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Cytotoxic Effect of the Alcoholic Extract of *Conocarpus erectus* Leaves on MDA-MB $_{231}$ and MCF7 Breast Cancer Cell Lines

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Abstract

Currently, there is a growing interest in medicinal plants extracts as some plants have shown antitumor potential. The goal of this study was to test the anticancer activity of methanol extract of *Conocarpous erectus* leaves in breast cancer cells. Cytotoxicity was tested *in vitro* on breast cancer cell lines, MCF_7 [Estrogen receptor + (ER+)] and MDA-MB_{231} [Estrogen receptor - (ER-)], in addition to normal fibroblast cells (REF). MTT assay was utilized to measure the growth inhibitory effects after 48 hours exposure to extracts. Viability results indicated that MDA-MB231 were sensitive (GI50 = 56.1µg/ml).However, no sensitivity was seen in both MCF7 and REF cells (GI50>100 µg/ml). Interestingly the sensitivity seen in MDA-MB231 cells was associated with a significant reduction in cell number and size. It can be concluded from this study that leaves extracts may provide a candidate therapy against breast cancer cells and sensitivity was not linked with ER expression. No effect was observed in normal cells.

Keywords: *Conocarpous erectus*, Methanol extract, Anticancer activity, MCF_7 and MDA-MB_{231}REF.

التأثير السمي الخلوي للمستخلص الكحولي لاوراق نبات الكونوكاربس في خطي خلايا سرطان الثديMCF_7 و MDA−MB $_{231}$

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الخلاصة

في الوقت الحالي ، هناك اهتمام متزايد بالمستخلصات النباتية الطبية حيث أظهر البعض إمكانات مضادة للأورام. كان الهدف من هذه الدراسة هو اختبار النشاط المضاد للسرطان لمستخلص الميثانول من أوراق الكونوكاربوس *Conocarpous erectus* في خلايا سرطان الثدي. تم اختبار السمية الخلوية في المختبر على خطوط خلايا سرطان الثدي ، MCF7 [مستقبل الاستروجين + (ER +)] و MDA−MB231 [مستقبل الاستروجين − (ER−)] بالإضافة إلى الخلايا الخلايا الليفية الطبيعية (REF). تم استخدام اختبار MTT لقياس التأثيرات المثبطة للنمو بعد 48 ساعة من التعرض للمستخلصات. أشارت النتائج المتعلقة بالحيوية إلى أن MDA−MB231 كانت حساسة (GI50 = 56.1 ميكروغرام / مل) ، ومع ذلك ، لم تظهر

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أي حساسية في كل من خلايا MCF7 و GI50) REF> 100 ميكروغرام / مل). ومن المثير للاهتمام أن الحساسية التي لوحظت في خلايا MDA–MB231 ارتبطت بانخفاض كبير في عدد الخلايا وحجمها ، يمكن الاستنتاج من هذه الدراسة أن خلاصات الأوراق قد توفر علاجًا مرشحًا لخلايا سرطان الثدي وأن الحساسية لم ترتبط بتعبير ER. لم يلاحظ أي تأثير في الخلايا الطبيعية.

Introduction

One of the common cancer types in women in the world is breast cancer, chiefly in Iraqi women [1]. In Iraq, breast cancer accounts32% amongst other types of cancers which infect women, depending on recent Board, I.C., Iraqi cancer registry.

Two-third of the population still depend on the plants as traditional drugs for the health care against the diseases [2]. Hence, it is very important to evaluate the plant extracts for discovering the compounds that may have a novelty to be drugs for treating the diseases. There are many extracts which record as having antibreast cancer effect that have been reported like *Zingiber officinale* [3], *Morus lba* L.[4], *Angelica archangelica*[5], *Aralia elata* [6], *Pithecellobium dulce*[7] *Abelmoschus esculentus* [8].

