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Investigation of Cytotoxic Effects of *Dichanthium Annulatum F.* And *Paspalum Distichum L.* On Cell Line SR. (Lymphoma) and L20B (Murine fibroblast)

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Abstract:

D. Annulatum F. and *P. Distichum L.*, are grasses belongs to Poaceae family, they grow without any effort in the western plateau of Iraq in general and particulary in the southern part, located in the province of Muthanna. These grasses are commonly used as a forage for livestock. The present study was designed to evaluate the cytotoxic effect of *D. Annulatum F.* and *P. Distichum* L. methanolic extracts in vitro by using MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) on two cell lines (SR and L20B) by using different concentrations (3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/ml) for an incubation period of 24 hours. We found cytotoxic effect for methanolic extract of *D. Annulatum F.* against SR cell line in low concentrations (3.125, 6.25µg/ml) which was (55%, 46%), and against the L20B cell line was (60%, 59%) in the same concentrations. Regarding methanolic extract of *P. Distichum* L. the best effect was found with the lower concentrations which was (45%,44%) against SR and (49%,48%) against L20B. In conclusion, the two grasses have a good potential anticancer effects.
Key words: Cancer, *Dichanthium Annulatum F.*, *Paspalum Distichum L.*, Cytotoxic effect.

الخلاصة:

تم اختبار مستخلصات عشبة الزمزوم (*Dichanthium Annulatum F.*) وعشبة السلهو (*Paspalum Distichum L.*) اللتان تعودان للعائلة النجيلية ومدى تاثيرها في اثنين من الخطوط السرطانية وهما من الاعشاب التي تنمو بدون بذل اي جهد يذكر في البادية الغربية للعراق بشكل عام وبشكل خاص في الجهة الجنوبية من محافظة المثنى، وهذه الاعشاب تستخدم عادة كعلف في الثروة الحيوانية. صممت الدراسة الحالية لاختبارالتاثير السمي لمستخلص الميثانول لعشبتي الزمزوم والسلهو في المختبر باستخدام اختبار الMTT ضد نوعين من خطوط الخلايا السرطانية (SR وL20B) وباستخدام تراكيز مختلفة (٣,١٢٥، ٦,٢٥، ١٢,٥، ٢٥، ٥٠، ١٠٠ و 200 µg/ml) ولفترة حضانة ٢٤ ساعة. اظهرت النتائج تاثير لمستخلص الميثانول لعشبة الزمزوم ضد خط الخلايا السرطانية SR في التراكيز الواطئة (٣,١٢٥، ٦,٢٥ µg/ml) فقد كان (٥٥%، ٤٦%). وكان تاثير نفس المستخلص ضد خط الخلايا L20B في نفس التراكيز هو (٦٠%، ٥٩%). اما التاثير الافضل للمستخلص الميثانولي لعشبة السلهو في التراكيز الواطئة (٣,١٢٥، ٦,٢٥ µg/ml) كان (٤٥%، ٤٤%) ضد SR و (٤٩%، ٤٨%) ضد L20B. وبالتالي نستطيع القول ان العشبتين لهما تاثيرات محتملة مضادة للسرطان.
الكلمات المفتاحية: السرطان، الزمزوم، السلهو، التاثيرات المضادة للسرطان.

Introduction

In accordance with The American Cancer Society, in the U.S.A just in 2016, there will be nearly 1,685,210 new cancer cases and 595,690 cancer deaths or approximately 3 cases and one death per minute. The development in our knowledge of cancer biology has led to remarkable progress in cancer prevention, early detection, and treatment. Scientists have learned more about cancer in the last 2 decades than had been learned in all the centuries preceding (American Cancer Society,2014), this doesn't change the fact, however, all types of cancer treatments cause a lot of side effects such as pain, nausea and vomiting, fatigue, anemia, infections etc. (American Cancer Society, 2016).

The focus of anticancer properties of plants was increased in the recent years where anticancer properties of plants have been examined for centuries. Scientists starting systematically examining natural organisms as a source of useful anti-cancer materials in the early of the fifties of twenty century (Cragg and Newman, 2005). We can say that the only successful strategy in the discovery of novel new medicines is by using natural products. (Tulp and Bohlin,2002). A number of natural products, and its diverse chemical structures, have been isolated as anticancer agents (Vandana *et al.*, 2005). For example the isolation of Podophyllotoxin and many compounds (known as lignans) from the common mayapple (*Podophyllum peltatum*) finally led to the development of drugs used to treat testicular and small cell lung cancer (Pettit *et al*., 1995).

