Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 35, Issue 1, 2024 DOI: http://doi.org/10.23851/mjs.v35i1.1387

Research Article Open Access

Evaluation of Effect of β-Glucan on Cancer Cell Lines *In vitro*

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Article Info

Received
05/05/2023

Revised
12/07/2023

Accepted
16/07/2023

Published
30/03/2024

Abstract

β-Glucan is linear polysaccharides containing d-glucose monomers connected by β-glycosidic linkages. Their structural variances are the result of several sources. This research project was designed to assess the anticancer activity by using β-glucan. The *in vitro* experiment employed breast cancer cell lines from Michigan Cancer Foundation-7 (MCF-7) and Ahmed, Murtudha, Jabriyah, 2013 (AMJ13). After 24, 48, and 72 hours in micro titration plate under completely sterile condition. Different concentrations of β-glucan (31.25, 62.5, 125, 250, 500, and 1000 µg/mL) were applied to the cancer cell lines. The MTT assay was used to check whether the cells had been inhibited. Cell viability in MCF7 and AMJ13 cells was significantly reduced by β-glucan. β-Glucan showed concentration and time-dependent growth inhibitory effects. The higher concentrations of β-glucan significantly ($P<0.05$) decrease the growth rate of cells, indicating that the higher concentrations were more effective at inhibiting growth.

Keywords: β-glucan; MTT; Anticancer; AMJ13; MCF-7.

الخلاصة

بيتا-جلوكان هو سكريات خطية يتكون من جزيئات الكلوكوز التي ترتبط بواسطة روابط جليكوسيدية. تبايناتهم الهيكلية هي نتيجة مصادره المختلفة. صممت هذه الدراسة للتحري عن التأثير السمي لمركب بيتا- جلوكان, شمل الفحص المختبري استخدام اثنان من الخطوط الخلوية السرطانية المزروعة في الزجاج (وهي خط سرطان الثدي MCF-7 وخط سرطان الثدي (AMJ13)وقد تم معاملة الخطوط الخلوية خلال 24 ساعة، 48 ساعة 72 ساعة وبظروف تامة التعقيم في أطباق المعايرة الخاصة بالزراعة النسيجية. تم تحضير تراكيز مختلفة (31.25، 62.5، 125، 250، 500، 1000) مايكروغرام/ مل واختبارها لكل من خطوط الخلايا السرطانية بمعدل ثلاث مكررات لكل تركيز، تم استخدام اختبار MTT للتحقق مما إذا كانت الخلايا قد تم تثبيطها، تم حساب السمية الخلوية لمركب بيتا- جلوكان، أظهر مركب بيتا- جلوكان تأثير تثبيطي في النمو متعلق بمقدار تراكيزه ومدة تعريضه وإن اعلى تأثير لوحظ عند التراكيز العالية (500,1000) مايكروغرام/ مل بعد مرور 48 ساعة من التعريض. أظهرت النتائج بأن التراكيز العالية أعطت فرقاً معنويا ($P<0.05$) وأن أعلى معدل لتثبيط نمو الخلايا ازداد عند التراكيز العالية.

INTRODUCTION

Cancer is a group of diseases spread over a lengthy period of time and destroy millions of lives globally [1]. Cancer is a significant global public health concern due to its incredibly aggressive nature, poor prognosis, and short survival rate [2]. A significant issue is the global prevalence of breast and liver cancer, which will account for 2.26 million and 2.21 million new cases, respectively, in 2020 [3]. Herbal medicine is one of the oldest medical practices in existence. All across the world, independent plant-based healing systems have developed over time, including Sa-sang in Korea, Ayurveda in India, Kampo medicine in Japan, and traditional Chinese medicine (TCM) [4]. In the 1930s, pure chemicals took the role of unpurified natural materials and crude extracts. The fast growth of chemistry in the 20th and 21st centuries, however, has made natural products less significant and stimulated high-throughput screens (HTS) of synthetic chemical libraries for drug discovery. However, the lack of chemical variety in synthetic compounds has led to a decline in therapeutic approvals and a return to medication development based on natural products [5]. β-Glucan has been shown to be extremely abundant and is one of the

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main fibers in algal, cell wall of plant, bacterial, and fungal. D-glucopyranosyl units are the primary component of the polysaccharides (Glcp) [6]. This Study aimed to determine the cytotoxic effect of purified β-glucan on the growth of cancer cell lines (MCF-7, and AMJ13).