*Conocarpus erectus*is is a genus of two species of Combretacea family that are flowering plants. Conocarpus have antioxidants, antimicrobial and anticancer properties.It has also showed high free radical scavenging activity towards DPPH radical in treating many disorders as folk remedy by using this plant like catarrh, orchitis, headache, anemia, prickly heat, diabetes, diarrhea, conjunctivitis, bleeding, tumors, gonorrhea, syphilis and as anti-pyretic [9, 10, 11]. Bark and fruits were used in the control of diabetes, wounds and hemorrhoids [12].

The aim of the search was to study the anticancer activity of methanolic extract of leaves from *Conocarpus erectus* with two breast cancer cell lines; MCF-$_7$ (ER+) and MDA-MB$_{231}$ (ER-).

Material and Methods

1. Plant Collection and Extract Preparation

Plant leaves collected from the gardens of Baghdad city, Iraq, were washed more than once with running water to get rid of the soil and dust and then dried at 40^{o}C for one day. Cleaned and dried plant material was blended with the help of a grinder [13].

Preparation of Alcohol Extract:

About 30 gm of ground leaves of *C. erectus* was taken with 300 ml of 70% methanol solvent and then put in a Soxhlet apparatus for 6 hours for alcohol leave extract. The organic solvent was isolated in vacuum using rotator evaporator. The alcohol extract was then put in dishes and was left in the oven overnight at 40°C to dry the extract and to turn it into powder [14].

2. Cell Culture:

Three cell lines were used, namely MCF$_7$which is estrogen receptor positive (ER+) and MDA-MB$_{231}$ that is estrogen receptor negative (ER-), both of them from VACSERA Company,Cairo, Egypt. The third cell line was rat embryo fibroblast (REF)(Iraqi Center for Cancer Research).

These cells were cultured in RPMI1640 culture medium (Capricorn scientific, Germany) and were then added with (10%) heated inactivated fetal bovine serum (Biowest, South America) at a humidified 37˚C temperature and (5%) CO2 [15].

Cytotoxicity Assay:

To test the cytotoxic effect of crude *C. erectus* leaf extract on cells growth and viability, MTT assay was utilized. MTT working solution (5mg/ml) was made by dissolving MTT powder in a sterile PBS. 7000 cells were seeded into each well before being exposed to the extract. Cells were then incubated at 37°C for 24 hours in 96 well plates to guarantee cells adherence. Following that, cells were co-cultured with escalating doses of plant extract (1µg/ml, 5µg/ml, 10µg/ml, 25µg/ml, 50µg/ml and 100µg/ml) for 48 hours versus null concentration (control).Three replicate wells were used per treatment. After 48 hours incubation, the wells were emptied from old and then washed with PBS. Later on 20µl of serum free media was added besides similar volume of MTT working solution (5mg/ml). The plate was then incubated in dark at 37˚C for 3 hours before adding 50µl of MTT formazan dissolvent (DMSO). Absorbance was measured by microplate reader (Expert Plus reader; Asys Hitech GmbH, Eugendorf, Austria) at 570 nm wavelength [16]. Viability percentage was determined by using this formula as was used by [17].

Viability % = (A test - A blank \ A control – A blank) X 100:

A: Absorbance Growth inhibitory concentration, at which viability is reduced to 50% (GI50), was determined from the viability curve, plotted by GraphPad prism software [1].

Results and Discussion

The cytotoxic effect of crud methanol extract against breast cancer cell line.v Methnol extract of *C. erectus* leaves crude was used to assess the cytotoxic effect on:

1. Rat embryo fibroblast (REF) as normale cell and two breast cancer cell lines.
2. Michigan Cancer Foundation (MCF_7).
3. MDA-MB_{231}

The results in Figure 1 shows that the viability % in MDA-MB231, MCF7 and REF at control was 100%, and MDA-MB231 at 100µg/ml concetration was 38.222%. In MCF7 at 100µg/ml, concetration was 73.877%, and in REF at 100µg/ml concetration was 69.745% .

