WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 2078-2099. Research Article ISSN 2277– 7105

EVALUATION OF THE ANTICARCINOGENIC EFFECT OF SOME PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR LIGANDS ON DIMETHYLBENZ (α) ANTHRACENE INDUCED MAMMARY TUMOR IN FEMALE RATS

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Article Received on
15 Sept 2015,

Revised on 07 Oct 2015,
Accepted on 29 Oct 2015,

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ABSTRACT

Introduction: Breast cancer is the leading cause of cancer death among females worldwide. Peroxisome proliferator activated receptors (PPARs) are one of several nuclear receptors involved in the biology of breast cancer. Aim: Compare the effect of fenofibrate (PPARα ligand), pioglitazone (PPAR γ ligand) and omega-3 (PPARα, γ ligand) and their probable mechanisms of action on 7, 12 dimethylbenz (α) anthracene (DMBA)-induced mammary carcinoma in female rats. Methods: Fifty female Waister albino rats were utilized, with ten serving as plain controls. The remaining were subjected to induction of mammary carcinomas by oral intubation with a single dose of 20 mg DMBA suspended in one ml of sesame oil. After the appearance of mammary tumors, rats were randomly assigned to 4 orally-treated groups: untreated, fenofibrate, pioglitazone and omega-3-treated for 28 days. Assessed parameters: Percentage change of tumor volume, serum and tumor tissue vascular endothelial growth factor levels, tumor caspase-3 and cyclooxygenase-2 concentrations, as well as immunohistochemical detection of Ki-67 expression. Results: The untreated rats had progressive increase in mammary tumor volume. Treatment with fenofibrate, pioglitazone or omega-3 significantly reduced the rate of tumor growth via antiangiogenic, proapoptotic, antiproliferative and anti-inflammatory effects. Conclusion:

Fenofibrate, pioglitazone and omega-3 exerted anti-tumor effects on breast cancer induced in rats via numerous mechanisms of action.

KEYWORDS: Peroxisome proliferator activated receptors, fenofibrate, pioglitazone, omega-3, cyclooxygenase-2, induced mammary tumor.

ABBREVIATIONS

PPARs: Peroxisome proliferator activated receptors. **COX-2:** Cyclooxygenase-2. **VEGF:** vascular endothelial growth factor. **DMBA:** 7,12 dimethylbenz(α)anthracene.

1. INTRODUCTION

Breast cancer is the leading cause of cancer death among females, and it represents one in four of all cancer cases in women.[1] A few dozen predisposing factors of breast cancer have been identified; however, all these diverse risks can be assigned to either of two major categories: excessive exposure to estrogens or deficiency in the maintenance of genomic integrity.[2] In addition to surgery and radiation therapy, the efficacy of endocrine therapy in breast cancer is limited by high rates of de novo and acquired resistances during treatment.[3] Moreover, traditional cancer chemotherapy acts against all actively dividing cells, resulting in toxicity.[4] Hence, there is a need for alternative therapies to treat breast cancer.

The discovery of reliable techniques to study nuclear receptors (NRs) has revealed the presence and potential importance of several NRs other than estrogen and progesterone in the biology of breast cancer, such as peroxisome proliferator activated receptors (PPARs).[5] PPARs are ligand-activated NRs that exert a transcriptional activity regulating energy homeostasis and other basic cellular processes. The addition of PPAR ligand causes dissociation of the corepressor proteins followed by recruitment of co-activators, leading to trans-activation or trans-repression of various genes. PPARs mainly exist in three subtypes; α, β/δ and γ, each of which mediates the physiological actions of a large variety of fatty acids and fatty acid-derived molecules.[6] We focused our study on 3 different PPAR agonists: fenofibrate, a well tolerated PPAR alpha agonist that is used as a lipid-lowering drug; pioglitazone, a PPAR gamma ligand that is used for treatment of type II diabetes and omega-3 polyunsaturated fatty acids ,dual agonists for PPARα and γ that are essential nutrients with additional beneficial effects on cardiovascular and inflammatory diseases.[7] Some studies have revealed the expression of PPAR receptors in tumor cells and that PPAR ligands

suppressed the growth of several cancer cell lines by affecting the expression and/or function of lipids, proteins and genes.[8-10]

Upregulation of angiogenesis is a key step in sustained tumor growth and metastasis, which is acquired by expressing growth factors, such as vascular endothelial growth factor (VEGF).VEGF regulates several aspects of angiogenesis; endothelial cell proliferation, migration, differentiation, tube formation and upregulation of vascular permeability.[11]

Cyclooxygenase-2(COX-2) is an inducible isoform of cyclooxygenase, a key enzyme in the synthesis of prostaglandins (PGs). COX-2 is expressed in response to certain stimuli such as growth factors, cytokines, tumor promoters and hypoxia. Various inflammation networks have been confirmed to play crucial roles in the microenvironment of carcinogenesis, and the most important network is the COX-2/PGE_2 pathway.[12]

Therefore, the present work was designed to investigate and compare the effect of fenofibrate (PPARα ligand), pioglitazone (PPARγ ligand) and omega-3 (dual PPARα, γ ligand) on the growth and proliferation of DMBA induced mammary carcinoma in female rats, and their possible mechanisms of action.

