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The Role of Beta Sitosterol on MCF7, local AMJ13 cell lines and Staph. Aureus

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Abstract:
Phytosterols are biological active compounds which are naturally present in plant with chemostructure similar to the eucayotic cell- derived cholesterol. They are highly available in lipid-rich plant foods such as nuts, seed, and olive oil. The results of effects β- Sitosterol, as a single agent used in breast cancer cells MCF-7 cells and AMJ13 showed treated with the following different concentrations: 0, 1, 10, 100, 1000, 10000, 100000 nm of β-sitosterol .The inhibition of viability showed much higher in MCF7 cell line than in AMJ13in β- Sitosterol 10000,100000nm, the data showed that β- Sitosterol has controversial effect compared with previous studies . This study explained simply the importance of β- Sitosterol on MCF7 cell line compared with AMJ13 Furthermore, It clarified the antimicrobial activity of β- Sitosterol on *Staphalococus aureus*. This led that β-Sitosterol may be used as a promising candidate drug for anti-cancer in further medical researches.
Key words: Beta sitosterol, cell lines, Staph. Aureus

الخلاصة:

الفايتوستيرول هي مركبات بيولوجية نشطة موجودة بشكل طبيعي في النبات مع بنية كيميائية مماثلة للكوليسترول المشتق من الخلايا الحقيقيه النواه وهي متوفرة بشكل كبير في الأطعمة النباتية الغنية بالدهون مثل المكسرات والبذور وزيت الزيتون. أظهرت نتائج التأثيرات بيتا سيتوستيرول كعامل منفرد يستخدم في خلايا سرطان بالتركيزات المختلفة التالية نانومتر: 0، 1، 10، 100، 1000، 10000، 100000. أظهرت تاثير التثبيط أعلى بكثير في خط مقارنةً بالدراسات السابقة AMJ13 من في تراكيز بيتا سيتوستيرول AMJ13 MCF-7 . وأظهرت البيانات أن سيتوستيرول له تأثير ممتاز في تثبيط الخلايا السرطانيه (10000100000) نانومتر مقارنة بالدراسات السابقة. أوضحت هذه الدراسة أهمية خلايا على خط خلية MCF7 مقارنةً بـ AMJ13 علاوة على ذلك، من جانب اخر على بدائيه النواه فقد أوضحت النشاط المضاد للميكروبات لـ ستاف .

الكلمات المفتاحية: بيتاستوسيول, السيل لاين, ستافلو اريوس

Introduction :

β-sitosterol is being noticed for its potential to effectively reduce benign prostatic hyperplasia (BPH) and decrease blood cholesterol levels.[1-8] β-Sitosterol extracted naturally as a powder of organic compound with a formula of $C_{29}H_{50}O$, and considered is one of the most abundant occurring phytosterols in many plants.[9] Phytosterols are bioactive compound naturally present in cell membranes of plants, (SIT) is the most abundant and widely distributed in lipid-rich foods of plants such as vegetables, seeds, nuts ,and olive oil.[3]

β-Sitosterol commonly used for lowering cholesterol levels and improving some bad symptoms of an enlarged prostate . It's also tried for rheumatoid arthritis, male-pattern baldness, heart disease and many other conditions, but there is not yet good scientific evidence to support these other uses.[4-8] In the united states, plant foods that contain at minimum 650 mg of beta-sitosterol are permitted to submid legally that they might reduce the risk for heart disease. βeta-sitosterol with sitostanol, a similar plant compound. For heart health, beta-sitosterol works via lowering the amount of LDL "bad" of cholesterol in the blood because it is thought to stop cholesterol absorption. However, research has not shown that β-Sitosterol also acts as a blood thinner.[8-11]

Material and methods:

Experimental Design: Objectives of primary study included determination of the dose and safety profile of β-Sitosterol. Secondary objectives included evaluation of β-Sitosterol as pharmacodynamics and preliminary clinical activity.

Cell line profile MCF7:

(Cell line**:** ECACC which has Catalogue no. 86012803) history of MCF7 was derived from a female 69 year old suffering a breast adenocarcinoma1 cell line isolated from the pleural effusion. It was named after the Michigan Cancer Foundation (MCF) besides, was the most studied human breast cancer cell line in the world. Applications of MCF7 are used primarily in Biology as an in vitro model to study breast cancer. Due to the number of variants available, it has many applications in development of chemotherapeutic drugs and understanding in resistance of drug.[12]

Cell culture

MCF-7 (The estrogen-sensitive human breast tumor cell lines) was obtained from the laboratory of Experimental therapy Department in Iraqi research center for cancer and medical genetics research, Al-mustansyriah. The cells were maintained in culture medium, RPMI media was supplemented with (10%) heat-inactivated fetal bovine serum, amino acid 2 mM L-glutamine. In these experiments, cells were grown in phenol red-free RPMI culture medium supplemented with (5%) dextran charcoal-treated FBS and amino acid 2 mM L-glutamine.