Figure1 also shows cancer cell line growh inhibition GI (IC) that MDA-MB_{231} (GI50 = 56.1µg/ml concentration), while MCF_7 (GI50 > 100µg/ml concentration), and the REF (GI50 > 100µg/ml concetration); that were after co-cultured with crude methanol extract for 48 hours.

In Figure 2, we can see the cell lines used inverted, also emphasising the inhibitory effect of leaves extract on cellular growth of MDA-MB_{231} and MCF-$_7$ treated for 48 hour. Figure 2 indicates the cells in culture that were treated with crude leaf methanol extract which indicates growth inhibition.

Rivero-Cruz *et al.,* [18] studied the cytotoxic effects of methanol crude extract of Conocarpous leaves that were rated contra two breast cancer cell lines (MDA-MB_{231},and MCF_7).Howover, the anti-cancer activity of this extract has yet not been investigated. Oxidation may play a role in cancer development.

Several studies have proven beneficial medical effects of *Typha domingensis* (Pers) while exploring the anti-tumor potential in vitro of this extract.

Khalaf & Abed [19] studied the Thyme essential oil (TEO) evaluation against two human cancer cell lines, HeLa and MCF-7.It showed the highest toxicity of TEO on both cell lines at 200 ppm concentration. The values of (IC50) of TEO against HeLa were 34.63 ppm and MCF-7 were 27.66 ppm.

Karbon and Alhammer [1] studied the cytotoxic effect tested on MCF7 and MDA-MB231 *in vitro* for 24 hours exposure, where (MTT) assay was utilized to test the effect of pollen extract on cells proliferation. The study found that MCF7 cells, estrogen receptor + (ER+), were sensitive (GI50 = 254µg/ml) to pollen extract and this effect was confirmed morphologically under a microscope. However, MDA-MB231 (ER-) cells were greatly resistant to the same extract.

Cytotoxic activity was also observed against MDA-MB_{231} ER- in that study which recorded the previous result and this growth inhibitory effect could attribute to the anti-oxidant and scavenging activity of *C.* leaves crude extract. However, low effect was seen in MCF_7 which ER+ cells, which could point to the mechanism of cytotoxicity is estrogen receptor dependent.