MATERIALS AND METHODS

Preparation of β-Glucan Concentrations

Standard β-1,3-glucan from *Euglena gracilis* (Sigma, USA) was purchased from Sigma lab, to make β-glucan stock, 0.2 g of β-glucan was dissolved in 10 mL of phosphate puffer saline before being filtered through a sterile Millipore filter (0.22 µm). Using sterile serum-free media, various concentrations were created, ranging from concentrations of (1000 g/mL) to (31.25 g/mL).

Cell Culture and Maintenance

The Iraqi center for cancer and medical genetics research (ICCMGR), Mustansiriyah University, kindly provided human breast cancer cells (MCF-7) and (AMJ13). Cells were maintained and grown in RPMI-1640 (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum, 100 U/mL^{-1} penicillin G, and 100 g/mL^{-1} streptomycin. Cells (3 x 104 cell mL^{-1}) were planted in tissue culture flasks and allowed to form an 80–90% confluent monolayer (24 to 48 h). Using a CO2 incubator, cultures were maintained at 37°C in a moist climate. Mild trypsinization (50 mg/mL trypsin) was used to extract the cells [7].

MTT cytotoxicity assay

The cytotoxic effect of β-glucan on MCF-7 and AMJ13 was calculated using the 3-(4,5-Dimethylthyiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test [8]. Cells were cultivated and incubated in 96-well plates until they achieved 80% confluence. After discarding the medium, 200 µL of various β-glucan concentrations were determined based on previous studies that examined the cytotoxicity of β-glucan (31.25, 62.5, 125, 250, 500, and 1000 µL/mL) were added to the appropriate wells containing the cells. As the adverse control in wells, cells that had not been treated were employed. After 24 hours, 10 µL of MTT (Sigma Aldrich, USA) were added to each well. At 37°C and 5% CO2, the plates underwent an additional four hours of incubation. Each well received 100 µL of dimethyl sulfoxide after the media had been carefully removed, and each was then given 5 minutes to incubate. The absorbance at 540 nm was measured using a microplate reader for an ELISA [7]. The inhibition % was calculated using the following formula:

$$IR\ (\%) = (ODc - ODt)/OD\ Control \times 100$$

ODc stands for optical density of control, ODt for optical density of test, and IR stands for inhibitor rate.

Statistical Analysis

The Statistical Analysis System- SAS (2018) [9] program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

RESULTS AND DISCUSSION

Cytotoxic effect of β-glucan extract on (MCF-7) and (AMJ13) cell lines

The results in Figure 1 revealed significant growth inhibition at level (P<0.05) of the MCF-7 cell line in all concentrations of β-glucan for 24, 48 and 72 hours. The greatest cytotoxic effect was noticed at the two highest concentrations (500 and 1000 µg/ml). The effect of β-glucan on AMJ13 cells are shown in figure 2, β-glucan showed a time and concentration dependent effect on viability of AMJ13 cells. The cytotoxic effect is significant at all concentrations at level (P<0.05). The highest concentration at the 48 hrs, produced the highest percentage of cytotoxic effect.

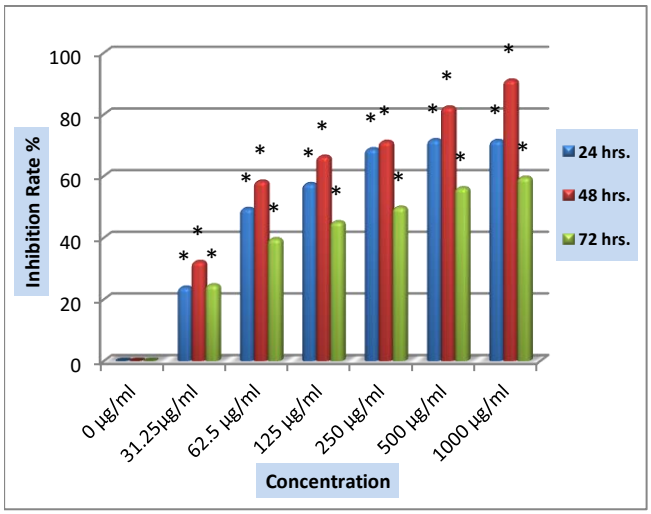

Figure 1. Percentage of inhibition of MCF-7 cell line by standard β- glucan during three periods of exposure.

