Clinical and Experimental Health Sciences

The Potent Cytotoxic and Oxidative Effects of β-2 Selective ICI-118,551 on Breast Adenocarcinoma Cell Lines with Different Aggressiveness

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ABSTRACT

Objective: Beta-blockers are a group of drugs used in the treatment of cardiovascular diseases. On the other hand, the potential anticancer effects of these drugs have become increasingly important in recent two decades. In this paper, the effects of beta-1 selective esmolol, beta-2 selective ICI-118,551 and non-selective nadolol on breast cancer cell lines with different aggressiveness were investigated for the first time.

Methods: A standard spectrophotometricMTT assay was used to determine cell viability. Catalase activities and malondialdehyde levels were measured spectrophotometrically based on the reduction of absorbance resulted from hydrogen peroxide decomposition and the formation of thiobarbituric acid – malondialdehyde product, respectively.

Results: It was found that beta-2 selective ICI-118,551 was the most effective one among investigated blockers against MCF-7 and MDA-MB-231 cell lines. Additionally, it was seen that 50-150 µM ICI-118,551 treatment for 48 hours significantly changed catalase activities and malondialdehyde levels in both breast cancer cell lines in favour of radical production.

Conclusion: The obtained results showed that beta-2 adrenergic receptor specific antagonism plays a significant role in beta-blocker induced breast cancer cell death. The outstanding suppression in catalase activities and concomitant increase in radical levels appear to contribute to potent cytotoxic effect of ICI-118,551 on breast adenocarcinoma. Consequently, it can be clearly interpreted that ICI-118,551 may be a valuable option in the treatment of breast cancer.

Keywords: Esmolol, ICI-118,551, nadolol, breast adenocarcinoma.

1. INTRODUCTION

Adrenergic receptors, also called as adrenoceptors, are the cell surface components that play a central role in the sympathetic nervous system and form a class of G protein-bound receptors.They are classified as alpha (α-1 and α-2) and beta (β-1 and β-2) based on the interactions with their agonists and antagonists.Adrenergic receptors are the targets of medications such as α – and β-blockers as well as catecholamines including norepinephrine and epinephrine. The classification of these α – and β-blockers is done based on the type of receptor affected and these drugs are used in the treatment of conditions such as high blood pressure, migraine, irregular heart rhythm, heart failure, heart attack and chest pain (1).In addition to the routine use of adrenergic receptor blockers in the treatment of the specified ailments, their effects on proliferations of various healthy and cancerous cell/tissue types have been also investigated, especially in studies conducted over the last 20 years. It should be noted that these researches are very valuable in terms of focusing both the side effects and possible off-label usages of the so-called medications with known pharmacology.For instance, it was found that while non-selective β-blockers carvedilol and propranolol, and β-1 selective atenolol suppressed endoplasmic reticulum stress, oxidative stress and cell death in human coronary artery endothelial and liver cancer cells (2), another β-1 selective blocker nebivolol inhibited cell proliferation and induced death in human coronary smooth muscle and endothelial cells (3).On the contrary, Uzar and his friends stated that the same blocker protected rat brain from ischemia-induced damage by preventing oxidative stress and cell death(4). The suppression in myocardial cell death was shown for β-1 selective blocker metoprolol (5-7).In a study conducted by Smith and Smith, propranolol and β-2 specific blocker ICI-118,551 were shown to induce death in peripheral lung capillary endothelial cells (8). It was found that longevity of patients with ovarian cancer who take atenolol, propranolol, metoprolol, non-selectivelabetolol and carvedilolblockers

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significantly increased compared to patients who did not use (9).In a similar study conducted by Powe et al., it was found that metastasis formation decreased in patients who started β-blocker treatment before breast cancer diagnosis (10). It was stated in another observationally-based study that while atenolol had no effect, propranolol significantly decreased the mortality rates of breast cancer patients (11).It was also shown for propranolol that this beta-blocker inhibited proliferation and induced significant death in endothelial cells from hemangioma (12-17). Besides hemangioma endothelial cells, propranolol was found to induce cell death in several cancer types including pancreatic and stomach carcinomas, neuroblastoma, and melanoma (18-21). From thisliterature view, it is clearly seen that adrenergic receptor blockers have reverse effects on cell viability depend on the type of affected cell/tissue. In this study, the effects ofβ-1 selective esmolol, β-2 selective ICI-118,551 and non-selective nadolol on breast cancer cell proliferation were examined for the first time. For this aim, two breast cancer cell lines having different aggressiveness were used. Our results showed that all three drugs, but especially β-2 selective ICI-118,551, have promising activities on breast adenocarcinoma. In addition, it was found that the most effective beta-blocker ICI-118,551 suppressed antioxidant system and caused the formation of oxidative stress, which were determined by catalase activities and malondialdehyde (MDA) levels, respectively.