According to World Health Organization (WHO), about three quarters of the world's population at present time use herbs and the other types of traditional medicines in the treatment of diseases. Even in the USA, the using of plants and phytomedicines has increased dramatically during the last two decades (Goel and Sairam,2002). According to the reports, more than 50% of all modern drugs in clinical use came from natural products (Rao *et al.*, 2000).

The western plateau of Iraq in general and the southern part, located in the province of Muthanna particularly consider distinctive geographical features, including possession of a variety of surface properties of large natural resources and though they have not been studied sufficiently (Raad, 2008).

Where there is a lot of common and rare plants spread in this region which are used for grazing animals but they never studied for their biological characteristics. In this research, two grasses were studied including *Dichanthium Annulatum F*. and *Paspalum Distichum L*. which grows in the Muthanna plateau. There is no effort to examine their cytotoxic effects against two cell line L20B and SR. These grasses belong to Poaceae family, which are larger and nearly ubiquitous family of monocotyledonous flowering plants known as grasses (Christenhusz and Byng,2016).The grasses-Poaceae (Gramineae) is the most important group of useful plants. The species of Poaceae contain bioactive components, including flavonoids (as C-glycosides of apigenin, luteolin, tricin), phenolic acids (as ferulic acid, caffeic acid, p -hydroxybenzoic acid) and triterpenes, saponins, sterols. In previous studies the rapeutic effect for some of the grass species have been proved (e.g. strong antioxidant properties) and they were effective in the treatment of inflammations and sclerosis (Rice-Evans *et al*., 1996; Adom *et al*., 2002).

Material and Methods

Collection of plants

Plants were compiled from the plateau of AL Samawah in the spring of 2016, where grass abound in this period. They have been diagnosed by Dr. Khalid Jamil, assist. Professor on plant classification in the College of Agriculture, University of Muthanna.

Plants extraction

The aerial parts of the two grasses *D. Annulatum F*. and *P. Distichum L*. were washed with tap water before use and dried in a cool and shade environment then pulverized into powder. Dried and powdered plants (5 g) for each plant were stirred in 250 ml methanol and placed on Soxhlet apparatus. Referred to the extracts, as DA and PD, the resulting extract has been left at room temperature to get rid of alcohol and the extracts were stored at 4 ° C (Handa *et al.,* 2008).

Cell line and cell culture

The two cell lines SR.(lymphoma) and L20B were provide gently by Dr. Mohammad Mahmoud Farhan, department of medical and molecular biotechnology research center.

The L20B cell line genetically engineered nonmalignant mouse cell line expressing the human poliovirus receptor (CD155) and SR established from the pleural effusion an 11-year-old boy with CD30+ (Ki-1) large T cell lymphoma in 1983 (also described as "SR") were used as cell models. It was maintained at molecular biotechnology research center laboratory Baghdad –Iraq. Secondary culture was prepared under sterilized conditions according to (Freshney, 2001).The cell culture medium was Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma/U.S.A) supplemented with: 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were seeded at a density of $1.5X10^4$ cells/well in Flat bottomed 96-well plate. The cells were incubated for 24 hours at 37°C under a humidified atmosphere containing 5% CO2 (Langdon, 2004).

Cytotoxicity test (MTT)

We weighed 0.1 g from plant extracts DA and PD then dissolved it in 1 ml from DMSO, after complete dissolving, 10 ml DMEM was added. 100 µL of the cell suspension was seeded on a Separate two flat bottom 96-well plate, one for each cell line with a concentration of 5×10^3 cell/well from SR and L20B cell lines, respectively, and incubated for 24 hr. at 37°C and 5% CO2. After incubation, serial triplicates dilutions of DA and PD were done for each 96-well plate, except the control wells, we don't add any extract on it, so that they have the cell lines in maximum growth. The plates were incubated for 24 hr. Then 25µl from MTT (3- (4,5- dimethylthiazol-2-yl) -2,5- diphenyltetrazolium bromide) was added to the all the wells. The plates were incubated for 4 hr. before addition of 100 µL from (10%) solubilization solution (sodium dodecyl sulfate SDS) and mix thoroughly. After 18 hr. incubation, the absorbance at 620 nm was measured for each well by ELISA. The absorption intensities were averaged and normalized to the untreated cells (negative controls) to generate the cell vi-ability (Carmichael *et al.*, 1987).

Data Analysis

The relative viability of the treated cells as compared to the control cells was expressed as the viability %, using the following formula (Mosmann, 1983):

Cell viability (%) =Sample absorbance /Control absorbance*100(Chih *et al*., 2004).

