EFFECT OF INDOLE EXTRACTED FROM UROPATHOGENIC *E. coli* ON SOME CELL LINES IN COMPARABLE WITH STANDARD INDOLE

Abdulazeez. H. Alqaisi **Mouruj A. Al aubydi**
Assis. Lecturer **Prof.**
Dept. Biotech., Coll. Sci., University of Baghdad, Iraq
E-mail: Azeez89kc@gmail.com

ABSTRACT

Indole mechanism as an interspecies signal particle to adjust various physiological actions, like virulence, antimicrobial and acid resistance. This study was aimed to use partial purified indole extracted from uropathogenic *E. coli* and study the cytotoxic effect on some cancer cell lines (A375, HepG2, PC3) and comparable with normal cell line (WRL- 68). Serial dilutions of natural and synthetic standard indole were tested (400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL). The results show a low cytotoxic effect for both natural and synthetic indoles in HepG2 and A375 cell lines as well as this degree of cytotoxicity was obviously appeared in normal cell line WRL-68 with no significant differences among them. While, both indoles effect was appeared high cytotoxic effect on PC3 cell line, this increasing has a significant difference with WRL-68. Thus, it can be suggesting that, this type of indole is not favorable for treatment of some types of cancer, and may has a specific biological activity against exact cancer cells.

Key words: heterocyclic, cytotoxicity, anticancer, effective compounds, PC3, A375, HepG2.

تأثير الإندول الطبيعي المستخلص من الإشريكية القولونية المسببة للمرض في المسالك البولية على بعض خطوط الخلايا السرطانية بالمقارنة مع الإندول القياسي

عبد العزيز حسين القيسي **مروج عبد الستار العبيدي**
مدرس مساعد **استاذ**
قسم التقنيات الاحيائية – كلية العلوم – جامعة بغداد – العراق

المستخلص

آلية الإندول يعمل كجسيم إشارة بين الأنواع لضبط الفعاليات الفسيولوجية المختلفة، مثل الفوعة ومقاومة الميكروبات ومقاومة الأحماض. تهدف هذه الدراسة إلى استخدام الإندول المنقى جزئياً والمستخلص من الإشريكية القولونية المسببة لأمراض المسالك البولية ودراسة التأثير السام للخلايا على بعض خطوط الخلايا السرطانية (PC3, A375, HepG2) ومقارنتها بخط الخلية الطبيعي (WRL–68). تم اختبار التخافيف التسلسلية للإندول الطبيعي والاصطناعي القياسي (400، 200، 100، 50، 25، 12.5، 6.25 ميكروغرام / مل). أظهرت النتائج تأثيرًا منخفضًا سامًا للخلايا لكل من الإندولات الطبيعية والاصطناعية في خطوط الخلايا HepG2 وA375 وكذلك ظهرت هذه الدرجة من السمية الخلوية بوضوح في خط الخلايا الطبيعي WRL–68 مع عدم وجود فروق ذات دلالة إحصائية فيما بينها. بينما ظهر تأثير كل من الإندولات السامة للخلايا على خط خلية PC3، وإن هذه الزيادة لها فرق معنوي كبير بالمقارنة مع WRL–68وبالتالي يمكن الإشارة إلى أن هذا النوع من الإندول غير مناسب لعلاج بعض أنواع السرطان، وقد يكون له نشاط بيولوجي محدد ضد خلايا السرطانية محددة.

الكلمات المفتاحية: المركبات الحلقية، مواد فعالة، منتج بكتيري، السمية الخلوية، سرطان البروستات، سرطان الكبد.