2. METHODS

2.1. Materials

All chemicals used were of analytical grade or higher where appropriate and obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA) unless otherwise stated.

2.2. Cell Culture

The human breast adenocarcinoma cell lines, MCF-7 and MDA-MB-231, were obtained from American Type Culture Collection (ATCC, USA). Both cell lines were cultured with high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 IU/mL penicillin, 100µg/mLstreptomycin and 20% fetal bovine serum (FBS) in a humidified atmosphere of 95% air with 5% CO_2 at 37°C.

2.3. Determination of Cell Viability

The effects of esmolol, ICI-118,551 and nadolol on the viability of MCF-7 and MDA-MB-231 cell lines were investigated through MTT assay which based on the reduction of the tetrazolium salt (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) to its insoluble formazan as a result of metabolic activity (22). In the assay, briefly, breast cancer cell lines were seeded into 96-well plates at a density of $1x10^4$ per 100 µLwell and allowed to attach for 24 h before drug treatment. Then, the cells were exposed to various concentrations of β-blockers (5–250 µM) for 24 and 48 h. After treatment period, 25 µL MTT solution (5 mg/mLphosphate-buffered saline, PBS) was added and the plates were located in an incubator with 5% CO_2 at 37 ^{0}C. After 4 h incubation time, insoluble formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and then cell growth was assessed by measuring the absorbance at 570 nm. Cell viability was expressed as percentage survival, with 100% survival taken as that observed in related control cells. Because DMSO was used as the drug solvent, control cells were treated with maximum 0.1% or lower concentrations of DMSO. The solvent in the used concentration range was non-toxic and did not influence the viabilities of both cell lines.

2.4. Crude Extracts Preparation For Biochemical Analysis

Commercial RIPA Buffer (Sigma, R0278, USA) was used for the cell lysis procedure. Briefly, growth medium was removed by aspiration and cells were washed two times with Dulbecco's phosphate-buffered saline (DPBS) to remove residual medium. After final washing step, an appropriate volume of RIPA Buffer (1 mL for $0.5\text{-}5x10^7$ cells) was added and cells were incubated on ice for five min. Then, the plates were scraped and the lysates were clarified by centrifugation at 12,000 rpm for 10 min at 4 ^{0}C. Supernatants were carefully transferred into clean tubes and stored at –70^{0}C for future use.

2.5. Biochemical Analysis

2.5.1. Catalase activity

Catalase activity was determined according to the Aebi method (23). The method is based on the reduction of absorbance at 240 nm resulted from hydrogen peroxide (H_2O_2) decomposition by catalase at 25 ^{0}C. 10.5 mM H_2O_2 prepared in 50 mM phosphate buffer (pH 7.0) was used in the assay. 1 U enzyme activity is defined as the amount of enzyme required to decompose 1 µmol H_2O_2 under standard conditions. Enzyme activity was calculated using the molar extinction coefficient of H_2O_2 (39.4 L $mmol^{-1}cm^{-1}$).

2.5.2. Membrane lipid peroxidation levels

MDAis a stable by-product of membrane lipid peroxidation. To assess the membrane lipid peroxidation levels, the formation of MDA was measured by using the thiobarbituric acid (TBA) reaction (24). Briefly, 500 µL cell lysate was incubated with 500 µL10% trichloroacetic acid (TCA) for 15 min at 90 ^{0}C. After 10 min centrifugation, 500 µL supernatant was mixed with 500 µLTBA and again incubated for 15 min at 90 ^{0}C. The absorbance of MDA-TBA product in 532 nm was recorded against blank. Lipid peroxidation levels were calculated using the molar extinction coefficient of MDA ($1.56x10^5$ mol L^{-1} cm^{-1}).

2.5.3. Total protein levels

Bradford method was used for the measurement of the total protein concentration in the cell lysates (25). Briefly, 100 µL sample was mixed with 900 µL Bradford reagent prepared by using Coomassie Brilliant Blue G-250 dye and after 2 min incubation, the absorbance in 595 nm was recorded against blank.Bovine serum albumin (BSA) was used asa standard.