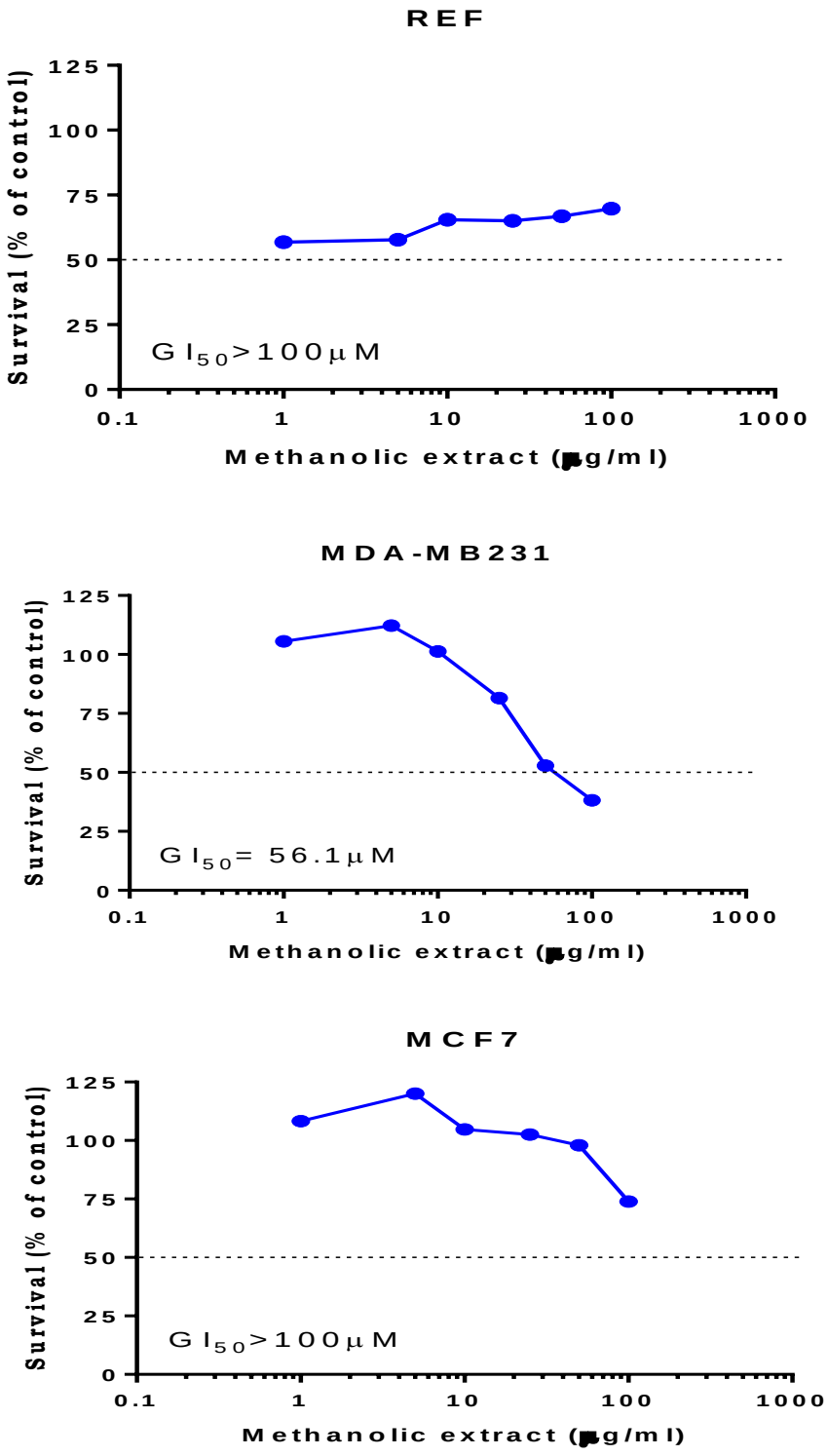
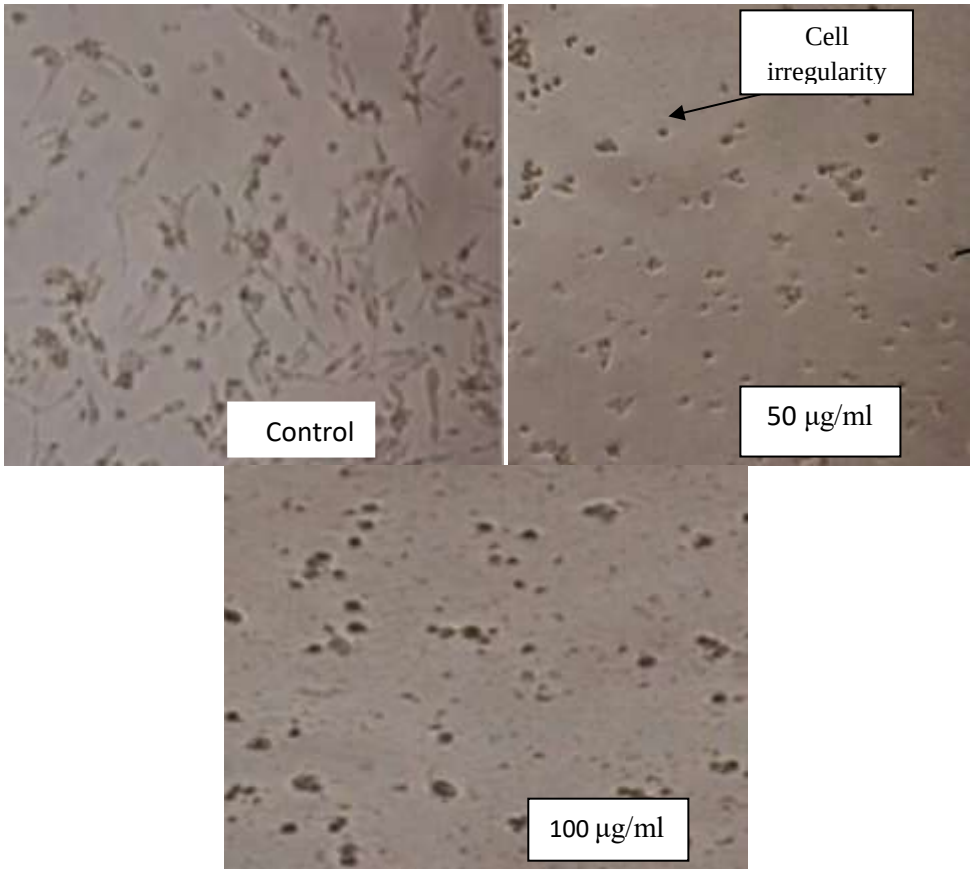
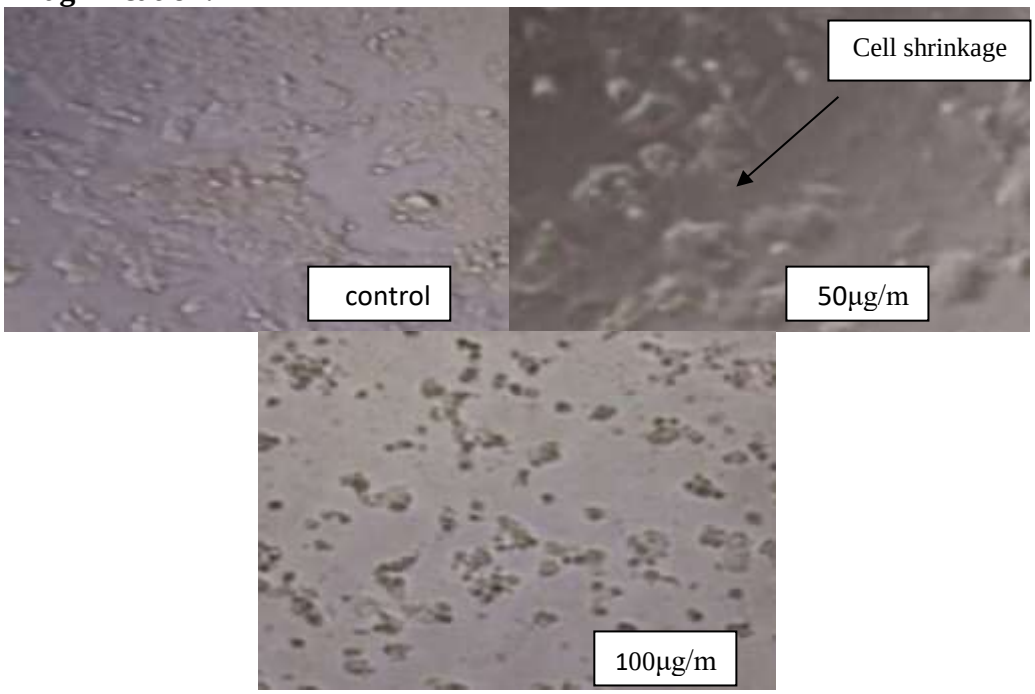