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INTRODUCTION

Cancer, a category of diseases characterized by the uncontrolled, fast, and pathological proliferation of aberrant cells, is one of the world's most dangerous diseases. If uncontrolled cell spread is not managed, it might lead to death (4). Cancer patients can be cure by treatment with surgery, immune treatment, radiation, hormone therapy, chemotherapy and targeted therapy (18). Targeted cancer therapies are medications or other substances that inhibit tumour cell growth and dissemination by interfering with specific molecules ("molecular targets") involved in cancer progression, growth and spread, as well as the signals that lead cancer cells to die naturally (20).Because of their structural characteristics, heterocyclic-compounds such as indole molecules, whereby the benzene ring is paired with the 2 and 3 sites of the pyrole ring, have a critical role in the development of pharmacological, chemical, medicinal and agricultural areas (23,1). In the presence of indole-3-glycerol phosphate and tryptophan, plants and microorganisms can generate indole via tryptophanase or its analogs; consequently, indole is found in plants and bacteria-rich habitats such as soil, rhizosphere, aquatic organisms, sludge, and intestinal tracts (15). Indole is a very prominent volatile biosynthesized by *Escherichia coli* and other some bacteria as well (e.g., *Loktanella*, *Vibrio cholera* …etc.). It derives from a one-step reaction of the enzyme tryptophanase of the aromatic amino acid tryptophan (21) and may use as biomarkers (25). Many natural and synthetic indole analogues have been shown to be effective anticancer drugs. Vinca alkaloids were discovered from the periwinkle plant *Catharanthus roseus* in the late 1950s, and it was a significant moment in the progress of cancer treatment (19). Although indole nucleus has a broad variety of pharmacological effects, including antiviral, anti-diabetic, CNS depressive, anti-inflammatory, antibacterial, and anticancer properties, it is widely used in the pharmaceutical industry (10). As a result, the indole core served as a framework for numerous anticancer drugs such as vincristine, vinblastine, vinorelbine, and vindesine (14). An extensive literature search revealed that indole derivatives have the traits of triggering apoptosis and disrupting tubulin assembly. Indoles have been linked to the suppression of NFkB/uPAR/mTOR/PI3K/AkT as well as the control of estrogen-mediated activity. Furthermore, indole compounds have been shown to affect key targets including topoisomerase and HDAC. These compounds have demonstrated strong anti-breast cancer cell action (13). A variety of pathways for indole-induced apoptosis have been suggested, including regulation of beta-catenin / FOXO3a / GSK-3beta/Akt/AR signaling, down-regulation of surviving (3) and activation of p75(NTR)-dependent programmed cell death via the p38 MAPK pathway. Indole-3-carbinol (I3C) one of important indole effective derivatives on cancer cells. among the goals of I3C and its metabolite, 3,3′-diindoylmethane (DIM), are receptor signaling, caspase activation, BRCA gene expression, Akt-NFB signaling, ER stress and cyclin-dependent kinase activity. Scientists and chemists alike are becoming more intrigued by the biological potential of a wide spectrum of indole compounds (21). Moreover, (8) it was reported that I3C induced G1 cell cycle arrest, cell growth inhibition and apoptosis in prostate carcinoma cells this study is aimed to use partial purified indole extracted from pathogenic *E. coli* and study its cytotoxic effect on some cancer cell lines (A375, HepG2, PC3) and normal cell line (WRL 68).

MATERIALS AND METHODS

Preparation of indole for biological assay

Partially purified bacterial indole prepared previously from uropathogenic *E. coli* and synthetic indole stock solutions were prepared at a concentration (2mg/ml) after dissolving them in dimethyl sulfoxide (DMSO) and added separately to the cultured cells. Serial dilutions were prepared from each indole stock solution separately as following; 400,200,100,50, 25, 12.5, 6.25 µg /ml.

Cell lines

The tumor cell lines included; A375, is a human melanoma cell line, HepG2, is a human liver cancer cell line, PC3, prostate cancer, and another normal cell line (WRL 68) were supplied from the Chemistry Analysis Center (CAC)/ Baghdad/ Iraq and initially been preserved in RPMI-1640 medium, inoculated

with 10% fetal bovine serum (FBS) (Capricorn, Germany), 100 IU/mL of streptomycin and 100 µg/mL of penicillin (Ajenta Pharm, India) We passaged the cell by, Trypsin,-EDTA, reseeded in50%, convergence double in a week then, incubated on 37 °C(16).

Determination of cytotoxic effect assay by using MTT: For investigated the cytotoxicity of the indole, the MTT cell viability assay was performed on 96-well micro-plates. The cell lines were cultured at ($1x10^4$–$1x10^6$ cells/mL) /well. After 2hrs or achievement of confluent monolayer, cells were treated with 10 µl of indole at concentrations (400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL) alongside with control (DMSO). Following culture, the plates were incubated at 37°C, 5% Co_2 for 20hrs. The MTT solution 5 mg/mL (Sigma, USA) then added 20 µl to each separate well (7,12). Subsequently, microtiter-plates were incubated at 37°C for additional 4 hours. Then supernatant medium was removed quietly and replaced with DMSO to solubilized the indicator. An ELISA reader system (ELISA reader, USA), measured the relative absorbance for each well at 590 nm. Each sample was tested three times to ensure accuracy (5,6).

Cell survival percentage (CSP) = C-A/C *100, Where C is the optical density of the control and A is the optical density of the test.