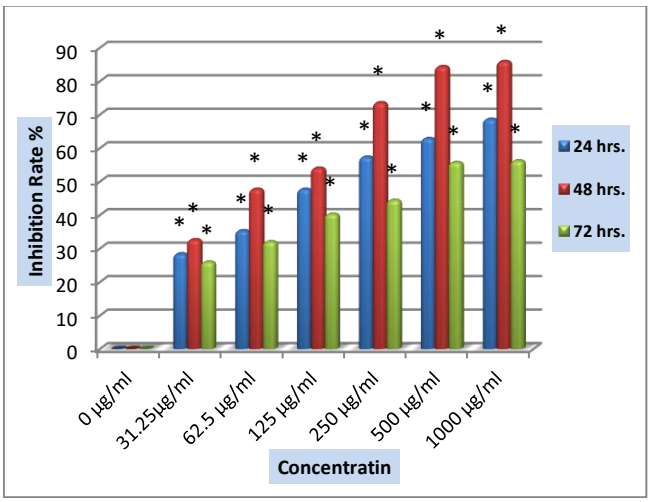

Figure 1. Percentage of inhibition of AMJ13 cell line by standard β- glucan during three periods of exposure.

The optical densities (OD) for the stained cell lines after treatment with different concentrations of the β-glucan for 24, 48, and 72 hrs, showed that there were differences of (OD) among the concentrations, with the high concentration giving low value of OD, demonstrating maximum response, and the low concentration giving high value of OD, indicating minimum response in compared to high percentage of viable cells**.** The results of this study demonstrated that β-glucan had a selective influence on the viability of various cell-lines, and that this selective effect of β-glucan may manifest itself in cell adhesion. These results indicate that β-glucan can prevent cells from adhering to a plate, causing them to separate from it.

β-Glucans can stimulate the immune system and have anticancer effects, the process by which β-glucan destroys cancer cells is complicated and poorly understood. There is many interesting research that support β-glucan's capacity to influence cancer cells *in vitro* and *in vivo*, even though there isn't any concrete proof that it can be used as an anti-cancer factor [10]**.** β-Glucan has garnered a lot of attention in recent years due to their anticancer effects throughout the world; nevertheless [11]**.** A numerous anticancer medications work through exposing tumor cells under oxidative stress, which is thought to be the primary cause of the most of macromolecular changes in the cell. Reactive oxygen species may damage proteins, membrane lipids, and macromolecules like DNA [12].

We observed a proportional decrease in cell viability in all examined MCF and AMJ cancer cell lines after β-glucan incubation. Hong and his colleagues [13] focused on the antitumor effect of β-glucan generated from microorganisms on four cancer line, which validated the cytotoxicity of β-glucan. Clearly harmful cells included Hela and Sarcoma 180**.** β-Glucan's cytotoxic properties were proven by Kim and his colleagues [14], they investigated colon cancer cells and hypothesized that the amount of β-glucan administered affects the viability of cancer cells. They discovered that a dose of 200 g/ml decreased the viability of cancer cells by about 50% when using the MTT assay . According to certain research β-glucan inhibit cancer cells, β-glucan stimulates the development of the caspase-3 enzyme, which causes cancer cells to undergo apoptosis. Additionally, β-glucan can affect morphology and result in the expression of proapoptotic genes [10]. The findings of this study and the majority of comparable studies indicate that β-glucan is an effective anticancer with no side effects that supports these properties; additional experiences and research on animal models are advised to achieve a better response to therapy.

CONCLUSIONS

In the present study, β-glucan exhibited a significant antitumor activity against MCF-7 and AMJ13 cell lines in concentration and time dependent manner. The cytotoxic effect of β-glucan was more on MCF-7 than on AMJ13.

Acknowledgment

We thank all the authors who contributed to this study and all the reviewers and invited editors who have helped to make this study.

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

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How to Cite

H. M. Al-Khuzaay, Y. H. . Al-Juraisy, A. H. Alwan, and E. Tousson, “Evaluation of Effect of β-Glucan on Cancer Cell Lines In vitro”, *Al-Mustansiriyah Journal of Science*, vol. 35, no. 1, pp. 17–20, Mar. 2024, doi: 10.23851/mjs.v35i1.1387.