2.6. Statistical Analysis

The data are presented as the mean ± S.E.M. The differences in variance were analyzed statistically using a one-way analysis of variance (ANOVA) test by Graphpad prism 5.0 statistics software (GraphPad, La Jolla, CA, USA). Tukey's test was used as a post hoc.

3. RESULTS

3.1. The Effects of Beta-Blockers on Breast Adenocarcinoma Cell Viability

The antiproliferative effects of 5-250 µM β-1 selective esmolol, β-2 selective ICI-118,551 and non-selective nadolol on breast cancer cell lines were investigated for 24 and 48 h. For this aim, two different cell lines with different aggressiveness, MCF-7 and MDA-MB-231, were used. MCF-7 cell line which has a normal expression of human epidermal growth factor receptor 2 (HER2) is estrogen – and progesterone-receptors positive. On the other hand, MDA-MB-231 cells are triple negative and therefore more aggressiveand less chemosensitive to conventional cytotoxic agentsthan the first one (26).We observed that there was no any significant difference between control and5-10µM esmolol treated groups after 24 h treatment in MCF-7 cell line.Although 25-250 µM esmolol could significantly inhibit cell viability compared to the control, the cytotoxicitydid not gradually increase with the increasing concentrations of the drug (Figure 1A).This cell line became sensitive to the lowest concentrations of esmolol after an additional 24 h of treatment. The same pattern for higher concentrations of the drug wasalso recorded but cell viability could not be reduced below 56% (Figure 1B). It was observed that MDA-MB-231 cell line was more resistant to esmolol (Figure 2). Whilecell viability could not be significantly reduced compared to the control group up to 150 and 250 µM of esmolol for 24 and 48 h, respectively, it was even insignificantly induced at lower concentrations of the drug. The viability percentage of MDA-MB-231cells treated with the highest concentration of esmolol was determined as 68.40±11.72. Unlike esmolol, ICI-118,551 was observed to be highly effective against breast cancer cell proliferation (Figures 3 and 4). As can be seen from Figure 3A, 25-250 µM ICI-118,551 treatment for 24 h caused significant inhibition of MCF-7 cell proliferation compared with the control group and this inhibition was generally correlated with the increasing concentration of the drug. This potent cytotoxic effect of ICI-118,551 was further enhanced by 48 h treatment and viability decreased up to 5.33±1.13 (Figure 3B). More aggressive breast cancer cell line MDA-MB-231 was again found more resistant to this blocker,but especially for highest concentrations and longer treatment period. Nevertheless, it was observed that cell viability significantly reduced below 50% from 100 µM onwards in both 24 and 48 h treatments. Despite it was observed that nadolol was slightly more effective than esmolol, viability values below 50% could not be obtained for both cell lines (Figures 5 and 6). As is the case with β-1 selective esmolol and β-2 selective ICI-118,551, triple negative MDA-MB-231 cell line showed more resistance to non-selective nadolol than MCF-7. Given all these results, it is obvious that β-2 selective ICI-118,551 is much more effective on breast cancer than other investigated blockers.

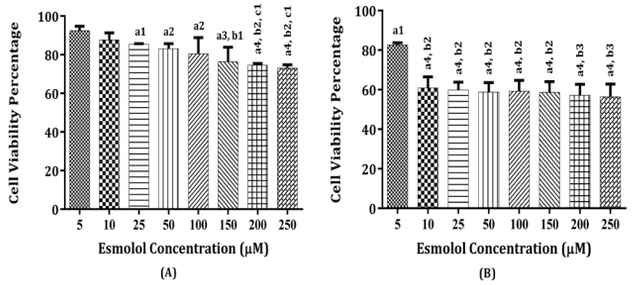

Figure 1. *The effects of different concentrations of esmolol on viability percentage of MCF-7 cells for 24 h (A) and 48 h (B). Data with error bars show the mean ± S.E.M of three experiments. adenotes significant differences between other studied groups and control group ([a1]p<0.05; [a2]p<0.01; [a3]p<0.001; [a4]p<0.0001), b denotes significant differences between other studied groups and 5 µM esmolol treated group ([b1]p<0.05; [b2]p<0.01; [b3]p<0.001), c denotes significant differences between other studied groups and 10 µM esmolol treated group ([c1]p<0.05) by Tukey's multiple range tests.*

