15.02 ^b	-4.16±16.29			
DMBA BW1000	150.00±0.00 ^{bc}	191.67±12.36 ^{bc}	41.66±16.91*	208.33±13.90 ^{bc}	16.66±16.94	220.00±15.00 ^b	11.66±16.91			

Values are expressed as Mean±SEM, values in a column with the same alphabetical superscript do not differ significantly (p<0.05) and values in a row with asterisks differ significantly (p<0.05)

Table 3: Concentration of CA 15-3 of DMBA-induced benign breast disease in female Wistar rats

Groups	Concentration of CA 15-3 (U mL ⁻¹)
Control	7.05±0.89 ^a
DMBA	15.56±0.32 ^b
DMBA STD	7.28±0.56 ^a
DMBA GLC500	9.66±0.62 ^a
DMBA GLC1000	10.91±0.38 ^a
DMBA <i>E. poly</i> 500	10.05±1.75 ^a
DMBA <i>E. poly</i> 1000	14.48±6.24 ^b
DMBA BW500	9.84±2.26 ^a
DMBA BW1000	32.07±11.77 ^{abc}

Values are expressed as Mean±SEM, values in a column with the same alphabetical superscript do not differ significantly ($p \leq 0.05$), means control, DMBA and DMBA STD is significantly different from other groups

Table 4: Hormonal profile of DMBA-induced benign breast disease in female Wistar rats, given various plant's extract

Groups	Estrogen (pg mL ⁻¹)	Progesterone (ng mL ⁻¹)	Prolactin (ng mL ⁻¹)	Luteinizing hormone (mIU mL ⁻¹)	Follicle stimulating hormone (mIU mL ⁻¹)
Control	61.33±4.37 ^a	9.35±0.78 ^a	1.55±0.15 ^a	2.66±0.19 ^a	0.80±0.28 ^a
DMBA	83.33±3.84 ^b	7.99±1.39 ^a	2.33±0.06 ^b	3.12±0.53 ^a	1.18±0.16 ^a
DMBA STD	68.66±7.21 ^a	6.31±0.53 ^a	1.58±0.16 ^a	2.04±0.66 ^a	0.76±0.29 ^a
DMBA GLC500	75.33±6.56 ^a	7.91±0.93 ^a	1.01±0.15 ^a	4.02±1.11 ^a	1.11±0.10 ^a
DMBA GLC1000	63.33±4.37 ^a	5.88±0.37 ^a	1.69±0.15 ^a	4.54±0.51 ^a	0.92±0.31 ^a
DMBA <i>E. poly</i> 500	74.33±8.66 ^a	5.15±0.18 ^a	2.18±0.19 ^b	2.97±1.16 ^a	0.67±0.16 ^a
DMBA <i>E. poly</i> 1000	72.33±6.35 ^a	7.77±0.39 ^a	2.00±0.61 ^b	3.82±0.89 ^a	1.81±0.44 ^a
DMBA BW500	57.00±0.57 ^a	3.87±0.37 ^{ab}	5.48±0.51 ^{abc}	14.36±0.01 ^{abc}	4.14±0.64 ^{abc}
DMBA BW1000	85.33±3.17 ^b	4.84±0.03 ^{ab}	5.07±0.10 ^{abc}	5.79±0.31 ^a	0.55±0.10 ^a

Values are expressed as Mean±SEM, values in a column with the same alphabetical superscript do not differ significantly ($p \leq 0.05$), means control, DMBA and DMBA STD is significantly different from other groups

and 220.83±11.93 g initial weight before inducement with tumour using DMBA) that had a significant decrease and DMBA BW1000 (191.67±12.36 g for 1 month after inducement and 150.00±0.00 g for initial weight before inducement with tumour using DMBA) that had a significant increase. At the end of the study, there was no significant difference across the months in all the groups except for the control (175.00±9.12 g for 1 month and 2 weeks after treatment with extract had started in some of the groups and 145.83±7.68 g for 2 months after inducement with tumour using DMBA in the other groups) that had a significant increase.

The concentration of CA 15-3 of DMBA-induced benign breast disease in female Wistar rats is shown in Table 3. The CA 15-3 concentration of DMBA (15.56±0.32 U mL⁻¹) and DMBA *E. poly*1000 (14.48±6.24 U mL⁻¹) was higher than the other groups except for DMBA BW1000 (32.07±11.77 U mL⁻¹) which was significantly higher than all the groups.

Table 4 shows the hormonal profile of DMBA-induced benign breast disease in female Wistar rats, given various plant's extracts. The estrogen concentration of DMBA (83.33±3.84 pg mL⁻¹) and DMBA BW1000 (85.33±3.17 pg mL⁻¹) was higher than the other groups. Progesterone concentration of DMBA BW500 (3.87±0.37 ng mL⁻¹) and DMBA BW1000 (4.84±0.03 ng mL⁻¹) was significantly lower than all the groups. The concentration of prolactin for DMBA (2.33±0.06 ng mL⁻¹), DMBA *E. poly*500 (2.18±0.19 ng mL⁻¹) and DMBA *E. poly*1000 (2.00±0.61 ng mL⁻¹) was higher than the other groups except for DMBA BW500 (5.48±0.51 ng mL⁻¹) and DMBA BW1000 (5.07±0.10 ng mL⁻¹) which significantly higher than all the groups. The LH and FSH concentrations of all the groups showed no significant difference except for DMBA BW500 (14.36±0.01 mIU mL⁻¹ for LH and 4.14±0.64 mIU mL⁻¹ for FSH), which was significantly higher than all the groups.