2. MATERIAL AND METHODS

2.1 Animals

Fifty healthy female Wistar albino rats of 55 days old and body weight of 90 to 110 g were used in the present study. They were housed under the same standard environmental conditions of light and temperature. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals.[13] The study protocol was approved by the Ethics Committee, Medical Research Institute, Alexandria University. They were housed on wood shavings in animal metal cages, four per cage, with free access to water and food.

2.2 Grouping of animals

Ten normal female rats received only 1 ml of sesame oil (single administration) via an oral intubation to serve as normal plain control (**Group I**). Mammary tumors were induced in the remaining 40 rats by oral intubation with a single dose of 20 mg dimethylbenz (α) anthracene (DMBA) (Sigma chemical Co. St. Louis, USA) suspended in one ml of sesame oil.[14] Starting from the 6^{th} week after DMBA intubation, rats were palpated at weekly intervals to assess the appearance of mammary tumors, which took 24 to 48 weeks to appear. Rats

bearing DMBA-induced mammary tumors are shown in figures (1,2). After the mammary tumor reached a considerable size (range 1.2 x1.4 cm), rats were randomly assigned to one of the following experimental groups for treatment (each of 10 rats).

Group II (Untreated tumor-bearing control group); rats in this group received an oral daily dose of 1ml of 2% gum acacia to serve as a positive control for the other treated animals.

Group III (Fenofibrate-treated group); in this group, rats were treated with fenofibrate (Lipanthyl , Minapharm, Egypt) orally in a daily dose of 100 mg/Kg.[15]

Group IV (Pioglitazone-treated group); in this group, rats were treated with pioglitazone (Actos , Takeda, Japan) orally in a daily dose of 120 mg/kg.[16]

Group V (Omega-3-treated group); in this group, rats were treated with omega-3(Omega-3 plus, Sedico, Egypt) orally in a daily dose of 1.5 g /rat.[17]

All the above mentioned drugs were obtained from their suppliers in crude form then dissolved, suspended or emulsified in 2% gum acacia, a total volume of 1 ml gum acacia contained the calculated dose of the drug to be administered daily for a treatment period of 28 days (cycle duration of chemotherapy).[18]

2.3 Determination of the total mammary tumor volume

Tumor volumes were determined at the beginning of treatment and assessed at weekly intervals till the end of the treatment period. At each time, both the shorter radius (r_1) and the longer one (r_2) were measured using a micrometer and the tumor volume was calculated according to the following equation: [Tumor volume = $(4/3)\pi (r_1)^2 X r_2$].[19] In animals bearing tumors at more than one site, the total tumor volume was calculated as the sum of the volumes of these tumors.

2.4 Serum samples

At the end of treatment period, rats of all groups were fasted overnight with free access to water. They were anesthetized by ether inhalation (which does not interfere with any of the studied parameters), blood samples were withdrawn from the retro-orbital venous plexus using capillary hematocrit tube, and left to coagulate at 37^0C in the incubator for 1 hour until clot retraction occurred. Sera were separated by centrifugation at 3000 r.p.m for 15 minutes, divided into aliquots and stored frozen at -80°C until being used for.

2.4.1 Estimation of the vascular endothelial growth factor (VEGF) level.[20] using **"Rat VEGF enzyme-linked immunosorbent assay (ELISA) Kit"** Glory Science Co., Ltd, USA The optical density was read from each well at 450 nm, and the concentrations were calculated by converting the optical density against a standard curve.

2.5 Tumor tissue samples

After collecting blood samples, animals were sacrificed by decapitation. Tumor tissues were then excised. Each excised tumor was divided into two parts.

An appropriate part was formalin fixed and paraffin embedded. Sections of 3-4 µm were stained by H&E stain for:

2.5.1 Histopathological examination.[21] to confirm tumor formation and to assess the effect of probed drugs on the tumor tissue in different treated groups of animals. figure (3: a,b). Other sections (4µm thickness) were cut from the paraffin blocks and used for:

2.5.2 Immune histochemical detection of the proliferative marker Ki-67.[22] using **ready to use Ki-67 rabbit monoclonal antibody** (CloneSP6) Kit.Positive cases for ki-67 immunostaining were scored as follows.