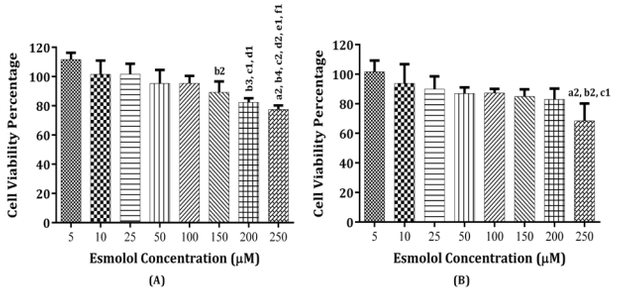

Figure 2. *The effects of different concentrations of esmolol on viability percentage of MDA-MB-231 cells for 24 h (A) and 48 h (B). Data with error bars show the mean ± S.E.M of three experiments. adenotes significant differences between other studied groups and control group ([a2]p<0.01), b denotes significant differences between other studied groups and 5 µM esmolol treated group ([b2]p<0.01; [b3]p<0.001; [b4]p<0.0001), c denotes significant differences between other studied groups and 10 µM esmolol treated group ([c1]p<0.05; [c2]p<0.01), d denotes significant differences between other studied groups and 25 µM esmolol treated group ([d1]p<0.05; [d2]p<0.01), e denotes significant differences between other studied groups and 50 µM esmolol treated group ([e1]p<0.05), f denotes significant differences between other studied groups and 100 µM esmolol treated group ([f1]p<0.05) by Tukey's multiple range tests.*

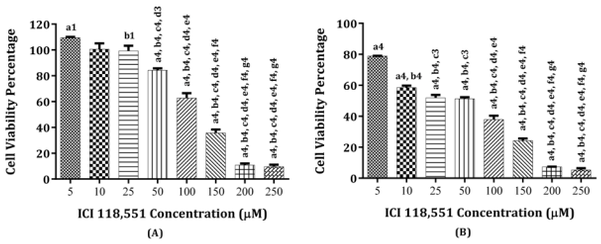

Figure 3. *The effects of different concentrations of ICI-118,551 on viability percentage of MCF-7 cells for 24 h (A) and 48 h (B). Data with error bars show the mean ± S.E.M of three experiments. adenotes significant differences between other studied groups and control group (^{a1}p<0.05; ^{a4}p<0.0001), b denotes significant differences between other studied groups and 5 μM ICI-118,551 treated group (^{b1}p<0.05; ^{b4}p<0.0001), c denotes significant differences between other studied groups and 10 μM ICI-118,551 treated group (^{c3}p<0.001; ^{c4}p<0.0001), d denotes significant differences between other studied groups and 25 μM ICI-118,551 treated group (^{d3}p<0.001; ^{d4}p<0.0001), e denotes significant differences between other studied groups and 50 μM ICI-118,551 treated group (^{e4}p<0.0001), f denotes significant differences between other studied groups and 100 μM ICI-118,551 treated group (^{f4}p<0.0001), g denotes significant differences between other studied groups and 150 μM ICI-118,551 treated group (^{g4}p<0.0001) by Tukey's multiple range tests.*

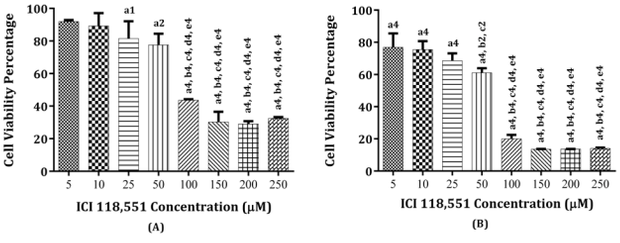

Figure 4. *The effects of different concentrations of ICI-118,551 on viability percentage of MDA-MB-231 cells for 24 h (A) and 48 h (B). Data with error bars show the mean ± S.E.M of three experiments. adenotes significant differences between other studied groups and control group (^{a1}p<0.05; ^{a2}p<0.01; ^{a4}p<0.0001), b denotes significant differences between other studied groups and 5 μM ICI-118,551 treated group (^{b2}p<0.01; ^{b4}p<0.0001), c denotes significant differences between other studied groups and 10 μM ICI-118,551 treated group (^{c2}p<0.01; ^{c4}p<0.0001), d denotes significant differences between other studied groups and 25 μM ICI-118,551 treated group (^{d4}p<0.0001), e denotes significant differences between other studied groups and 50 μM ICI-118,551 treated group (^{e4}p<0.0001) by Tukey's multiple range tests.*

