DOI: 10.29409/ijcmg.v5i1.79

Iraqi Journal of Cancer and Medical Genetics

THE EFFECT OF Beta glucan EXTRACT OF Saccharomyces cerevieses ON CANCER CELL GROWTH (In vitro)

Ali J. Jabber[1] , Nidhal R. Mahdi[2] , Nahi Y. Shahin[3]

1Department of Microbiology / Medicine collage / missan university
2Department of Microbiology ,Collage of Veterinary medicine ,Baghdad university
3Iraqi Center for Cancer & Medical Genetic Research / University of AL-Mustansiriy

Abstract :

This study was designed to evaluate the anticancer Effects of the beta glucan (ß- glucan) extract of the Saccharomyces cerevieses on cancer cell lines .

In vitro study was performed on two cancer cell lines (murine mammary adenocarcinoma AMN-3 cell line) and rat emberyogenic fibroblast (Ref) as normal cell line. Periods of exposure of cell lines were measured at 24-hours, 48-hr, and 72-hr in a microtitration plate under complete sterile conditions. Different concentrations starting from (5,50,500, 1000) µg/ml of two fold dilution for extract were prepared and tested on each cell lines, The extract showed concentration and time dependence growth inhibitory effects, and the highest effect was obtained from beta glucan extract at higher concentrations after 48 hr. of exposures on AMN3, that the higher concentrations gave a significantly ($P<0.05$) and the higher inhibition growth rate of cells were increased at 24 hours.

Key word: *ß- glucan , MTT , AMN-3 , Ref .*

Introduction:

Cancer is one of the leading causes of death in the world. The main cause is that they damage immune systems in tumor treatment. So, it is necessary to develop novel anti-tumor agents with administrating immunity potential. Polysaccharides have attracted more attention recently in the biochemical and medical because of their anti-tumor and immunomodulating properties (1).Some polysaccharides extracted in medicines Laboratory have been reported to possess anticancer activities (2) .

Recent developments of modern techniques of targeted tumor cell elimination (3), include Immunotherapy, which also called biological therapy, that uses the body's own immune system to fight cancer (4), and gene therapy, as a new trials to treat cancer (3). However, there is a continuing need for development of new anticancer drugs, drug combinations and chemotherapy strategies, by methodical and scientific exploration of enormous pool of synthetic, biological and natural products (5). A safe and effective cancer treatment has been the goal of scientists for many decades. Such a technique must be selective in destroying the cancer cells without irreversibly damaging normal cells (6).

Beta glucan is a scientifically proven biological defence modifier (BDM) that nutritionally potentiates and modulates the immune response. through immune response potentiation and modulation, in many instances various therapeutic healing effects generated by the immune cells. For many years Glucans have been investigated (History) for these immune enhancing properties (7,8) .

β-Glucans (beta-glucans) are polysaccharides of D-glucose monomers linked by β-glycosidic bonds. β-glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. They occur most commonly as cellulose in plants, the bran of cereal grains, the cell wall of baker's yeast, certain fungi, mushrooms and bacteria.(9).

Polysaccharides from fungi have attracted attention in the fields of biochemistry and pharmacology for their immunopotentiation and anti-tumor effects (10 , 11) . The anti-tumor activities of polysaccharides are mostly resulted from their immunopotentiation effects (12). Polysaccharides can stimulate immune cells such as granulocytes,monocytes, macrophages and nature killer cells to trigger the secretion of cytokines that will stimulate the immune system (13,14).

Carboxymethylated derivatives from both a- and b-D-glucans show higher water solubility along with antitumor

Corresponding Address:
Ali J. Jabber
Department of Microbiology/ Medicine collage / missan university
Email: jwad ali52@yahoo.com

activity against cell lines,(15) Moreover, hydroxyethylation, hydroxypropylation, and methylation can also increase the water solubility and antitumor activity of certain polysaccharides.(16) In addition, the introduction of suitable ionic groups with appropriate degrees of substitution (DS) can also cause the polymer chain to adopt certain conformations in aqueous solution.15 The effects of different substitution groups, their positions, and DS, on the bioactivities of polysaccharides have been reported one of the most important traditional medicines in China and Japan, and exhibits various biological activities.(17).

Therefore this study has been designed to assess the cytotoxic activity of fungal extract of Saccharomyces cerevisiae though performs the following aims:

* - Study the effect of extracts on the growth of :
1 - cancer cell lines (AMN-3) .
2 - normal cell line (REF) in vitro.