- Mild positivity (+) if they contain from 5% to 10% of their cells expressing Ki-67 immunostaining.
- Moderate positivity (++) if they contain from 10% to 20% of their cells expressing Ki-67 immunostaining.
- High positivity (+++) if they contain 20% or greater of their cells expressing Ki-67 immunostaining.

A second part of the tumor was immediately chilled on ice, cut, homogenized in phosphate buffered saline (PBS, pH 7.4), divided into aliquots and stored at -80°c until used for.

2.5.3 Estimation of tumor tissue VEGF level,.[20] using **"Rat VEGF ELISA Kit"** Glory Science Co., Ltd, USA.

2.5.4 Estimation of tumor tissue cyclooxygenase-2 (COX-2) level.[23] using **"Rat COX-2 ELISA Kit"** Glory Science Co., Ltd, USA.

2.5.5 Estimation of tumor tissue levels of Caspase-3 (marker of apoptosis).[24] using **"Rat caspase-3 ELISA Kit"** Glory Science Co., Ltd, USA**.**

The optical density for each parameter was read from each well at 450 nm, and the concentrations were calculated by converting the optical density against a standard curve.

2.6 Statistical Analysis

Statistical analyses were done using the Statistical Package of Social Sciences (SPSS) version 20. Quantitative data were described using mean ± SD as well as median, minimum and maximum. For normally distributed quantitative data comparisons among the different groups were done using analysis of variance **(ANOVA; F test)** followed by a Post Hoc test **(Games-Howell)** for pair wise comparison. For abnormally distributed quantitative data, the **Kruskal Wallis (KW; chi^2 test)** and **Mann-Whitney test** were used to compare between different groups to compare two independent populations. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using **the Monte Carlo test (MC)**, then **the Fisher Exact test (FE)** for pair wise comparison.

Values for $P < 0.05$ were considered statistically significant.

Table (I): Comparison of serum and tumor tissue levels of the vascular endothelial growth factor (VEGF) in the different studied groups.

Parameter	Group I (plain controls) (n = 10)	Group II (untreated rats bearing DMBA-induced mammary carcinoma receiving 1 ml gum acacia 2%/d) (n = 10)	Group III (rats bearing DMBA-induced mammary carcinoma treated with fenofibrate 100mg/kg/d) (n = 10)	Group IV (rats bearing DMBA-induced mammary carcinoma treated with pioglitazone 120mg/kg/d) (n = 10)	Group V (rats bearing DMBA-induced mammary carcinoma treated with omega- 3 1.5 g/rat/d) (n = 10)	F and p
Serum VEGF(ng/L)						
Min.-max.	62.15-74.29	76.02-123.79	67.15-86.43	63.57-75.0	61.43-81.43	21.688* and <0.001*
Mean±SD	67.54±4.20	100.35[a] ±16.11	74.25[b]±7.41	70.16 [b] ±3.46	69.60 [b] ±5.48	
Tumor tissue VEGF (ng/g tissue)						
Min.-max.	-	796.88-975.0	362.5-668.75	512.5-718.75	531.25-756.25	49.033* and <0.001*
Mean±SD	-	923.05±55.51	545.14[b] ±86.36	595.83 [bc] ±72.48	642.36 [bc] ±72.0	

* : Statistically significant at $p \leq 0.05$.

n : number of rats

SD : standard deviation

a, b, c : Letters indicate statistically significant difference

Table (II): Comparison of tumor tissue levels of COX-2 and caspase-3 in the different studied groups.

Parameter	Group I (plain Controls) (n = 10)	Group II (untreated rats bearing DMBA-induced mammary carcinoma receiving 1 ml gum acacia 2%/d) (n = 10)	Group III (rats bearing DMBA-induced mammary carcinoma treated with fenofibrate 100mg/kg/d) (n = 10)	Group IV (rats bearing DMBA-induced mammary carcinoma treated with pioglitazone 120mg/kg/d) (n = 10)	Group V (rats bearing DMBA-induced mammary carcinoma treated with omega- 3 1.5 g/rat/d) (n = 10)	F and p
Tumor tissue COX-2 (U/g tissue)						
Min.-max.	-	282.4-345.0	225.0-283.35	186.10-266.65	208.35-258.35	19.621* and <0.001*
Mean±SD	-	307.29±24.48	260.35[b] ±21.37	240.43[b] ±25.21	236.11[b] ±16.01	
Tumor tissue Caspase3 (ng/g tissue)						
Min.-max.	-	30.78-43.29	42.62-51.68	41.95-47.99	41.28-49.66	24.213* and 0.001*
Mean±SD	-	37.51±3.39	47.13[b]±2.93	45.13[b] ±1.79	46.25[b] ±2.36	

* : Statistically significant at $p \leq 0.05$.

n : number of rats

SD : standard deviation

a, b Letters: indicate statistically significant difference

Table (III): Comparison of tumor tissue Ki-67 expression in the different studied groups.