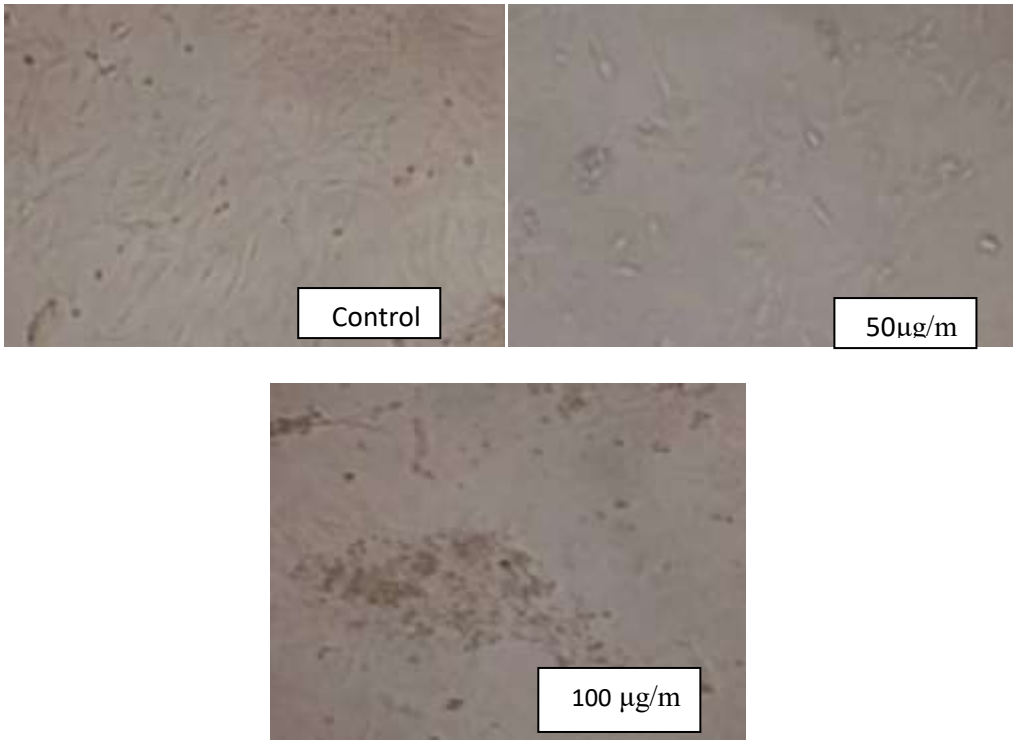
Figure 1: Viability and GI of REF, MDA-MB_{231}, and MCF_7 cancer cell line that incubated with a concentration increasing of methanol crude extract for48 hours by using microscopy at 100x magnification.