β- Sitosterol preparation:

β- Sitosterol was purchased from KRK American company, and several concentrations were used 0,1,10,100,1000,10000,100000nm in exposing to MCF7cell lines than AMJ13 cell lines

Cytotoxicity: In first plate, the materials of experiment media were discarded from each well of microplate after washing. Then, 50 µl of MTT reagent (5 mg/ml) was added in each well and incubated for two hour at 37 °C in the incubator supported by CO_2. Then The MTT solution was discarded, and then 100 µl of room temperature isopropanol was added. The plates were kept on a shaker for solubilizing the purple crystal formazan formations. The absorbance measured using a microplate reader machine at a wavelength 570 nm. The results were gave numbers of cell viability and we calculated the percentage against extract concentrations. β-Sitosterol exhibits greatest efficacy in MCF7cell lines than AMJ13 cell lines. The impact of β- Sitosterol 0, 1, 10, 100, 1000, 10000, 100000 nm on cell viability was first assessed in MCF7cell lines cell lines. The IC50s after exposure for 72h is shown in Figure (2), The β- Sitosterol 0, 1, 10, 100, 1000, 10000, 100000 nm on cell viability was second assessed in AMG13 cell lines. The measurement results were repeated 3 times and averaged in figure (3).

Exposing samples on microorganism: The antimicrobial activity was determined in the following steps, after incubation the microorganism overnight, 0.5 mL from each tube was pulled and put in the Eppendorf tube (Before treatment). After that, the media was discard from all tube (, Chloro hydroxyl and 1000, 10000, 100000 nm of β-Sitosterol as showed in figure 2 with different concentrations and left it for 30 minutes with mixing at each 2 minutes. After the standing time, the tubes were rinsed with D.W and then addition 4 mL from D.W for each tube and stand for 2 minutes. After that, 0.5 mL from each tube was taken and put it on the Eppendorf tube.

The efficiency of β- Sitosterol on *S.aureus*

Mannitol Salt media was used to identify the *S.aureus* bacteria. Specific dilution was used in this experiment , D.W : Stock as (1000:1) was prepared from each tube then 50 µL form each dilution was taken and spread on the media using L. Shape loop, and then incubate for 24 -48 hrs for determining the efficiency of samples on microorganisms.

Results and discusions:

The results of effects β- Sitosterol, as a single agent showed individually in each one of breast cancer cells MCF-7 cells and/or in AMJ13 cell line calculated as (Ratio) treated with β- Sitosterol with different concentrations 0, 1, 10, 100, 1000, 10000, 100000 nm on cell viability was superior in treatment via inhibition cell line MCF7 compared with AMJ13, in figures (3 and 4Data showed that β-Sitosterol significantly reduced cellular viability of Cancer cell (IC_{50}, 10000-100000 nM). β-Sitosterol. In this study, We simply explained the importance of β- Sitosterol on MCF7 cell line compared with AMJ13 Furthermore, we showed antimicrobial activity of β-Sitosterol on Staph. aureus. This elucidates that β-Sitosterol can be used as a best candidate product in further medical researches.

The pure β-sitosterol compound was prepared with several concentration as prepared the results from exposing β-sitosterol compound with different concentrations to eukaryotic cell line MCF-7 cells and AMJ13 showed in 10.000 nm and 1000.000 nm reduction in cancer cell lines viability, while the previous results of a study said that β-sitosterol showed a stimulatory effect on MCF-7 cells In vitro.[9] Although β-sitosterol compound has significant anti-tumor activity, natural phytosterols compound undergoes enzymatic oxidation, auto-oxidation or may stimulated by different of factors like ROS (such as ozone), O_2, light, heat, or enzymes[11] resulting in the formation of oxidation products in phytosterol called oxygenated phytosterols. The beneficial and detrimental side effects of these compounds may be harmful on human health and may still remain controversial[5] and their poor solubility of phytosterol in water limits their therapeutic effect and bioavailability.[12-14] Therefore, scholars and scientists believe that modifying the structure or changing the dosage forms of monomeric compounds is important to improve drug release level, solubility and targeting,.

It is necessary to develop phytosterol derivatives with significant anti-tumor effects as anti-tumor drugs.[2] We demonstrated here antimicrobial activity of β-Sitosterol on Staph. aureus. compared with previous studies.[15] This led that β-Sitosterol can be used as an anti-tumor in further medical researches.

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