Statistical data	Group II (untreated rats bearing DMBA-induced mammary carcinoma receiving 1 ml gum acacia 2%/d) (n = 10)		Group III (rats bearing DMBA-induced mammary carcinoma treated with fenofibrate 100mg/kg/d (n = 10)		Group IV (rats bearing DMBA-induced mammary carcinoma treated with pioglitazone 120mg/kg/d) (n = 10)		Group V (rats bearing DMBA-induced mammary carcinoma treated with omega-3 1.5 g/rat/d (n = 10)	
	No.	%	No.	%	No.	%	No.	%
Negative	0	0.0	5	50.0	0	0.0	0	0.0
Positive+	0	0.0	4	40.0	2	20.0	1	10.0
Positive++	2	20.0	1	10.0	5	50.0	7	70.0
Positive+++	8	80.0	0	0.0	3	30.0	2	20.0
MCp	<0.001*							
FEp$_1$			<0.001*		<0.001*		<0.001*	
FEp$_2$					0.008*		0.002*	
FEp$_3$							0.697	

MCp : p for Monte Carlo test

FEp$_1$: p value of Fisher Exact test for comparing between plain control with each other groups.

FEp$_2$: p value of Fisher Exact test for comparing between positive control with each other groups.

FEp$_3$: p value of Fisher Exact test for comparing between fenofibrate with each other groups.

FEp$_4$: p value of Fisher Exact test for comparing between omega-3 and pioglitazone.

* : Statistically significant at $p \leq 0.05$.

n : number of rats

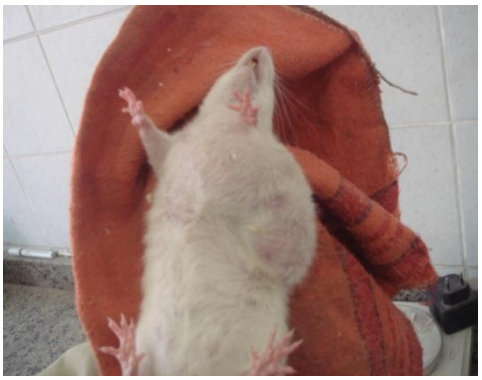


Figure (1): Left thoracic and abdominal mammary carcinomas

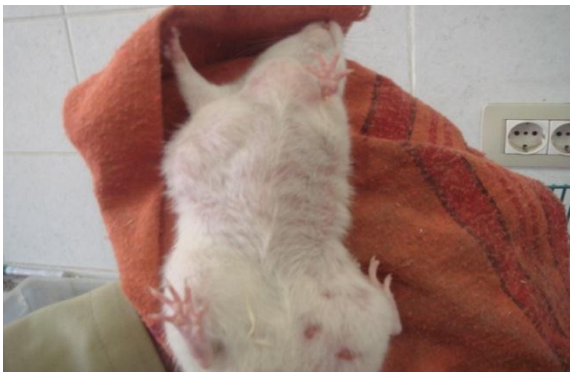
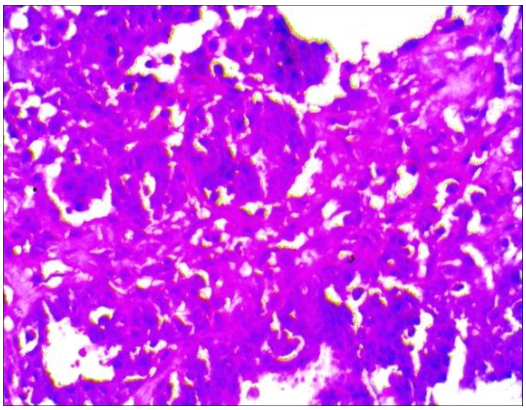
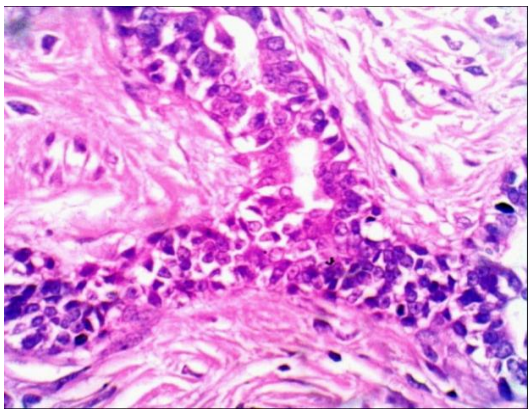


Figure (2): Right thoracic and left inguinal mammary carcinomas



(a)



(b)

Figure (3): H&E stained section from the mammary gland of untreated rats bearing DMBA- induced mammary carcinoma showing invasive ductal carcinoma grade III. a: (H&E x 20), b: (H&E x 40)