THE RESULTS

Tables (1,2) and figure (1,2) show the effect of *D. Annulatum F*. methanolic extract (DA) on the two cell line SR and L20B by using the following concentrations (3.125, 6.25, 12.25, 25, 50, 100 and 200 µg/ml) compared with the control. It has been noticed that the low concentrations (3.125 and 6.25µg/ml) had the best results in cytotoxic effects which was 55% and 45%, respectively against SR cancer cell line while there was no outcome of the highest concentrations. In addition the cytotoxic effects of DA against L20B cell line in low concentrations (3.125 and 6.25 µg/ml) had the best results which was 60% and 59% respectively, while the outcome of the higher concentrations was 41% and 32%.

Sample Concentration µg/ml	Cytotoxicity %	Viability %
3.125	55	45
6.25	46	54
12.25	43	57
25	30	70
50	5	95
100	0	108
200	0	121
Control (maximum growth)		100

Table 1: Effect of methanolic extract of DA against cancer cell line SR. (lymphoma)

Sample Concentration µg/ml	Cytotoxicity %	Viability %
3.125	60	40
6.25	59	41
12.25	56	44
25	53	47
50	49	51
100	41	59
200	32	68

Table 2: Effect of methanolic extract of DA against cancer cell line L20B

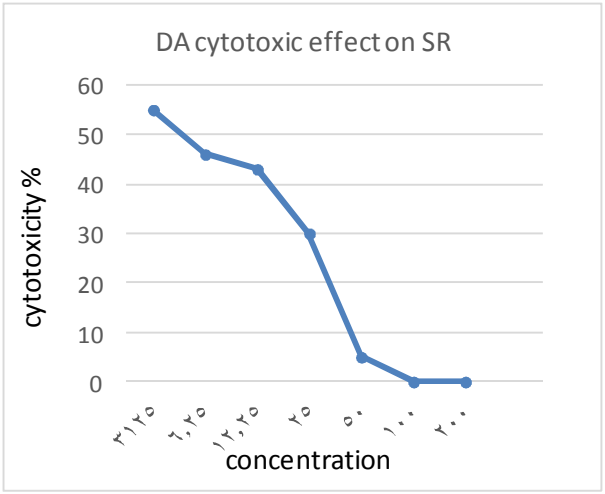


Figure 1: **Effect of methanolic extract of DA against against cell line SR. (lymphoma)**

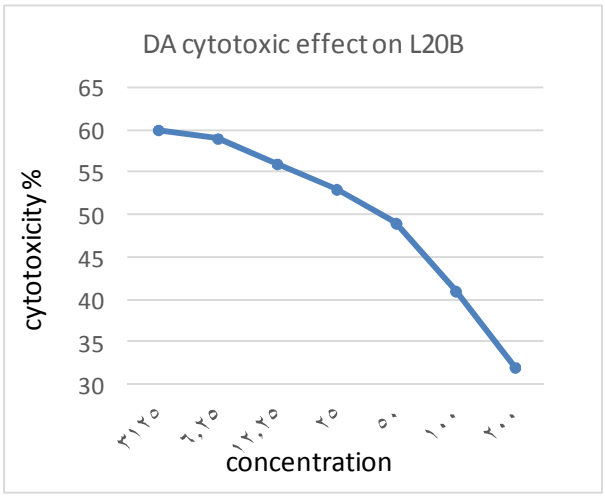


Figure 2: Effect of methanolic extract of DA cell line L20B

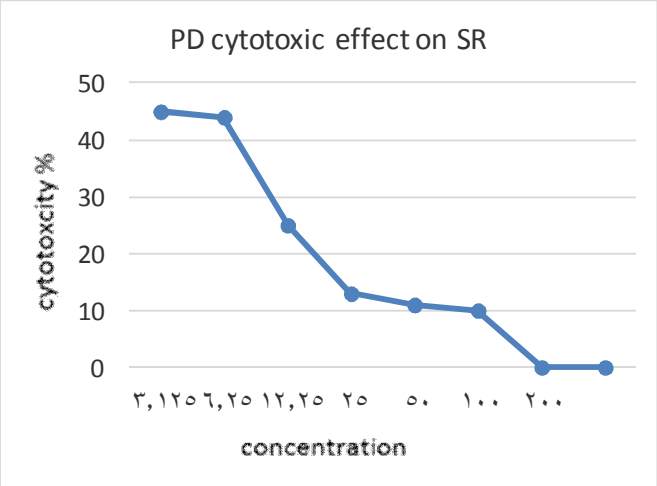
Table (3,4) and figure (3, 4) show the effect of *P. Distichum L.* methanolic extract (PD) on the two cell line SR and L20B by using the following concentrations (3.125, 6.25, 12.25, 25, 50, 100 and 200µg/ml). It has been noticed that the low concentrations (3.125 and 6.25 µg/ml) had the best cytotoxic effect which was 45% and 44% respectively against SR cell line while there was no outcome of the highest concentrations. In addition the cytotoxic effects of PD against L20B cell line in low concentrations (3.125 and 6.25 µg/ml) had the best results which was 49% and 48% respectively, while the outcome of the higher concentrations was 17% and 12%.