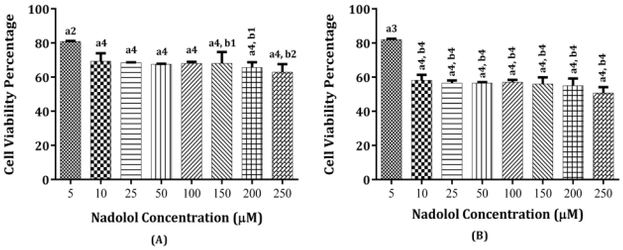

Figure 5. *The effects of different concentrations of nadolol on viability percentage of MCF-7 cells for 24 h (A) and 48 h (B). Data with error bars show the mean ± S.E.M of three experiments. adenotes significant differences between other studied groups and control group (^{a2}p<0.01; ^{a3}p<0.001; ^{a4}p<0.0001), b denotes significant differences between other studied groups and 5 μM nadolol treated group (^{b1}p<0.05; ^{b2}p<0.01; ^{b4}p<0.0001) by Tukey's multiple range tests.*

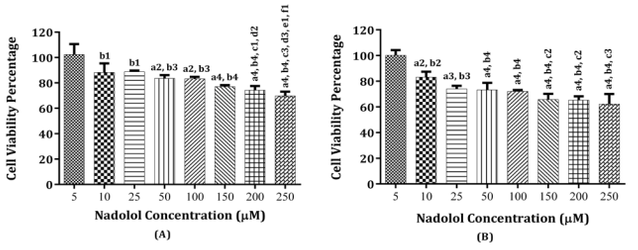

Figure 6. *The effects of different concentrations of nadolol on viability percentage of MDA-MB-231 cells for 24 h (A) and 48 h (B). Data with error bars show the mean ± S.E.M of three experiments. adenotes significant differences between other studied groups and control group (^{a2}p<0.01; ^{a3}p<0.001; ^{a4}p<0.0001), b denotes significant differences between other studied groups and 5 μM nadolol treated group (^{b1}p<0.05; ^{b2}p<0.01; ^{b3}p<0.001; ^{b4}p<0.0001), c denotes significant differences between other studied groups and 10 μM nadolol treated group (^{c1}p<0.05; ^{c2}p<0.01; ^{c3}p<0.001), d denotes significant differences between other studied groups and 25 μM nadolol treated group (^{d2}p<0.01; ^{d3}p<0.001), e denotes significant differences between other studied groups and 50 μM nadolol treated group (^{e1}p<0.05), f denotes significant differences between other studied groups and 100 μM nadolol treated group (^{f1}p<0.05) by Tukey's multiple range tests.*

3.2. The Effects of ICI-118,551 on Catalase Activities of Breast Adenocarcinoma

Catalase is one of the main antioxidant enzymes that catalyzes the dismutation of H_2O_2 to molecular oxygen and water. In this study, catalase activities of MCF-7 and MDA-MB-231 cells treated with 50-150 μM ICI-118,551 for 48 h were determined. It was found for all treatments that catalase activities were significantly decreased compared to related controls (p<0.0001) (Figure 7A-B). These decreases were reached to about 14.7 – and 11.6-folds at 150 μM in MCF-7 and MDA-MB-231 cell lines, respectively.

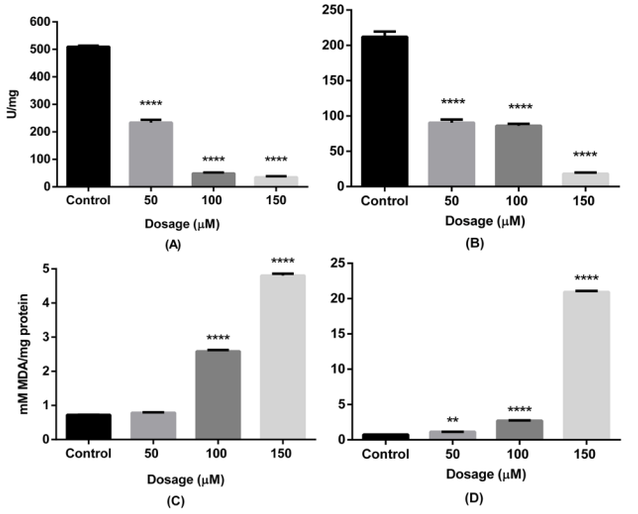

Figure 7. *The effects of different concentrations of ICI-118,551 on catalase activities of MCF-7 (A) andMDA-MB-231 (B) and MDA levels of MCF-7 (C) andMDA-MB-231 (D) cells for 48 h. Data with error bars show the mean ± S.E.M of three experiments. **=p<0.01; ****=p<0.0001 denotes significant differences between control and other studied groups by Tukey's multiple range tests.*