a. **Pictures show the effect of incubating of MDA-MB_{231} cells treated with concentration 0, 50 and 100 µg/ml of *Conocarpous* methanolic extract for 48 hrs. by using microscopy at 100x magnification.**



b. **Pictures show the effect of incubating of MCF_7 cells treated with concentration 0, 50 and 100 µg/ml of *Conocarpous* methanolic extract for 48 hrs. by using microscopy at 100x magnification.**



c. **Pictures show the effect of incubating of REF cells treated with concentration 0, 50, and 100 µg/ml of *Conocarpous* methanolic extract for 48 hrs. by using microscopy at 100x magnification.**

Figure 2: Pictures a, b, c show the effect of incubating of MDA, MCF7, REF cells treated with 0, 50 and 100 µg/ml of *Conocarpous* methanolic extract for 48 hrs. by using microscopy at 100x magnification.

Conclusions

Although a few studies in Iraq have proven anticancer effects of *C. erectus* plant leaves while exploring the anticancer activity *in vitro* of this extract. It can be concluded that the effect of *C. erectus* leaves extract has cytotoxic effect on breast cancer cells on the cancer cell line ER- cells (MDA-MB_{231}). Due to the antioxidant activity, this effect might be related with ER expression. While this extract did not affect the normal cell lines REF, on the contrary the growth continued in the normal cells. Therefore, it considers this extract as an alternative treatment for chemotherapy that affects all cancerous and normal cells. Hence, the results of the extract impact against other cancer types may offer new therapies. In addition, more studies are recommended to recognize the active compounds that directly interest subsequent cytotoxicity, both *in vitro* and *in vivo.*

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