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Research Article

Potential Effects of Hypoglycemic and Non-Steroidal Anti-Inflammatory Drugs on Breast Cancer (MCF-7) Cell Line

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ABSTRACT
Breast cancer represents the most common non-cutaneous cancer and the second disease leading to cancer death for women in the world. Anti-diabetic and NSAIDs drugs have become as potential chemo preventive agents. This study is attentive to investigate the impact of metformin as oral hypoglycemic drug on breast cancer call lines as well as the effect of NSAIDS namely aspirin and ketorolac separately and compared the outcome. In vitro method was used to investigate the influence of metformin, aspirin and ketorolac on human breast cancer cells lines MCF7 and HBL100 cell lines as normal breast tissue and both maintain with minimal essential media. The results showed 0.0025 g/ ml of aspirin inhibits significantly viability of MCF7 cell line 14.3% compared with HBL100 cell line (represents normal breast tissue)91% viable cells. In metformin cell viability 48% in MCF7 cell line at 2 mg/ml in comparison with HBL100 cell line with same concentration to be 89% availability. In case of ketorolac is only effective at high concentration of 15mg /ml which clinically not applicable. The obtained results might be useful to treat and prevent reoccurrence breast cancer with women at high risk especially aspirin drug.

Keywords: MCF7, Aspirin, Metformin, NSAIDS drugs, Breast cancer

INTRODUCTION
Breast cancer typically represents the most common non-cutaneous cancer, as well as the second disease leading to cancer-related death for women in the world [(1)]. It was found that every 1 in 8 women might be undergoing development for breast cancer over her lifetime [(2)]. In the past 30 years, the rate of breast cancer occurrence has remained relatively unchanged, and the results of death caused by breast cancer continue to be more than 40% worldwide [(1,3)]. It was known that breast cancer significantly arises when cells lose their capability to stop the process of proliferation and the resistance or reduction of cell death by apoptosis [(4)]. Antidiabetic and Non-steroidal anti-inflammatory (NSAIDs) drugs have become substantial interest as potential chemo-preventive agents for cancer treatment [(5-7)]. Metformin is an antidiabetic drug. It affects cancer through a direct and indirect mechanism. Directly metformin can minimize the proliferation of many breast cancer cell lines. The indirect mechanism of metformin on cancer cell lines is a reduction in the insulin level and bioavailability of sexual hormones. It had been exhibited that metformin inhibits the proliferation of breast cancer cell lines, maybe the promotion of cell cycle arrest, and induce cell apoptosis and even necrosis, also associated with increase oxidative stress of the cells. A retrospective study suggested that the uses of non-steroidal anti-inflammatory drugs (NSAIDs) may be related to a better outcome after doing the mastectomy. Ketorolac was approved by FDA in 1989. It is available as a generic drug. It is not narcotic but provides opioid level pain management, therefore reduced narcotic requirement. It had been reported about 55% of women with breast cancer who received ketorolac drug; their risk was decreased of a breast cancer relapses in the first 24 months of follow up. While other analgesics (ketamine, clonidine, and sufentanil) did not show this benefit. Aspirin is an anti-inflammatory antipyretic that belongs to NSAID. Aspirin is mainly used as pain killers; also, it has an anticoagulant effect. For that reason, it became the most wildly used drug around the world. there is evidence that chronic treatment with aspirin is associated with decreased risks of breast cancer recurrence and mortality. Aspirin may exert an anti-cancer effect by blocking the interaction between breast cancer tumor cells and platelets. Based on above information, this study is attentive to investigate the impact of metformin on the oral hypoglycemic drug on breast cancer cell lines and also the

effect of NSAID, namely ketorolac and aspirin separately and compared the outcomes.

MATERIALS AND METHODS

This study was carried out in the Iraqi Center of Cancer and Medical Genetics Research (ICCMGR) 2019.

