

Damper Effect of Carvacrol on T47-D, A Human Breast Cancer Cell Line.

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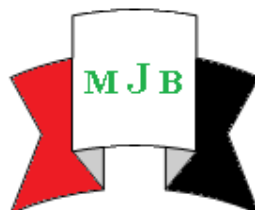
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Received 23 June 2014

Accepted 18 August 2014

Abstract

Background: Carvacrol is predominant monoterpene presents in the volatile oils of oregano, thyme and some other herbs.

Objectives: The present study was carried out to determine the *in vitro* cytotoxic effects of carvacrol on breast cancer cell line.

Materials and Methods: The cytotoxic effect of carvacrol was evaluated by measuring absorbance of crystal violet stain by ELISA technique and determining the percentage of growth inhibition.

Results: The study clearly showed the anti-proliferative effect of carvacrol on T47-D cells in dose dependent manner.

Conclusion: These data demonstrated the cytotoxic effect of carvacrol on human breast cancer cells, T47-D, and that compound could have a potential therapeutic significance in treating cancer.

Key words: carvacrol, breast cancer, T47-D cell line.

تأثير الكارفاكروول المثبط لنمو خلايا سرطان الثدي البشريه T47-D

الخلاصة

الخلفية: يعتبر الكارفاكروول الاحادي التريين السائد الموجود في الزيوت الطيارة المستخلصة من الزعتر البري والزعتر وبعض الاعشاب الاخرى.

الأهداف : أجريت هذه الدراسة لتبيان فيما اذا كان للكارفاكروول تأثير مثبط لنمو خلايا سرطان الثدي في الزجاج.

المواد والطرق: تم تقييم فعالية الكارفاكروول بقياس امتصاصيه صبغه الكريستال البنفسجية بتقنيه الاليزا وتحديد النسبه المئوية لتثبيط نمو الخلايا.

النتائج: اظهرت النتائج ان للكارفاكروول تاثيرا مثبطا واضحا على نمو خلايا T47-D مرتبطا بالجرعه المستخدمه.

الاستنتاج: اوضحت البيانات فعالية الكارفاكروول في الحد من نمو خلايا سرطان الثدي الامر الذي قد يعطي اهميه مستقبلية لذلك المركب في علاج مرض السرطان.

مفاتيح الكلمات: كارفاكروول, سرطان الثدي, خط خلايا T47-D.

Introduction

Despite continual advances in breast cancer care, it remains the main leading cause of death among most women [1], [2]. In the few past years, breast cancer morbidity appears to be declining in the west, due to early detection and advances in cancer medicine [3]. The pattern of breast cancer in Iraq is rather different from the pattern in other countries like USA and European countries [4], since the future number of breast cancer incidence and mortality are estimated to be increased, generally due to increased hazards and decreased awareness [5].

Recently a significant rebirth and attention have seen in botanical remedies, shown by extracts of plants, fruits, and vegetables, depending on the impression that cancer treatment need to be administered over protracted periods of time [6].

However the study of these extracts was considered to be fraught with

difficulties. Since most of these contain a complex mixture of terpenes, phenolic compounds and alkaloids, also plants may produce a wide variety of secondary metabolites which have no apparent role in their effect [7].

Research on biologically active compounds from essential oils has proved them to be potent anti-bacterial, anti-fungal and anti-oxidant agents. Carvacrol is one of main substances of essential oil also possess anti-tumor activity [8]. It is the major component of the essential oil of oregano and thyme [9], [10], and to lesser extent pepperwort, wild bergamot, and other herbs [11], which was isolated from essential oils as fractions of hydrodistillated oil [10].

Carvacrol is a phenolic compound (5-isopropyl-2-methylphenol), belongs to a chemical group called monoterpenes, $C_{10}H_{14}O$ represents its formula (Figure 1).