RESULTS AND DISCUSSION

Indole and its derivatives belong to a significant category of heterocyclic compounds that have been used as a crucial component for treatment several diseases. In the same context, the results of natural extracted Indole from uropathogenic *E. coli* against several cancer cell lines as the following; Firstly, Hep G2, is one of predominantly cell line used in many experimental test, the results of a serial dilutions of natural and synthetic standard indole (Table 1and Figure 1a,b) showed a low cytotoxic effect for both natural and synthetic indole as well as this cytotoxicity was obviously appeared in normal cell line WRL-68. Thus, it can be suggesting that, this type of indole is not favorable for treatment of this type of cancer in these concentrations which are used, or indole is not has specification for HepG2. Since several global researchers confirmed the effect of indole derivatives against this type of cell line, which they explained the mechanisms of indole derivative effect throughout either activated by the process of metabolic bio-activation or genotoxicity induced by indole derivative and the correlation indicated to the metabolic bio-activation might result in oxidative DNA damage (17). On the other hand, the results suggest the effect of indole either natural or synthetic one is dose dependent, the cytotoxic effect was increases with increasing dose. Simultaneously, this cytotoxic effect was included normal cell line as well. As Wong *et al.* mentioned these results may be due to the anti-proliferative activity that indole characterized (24).

Table 1. Results of viable cells count for HepG2 compared with WRL-68 cell lines treated with indole (Natural and synthetic)

Indole Conc.	(Natural Indole) Mean ± SD		(synthetic Indole) Mean ± SD	
µg/mL	* HepG2	* WRL-68	HepG2	WRL-68
400	63.46 ± 1.40	69.98 ± 2.91	64.16 ± 8.97	75.35 ± 2.87
200	75.11 ± 1.27	86.81 ± 1.51	65.63 ± 2.46	86.03 ± 0.85
100	92.94 ± 0.83	94.29 ± 1.83	77.62 ± 2.41	92.13 ± 1.56
50	93.29 ± 1.21	95.83 ± 0.31	89.82 ± 2.34	96.18 ± 1.25
25	96.80 ± 0.94	95.37 ± 0.90	94.79 ± 1.33	96.95 ± 1.14
12.5	93.87 ± 5.03	96.64 ± 0.70	95.41 ± 1.41	94.91 ± 2.20
6.25	95.68 ± 0.57	95.68 ± 0.41	96.14 ± 1.05	96.10 ± 0.48

*** HepG2: Human liver cancer cell line, * WRL-68: Normal cell line**

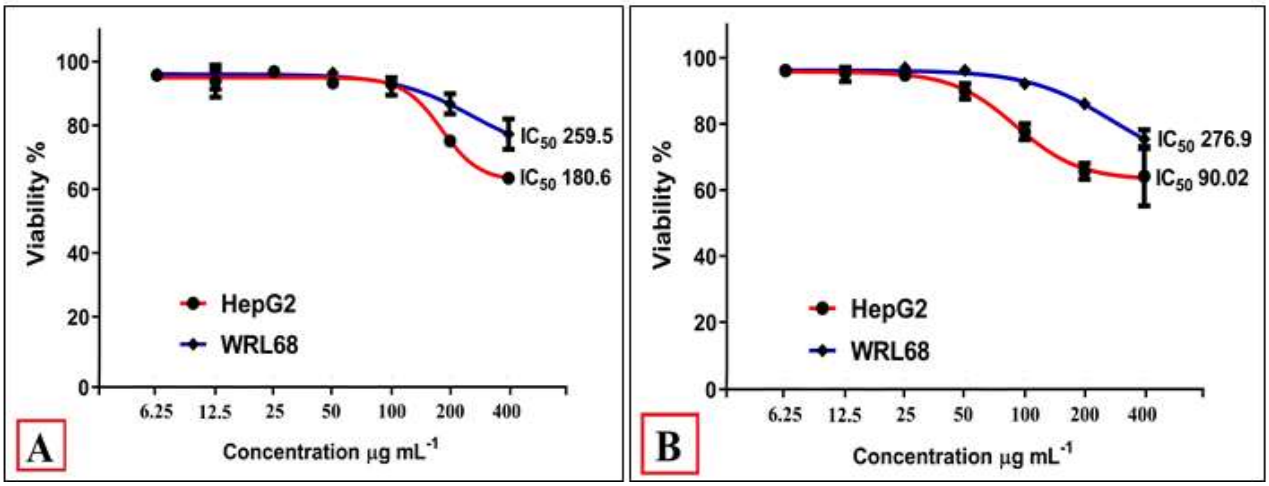

Figure 1. A comparable result treated HepG2 and WRL68 cell lines with, A) natural and B) synthetic standard