Drugs preparation:

Metformin drug (1,1-dimethylbiguamidehydrochlloride) have purchased form Sigma Aldrich were dissolved and diluted in dimethyl sulfoxide (DMSO) solvent. Serial dilution of concentrations 0.0164, 0.0082, 0.0041, 0.0020, 0.001, 0.0005, 0.00025, and 0.000128 g/ml freshly were prepared. Aspirin drug (acetylsalicylic acid) (CDH company) were also used and diluted using phosphate buffer saline (PBS) to prepare the following concentrations 0.0025, 0.00125, 0.000625, 0.0003125, 0.000156, 0.000078, 0.000039, and 0.0000195 g/ml. Ketorolac ((±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid) (Huons O.Ltd.) were additionally used and dissolved in PBS buffer. The serial dilutions are as the following 0.015, 0.0075, 0.00375, 0.001875, 0.0009375, 0.000468, 0.0002343, 0.000117, and 0.0000585 mg/ml.

Cell Culture:

The effect of the Metformin, Aspirin and Keorolac drugs was investigated with In vitro method on human breast cancer cell line MCF7 (provided from the experimental therapy department/ ICCMGR) and HBL-100 cell line that obtained from a milk nursing mother (three days after labour with no evidence of breast lesion and no family history of breast cancer). These cell lines was maintained in MEM (Minimal Essential Media) (GIBCO chemicals) supplemented with 10% Fetal Bovine Serum (F.B.S.), and used in cytotoxicity assay.

Cell line Preparation and Cytotoxicity:

According to Freshney [8]. The growth medium was poured off. The trypsin- versene (U.S. Biological, USA) as 2-3 ml was added to the cell-sheet and the flask racked gently. After about 30 seconds most of the tyrosine-versene was dispensed off and next, cells were incubated at 37°C for 10 min. until cells ensured detached from the plate. Cells were further soaked by pipetting in growth medium.

Next, (200 μl) of cells with concentration (1×10^4) in growth medium were added to the sterile 96-well black plates. The 96-well plates were wrapped with a self-adhesive film; lid placed on, and for 24hrs at 37°C were incubated. When the cells are in exponential growth, (Lag phase), the medium was soaked and serial dilutions of Metformin, Aspirin and Ketorolac drugs in serum free media (SFM) were added (for each concentration of each drug, six replicates were added). Then, the plates were re-incubated for 72 hrs at 37°C exposure time.

Next, the well plates were decanted off from supernatants after ending of each exposure period, while sterile conditions was maintained. 100 μl of crystal violate stain was added. After that, plates were covered and incubated for 20 min. at 37°C. Finally, plate was washed in tap water and let to dry. The Optical density (OD) was measured at 492 nm. using microplate reader.

The obtained results were then compared with control which was incubated in the absence of the above drugs. Cell viability % was estimated using the following formula

$$\text{cell viability } \% = \frac{\text{mean OD}}{\text{control OD}} \times 100\%$$

RESULTS

An ideal method for cancer treatment needs a drug that showed high cytotoxicity with a lower concentration and induces a high percentage of cell death when incubated with cancer cells. In order to evaluate the intrinsic toxicity of the Asprin, Metaforin, and ketorolac drugs on the MCF7 cell line, an MTT assay of cell viability was done. MCF7 were incubated with different concentrations of Asprin 0.0025, 0.00125, 0.000625, 0.0003125, 0.000156, 0.000078, 0.000039, and 0.0000195 g/ml dissolved with BPS and 0.0164, 0.0082, 0.0041, 0.0020, 0.001, 0.0005, 0.00025, 0.000128 g/ml of Metaformin, and 0.015, 0.0075, 0.00375, 0.001875, 0.0009375, 0.000468, 0.0002343, 0.000117, 0.0000585 mg/ml of Ketorolac in medium for 48 hrs. at 37°C. Cell viability evaluation of breast cancer cells (MCF7), which treated with different concentrations of Asprin, was done. The results showed that (0.0025 g/ml) concentration of Aspirin exhibited significantly lower cell viability (14.3%) comparing to the HBL100 cell line (normal cell line) that treated with the same concentration (cell viability 91%) Figure 1-A, B. The same was true for (0.00031, 0.00062, and 0.00125 g/ml) concentrations of Asprin drug for MCF7 cell line which were also showed sharp decreasing in cell viability (65, 25.67, and 15.47 %) respectively in comparison with the identical concentrations of Aspirin that have been treated to HBL100 and presented cell viability (88, 85, and 92%) respectively.