Table 5: Oxidative profile of DMBA-treated albino rats, given an aqueous extract of the various plants

Groups	Malondialdehyde MDA ($\mu\text{mol mL}^{-1}$)	Glutathione GSH (U L^{-1})	Glutathione s-transferase GST (U L^{-1})	Glutathione peroxidase GPx (U L^{-1})	Catalase CAT (U L^{-1})	Superoxide dismutase SOD (U L^{-1})
Control	0.11±0.03 ^a	6.16±1.03 ^b	0.12±0.01 ^a	0.09±0.00 ^a	11.76±0.92 ^a	1.39±0.10 ^a
DMBA	0.19±0.07 ^{ac}	6.86±1.17 ^b	0.12±0.01 ^a	0.12±0.01 ^a	14.56±0.63 ^b	0.78±0.06 ^a
DMBA STD	0.12±0.07 ^a	7.03±0.17 ^b	0.10±0.00 ^a	0.14±0.02 ^{ab}	15.13±1.01 ^b	0.87±0.15 ^a
DMBA GLC500	0.15±0.01 ^a	8.73±0.08 ^b	0.16±0.01 ^c	0.11±0.01 ^a	17.63±0.21 ^b	1.31±0.11 ^a
DMBA GLC1000	0.15±0.02 ^a	3.36±0.08 ^{bc}	0.45±0.02 ^{abc}	0.04±0.00 ^{abc}	10.93±0.17 ^a	0.65±0.08 ^a
DMBA <i>E. poly</i> 500	0.13±0.01 ^a	3.66±0.08 ^{bc}	0.11±0.02 ^a	0.06±0.00 ^{abc}	11.96±1.01 ^a	0.60±0.13 ^a
DMBA <i>E. poly</i> 1000	0.14±0.02 ^a	3.46±0.31 ^{bc}	0.075±0.01 ^a	0.11±0.01 ^a	13.00±1.00 ^a	0.65±0.14 ^a
DMBA BW500	0.20±0.01 ^{ac}	7.53±0.08 ^b	0.082±0.01 ^a	0.11±0.00 ^a	12.40±1.27 ^a	0.56±0.03 ^a
DMBA BW1000	0.14±0.01 ^a	7.40±0.11 ^b	0.096±0.01 ^a	0.12±0.01 ^a	8.03±0.08 ^{bc}	1.07±0.22 ^a

Values are expressed as Mean±SEM, values in a column with the same alphabetical superscript do not differ significantly ($p \leq 0.05$), means control, DMBA and DMBA STD is significantly different from other groups

Table 6: Lipid profile of DMBA-treated albino rats, given an aqueous extract of the various plants

Groups	Total cholesterol (TC) (mmol L^{-1})	Triglyceride (TG) (mmol L^{-1})	High-density lipoprotein (HDL) (mmol L^{-1})	Low-density lipoprotein (LDL) (mmol L^{-1})	Very low-density lipoprotein (VLDL) (mmol L^{-1})
Control	3.30±0.47 ^a	0.95±0.36 ^a	1.03±0.23 ^a	1.70±0.30 ^a	0.43±0.16 ^a
DMBA	3.48±0.24 ^a	0.73±0.06 ^a	1.22±0.13 ^a	2.24±0.43 ^a	0.33±0.03 ^a
DMBA STD	3.39±0.28 ^a	0.90±0.13 ^a	1.12±0.18 ^a	1.94±0.10 ^a	0.41±0.06 ^a
DMBA GLC500	2.96±0.29 ^a	0.72±0.07 ^a	1.52±0.53 ^a	1.46±0.37 ^a	0.33±0.03 ^a
DMBA GLC1000	3.18±0.17 ^a	0.70±0.06 ^a	1.28±0.18 ^a	1.93±0.17 ^a	0.32±0.03 ^a
DMBA <i>E. poly</i> 500	2.63±0.85 ^a	0.89±0.21 ^a	1.13±0.68 ^a	1.54±0.37 ^a	0.41±0.09 ^a
DMBA <i>E. poly</i> 1000	2.70±0.23 ^a	0.64±0.09 ^a	0.90±0.28 ^a	1.78±0.13 ^a	0.29±0.04 ^a
DMBA BW500	2.81±0.11 ^a	0.76±0.11 ^a	1.24±0.26 ^a	1.07±0.56 ^b	0.44±0.05 ^a
DMBA BW1000	2.40±0.03 ^{bc}	0.76±0.01 ^a	0.72±0.01 ^a	1.49±0.14 ^a	0.34±0.00 ^a