Secondly; The results in Table 2 and Figure 2(a,b) show the highly toxic against PC3 whether natural or synthetic one in compared with normal cells, in spite of natural indole is more cytotoxic than synthetic one, the results revealed no significant differences between them, whereas the results recorded significant differences between both indoles and the normal cell lines. These results were confirmed to that concluded by (9), study has shown that indole-3-carbinol (I3C), a common phytochemical in cruciferous vegetables with decreasing prostate cancer, suggesting increased capacity for detoxification and inhibition of carcinogens. Their study found that I3C can stimulate G1 cell-cycle arrest and apoptosis in prostate cancer cells. In addition, I3C regulate many genes that are significant for the control of cell cycle, signal transduction, cell propagation, and other cellular processes, suggesting the pleiotropic effects of I3C on prostate cancer cells (9).

Table 2. Results of viable cells count for PC3 compared with WRL-68 cell lines treated with indole (Natural and synthetic)

Indole Conc.	(Natural Indole) Mean ± SD		(Synthetic Indole) Mean ± SD	
µg/mL	* PC3	* WRL-68	PC3	WRL-68
400	35.07 ± 6.99	69.98 ± 2.91	42.44 ± 1.47	75.35 ± 2.87
200	40.28 ± 3.61	86.81 ± 1.51	48.03 ± 2.55	86.03 ± 0.85
100	54.48 ± 2.70	94.29 ± 1.83	73.03 ± 4.64	92.13 ± 1.56
50	63.97 ± 5.21	95.83 ± 0.31	90.70 ± 3.18	96.18 ± 1.25
25	75.27 ± 4.21	95.37 ± 0.90	95.72 ± 0.81	96.95 ± 1.14
12.5	85.30 ± 0.99	96.64 ± 0.70	95.18 ± 1.28	94.91 ± 2.20
6.25	96.10 ± 1.71	95.68 ± 0.41	95.95 ± 0.53	96.10 ± 0.48

*** PC3: prostate cancer, * WRL-68: Normal cell line**

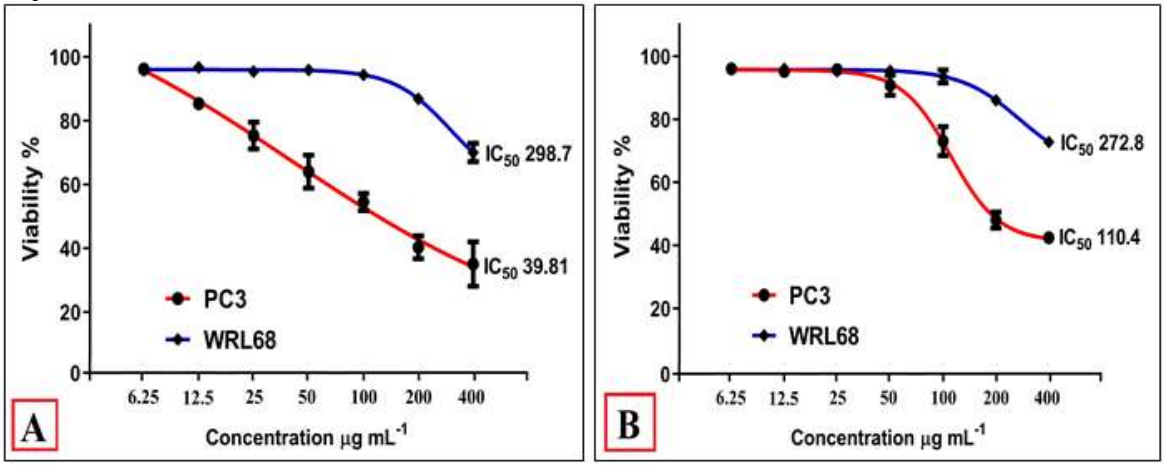

Figure 2. A comparable result treated PC3 and WRL68 cell lines with, A) natural and B) synthetic standard