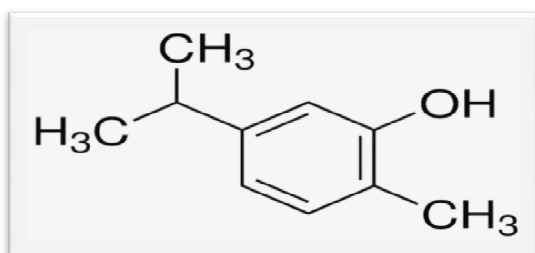


Figure (1): The structural formula of carvacrol [12]

The antibacterial and antioxidant activity of carvacrol [13] as a component of essential oils makes it suitable as a novel food preservative [14] and its protective actions against induced hepatic steatosis in mice had been also mentioned [11].

Another study suggested that carvacrol has selective stimulatory effect of on β_2 -adrenoceptors and a possible inhibitory effect on histamine (H_1) receptors, precisely; further studies are required to improve this selectivity [15], while its antidepressant effects in mice

seem to be dependent on its interaction with the dopaminergic system [9].

Anti-inflammatory, analgesic and partially antithrombotic effects, of traditionally used carvacrol-rich plant drugs, might be some of the carvacrol biological effects which mediated by inhibition of prostaglandin production via arachidonic acid pathway [16], also its inhibitory effect on leukocyte migration could contribute to the aforementioned anti-inflammatory action [10].

Carvacrol has a strong antimutagenic effect [17]. In the recent past, its antitumor effect has been raised as concern of investigators. It was revealed that carvacrol inhibited viability and proliferation of human non-small cell lung cancer cell line, and induced early apoptotic features in a dose-dependent manner on these cells [18]. The same effect also had been seen on human metastatic breast cancer cells MDA-MB 231 [19]. It has anti-proliferative properties on human hepatocellular carcinoma cell line [20] and increases apoptosis proportion in the human cervical cancer cell lines [8].

Carvacrol is relatively safe and nontoxic. Several studies have taken into consideration its acute toxic effect and concluded that there were no apparent behavioral side effects on lab animals during experiments. Fasted mice or rats were orally treated with carvacrol, the high median lethal dose (LD_{50}) values, which were found to be greater than the therapeutic doses, provided preliminary positive evidence for the safety of carvacrol [21], [10].

Conversely, there were no data available on chronic toxicity, carcinogenicity, teratogenicity, mutagenicity, and reproductive toxicity [22], [12].

Materials and Methods

Chemicals:

Carvacrol (99%), RPMI-1640 liquid media, and crystal violet crystals were purchased from Sigma–Aldrich (USA). All the other chemicals and reagents were purchased from local companies and were evaluated before use.

Cell culture and treatment:

T47-D cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum, 40 µg/ml gentamycin and 0.5 µg/ml amphotericin B. Cultures were incubated at 37°C, the cultured cells were subcultured when 80-90% confluence was reached. Cell viability was

determined by the trypan blue dye exclusion method.

Cytotoxicity assay:

The effect of carvacrol on the viability of T47-D breast cancer cells was determined by crystal violet staining method. Briefly, 200 µl of 1×10^6 cells/ml were seeded in triplicate in 96-well plates allowed to attach and grow for 24 hours [23]. Appropriate serial dilutions of carvacrol were carried out in culture medium before their addition to cultured cells to obtain five different concentrations (25-400 µg/ml). Cells were treated with those concentrations and incubated at 37°C for 24 hours.

At the end of the treatment, the medium was carefully removed and washed two times with 100 µl phosphate buffer saline, then fixation was done with 10% buffered formalin at room temperature for 20 minutes. The fixative solution was removed and each well was stained with 100 µl of 0.1% aqueous crystal violet solution for 20 minutes at room temperature. The plate was washed and allowed to be air dried. Crystal violet stain was solubilize by adding 200 µl of 95% ethanol to each stained well with simple shaking from time to time. Then the absorbance was measured for each well using ELISA reader (an analytical biochemistry assay involves the detection of analyte in a liquid media) at a wavelength 540 nm.

The cytotoxic effects of carvacrol could be measured compared to the viability of untreated cells according to the following formula:

$$\text{Percentage of growth inhibition} = 100 \times \frac{(1 - A_t/A_o)}{A_o}$$

Where A_t and A_o are the absorbance values for treated cells and untreated cells, respectively [24].