Values are expressed as Mean±SEM, values in a column with the same alphabetical superscript do not differ significantly ($p \leq 0.05$), means control, DMBA and DMBA STD is significantly different from other groups

Table 5 shows the oxidative profile of DMBA-treated albino rats, given an aqueous extract of the various plants. The MDA concentration of DMBA ($0.19 \pm 0.07 \mu\text{mol mL}^{-1}$) and DMBA BW500 ($0.20 \pm 0.01 \mu\text{mol mL}^{-1}$) was higher than the other groups. The GSH concentration of DMBA GLC500 ($8.73 \pm 0.08 \text{U mL}^{-1}$) was higher than the control ($6.16 \pm 1.03 \text{U mL}^{-1}$) and DMBA ($6.86 \pm 1.17 \text{U mL}^{-1}$) groups although not significant. The GST level of DMBA GLC1000 ($0.45 \pm 0.02 \text{U mL}^{-1}$) was higher than all the other groups. The GPx activity of DMBA GLC1000 ($0.04 \pm 0.00 \text{U mL}^{-1}$) and DMBA *E. poly*500 ($0.06 \pm 0.00 \text{U mL}^{-1}$) was significantly lower than all the groups while, DMBA STD ($0.14 \pm 0.02 \text{U mL}^{-1}$) was significantly higher than the control ($0.09 \pm 0.00 \text{U mL}^{-1}$) and DMBA ($0.12 \pm 0.01 \text{U mL}^{-1}$) groups. Catalase activity of DMBA ($14.56 \pm 0.63 \text{U mL}^{-1}$), DMBA STD ($15.13 \pm 1.01 \text{U mL}^{-1}$) and DMBA GLC500 ($17.63 \pm 0.21 \text{U mL}^{-1}$) were higher than all the groups except for DMBA BW1000 ($8.03 \pm 0.08 \text{U mL}^{-1}$) which was significantly lower than all the groups. The SOD activity of all the groups showed no significant difference.

The lipid profile of DMBA-treated albino rats, given an aqueous extract of the various plants is shown in Table 6. The total cholesterol concentration of DMBA BW1000 ($2.40 \pm 0.03 \text{mmol mL}^{-1}$) was significantly lower than that of the DMBA ($3.48 \pm 0.24 \text{mmol mL}^{-1}$) and DMBA STD ($3.39 \pm 0.28 \text{mmol mL}^{-1}$) groups. For TG, HDL, LDL and VLDL concentrations, there was no significant difference in all the groups.

Histopathology of the breast tissue: Figure 1a-i shows the histopathology of the breast tissue of the animals induced with tumours using DMBA and the various groups treated with the aqueous extract of the different plants. The data in Fig. 1a is the control and it shows normal histology of mammary ducts and stroma. The data in Fig. 1b is the DMBA group that was induced with tumour and not treated. It shows increased ducts with hyperplasia. The data in Fig. 1c is the DMBA STD group which was induced with tumour using DMBA and treated with a standard drug (tamoxifen). It showed increased ducts with