3.3. The Effects of ICI-118,551 on MDALevels of Breast Adenocarcinoma

In this paper, we also dealt with MDA levels of the samples treated with 50-150 µM ICI-118,551 for 48 h. As very well known, MDA level is a reliable indicator of lipid peroxidation resulting from oxidative stress. As can be seen from Figure 7C-D, all treatments caused the significant increases in MDA levels, except 50 µM ICI-118,551 treated MCF-7 cells. MDA levels of MCF-7 and MDA-MB-231 cells gradually increased with the increasing concentrations of the blocker and reached to 4.81±0.06 and 20.93±0.18 by increasing about 6.68 – and 28.67-folds compared to related controls, respectively. There were moderate negative correlations (r_{MCF-7}=-0.778; $r_{MDA-MB-231}$=-0.731) between catalase activities and MDA levels of both cell lines. These findings clearly showed for the first time that the significant suppressions in catalase activitiesand concomitant increases in radical levelscontributed to potent cytotoxic effect of ICI-118,551.

4. DISCUSSION

In this paper, the effects of β-1 selective esmolol, β-2 selective ICI-118,551 and non-selective nadolol blockers were examined on MCF-7 and MDA-MB-231 cell lines. According to the obtained findings, it was seen that ICI-118,551 rather than esmolol and nadolol has very potent cytotoxic effects on both breast adenocarcinoma lines. As mentioned previously, β-blockers exert their intracellular effects via interaction with theassociated adrenergic receptors. Hence, the apparent cytotoxicity of β-2 selective ICI-118,551 on the stated cell lines indicate in a sense that β-2 adrenergic receptor-specific antagonism plays an important role in β-blocker induced breast cancer cell death. As a matter of fact, similar results have been reported in some other studies. For instance, Wolter et al. compared the effects of β-1 and β-2 specific blockers on neuroblastoma cell line with non-selective blocker propranolol and showed that this non-selective blocker mainly induced death with β-2specific antagonism (20). It was stated in other recent studies that non-selective propranolol and β-2 selective ICI-118,551 but not β-1 selective metoprolol and atenolol were found to be quite effective against human breast and colorectal cancers (27,28). However, there are some data that contradict these results (29). The variations in the expression levels of differentβ-adrenergic receptor subtypesin different cell types can be demonstrated as the responsible for this situation. On the other hand, off-target effects of ICI-118,551 on human breast cancer cells should be considered and further investigated due to the inability to reach similar results with non-selective nadolol.

It is known from other studies that β-blockers have some anti-oxidative effects on healthy/non-cancerous cells and tissues (30,31). On the other hand, according to our literature view, there is no any other research dealing with the effects of β-blockers on antioxidant system of cancerous cells. Our results indicated that oxidative stress, which wascharacterized by MDA levels, increased as a result of β-2 selective ICI-118,551 treatment in both cancer cell lines. As it is known, increase in intracellular radical levels, occurrence of oxidative stress and resultant damage in biomolecules is a cascade-like process leading the cell to death. Hence, it is clear that increased oxidative stress status as one of the results of significant decreases in catalase activities contributed to ICI-118,551 induced toxicity.

5. CONCLUSION

As a conclusion, all these results reveal the potential cytotoxic and oxidative effects of ICI-118,551 on human breast cancer cell lines. The findings obtained on the aggressive MDA-MB-231 model which is resistant to chemotherapy are particularly important. Although high concentrations seem to be required to reach the effective doses in *in vitro* studies, it is well known that there are significant differences in these doses when compared to *in vivo* models which require much lower doses of β-blockers. Therefore, β-2 selective ICI-118,551 may be seen as a potential candidate in the treatment of human breast cancer. Considering the difficulties of the new drug development stages, it is extremely important to find the usability of β-blockers having no side effects and with known pharmacology in cancer treatment. Finally, we must state that these results should be supported by further *in vivo* studies.

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