Regarding to Metformin, MCF7 cell line that treated with concentrations of (0.004, 0.008, and 0.016 g/ml) (cell viability 45.2, 46.9, and 48 % respectively) were significantly lower than that of

same concentrations of Metformin incubated with HBL100 cell line (cell viability (90, 89, and 89% respectively) (Figure 2 A,B).

For Ketorolac, MCF7 cell line were incubated with 0.0 15 g/ml concentration indicated significantly lower cell viability (42.7%) in compared with HBL100 cell line of the same concentration with cell viability percentage (86%) and non-significant differences were indicated with the other concentrations as shown in Figure 3 A,B.

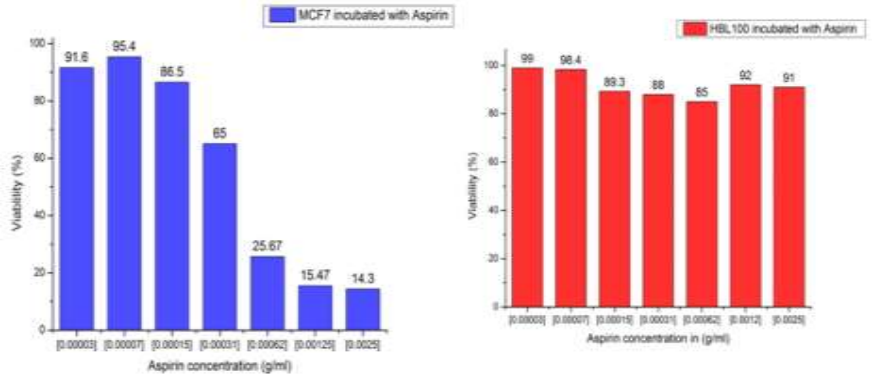

Fig.1: A- Cell viability percentage of MCF7 cell line incubated with Aspirin different concentrations, B- Cell viability percentage of HBL100 (normal cell line) incubated with Asprin different concentrations

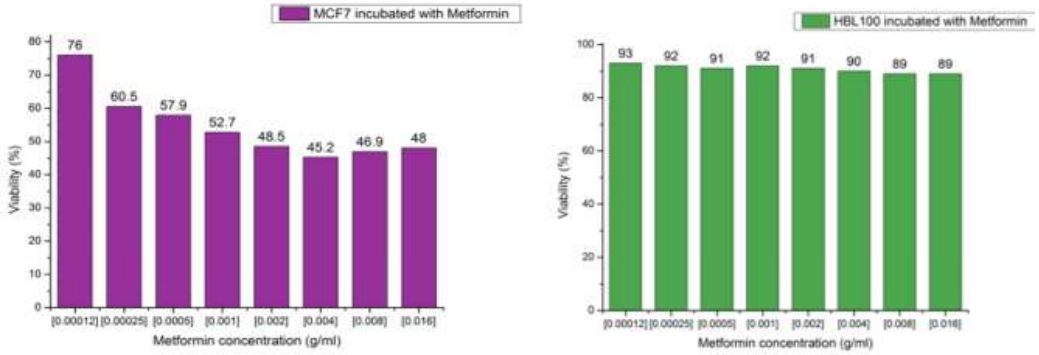

Fig.2: A- Cell viability percentage of MCF7 cell line incubated with Metaformin different concentrations, B- Cell viability percentage of HBL100 (normal cell line) incubated with Metaformin different concentrations

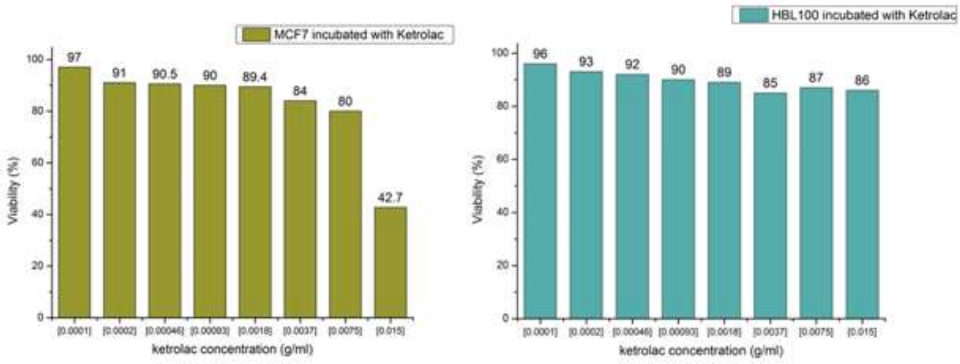

Fig.3: A- Cell viability percentage of MCF7 cell line incubated with Ketrolac different concentrations, B- Cell viability percentage of HBL100 (normal cell line) incubated with Ketrolac different concentrations