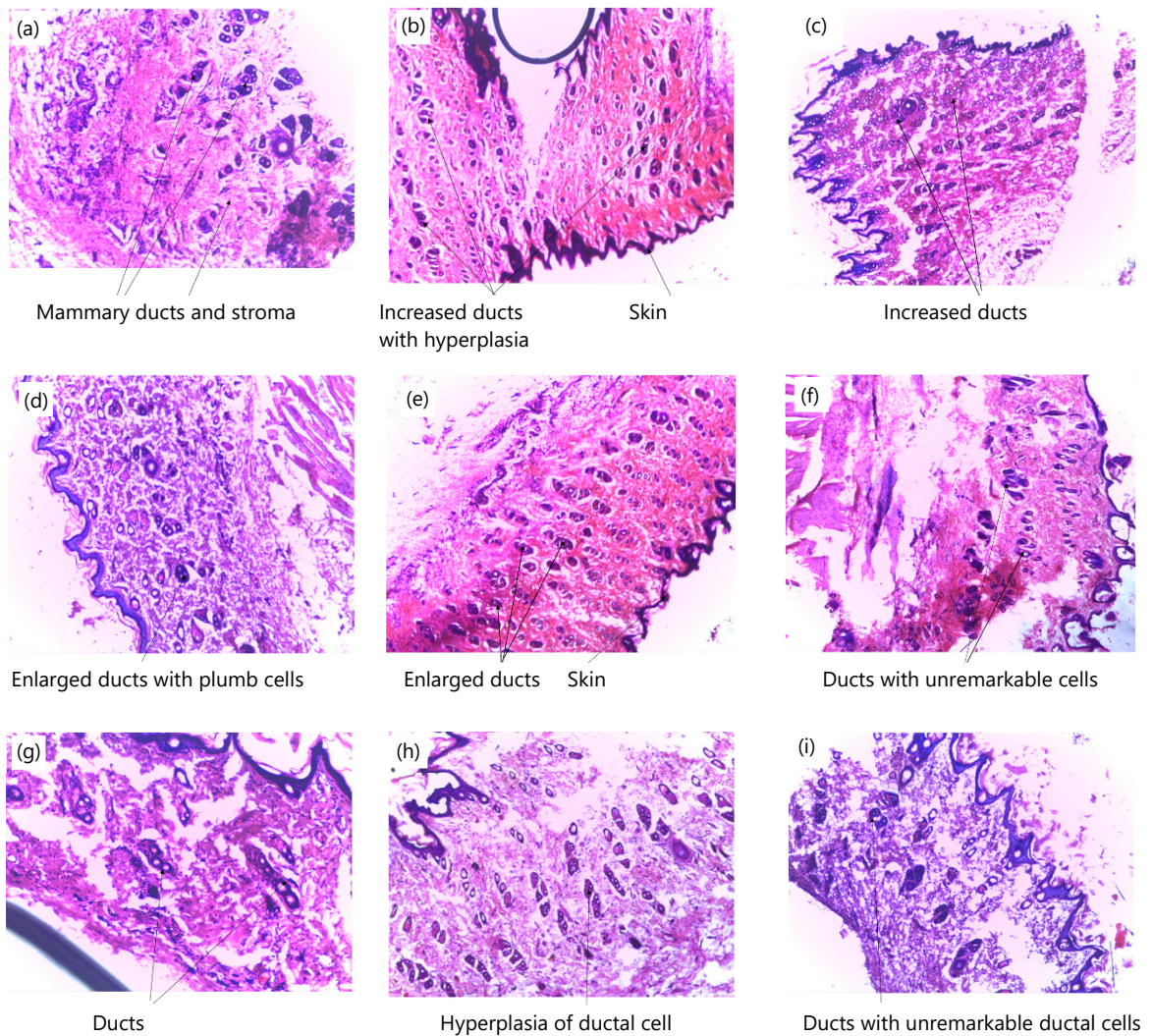


Fig. 1(a-i): Histopathology analysis of the breast tissue of rats with different groups, (a) Control (H&E×100) (Histologic slide shows normal histologic features, mammary ducts are normal), (b) DMBA rats (animals treated with 20 mg kg⁻¹ of DMBA only) H&E×100 (Histologic slide shows increased ducts in the mammary lobules), (c) DMBA STD rats (animals given 20 mg kg⁻¹ of DMBA and treated with 6.6 mg kg⁻¹ of TAMOXIFEN) H&E×100 (Histologic slide shows increased ducts with hyperplasia but the ductal cells are not enlarged when compared to the DMBA untreated group), (d) DMBA GLC500 (animals given 20 mg kg⁻¹ of DMBA and treated with 500 mg kg⁻¹ of *Sorghum vulgare* leaf sheath) H&E×100 (Histologic slide shows enlarged ducts with plumb cells but fewer multiplications of ducts compared to the DMBA untreated group), (e) DMBA GLC1000 (animals given 20 mg kg⁻¹ of DMBA and treated with 1000 mg kg⁻¹ of *Sorghum vulgare* leaf sheath) H&E×100 (The histologic slide showed enlarged ducts with lining cells but no multiplication of the cells), (f) DMBA *E. polysperma*500 (animals given 20 mg kg⁻¹ of DMBA and treated with 500 mg kg⁻¹ of *E. polysperma* leaf) H&E×100 (Histologic slide shows ducts with no obvious histologic change), (g) DMBA *E. polysperma*1000 (animals given 20 mg kg⁻¹ of DMBA and treated with 1000 mg kg⁻¹ of *E. polysperma* leaf) H&E×100 (A histologic slide showing unremarkable ducts and lining cells), (h) DMBA *B. owariensis* 500 (animals given 20 mg kg⁻¹ of DMBA and treated with 500 mg kg⁻¹ of *B. owariensis* leaf) H&E×100 (Histologic slide shows hyperplasia of ductal cells) and (i) DMBA *B. owariensis*1000 (animals given 20 mg kg⁻¹ of DMBA and treated with 1000 mg kg⁻¹ of *B. owariensis* leaf) H&E×100 (Histologic slide shows unremarkable ductal cells)

hyperplasia but the ductal cells are not enlarged when compared to the DMBA untreated group. The data in Fig. 1d is the DMBA GLC500 group, which showed enlarged ducts with plumb cells but fewer multiplications of ducts compared to the DMBA untreated group. The data in Fig. 1e is the DMBA GLC1000 group and it showed enlarged ducts with lining cells but no multiplication of the cells. The data in Fig. 1f is the DMBA *E. poly*500 group which showed ducts with no obvious histologic change. The data in Fig. 1g is the DMBA *E. poly*1000 group, which showed unremarkable ducts and lining cells of the breast. The data in Fig. 1h is the DMBA *B. owariensis* 500 group which showed hyperplasia of ductal cells and lastly Fig. 1i is the DMBA *B. owariensis* 1000 group, which showed unremarkable ductal cells.