Sample Concentration µg/ml	Cytotoxicity %	Viability %
3.125	45	55
6.25	44	56
12.25	25	75
25	13	87
50	11	89
100	10	90
200	0	156
Control (maximum growth)		100

Table 3: Effect of methanolic extract of PD against cell line SR. (lymphoma)

Sample Concentration µg/ml	Cytotoxicity %	Viability %
3.125	49	51
6.25	48	52
12.25	46	54
25	42	58
50	32	68
100	17	83
200	12	88
Control (maximum		100

Table 4: Effect of methanolic extract of PD cell line L20B



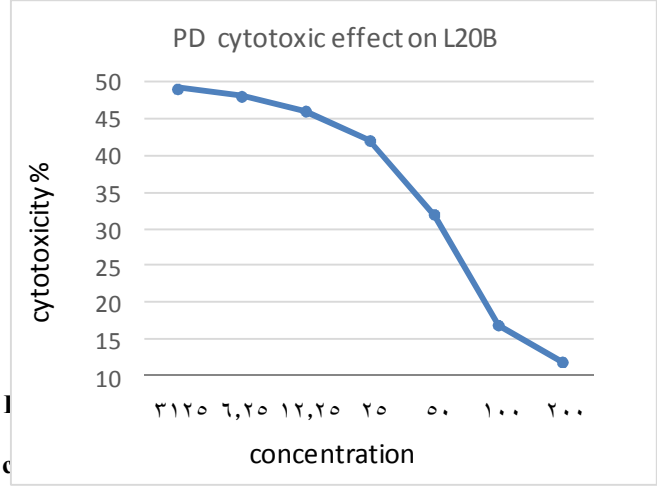


Table 3: Effect of methanolic extract of PD cell line SR. (lymphoma)

Table 4: Effect of methanolic extract of PD against cell line L20B

The discussion

The cytotoxic effect of different concentrations of the two grasses (*Dichanthium Annulatum F.* and *Paspalum Distichum L.*) methanolic extract was studied by using SR cancer cell line and L20B murine fibroblast cell lines. These cells were examined after 24 hr. of incubation. The high scale of cytotoxicity was found in low concentration (3.125 and 6.25 µg/ml) of the two plants while in high concentration (100 and 200 µg/ml) there was a minimum effects and these statuses was very general biologic phenomenon in toxicology known as Hormetic effect characterized by inversion the work of low doses compared with high doses (Calabrese and Baldwin,2002). The ratios showed an observed cytotoxic effects of DA against SR cell line (55%, 45%) and against L20B cell line (60%, 59%). Furthermore the ratios of PD against SR cell line (45%, 44%) and against L20B were (49%, 48%). These relatively ratios may return to the phenolic compounds that found in the Poaceae family according to (Mohammed *et al.*, 2015) who reported that *D.*

annulatum has flavonolignan and flavonoid which have multiple effects, including antioxidants effect against cancer cell line.

In general the phenolic compounds known to be possess the ability to scavenging the free radicals generated when normal cells become cancerous, these compounds containing hydroxyl group (3-hydroxyl group) that would increase the ability on a scavenging free radicals that generated in the cancer cells and can act in all stages of carcinogenesis (Klaunig and Kamendulis, 2004) .

This the most likely explanation for the toxic effects shown by plant extracts against cancer cells is their ability to stimulate programmed cell death, and its attempt to stop the proliferation those cells because it's found that many of the nutrients and herbs medical toxic effects in cancer cells possess through urging them to apoptosis (Thatte *et al*., 2000).

In another study provides an evidence for the strong cytotoxic activity of the ethyl acetate and n-hexane extracts of *Dichanthium annulatum* against several of cell lines (HepG-2, HCT-116 and MCF-7) (Mohammed *et al*., 2015).

While the other grass *Paspalum Distichum L*. we don't find any study that deals with its extract effect on any biological level so our study consider one of its kind in this area.

Conclusion

We conclude from this study that *Dichanthium Annulatum F*. and *Paspalum Distichum L*. methanol extract has a good evident impact despite the variation from concentration to another. This property could be capitalized to develop other researches to get a clear results of having natural antitumor for the prevention/control against cancer. More research on plants and plant-derived chemicals may result in the discovery of potent anticancer agents.

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