DISCUSSION

This study includes three FDA approved, cheap and shelf available drugs, that have been used for many years for treating certain diseases namely are Aspirin, Metaformin and Ketrolac. The mentioned drugs were used in this study to find

their potential effect on breast cancer cell line MCF7 which is carrying estrogen receptors. Approximately 50-70% of breast cancer are estrogen receptor positive; so, estrogen signaling play crucial role in estrogen receptor breast cancer that involved in cell proliferation and survival [9,10]. HBL100 cell line as normal breast tissue was used in this study as a negative control. According to studies on anti-proliferative effect of aspirin on breast cancer which is in agreement with recent records [11-15], our finding reported that aspirin at concentration of 0.62 mg/ ml has viability of only 25.6% of MCF7 cell line while the viability of cell of HBL100 cell line is 85% (normal cells) at the same concentration of aspirin, which means aspirin has selective effect on cancer cells. Aspirin had been illustrated to reduce the incidence, progression also the mortality of several forms of cancer in many randomized studies [16-19]. At molecular level, it had been identified that the activity of (NSAIDs) is attitudes to inhibition of the COX-2. Enzyme that overexpressed in certain form of breast tumors [20,21]. Moreover, there is clear evidence had suggested that the ability of aspirin to reduce breast cancer invasion can be platelets mediated. As demonstrated by a study investigate the effect of aspirin on embryonic induced angiogenesis. Aspirin is embedded on agarose reduce the number of capillaries comparing to the control group and this procedure indicated that aspirin has strong antiangiogenic effect [22].

Platelets had been associated with cancer metastasis and regarded as central component micro environment of the tumor and Aspirin was known to have anti platelets activity [23,24].

It is well known that lL8 increases tumor cell invasion, angiogenesis and expansion of cancer stem cells. It has been noticed that platelets activate endothelial Cells to release about 50 fold of lL8. concerning to that, Aspirin was used to decreases circulating IL8 and tumor IL8 levels [25]. Regarding, metformin that in controlled clinical studies of metformin maximum plasma concentration doesn't exceed 5 mg/ ml. Our data showed that at level of 2mg/ml only 48.5% of MCF7 cell line was viable compared with 91% viable cells HBL100 cell line (normal breast tissue). This means that metformin selectively inhibit cancer cells. In this article, the effect of different concentrations of metformin on MCF7 cell line were investigated to to if the effect of metformin could be achieved using clinically relevant concentration or only at higher concentration (i.e experimental concentration) [26]. At molecular levels metformin is responsible for induction of P53. P53 is transcription factor that reduces tumorogenesis by regulating transcription of large number of genes that responsible for control of cell cycle arrest, apoptosis, protein translation and cell growth [27, 28]. Moreover, Metformin induced increase in the level of TGF-B-1 contributed to induction of apoptosis in response to the damage of DNA. Also, metformin had been stated to have cytotoxic activity. Hirsch et. al. demonstrate that metformin reduces cellular transformation that selectively kills cancer stem cells in different breast cancer cell lines [29]. Furthermore, Song et al, identified that metformin is cytotoxic to cancer stem cells comparing to non-cancer stem cells. Higher concentration of metformin may bring cancer cells to death but this doesn't fit with clinical data that patients exposed to a constant micromoloar concentration of metformin for treatment of diabetes which respond better to chemotherapy. Therefore, diabetics patients will get benefits from therapeutic levels of metformin [30]. Considering ketorolac as potent NSAIDs at very high level (15 mg/ ml) achieved 42.7% viable cancer cells that is clinically not applicable. Additionally, this drug can't be given for more than five days.

CONCLUSION

Further research is recommended to adjust the doses of metformin for diabetic patients with breast cancer (therapeutic level). Moreover aspirin is promising drug for breast cancer and to adjust the dose to clinically relevant dose. Further studies for combination of these two drugs together to investigate their effect on breast cancer cell lines.,

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