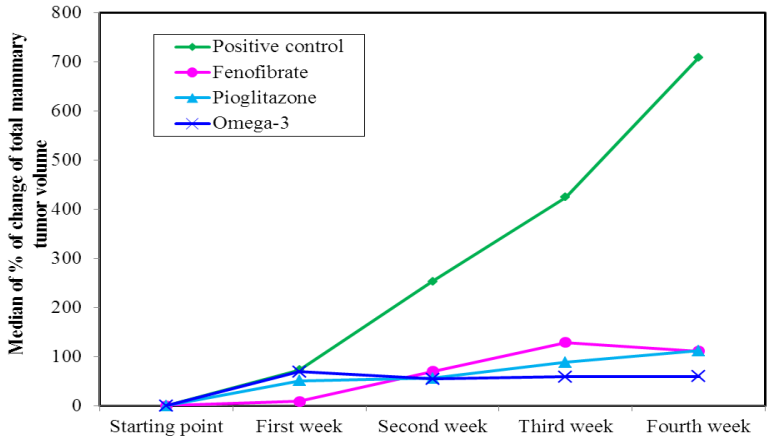


Figure (4): Line graph for comparison of median values of percentage changes of total mammary tumor volume (%) relative to starting volume, along the 4 weeks of therapy in the studied groups

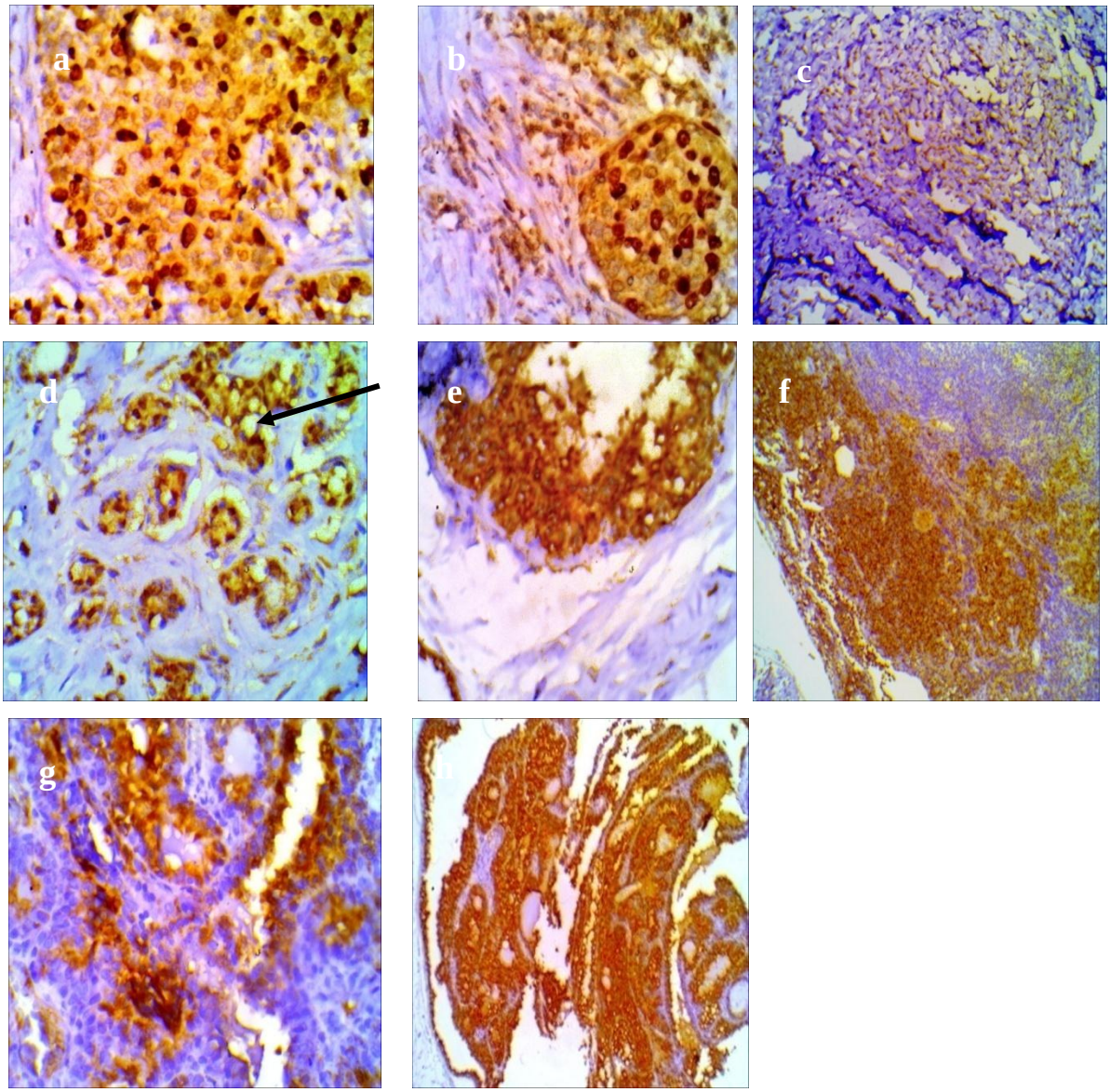


Figure 5 (a-h): DMBA-induced mammary tumor sections stained with anti-Ki-67 antibody in untreated rats and different drug- treated groups;