Material & Methods:

Cell lines:

The cell lines that used in this study were supplied by experimental therapy department , tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) maintaintained in RPMI- 1640.

Ahmed-Mohammed-Nahi-2003 (AMN-3 cell line) :

The cell line was supplied by tissue culture unit / ICCMGR, Baghdad, Iraq (passage number 162). The origin and description of this cell line was first mentioned by Al-Shamery, (18). The specimen was taken from murine mammary adenocarcinoma.

Embryo Fibroblast (REF):

The normal culture of the rat embryo is the most important source for the undifferentiated fibroblastic culture. This cell line was supplied by experimental therapy department , tissue culture unit / Iraqi Center for Cancer and Medical Genetic Research (ICCMGR), Baghdad, Iraq (passage 48). The specimen was taken from rat embryo then killed and Trypsinized , then it was maintained in RPMI-1640 medium with 20% bovine calf serum, when it becomes confluent monolayer, the cells treated with Trypsin-Versine mixture in order to pursue subculture process(19) .

Extraction of beta glucan:

Laboratory extract of Beta-glucan in our experiment, by Extraction Procedure of beta-glucan from baker yeast (Saccharomyces cerevisiea) . a combination of (20) and (21).

Processing of yeast glucan

The starting S. cerevisiae -glucan material was obtained from the Market, This material was processed from common baker's yeast using the following procedure. Active dry yeast (300gm) was added to one liter 0.1 mol of NaOH and stirred for 30 min at 60 °C. The material was then heated to 115 °C at 8.5 psi for 45 minute and then allowed to settle for 72 h. The sediment was resuspended and washed in distilled H2O by centrifugation (350 g for 20 min). The alkali insoluble solids were combined with 0.1 mol of 1L of acetic acid and heated to 85 °C for 1 h, then allowed to settle at 38 °C. The acid insoluble solids were drawn off and centrifuged as above. The compacted solid material was mixed with 3% H2O2 and refrigerated for 3 h with periodic mixing. The material was then centrifuged and the pellet washed twice with distilled H2O followed by two washes in 100% acetone. The harvested solid material was dispersed on drying trays and dried under vacuum at 38 °C for 2 h in the presence of Ca2SO4, and then further dried overnight under vacuum at room temperature. This procedure yielded a white powder .

were phosphorylated individually by the improved method for (21) ,The fraction (4gm) of powder was dissolved in (200) ml of Me2SO containing (72)gm of urea. With stirrer, About (40) mLof H3PO4 (85%) was added dropwise slowly to the above solution at ambient temperature. Then the solution was heated to 100 ◦C , and the reaction was carried out for 6h with stirring. A crystalline precipitate (presumed ammonium phosphate) formed at 1–2 h of reaction. Following heating, the reaction mixture was cooled to ambient temperature and diluted in distilled-water to form a yellow clear solution. Finally, the resulting phosphate derivative was dialyzed (3000 – 5000) Millipore in size against distilled water for seven days to remove endotoxin (includingMe2SO,H3PO4 and salt).

Phenol-Sulphuric Acid Assay to Determine Composition of Starch-Oil Composites

The phenol-sulphuric acid procedure was conducted as described by Dubois (22), with the modification that the solvent for samples at final dilution (at concentrations low enough for absorbance measurement Samples containing 1 mL of solvent were mixed with 1 mL of 5% phenol in a test tube cuvette with a 19-mm path length; 5 mL of concentrated sulphuric acid was added rapidly to generate heat to drive the reaction. The reaction mixture was allowed to cool to room temperature, and absorbance was measured at 490 nm in a spectrophotometer (Sequoia-Turner, model 690) against a water blank.

MTT Staining Assay:

The colorimetric MTT method (23) was used for measuring, the proliferation of tumor cells. and Detection of tumoricidal activity of immunotherapeutic effect of beta-glucan.Colorimetric 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used for measuring the proliferation of adherent tumor cells. The tumor cells were inoculated on a 96-well cultivation plate at a concentration of 1 x 10^6 cells/mL. Each well was inoculated with 100 µl UL Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10 % fetal bovine serum solution containing the tumor cells and 20µL samples (at concentrations of 0.005 mg, 0.05 mg ,0.5mg ,and 1 mg/mL in PBS, at 37 ℃ for 24 - 36h . The tumor cells were continuously inoculated for another, 4h after 10 µL MTT (5mg/mL) had been added. The supernatant was removed by centrifuging, and then 100 µl Me2SO was added to terminate the reaction. The optical density of each well was read by using a micro-ELISA reader at a transmitting wavelength on at 550nm. The sample groups were compared with control group in the absence of the tested samples. All in vitro results were expressed as the inhibition ratio of tumor cell proliferation as follows:

$$IR. = (ODc - ODt) / ODc \times 100$$

IR= inhibitor rate , ODc = the optical density of control , ODt = the optical density of test. IR

were converted for arsine transformation for statically analysis

Results and Discussion:

In vitro study:

Table (1): The percentage of crude extraction of beta glucan

Weight of baker›s yeast (gm)	Weight of extract (gm)	Percentage of extraction %
300	37	12.3

Table (1): The percentage of crude extraction of beta glucan

chemical detection of active compounds:

samples containing carbohydrate developed a red-orange colour rather than the amber colour typical of the phenol-sulphuric acid assay. Intensity of the red colour increased with increasing the concentration, absorbance at 490 nm (the wavelength of maximum absorbance for glucose and starch).

Growth inhibitory effect:

Cancer cell lines in invitro study (AMN-3 and REF) were exposed to four concentrations, of dilutions between (5 ,50, 500 ,1000 µg/ml) of Saccharomyces cerevieses extracts for 24, 48 and 72 hours durations, and optical density was measured under wavelength 530 - 550 nm with micro-ELISA reader after their staining with MTT assay . The growth inhibitory effect by the extracts of Saccharomyces cerevieses that the extract had the greatest inhibitory effect on both AMN3 and Ref .

Cytotoxic effect of beta glucan extract on (AMN3), & (REF) cell lines:

The effect of different concentrations of extract from (5,50,500 to 1000 µg/ml) on tumour cell lines after (24, 48 &72) hrs. of exposure in table (2). The results revealed significant cytotoxic effect at levels ($P<0.05$) for all concentration , all extracts inhibited cell growth at highest concentrations and have no effect at the lower concentrations. The extract had highest inhibitory growth on AMN3 cell lines at the concentrations (500 and 1000 µg/ml) for the period of 48 hrs. Some of concentrations increase in their inhibitions over the previous one, like (500 µg/ml) for 24 hrs, 48 hrs . and (50 µg/ml) for 72 hrs on AMN3 cell line , figure(1)

while on REF cell line were (500 µg/ml) & (1000 µg/ml) on 24 hrs, and (5 µg/ml), (1000 µg/ml) on 48 hrs and (50 µg/ml), (500 µg/ml), for 72 hrs., table (2) shows the effect of beta glucan extract on proliferation of the cell lines. The results revealed significant cytotoxic effect for all concentration .

Cancer cell lines control cells for AMN3 exhibited confluent monolayer of cohesive malignant cell as shown of AMN-3 cell line with wall differentiation, there are no empty spaces and in (Fig-2)

AMN-3 cell line showed cellular swelling, vaculation and lyses of nucleus after their exposure to beta glucan extract of Saccharomyces cerevisiea as seen in (Fig-3).

REF cell line showed very low growth inhibition after exposure to 50 µg concentration of extract.

the outline cellular feature has been lost and the changes progressed with highly effect of high concentration and this can be seen in (Fig-3). The dead cells became prominent more than what seen in low concentration for beta glucan extract of Saccharomyces cerevisiea.

Extraction:

The dried leaves of Beta- glucan extracts of Saccharomyces cerevisiea as in the table (1) gave a bright yellow product, which became powder upon drying.

Table (2): Mean values of inhibition rate percentage (IR%) of (AMN3,& REF) cell lines after treatment with different concentrations of Beta glucan extract of Saccharomyces cerevisiea for (24, 48 & 72) hours.

Concentration µg/ml	IR% AMN3				IR% REF			
	24 hrs	48 hrs	72 hrs	LSD	24 hrs	48 hrs	72 hrs	LSD
5	26	21.4	33.3	* 5	7.3	12.9	11.9	*1.2
50	33	35.4	50.7	*7.6	3	9.5	24.7	*2.4
500	60	79.7	53	*8.1	11	6.4	15.4	*3.3
1000	51	84.7	56.3	*7.4	12.8	20.5	11.5	*2.8

(*) mean significant difference between means comparation with columns groups and in comparation with arrows groups at levels ($P<0.05$).