DISCUSSION

Our study looked at the relationship between breast cancer and bodyweight of the animals because, in persons who are obese, there is a higher threat of developing breast cancer, predominantly in postmenopausal women²⁰. Table 2 actual shows the weight of DMBA-induced benign breast disease in female Wistar rats. We found a reduction in the weight of some of the animals treated with the various plant's extract when compared to the untreated animals. This reduction might be due to some phytochemicals present in the plants. This is following the works of Minari *et al.*²¹, which showed a reduction in body weights of DMBA-induced breast cancer female rats when treated with 300 mg kg⁻¹ of *Mucuna pruriens* extract compared to the DMBA-untreated group.

Since breast cancer is one of the utmost recurrent causes of cancer death in women in both developing and developed countries, looking at various ways to reduce its menace is of great importance. Serum tumour markers have been widely used as non-invasive tools for assessing treatment response, early diagnosis of recurrence and predicting the prognosis of a disease. Cancer antigen 15-3 (CA 15-3) is the commonly used tumour marker for the early diagnosis of cancer of the breast²². The CA 15-3 plays a role in cell adhesion and elevated levels present in cancer may pose metastasis²³. So, the study looked at the concentration of this tumour marker in animals treated with various extract concentrates. The result is shown in Table 3 is the Concentration of CA 15-3 of DMBA-induced benign breast disease in female Wistar rats, given an aqueous extract of the various plants. We found out that those animals induced with tumour using DMBA and left untreated and those treated with *E. polysperma* (1000 mg kg⁻¹) had significantly higher ($p \leq 0.05$) CA 15-3 concentration than the other groups excluding those treated with *B. owariensis* (1000 mg kg⁻¹) which had a significantly higher value ($p \leq 0.05$) than all the groups. The plants which had a positive reduction of this tumour marker were *Sorghum vulgare* leaf sheath (500 mg kg⁻¹) and *Eremomastax polysperma* (500 mg kg⁻¹), suggesting they might have anti-cancer activity since they were able to reduce CA 15-3 concentration of the DMBA induced tumour in animals after treatment. This is similar to the works of Gurudatta *et al.*²⁴, which demonstrates that CA 15-3 levels were significantly reduced when treated with *Carica papaya* on DMBA induced mammary tumour rats, compared to the untreated group.

The study also looked at the Hormonal profile of DMBA-induced benign breast disease in female Wistar rats, given various plant's extracts which are shown in Table 4. The estrogen concentration of all groups excluding those treated with *Brillantaisia owariensis* (1000 mg kg⁻¹) was lesser than DMBA-untreated (significant difference, $p \leq 0.05$). Estrogens have an important role, in female sex organs and secondary sex characteristics development, the regulation of the menstrual cycle and reproduction. Increased concentrations of endogenous estrogens are strongly associated with increased risk for breast cancer in postmenopausal women²⁵. The result shows a reduction in some of the treated groups compared to the DMBA-untreated group. In comparison to the works of Kaskurthy *et al.*¹⁴, this study was similar to theirs, who stated that estradiol concentration was reduced when treated with 100 mg kg⁻¹ of saponins of *Momordica cymbalaria* of DMBA induced mammary tumour albino rats. Although, estradiol concentration was a bit lower in this present study when related to theirs.

The progesterone concentration showed no significant difference ($p \leq 0.05$) excluding those animals treated with *B. owariensis* (500 mg and 1000 mg kg^{-1}) which had significantly reduced ($p \leq 0.05$) values when compared to the control and DMBA-untreated groups.

The prolactin concentration of the animals treated with *S. vulgare* (500 mg and 1000 mg kg^{-1}) was decreased when compared to the DMBA-untreated animals. Prolactin has effects on reproduction, lactation and metabolism²⁶. High circulating prolactin concentration increases breast cancer risk in post and pre-menopausal women²⁷ and can also be increased in benign breast disease. The prolactin concentration of this present study was lower than those of Kaskurthy *et al.*¹⁴, although, the prolactin concentration of *Brillantaisia owariensis* was higher than theirs.

The FSH and LH concentration of the treated animals showed no significant difference ($p \leq 0.05$) excluding the animals treated with *Brillantaisia owariensis* (500 mg kg^{-1}) which had significantly higher values when compared to the control, DMBA-untreated group and the those treated with the standard drug (Tamoxifen). From this study, *Eremomastax polysperma* (500 mg kg^{-1}) and *Sorghum vulgare* leaf sheath (500 mg kg^{-1}) were able to reduce estrogen and prolactin concentrations, making these plants at this concentration possibly reducing benign breast disease and probably cytotoxic to breast cancer.

Oxidative stress involves the production of free radicals which are injurious to the body system and the body not being able to reduce the free radicals. Radical induced lipid peroxidation products such as dialdehydes (Malondialdehydes) can also modify bases in DNA in the cell membranes²⁸. Oxidative stress has been implicated in several diseases which include atherosclerosis, pulmonary fibrosis, cancer and aging²⁹. The oxidative markers of DMBA-induced mammary tumour in female albino rats, given an aqueous extract of the various plants, is shown in Table 5. There was a significant reduction ($p \leq 0.05$) in the Malondialdehyde (MDA) concentration of all treated groups when compared to the DMBA-untreated group except for those animals treated with *Brillantaisia owariensis* (500 mg kg^{-1}), showing the usefulness of these plants in reducing oxidative stress and its related disease. The result from this study is commensurate with those of Soliman and Elfeky³⁰, who demonstrates that the MDA concentration of mammary tumorigenic rats was lower in the animals treated with ginger and cinnamon when compared to the untreated animals.