a: Untreated mammary carcinoma- bearing female rats showing strong positive nuclear staining of most ductal carcinoma cells. (Ki-67 x 40) **b:** Untreated mammary carcinoma-bearing female rats showing prominent intra-ductal component with strong positive nuclear staining of most ductal carcinoma cells. (Ki-67 x 40) **c:** Fenofibrate-treated female rats showing weak staining of nuclei of tumor cells. (Ki-67 x 20) **d:** Fenofibrate-treated female rats showing adenosis and areas of benign ductal hyperplasia (arrow) with strong nuclear staining. (Ki-67 x40) **e:** Pioglitazone-treated female rats showing areas of atypical ductal hyperplasia with strong staining. (Ki-67 x40) **f:** Pioglitazone- treated female rats showing strong staining in areas of inflammation. (Ki-67 x20) **g:** Omega-3-treated female rats

showing strong expression of Ki-67 in ductal hyperplasia. (Ki-67 x40) **h:** Omega-3-treated showing moderate staining in intraductal papillary lesions. (Ki-67 x20)

3. RESULTS

3.1 Comparison between the median values of the percentage changes in total mammary tumor volume, induced by DMBA in female rats, relative to starting volume along the 4 weeks of therapy in the studied groups. (figure 4)

The current work revealed that the untreated group of rats bearing mammary tumors (group II) had a progressive increase in breast tumor growth throughout the course of the experiment until the end, relative to the starting volume. On the other hand, mammary tumor bearing rats of group III (fenofibrate-treated), group IV (piogitazone-treated) and group V(omega 3-treated) succeeded to significantly decrease this steep increase in tumor growth as compared to untreated rats ($^{KW}\chi 2$ =25.939, P< 0.05).The most favorable response was obtained in group V with a significant difference between this group and all other treated groups (P< 0.05). Surprisingly, group III showed a marked decrease in tumor growth rate within the 4th week of treatment. However, no significant difference was detected between group III and IV (P> 0.05).

3.2 Serum and tumor tissue levels of the vascular endothelial growth factor (VEGF) (ng/L) and (ng/gm tissue) respectively, in the studied groups. (Table I)

Results of the present work revealed that the untreated group of rats bearing DMBA -induced mammary tumor (group II), after 4 weeks treatment period, showed a significantly higher serum VEGF concentration compared to normal control rats (group I).Serum and tumor tissue levels of the VEGF were significantly decreased in mammary carcinoma bearing rats of groups III (fenofibrate-treated), IV (piogitazone-treated) and V(omega 3-treated) as compared with the non-treated rats of group II. (F=21.688, P< 0.001) and (F=49.033, P< 0.001) respectively. Furthermore, no significant difference was found in serum VEGF levels between group III, IV or V (P> 0.05). The least value of tumor VEGF level was found in fenofibrate-treated group, with a significant difference between this group and all other treated groups. Nevertheless, there was no significant difference found in tumor VEGF concentration between pioglitazone-treated group (group IV) and omega-3-treated group (group V) (P> 0.05).

3.3 Tumor tissue levels of cyclooxygenase-2 (U/g tissue), caspase-3 (ng/g tissue) and Ki-67 expression (%of positive staining cells) in the studied groups. (Table II, III; figure 5:a-h)

Data of the current study revealed that daily oral treatment of breast cancer bearing rats with fenofibrate (group III), pioglitazone (group IV) or omega-3 (group V) for successive 4 weeks resulted in a significant decrease in COX-2 level and a significant increase in caspase-3 level as compared to the untreated control group (group II) ($F=19.621$, $P< 0.\ 0.001$) and ($F=24.213$, $P< 0.001$) respectively. However, no significant difference in COX-2 or caspase-3 levels was detected between group III, IV or V ($P> 0.05$).

Regarding Ki-67 expression, after 4 weeks of therapy, the histopathologic examination of Ki-67-immunohistochemical stained tissue sections of untreated female rats bearing mammary carcinoma (group II) showed a strong nuclear staining in areas of invasive ductal carcinoma as well as in areas of in situ components. Oral treatment with fenofibrate, pioglitazone or omega-3 for 4 successive weeks to breast cancer-bearing female rats resulted in negative to weak, moderate and moderate to strong proliferative activity in the mammary tissue, respectively, with a significant decrease in Ki-67 expression in comparison to group II (MCp <0.05). Among all treated groups, group III has shown the least proliferative activity with a significant difference in Ki-67 expression between this group and all other treated groups ($P< 0.05$). However, no significant difference was detected between group IV and group V ($P> 0.05$).