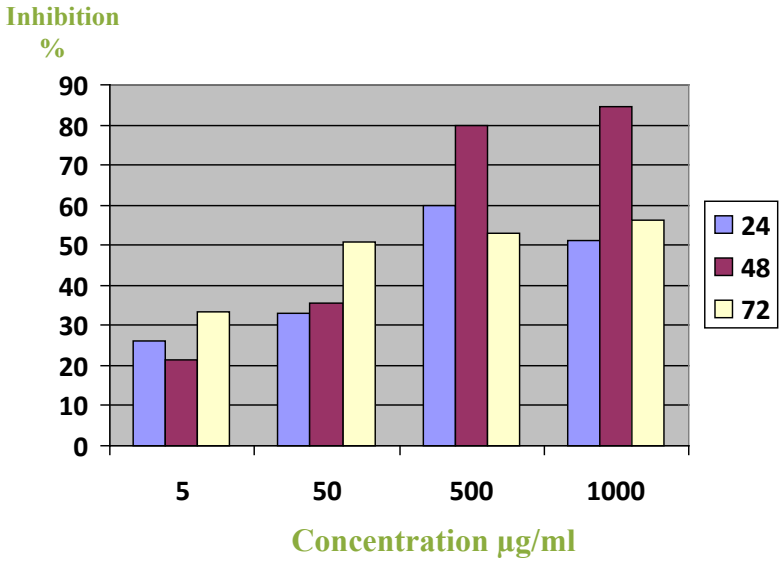


Figure (1) : The inhibitory effects of beta glucan extract of Saccharomyces cerevieses on AMN3 cell line growth during different periods of exposure.

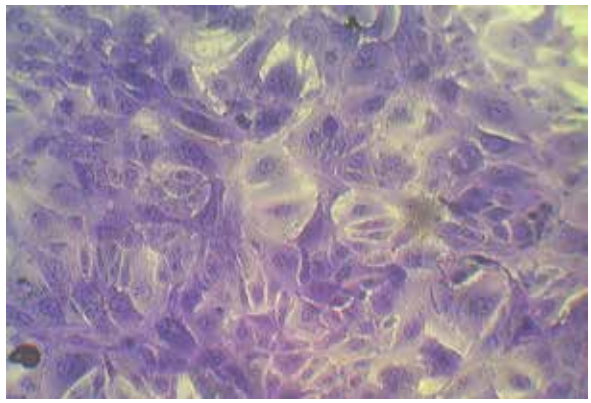
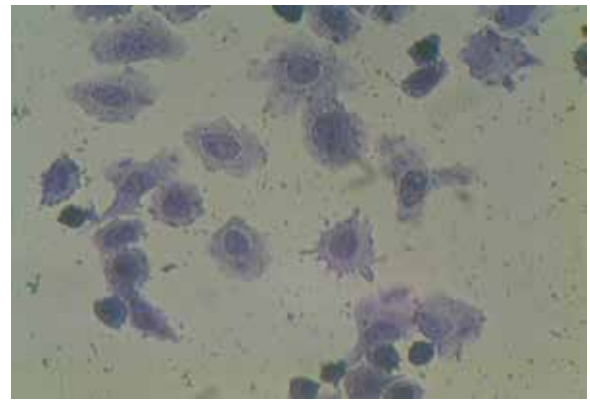


Figure-(2)- Confluent monolayer in control group of AMN-3 cell line with wall differentiation, no empty spaces (X200), (crystal violet stain).



Figure(3): The cell line of AMN-3 show cellular swelling, vacuolation and (lysis of nucleus) after their exposure 500 µg of beta glucan extract of Saccharomyces cerevieses (X200), (crystal violet stain)

Discussion:

The extractions of Saccharomyces cerevisiea yield crude extract (12.3%) which was the greater than (8.7%) of beta glucan extracts of Poria cocos (17). Differences between percentage of extracts may be due to some lost during processing of extraction depending on the type of preparation to each one. The extractions showed fine bright yellow powder and sticky extract, but was dark brown sticky for hot aqueous extract of Poria cocos.

After treatment with different concentration of the extracted of Saccharomyces cerevisiea during 24,48,72 hours the optical densities (OD) for the stained cell lines , revealed that differences of (OD) between the concentration , that the high concentration gave low value of OD , indicating maximum response ,Whereas the low concentration gave high value of OD which indicate minimum response in proportional to high percentage of viable cells.

The result of this study showed that Saccharomyces cerevisiea extracts have selective effect on the viability of different cell-lines, this selective effect of extractions may appears on the cell adhesion. These results indicate that Saccharomyces cerevisiea extracts have one or more constituents capable to interfere with the adhesion process of cells leading to detach from plate and not involved in the measurement of O.D. Most cells in culture need a period of lag phase to attach on the substratum and adapt to medium conditions before they will start to proliferate (19). The other factor may be osmolality effect, in which extracts of Saccharomyces cerevisiea rich in proteins, carbohydrates, minerals and other constituents that make it hypertonic solution (24, 35) and may cause dose-dependent osmotic shock to cell-lines.