The GSH concentration of animals treated with 500 mg kg^{-1} of *Sorghum vulgare* leaf sheath was significantly higher ($p \leq 0.05$) than the control and DMBA untreated animals. Indicating the plant has a high antioxidant property. This review is following Sarvanan *et al.*³¹ stating that thymoquinone isolated from *Nigella sativa* seeds was able to increase GSH concentration in DMBA-induced experimental cancer, although it was lower than *Sorghum vulgare* and *Brillantaisia owariensis* but higher than those of *Eremomastax polysperma*.

For the GST activity, animals treated with 1000 mg kg^{-1} *Sorghum vulgare* leaf sheath had significantly higher values ($p \leq 0.05$) when compared to the control, DMBA-untreated and DMBA STD groups. For GPx activity animals treated with 1000 mg kg^{-1} *Sorghum vulgare* leaf sheath and those treated with 500 mg kg^{-1} of *Eremomastax* had significantly reduced values ($p \leq 0.05$) when compared to the control, DMBA and DMBA-STD groups. Catalase activity was significantly increased in animals treated with 500 mg kg^{-1} *Sorghum vulgare* when compared to the control and also higher than the DMBA-untreated animals, though not significant ($p \leq 0.05$). There was an increase in superoxide dismutase activity of the control group and animals treated with 500 mg kg^{-1} *Sorghum vulgare* leaf sheath and 1000 mg kg^{-1} *Brillantaisia owariensis*. These results indicate that the plants extract especially 500 mg kg^{-1} *Sorghum vulgare* leaf sheath might have an antioxidant protective effect against breast damage.

There is an association between increased Total Cholesterol (TC), Low-Density Lipoprotein (LDL-C) and decreased High-Density Lipoprotein (HDL-C levels) with increasing tumour. Also from *in vitro* studies, exogenous LDL-C has been found to promote proliferation and migration of cancer cells. High cholesterol levels induce a pro-inflammatory microenvironment that contributes to breast cancer initiation and progression³². The lipid profile of DMBA-induced benign breast disease in female Wistar rats, given an aqueous extract of the various plants, is shown in Table 6. There was no significant difference ($p \leq 0.05$) in all the groups, although, there was a decrease in TC, LDL and TG concentrations and an increase in HDL concentration (not significantly different, $p \leq 0.05$) of those animals induced with tumour using DMBA and treated with 500 mg kg^{-1} of *S. vulgare* when compared to the DMBA-untreated animals. Indicating that some of the plant's extract might have breast cancer regression properties by reducing cholesterol and low-density lipoprotein concentrations.

Breast cancer is a malignant tumour that arises in the breast. Other tumours are not malignant. Those non-malignant lesions (benign diseases) predispose patients to increased risk of developing breast cancer and they include sclerosing adenosis, radial scars, inductive papillomas, hyperplasia without atypia, fibroadenomas, proliferative lesions with atypia and lobular carcinoma *in situ*³³. Hyperplasia is an overgrowth (proliferation) of cells that line the ducts or lobules inside the breast. Atypical hyperplasia is a proliferation of dysplastic, monotonous epithelial-cell populations and it is found in approximately 10% of biopsies with benign findings with a high-risk rate of developing breast cancer³⁴. Sclerosing adenosis is a tumour-like lobulocentric proliferation that consists of enlarged and distorted lobules, containing duplicated and crowded acini, with prominent myoepithelium and stromal fibrosis (which means in adenosis, the lobules are enlarged and there are more glands than usual)³⁵.

The results of the histopathology of DMBA-treated Wistar-albino rats treated with the various extract is shown in Fig. 1a-i. The control had normal mammary lobules and ducts (Fig. 1a). The DMBA-untreated group (Fig. 1b) showed increased ducts and enlargement of cells. The DMBA STD (Fig. 1c) had increased ducts with hyperplasia but no enlargement of cells when compared to the DMBA-untreated group. The DMBA GLC500 (Fig. 1d) and DMBA GLC1000 (Fig. 1e) had enlarged ducts with less multiplication when compared to the DMBA-untreated group. The DMBA *E. poly*500 group showed ducts with no histologic changes (Fig. 1f). The DMBA BW500 (Fig. 1g) showed hyperplasia of ducts similar to the DMBA-untreated group. The DMBA *E. poly*1000 (Fig. 1h) and DMBA BW1000 (Fig. 1i) showed no remarkable changes.

The histologic result showed that the extracts might be effective in reducing hyperplasia. This tallies with the study of Minari *et al.*²¹, which showed that *Mucuna pruriens* had anti-proliferative properties on hyperplasia of the serous and mucinous glands of DMBA induced mammary tumour.