These results were used to plot a curve showed percentage of cell killing vs. concentration, and the cytotoxicity of carvacrol was expressed as IC_{50} (concentration produced 50% growth inhibition).

Statistical analysis:

Data were presented as mean $\pm$ SD with 95% confidence interval. Values with $p < 0.05$ were considered as significant.

Results

Carvacrol reduced the viability of T47-D cells, that effect was estimated by crystal violet staining for those cells. A wide range of concentrations represented by (25, 50, 100, 200, and 400 $\mu\text{g/ml}$)

were used. After 24 hours incubation the absorbance was measured for each well by ELISA reader and the percentage of growth inhibition was determined. Data in table (1) revealed the mean percent of growth inhibition of T47-D cells, while figure (2) was used to determine IC_{50} .

Discussion

Over the past decades, and despite the advance in our understanding

Figure (2): Concentration vs. percentage of growth inhibition after 24 hours incubation.

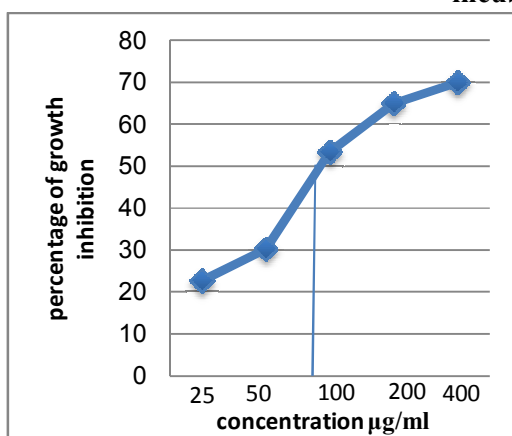


Table (1): Mean differences in percentage of growth inhibition produced by different concentrations of carvacrol

*means significant difference ($p < 0.05$) as compared to control group

Concentration $\mu\text{g/ml}$	Percentage of growth inhibition (mean $\pm$ SD)
25	22.76 $\pm$ 0.71*
50	30.33 $\pm$ 0.61*
100	53.43 $\pm$ 0.83*
200	65.03 $\pm$ 0.47*
400	70.70 $\pm$ 0.75*

of the molecular basis of cancer and the approval of a number of molecularly targeted agents, the clinical drug development process remains slow, costly and inefficient [25] due to the fact that carcinogenesis is generally

recognized as a complex and multistep process in which oxidative stress and inflammation plays a crucial role and distinct molecular and cellular alterations occur. Carvacrol possesses many actions beside its cytotoxic effect which makes it

a novel anticancer therapy like its antimutagenic [17], anti-inflammatory [16], and antioxidant effect [11].

In vitro cytotoxicity study can be used to expect the effect of chemicals and plant extracts [8]. The results obtained from the cytotoxicity assay revealed that T47-D cell line is susceptible to carvacrol and the percentage of growth inhibition increased with increasing carvacrol concentration.

The significant increase ($p < 0.05$) in the percent of growth inhibition of all applied concentrations of carvacrol seen after 24 hours incubation (table 1) signify directly the dose dependent cytotoxic effect of carvacrol. These results coincide with the known cytotoxic effect of carvacrol on human breast cancer cell line, MDA-MB 231 [19] and also represented good agreement for the dose dependent carvacrol induced anti-proliferation in HepG-2 cells [20]. As seen also in HeLa and SiHa cervical cancer cell lines [8] and with the induction of early apoptotic features in A549 cells in a dose-dependent manner [18]. However, these results were controversial with findings of Gohari *et al.*, who concluded that CVL had no effect on the viability of T47-D cells [26].

According to published guidelines, the IC_{50} 10-100 $\mu\text{g/ml}$ represents potentially cytotoxic [27]. The IC_{50} value, obtained from figure (2), indicated that carvacrol showed prominent cytotoxicity against T47-D cell line (IC_{50} value was 90 $\mu\text{g/ml}$).

In conclusion, carvacrol has an *in vitro* cytotoxic effect against T47-D human breast cancer cells with $IC_{50} = 90$ $\mu\text{g/ml}$ after 24 hours exposure. Prospective studies are required on laboratory animals to prove its *in vivo* efficacy.

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