The sensitivity of mammary gland adenocarcinoma (AMN3) may be due to the natural bioactive structures of beta glucan which inhibit the tyrosine kinase activity of

growth factor receptors and oncogene products, as well as the in vitro growth of some tumor cell lines(26).

The sulfation and carboxymethylation significantly enhanced the antitumor activities of the b-glucan against cell lines (AMN3, Sarcoma 180 and gastric carcinoma tumor cell in vivo and in vitro). Considering the molecular parameters and bioactivities, good water solubility, relatively high chain stiffness, and moderate molecular mass of the derivatives in aqueous solution are shown to be beneficial to enhancement of antitumor activity (15) .

References:

1. Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. Trends in Food Science & Technology, 18, 4–19.
2. Chen, Y., Cheng, P., Lin, C., Liao, H., Chen, Y., Chen, C., et al. (2008). Polysaccharides from Antrodia camphorata mycelia extracts possess immunomodulatory activity and inhibits infection of Schistosoma mansoni. International Immunopharmacology, 8, 458–467.
3. Sobol, R. E. and Scanlon, K. J. (2008). Cancer Gene Therapy. Journal Citation Reports. 15: 10.
4. Bauer, T.R Jr; Allen, J.M; Hai, M; Tuschong, L.M; Khan, I.F; Olson, E.M; Adler, R.L; Burkholder, T.H, Gu Y.C; Russell, D.W; Hickstein, D.D. (2008). Successful treatment of canine leukocyte adhesion deficiency by foamy virus vectors. Nat. Med. 14:93-7.
5. Mukherjee, A.K; Basu, S.; Sarkar, N. and Ghosh, A.C. (2001). Advances in Cancer Therapy with Plant Based Natural Products. Current Medicinal Chemistry. 8: 1467-1486.
6. Hunt, K; Vorburger, S; and Swisher, S. (2008). Gene Therapy for Cancer. AMA. 299: 1367-1368.
7. Saxena, A., McMeekin, J. D., & Thomson, D. J. (2002). Expression of Bcl-x, Bcl-2, Bax, and Bak in endarterectomy and atherectomy specimens. The Journal of Pathology, 196, 335–342.
8. Zhaocheng M., Jianguo W., Lina Z. a, Yufeng Z. b, Kan D. c (2010). Evaluation of water soluble b-D-glucan from Auricularia auricular-judae as potential anti-tumor agent b School of Stomatology, Wuhan University, Wuhan 430079, PR China.
9. Godfrey Chi-F., Chan, Wing K. C. and Daniel M. (2009). Review The effects of beta-glucan on human immune and cancer cells.
10. Jarek B., Daniel J. Allendorf, Feng H. and Gordon D. R. (2007). Oral β-glucan adjuvant therapy converts nonprotective Th2 response to protective Th1 cell-mediated immune response in mammary tumor-bearing mice, Tumor Immunobiology Program.
11. Valmori D (2005). SSX antigens as cancer vaccines. Cancer Immun. 5 Suppl 1: 18. URL: http://www.cancerimmunity.org/v5suppl1p18/041239_abs.htm.
12. Strong , V., Theresa (2000) Gene therapy for carcinoma of the breast: Genetic immunotherapy ,The electronic version of this article is the complete one and can be found online at: http://breast-cancer-research.com/content/2/1/015.
13. GWEN , v., chi (2009). In situ hybridization of wild Drosophila embryo at different developed stages for gene., , China Carbohydrate Research 344 2209–2216 .
14. Letters, J, Baell, J. B., & Huang, D. C. (2002). Prospects for targeting the Bcl-2 family of proteins to develop novel cytotoxic drugs. Biochemical Pharmacology, 64, 851–863.
15. Wang ,Y ; Lina ,Z. ; Yunqiao L. ; Xiaohua H. and Fanbo Z. (2004) Correlation of structure to antitumor activities of five derivatives of a b-glucan from Poria cocos sclerotium ,Carbohydrate Research 339 2567–2574,Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China .
16. Xiaohua W. and Lina Z. (2009) Physicochemical properties and antitumor activities for sulfated derivatives of lentinan ,Department of Chemistry, Wuhan University, Wuhan 430072, China Carbohydrate Research 344 2209–2216 .
17. Jianhui W. ; Chunyu L.; Ming Z.; Wenjing W.; Yuji W.; Shiqi P. (2010) A class of novel carboline intercalators: Their synthesis, in vitro anti-proliferation, in vivo anti-tumor action, and 3D QSAR analysis Bioorganic & Medicinal Chemistry 18 6220–6229.
18. Al-Shamery, A. M. H. (2003). The study of Newcastle disease virus effect in the treatment of transplanted tumour in mice. M.Sc. Thesis. College of veterinary medicine, University of Baghdad, Iraq.
19. Freshney, R. S. (1994). Culture of animal cells, (3 th. ed.). A manual of Basic Technique Wileg. Liss Inc 605 Third Avenual, New York, Press. P: 287.
20. Hunter ,K. W. ,Gault, R. A. , Berner , M. D. (2004) Preparation of micropartical B-glucan from S. cerevisiae for use in immune potentiation ,Letters in Applied Microbiology .Volume 35 issue 4 page :268 .
21. William ,D. l. , Mcnamee , R. B. , Jones , E. L., Pretus , H. A. , Ensley , H. E. , Browder , I. W. , Diluzio , N. R. (1991) Amethod for the solubilization of a (1-3) beta glucan isolate from S. cerevisiae . Carbohyd. Res. 219, 203 – 213.
22. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
23. Xiaoyu C. , Lina Z., Peter C. (2010) Immunopotentiation and anti-tumor activity of carboxymethylated-sulfated â-(D-glucan from Poria cocos ,International Immunopharmacology ,journal homepage: www.elsevier.com/ locate/i n t imp
24. Karakaya, S. (2004). Radical scavenging and iron- chelating activities of some greens used as traditional dishes in Mediterranean diet. Int.J. Food Sci. Nutr. 55: 67-74.
25. Huange, b. Q. , Zhanga ,L. (2011) Preparation, chain conformation and anti-tumor activities of water-soluble phosphated beta-glucan from Poria cocos mycelia,a Department of Chemistry, Wuhan University, Wuhan 430072, China
26. Pagliacci, M.; Smacchia, M; Migliorati,G.;Grignani ,F.; Riccardi , C .; Nicoletti, I. (1994): Growth-inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells. Eur J Cancer ; 30 A (11):1675-82.