In vitro study (1), mentioned that I3C has been shown to reduce the proliferation of tumor cells such as prostate cancer, breast cancer, colon cancer, endometrial cancer, and leukemic cells, as well as induce G1/S cell cycle arrest and apoptosis. Amongst different heterocyclic compounds, nitrogen-based heterocycles as they establish the core structures of many biologically applicable molecules and have been found to be active against different types of cancers. Due to the versatility of indole, it has been a highly privileged motif for the target-based design and development of anticancer agents (24). Thirdly; A375 is one of melanoma cell line, the results in Table 3 revealed the low cytotoxicity of indole whether for natural or synthetic one. Also, the results revealed no significant differences between two types of indole, as well as with the normal cell line. These results disagreed with Wan-Ping et al., who they demonstrated and explained the mechanism of anticancer effect of hybrid indole agent (IN6CPBD) on human melanoma A375 cells. IN6CPBD-treated cells exhibited higher cytotoxicity and exhibited several features of apoptosis, including an expressively increased annexin V binding, a rise in the sub-G1 population, poly (ADP-ribose) polymerase cleavage, and a degradation of caspase-3. Because degradative variations related with apoptosis are frequently preceded by the distraction of mitochondrial function (11). Treatment of A375 cells with hybrid indole resulted in the loss of mitochondrial membrane probable, a reduction in intracellular pH (pHi), a decrease of ATP synthesis, augmented reactive oxygen species (ROS) development, and cytochrome c release, and concluded that an induces apoptosis in A375 cells through a mitochondrial dysfunction pathway, leading to caspase-3 substrate cleavage and following apoptotic cell death.

CONCLUSION

Indole and its derivatives have multiple activities specially against cancer cell lines, and any chemotherapy agents has an effect on both cancer and normal cells. The recent results revealed owing to their specific biological reactivity, its effect was varying among different cell lines, and may has a specification in effect as that obviously appeared it cytotoxicity against PC3 than HepG2 and A375, as well as WRL-68.

Table 3. Results of viable cells count for A375 compared with WRL-68 cell lines treated with indole (Natural and synthetic)

Indole Conc.	(Natural Indole) Mean ± SD		(synthetic Indole) Mean ± SD	
µg/mL	* A375	* WRL-68	A375	WRL-68
400	73.69 ± 4.92	69.98 ± 2.91	73.38 ± 4.42	75.35 ± 2.87
200	79.44 ± 1.60	86.81 ± 1.51	80.67 ± 1.98	86.03 ± 0.85
100	90.39 ± 1.45	94.29 ± 1.83	93.60 ± 3.90	92.13 ± 1.56
50	93.83 ± 1.29	95.83 ± 0.31	94.29 ± 0.82	96.18 ± 1.25
25	94.79 ± 3.24	95.37 ± 0.90	95.95 ± 0.90	96.95 ± 1.14
12.5	96.76 ± 1.14	96.64 ± 0.70	94.87 ± 0.29	94.91 ± 2.20
6.25	96.30 ± 0.81	95.68 ± 0.41	94.83 ± 0.71	96.10 ± 0.48

*** A375: Human melanoma cell line, * WRL-68: Normal cell line**

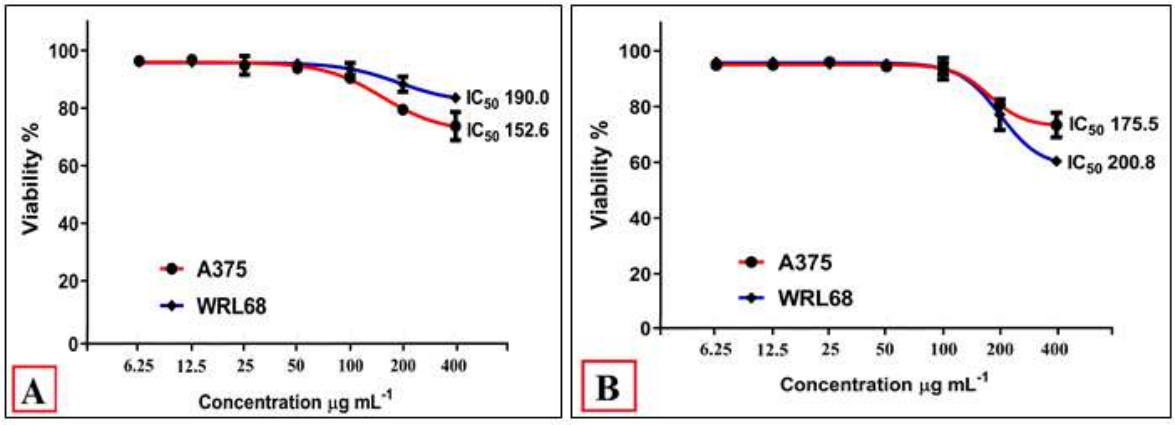

Figure 3. A comparable result treated A375 and WRL68 cell lines with, A) natural and B) synthetic standard

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