4. DISCUSSION

The widely used breast cancer comprehensive treatments of surgery, chemotherapy, hormonal and radiation therapy have yielded positive results; however, their efficacy is beginning to plateau.[4,25] Studies concerning nuclear receptors have revealed the existence and implication of several additional nuclear receptors other than estrogen and progesterone in the biology of breast cancer. Peroxisome proliferator activated receptors (PPARs) are one of them. Therefore, PPAR ligands may be used as alternative or adjuvant therapy to treat breast cancer.[5]

DMBA is a well-known potent carcinogen which has been used to induce carcinogenesis in the mammary gland of experimental rodents such as rat and mouse. The mechanism of action of DMBA involves up-regulation of cytochrome P450 enzymes that metabolize DMBA into a mutagenic epoxide intermediate which readily forms DNA-adducts that are associated with DNA mutations and the malignant transformation that leads to carcinogenesis.[26]

Therefore, we examined the effect of PPARα ligand; fenofibrate, PPARγ ligand; pioglitazone and PPARα,γ dual ligand; omega-3 on the growth and proliferation of DMBA-induced mammary carcinoma in female rats as anticarcinogenics.

Findings from the current study revealed that, treatment of mammary tumor-bearing rats with fenofibrate, pioglitazone or omega-3, significantly decreased the steep increase of tumor growth rate as compared to the untreated mammary tumor-bearing rats. This finding confirmed previous reports that found that different PPAR agonists slowed down tumor growth effects in numerous breast cancer animal models and cell lines.[27-29] Fenofibrate alters cancer cell energy metabolism via two seemingly independent mechanisms: PPARα-dependent, which shifts cell metabolism from glycolysis to fatty acid β-oxidation and PPARα-independent, which causes accumulation of fenofibrate in cell membranes and inhibition of oxidative phosphorylation which leads to suppression of tumor growth.[30] Moreover, PPARα ligands can induce apoptosis in tumor as well as in endothelial cells.[31, 32] Pioglitazone probably exerts its ability to reduce tumor size via many mechanisms that may include inhibition of proliferation, induction of apoptosis and necrosis, inhibition of metastasis, migration and inflammation.[27,33,34] Furthermore, pioglitazone exerts broad spectrum anti-stromal, antiangiogenic and immuno-modulating activities.[35] Omega-3 affects breast cancer via many overlapping mechanisms that suggest their powerful inhibition of tumor growth.[36] These mechanisms include alterations in the properties of cancer cells; decrease proliferation, invasion, metastasis and increase apoptosis) as well as those of host cells; inflammation, immune response and angiogenesis.[37]

Inconsistent with our work, Suchanek et al reported that agonism of PPARα showed an increase in proliferation of MCF-7 breast cancer cells.[38] Also, Saez et al demonstrated that once an initiation event has occurred in breast tissue, increased PPARγ signaling might promote tumor progression in mammary gland tissue.[39]

Regarding serum and tumor tissue levels of VEGF, data presented in our study demonstrated that serum VEGF was significantly higher in untreated breast carcinoma- bearing rats when compared to normal rats. However, finofibrate, pioglitazone and omega-3 therapy inhibited angiogenesis, as evident from the significant reduction in serum as well as tumor VEGF concentrations in comparison to the untreated mammary carcinoma bearing rats. Angiogenesis enhancement and elevated levels of VEGF in serum and tumor tissue associated with breast cancer are due to hypoxia, inflammatory cytokines in the tumor

microenvironment and estrogens or progesterone in hormone-dependent tumors that stimulate the expression of hypoxia inducible factor-α, which in turn stimulates the release of VEGF from various sources, including tumor cells, inflammatory cells, stromal cells, platelets, vascular cells, fibroblasts and tumor-infiltrating macrophage.[40,41] However, suppression of angiogenesis by PPAR agonists was confirmed by previous reports which suggested that PPAR ligands down-regulate different proangiogenic factors (VEGF, basic fibroblast growth factor, matrix metalloproteinases, endothelin-1, nitric oxide, beta-catenin and platelet derived growth factors) and up-regulate endogenous angiogenesis inhibitors; such as thrombospondin-1, plasminogen activator inhibitor-1 and maspin.[15,42,44] In addition, fenofibrate, pioglitazone and omega-3 cause inhibition of endothelial VEGF receptor-2 expression and VEGF-mediated signaling via inhibition of the PI3K/Akt, MAPK/Akt signaling pathway and ERK1/2 phosphorylation, respectively.[31,45] Contrary to our results, a proangiogenic effect of pioglitazone has been reported in adipose tissue.[46] In addition, Jaminet et al reported that oxidation products of docosahexaenoic acid can promote angiogenesis.[47]