دراسة تأثير المستخلص الخام لخميرة الخبز Saccharomyces cerevieses على نمو الخلايا السرطانية في الزجاج (خارج الجسم الحي)

علي جواد جابر1, نضال رؤوف مهدي2 , ناهي يوسف ياسين3

1 فرع الاحياء المجهرية / كلية الطب / جامعة ميسان
2 فرع الاحياء المجهرية /كلية الطب البيطري /جامعة بغداد
3 المركز العراقي لبحوث السرطان والوراثة الطبية

الخلاصة:

صممت هذه الدراسة للتحري عن التأثير السمي للمستخلص خميرة الخبز Saccharomyces cereviasie للخطوط الخلوية السرطانية والخلايا الطبيعية خارج الجسم الحي .

شمل الفحص المختبري استخدام اثنان من الخطوط الخلوية السرطانية المزروعة في الزجاج (وهي خط سرطان الغدة اللبنية الفأري AMN3) و خط الخلايا الجنينية الليفية الفأري الطبيعي (Ref) , وقد تم معاملة الخطوط الخلوية خلال 24 ساعة ،48 ساعة و72 ساعة وبظروف التعقيم التامة في أطباق المعايرة الخاصة بالزراعة النسجية. تم تحضير تراكيز مختلفة ثنائية التخفيف من المستخلصات الخام ابتدائا من التركيز 500,50, 5 مايكروغرام\ملليتر وإنتهاءً بالتركيز 1000 مايكروغرام\ملليتر واختبارها لكل من خطوط الخلايا السرطانية بمعدل ثلاث مكررات لكل تركيز.

أظهر المستخلص تأثيرات تثبيطية في النمو متعلقة بمقدار تراكيزه ومدة تعريضه وإن اعلى تأثير لوحظ عند التراكيز العالية(500,1000) مايكروغرام\ملليتر للمستخلص بعد مرور48 ساعة من التعريض خطوط الخلايا السرطانية (الغدة اللبنية الفأري), أظهرت بأن التراكيز العالية أعطت فرقآ معنوياً ($P<0.05$) وأن أعلى معدل لتثبيط نمو الخلايا ازداد خلال الفترة 24ساعة.