The study implies that medicinal plants such as *Sorghum vulgare* leaf-sheath, *Eremomastax polysperma* and *Brillantaisia owariensis* can have an anti-cancer effect and it can be applied for possibly chemotherapeutic medication in a more purified form. It is recommended that a clinical study should be carried out using these plants and more study at the molecular level be done since this study didn't look at those areas.

CONCLUSION

The present study was undertaken to assess biochemical studies of aqueous extract of selected plants on 7,12-dimethylbenz(a)anthracene (DMBA)-induced benign breast disease in female Wistar rats. The finding suggests anti-hyperplasia capacity of the plants or ability to reduce benign breast disease (especially *Sorghum vulgare* leaf-sheath (500 mg kg^{-1}) and *Eremomastax polysperma* (500 mg kg^{-1})). Histological findings also showed normal histology with some of the treated groups. This study indicates the usefulness of these plants as a potential reducer of benign breast disease.

SIGNIFICANCE STATEMENT

This study discovers the antihypersia activity of some medicinal plants (*Sorghum vulgare* leaf-sheath, *Brillantasia owariensis* and *Eremomastax polysperma*) on benign breast disease. This study will help the researcher to uncover the critical areas of these plant's extract on oxidative damage, lipid profile, hormonal profile and histopathology of the breast tissue. Thus a new theory on these plants used to treat benign breast disease may be arrived at.

REFERENCES

1. Amin, A.L., Purdy, A.C., J.D. Mattingly, A.L. Kong and P.M. Termuhlen, 2013. Benign breast disease. *Surg. Clin. North Am.*, 93: 299-308.
2. Lagiou, P., E. Samoli, A. Lagiou, P. Zourna and A. Barbouni *et al.*, 2013. A comparison of hormonal profiles between breast cancer and benign breast disease: A case-control study. *Ann. Oncol.*, 24: 2527-2533.
3. Nounou, M.I., F. ElAmrawy, N. Ahmed, K. Abdelraouf, S. Goda and H. Syed-Sha-Qhattal, 2015. Breast cancer: Conventional diagnosis and treatment modalities and recent patents and technologies. *Breast Cancer: Basic Clin. Res.*, 9s2: 17-34.
4. Sanchez, A.M., M.I. Flamini, S. Zullino, E. Russo and A. Giannini *et al.*, 2018. Regulatory actions of LH and follicle-stimulating hormone on breast cancer cells and mammary tumors in rats. *Front. Endocrinol.*, Vol. 9. 10.3389/fendo.2018.00239.
5. Hernández, L., M.J. Nuñez-Villar, F. Martínez-Arribas, M. Pollán and J. Schneider, 2005. Circulating hormone levels in breast cancer patients, correlation with serum tumour markers and the clinical and biological features of the tumour. *Anticancer Res.*, 25: 451-454.
6. Liang, J. and Y. Shang, 2013. Estrogen and cancer. *Annu. Rev. Physiol.*, 75: 225-240.
7. Russo, J. and I.H. Russo, 2006. The role of estrogen in the initiation of breast cancer. *J. Steroid Biochem. Mol. Biol.*, 102: 89-96.
8. Brisken, C., 2013. Progesterone signalling in breast cancer: A neglected hormone coming into the limelight. *Nat. Rev. Cancer*, 13: 385-396.
9. Arora, M.K., A.S. Trehan, S. Seth and A. Chauhan, 2012. Role of endogenous hormones in premenopausal females with breast carcinoma-A pilot study in North Indian population. *Internet J. Med. Update*, 7: 25-31.
10. Ahmed, M., M.I. Khan, M.R. Khan, N. Muhammad, A.U. Khan and R.A. Khan, 2013. Role of medicinal plants in oxidative stress and cancer. *Open Access Sci. Rep.*, Vol. 2. 10.4172/scientificreports.641.
11. Desai, A.G., G.N. Qazi, R.K. Ganju, M. El-Tamer and J. Singh *et al.*, 2008. Medicinal plants and cancer chemoprevention. *Curr. Drug Metab.*, 9: 581-591.
12. Dahiru, D., J. Onubiyi and H. Umaru, 2006. Phytochemical screening and antiulcerogenic effect of *Moringa oleifera* aqueous leaf extract. *Afr. J. Traditional Complementary Alt. Med.*, 3: 70-75.
13. Barros, A.C.S.D., E.N.K. Muranaka, L.J. Mori, C.H.T. Pelizon, K. Iriya, G. Giocondo and J.A. Pinotti, 2004. Induction of experimental mammary carcinogenesis in rats with 7,12-dimethylbenz(a)anthracene. *Rev. Hosp. Clínicas*, 59: 257-261.
14. Kaskurthy, R.L., R.B. Koneri and S. Samaddar, 2015. Evaluation of anti-tumor activity of *Momordica cymbalaria* fenzl. *Int. J. Basic Clin. Pharmacol.*, 4: 779-786.
15. Nwinyi, F.C., H.O. Kwanashie, A.A. Ahmad and L.E. Odama, 2009. Evaluation of toxicity profile of leaf base extract of sorghum bicolor in rat. *Afr. J. Biotechnol.*, 8: 334-342.
16. Okokon, J.E., B.S. Antia, A.E. Udoh and M.M. Akpan, 2007. Antianaemic and antimicrobial activity of *Eremomastax speciosa*. *J. Pharmacol. Toxicol.*, 2: 196-199.
17. Akah, P.A., C.E. Okolo, T.C. Okoye and N.V. Offiah, 2010. Aqueous extract and methanol fractions of the leaves of *Brillantaisia nitens* Lindau. reverse phenylhydrazine induced anaemia in rats. *J. Med. Plants Res.*, 4: 271-277.