Focusing on COX-2 level, it was reported in our study that tumor tissue level of COX-2 was significantly lower in fenofibrate, pioglitazone or omega-3 treated groups as compared to breast cancer untreated rats. The decreased tumor COX-2 level associated with fenofibrate, pioglitazone or omega-3 treatment is compatible with previous studies. Moraes et al denoted that PPAR agonists suppressed COX-2 promoter activity by interfering with the nuclear factor-κB (NF-κB) signaling pathway and through inhibition of activated protein-1 (AP-1) binding to the cyclic AMP response element site of the COX-2 promoter.[48] In addition, binding of PPAR agonists to their receptors causes the activation of the MAPK pathways that enhances PPAR ligands-dependent transcriptional activity.[49] Moreover, n-3 Polyunsaturated fatty acids (PUFAs) may act as a natural COX "inhibitor" by inhibiting n-6 series eicosanoid biosynthesis, particularly prostaglandin E2; n-3 PUFAs become incorporated into membrane phospholipids, where they partially replace arachidonic acid and reduce its pool. Thus, they compete with n-6 PUFAs for desaturases, elongases, COXs and lipooxygenases.[37] Although the majority of studies support the anti-inflammatory role for PPAR activation, a few studies report that PPAR ligands have no anti-inflammatory activity or even exert a pro-inflammatory response.[50-52]

With respect to the marker of apoptosis;caspase-3, this study revealed that rats bearing mammary carcinoma treated with fenofibrate, pioglitazone or omega-3 showed a significant elevation of tumor tissue caspase-3 level relative to the untreated rats. This result is in agreement with previous studies that demonstrated the promotion of apoptosis by these PPAR ligands in different human breast cancer and other cancers cell lines by disrupting the net balance between pro- and anti-apoptosis factors that leads to promotion of the intrinsic pathway of apoptosis and activation of downstream effector caspases.[29,53,54] Further investigations have shed light on the exact PPAR agonists-induced effects on this apoptotic pathway. For instance, the up-regulation of different pro-apoptotic proteins (such as Bad, Bim, Bax and BID) via the inhibition of Akt1 and/or Erk1/2 pathways,[28,29,55,56] and the down-regulation of anti-apoptosis Bcl-2 family proteins such as Bcl-xl, Bcl-2, Mcl-1, Bcl2A1 and Bcl2L1 by down regulating NF-κB have been noted.[56-58]

Regarding ki-67 expression; a marker of proliferation, findings from our study revealed that fenofibrate, pioglitazone or omega-3 treatment for mammary carcinoma-bearing rats produced a significant reduction in tumor tissue Ki-67 expression as compared to the untreated rats. In agreement with our results fenofibrate, pioglitazone and omega-3 suppressed Ki-67 expression in several in vitro models of breast cancer.[27,29,59] In addition, omega-3 decreased proliferation of hyperplastic lesions of MNU-induced and DMBA-induced rat mammary carcinogenesis models.[60,61] Most probably, the antiproliferative effect of different PPAR agonists is through accentuated G_1 phase arrest by fenofibrate or pioglitazone. This arrest results from down-regulation of different cyclins (D1, E1, E2F1and A) and cyclin-dependent kinase 4 while up-regulating cyclin-dependent kinase inhibitor proteins (p21 and p27/Kip1) and (p16, p18, p21 and p27) by fenofibrate and pioglitazone respectively.[33,34,62,63] Moreover, the expression of cyclin A, cyclin B1 and cyclin-dependent kinase 1, the regulators required for the progression from G2 to mitosis, were decreased by omega-3.[64] In addition, PPAR agonists attenuated different growth factors and oncogenes which resulted in a severe retardation of the clonogenic tumor growth.[34,44,65,66]

5. CONCLUSION

From the aforementioned results, it could be concluded that modulating the activity of some nuclear receptors may provide a novel, nontoxic and selective therapeutic approaches for breast cancer patients. Peroxisome Proliferator Activated Receptor (PPAR) agonists are promising agents as they exert both a direct antitumoral and a broad spectrum of

proapoptotic, antiangiogenic, antiproliferative and anti-inflammatory activities. Moreover, fenofibrate, pioglitazone and omega-3 affect breast cancer via an orchestrated action on several signaling pathways that alter the expression of genes involved in breast tumorigenesis, making PPAR ligands candidate therapies for the treatment of breast cancer.

ACKNOWLEDGEMENTS

The authors are thankful to Staff members of Pharmacology and Experimental Therapeutics Department for support and cooperation.

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