18. Johnkennedy, N., D.N. Joy, E.H. Ndubueze, N. Melvina, E. Richard and O. Vitus, 2013. Antioxidant and cardioprotective effect of coconut water against doxorubicin induced cardiomyopathy. *J. Krishna Inst. Med. Sci. Uni.*, 2: 37-41.
19. Duffy, M.J., 2006. Serum tumor markers in breast cancer: Are they of clinical value? *Clin. Chem.*, 52: 345-351.
20. Picon-Ruiz, M., C. Morata-Tarifa, J.J. Valle-Goffin, E.R. Friedman and J.M. Slingerland, 2017. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. *CA: Cancer J. Clinicians*, 67: 378-397.
21. Minari, J.B., G.O. Ogar and A.J. Bello, 2016. Antiproliferative potential of aqueous leaf extract of *Mucuna pruriens* on DMBA-induced breast cancer in female albino rats. *Egypt. J. Med. Hum. Genet.*, 17: 331-343.
22. Osman, A.M., M.E. Hamid, A.H. Satti and I.A. Goreish, 2016. High concentration of tumor marker cancer antigen CA15-3 in breast cancer patients after surgery. *Breast Cancer: Curr. Res.*, Vol. 1. 10.4172/2572-4118.1000114.
23. Duffy, M.J., S. Shering, F. Sherry, E. McDermott and N. O'Higgins, 2000. CA 15-3: A prognostic marker in breast cancer. *Int. J. Biol. Markers*, 15: 330-333.
24. Gurudatta, M., Y.A. Deshmulch and A.A. Naikwadi, 2015. Anticancer effect of *Carica papaya* in experimental induced mammary tumours in rats. *Int. J. Med. Res. Health Sci.*, 4: 667-671.
25. Travis, R.C. and T.J. Key, 2003. Oestrogen exposure and breast cancer risk. *Breast Cancer Res.*, 5: 239-247.
26. Hamoda, H., Y. Khalaf and P. Carroll, 2012. Hyperprolactinaemia and female reproductive function: What does the evidence say? *Obstetrician Gynaecol.*, 14: 81-86.
27. Bernichtein, S., P. Touraine and V. Goffin, 2010. New concepts in prolactin biology. *J. Endocrinol.*, 206: 1-11.
28. Kennelly, P.J., R.K. Murray, M. Jacob and J. Varghese, 2016. Plasma Proteins and Immunoglobulins. In: Harper's Illustrated Biochemistry, Rodwell, V.W., D.A. Bender, K.M. Botham, P.J. Kennelly and P.A. Weil (Eds.), McGraw Hill, New York, USA, ISBN: 978-0-07-182534-4, pp: 1-28.
29. Kerkick, C. and D. Willoughby, 2005. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *J. Int. Soc. Sports Nutr.*, Vol. 2. 10.1186/1550-2783-2-2-38.
30. Soliman, M.M. and A.M.S. Elfeky, 2016. Studies on the biochemical and molecular effects of some natural herbs on experimental-induced breast cancer in Wistar rats. *Nat. J. Physiol. Pharm. Pharmacol.*, 6: 349-358.
31. Saravanan, D., K. Baskaran and D. Sakthisekaran, 2016. Protective effect of thymoquinone on the liver tissues of 7, 12-dimethylbenz (A) anthracene induced experimental breast cancer rats. *Asian J. Pharm. Clin. Res.*, 9: 197-201.
32. Arroyo-Acevedo, J., R.J. Chávez-Asmat, A. Anampa-Guzmán, R. Donaires and J. Ráez-González, 2015. Protective effect of piper aduncum capsule on DMBA-induced breast cancer in rats. *Breast Cancer: Basic Clin. Res.*, 9: 41-48.
33. Kiluk, J.V., G. Acs and S.J. Hoover, 2007. High-risk benign breast lesions: Current strategies in management. *Cancer Control*, 14: 321-329.
34. Hartmann, L.C., A.C. Degnim, R.J. Santen, W.D. Dupont and K. Ghosh, 2015. Atypical hyperplasia of the breast-risk assessment and management options. *New Engl. J. Med.*, 372: 78-89.
35. Visscher, D.W., A. Nassar, A.C. Degnim, M.H. Frost and R.A. Vierkant *et al.*, 2014. Sclerosing adenosis and risk of breast cancer. *Breast Cancer Res. Treat.*, 144: 205-212.