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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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PURIFICATION, CHARACTERIZATION, AND IN VITRO ANTITUMOR ACTIVITY OF A NOVEL GLUCAN FROM PHOENIX DACTYLIFERA L. FRUITS

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Abstract.

In this study, β- glucan was extracted by the hot water extraction method followed by ethanol precipitation and purified using ion and gel filtration chromatography, then evaluate the anticancer effects of β- glucan that purified from *Phoenix dactylifera* on cancer cell line. Ahmed Nahi Glioblastoma Multiform (ANGM) cancer cell line was used in the in vitro study. Cell line exposure times were calculated after 24, 48, and 72 hours in a micro titration plate under absolutely sterile conditions. High molecular weight β-glucans can be obtained using the hot water extraction method without having to use strong agents to change their structure, like alkalis or acids. Anti-cancer property of β-glucan derived from *Phoenix dactylifera* fruits on cancer cell lines has been reported. In this work, the ANGM cell line was treated with different concentrations of β-glucan (31.25, 62.5, 125, 250, 500 and 1000 µg/mL). and the inhibition of the cells was investigated using the MTT assay after 24, 48 and 72 hours. The result obtained showed time and concentration dependent cytotoxic effect, and the higher concentrations at 48 hrs of exposure gave significantly ($p<0.05$) higher cytotoxic effect.

Key words. β-glucan, *Phoenix dactylifera*, MTT, Cytotoxicity, Cancer, Chromatography.

Introduction.

Cancer is a group of illnesses known as cancer that spread over a lengthy period of time and claim millions of lives worldwide [1]. Because of its extraordinarily aggressive nature, poor prognosis, and low survival rate, cancer continues to be a major global public health concern. With 2.26 million and 2.21 million new cases of breast and liver cancer, respectively, in 2020, these two malignancies are among the most dangerous in the world [2].

Phoenix dactylifera (date palm) belonging to the family Arecaceae it is indigenous to Southwest Asia and North Africa and is regarded as one of the oldest plants. Around the world, it has historically been valued for its nutritional qualities. Recently, the date palm tree's entire genome was once more sequenced to improve its growth, The plant's fruits are where it gets the majority of its nutrients. Fruits are rich in vitamins, minerals, amino acids, and carbohydrates [3]. β-glucans are a type of polymer comprised only of glucose monomers are known as a heterogeneous class of polysaccharides These glucose polymers differ from one another in some aspects, including chain length, the presence of branches, whether they are alpha or beta isomers, and their capacity to dissolve [4].

Ahmed Nahi Glioblastoma Multiform (ANGM) cell line is a human brain glioblastoma multiform (GBM) that was taken after surgery for an intracranial tumor on a 72-year-old Iraqi male. In this study, β- glucan was isolated from *Phoenix dactylifera* by water extraction and ethanol precipitation then purified by DEAE and Sephadex G-100 column chromatography. The structural characteristics of β-glucan were determined. Further, the cytotoxic effects of date fruits-derived β-glucan on malignant cell line were investigated.

Materials and Methods.

1. Extraction and Purification of β-glucan from *Phoenix dactylifera*

Water Extraction Method: A water extraction method was used to extract β-glucan from date fruits [5]. Dates (500 g) were mechanically crushed, cut into smaller pieces, and dried. The powder was mixed with double distilled water in ratio of 1:20 (wt/v). The mixture was heated to 90°C for 6 hours in a shaker water bath. At 4°C, the mixture was centrifuged for 20 minutes at 5000 rpm. The particle was discarded, and the supernatant was obtained. The suspension was centrifuged at 5000 rpm for 20 minutes at 4°C after the supernatant solution (500 mL) was diluted with ethanol absolute (500 mL) in a ratio of 1:1. The supernatant was removed, and the pellet was collected. The pellet was washed with acetone before the suspension performed a 20-minute centrifugation at 5000 rpm at 4°C, then it was dried. An ethanol 100% solution was used to dilute a 200 mL solution of the polysaccharide in water, and centrifugation was used to collect the precipitate (5000 rpm for 15 min). Four times of this procedure were done to precipitate β-glucan (glucan-p1). The phenol-sulfate acid method was used to determine the polysaccharide content, and protein content was measured according to Bradford, (1976) by using a UV, Visible Spectrophotometer that measure the absorbance at 595nm.

2. Purification of β- glucan by DEAE cellulose- 52 column

Glucan-P1 was dissolved in distilled water, and a dropper was used to add the solution gradually along the balanced DEAE cellulose-52 column wall. The phenol-sulfate acid method was used to determine the polysaccharide content of each fraction by using a UV, Visible Spectrophotometer that measured the absorbance at 490 nm, and protein content was measured at 595 nm. The eluent's main sugar-containing fractions were collected, mixed, and dried in an oven at 60 C. (glucan-p2).

3. Gel filtration chromatography by using Sephadex G-100 Column

The previous step's glucan-P2 fraction was re-dissolved in deionized water and gradually added to the Sephadex G-100 column which had been set up for equilibration. Deionized water was used for elution at a flow rate of 18 ml/hr with a yield of 5 ml for each tube. At 490 nm and 595 nm, the absorbance of the samples that were collected and the elution gradient curve were both calculated. The sample with the highest concentration

of glucan was chosen, and it was dried in an oven (at 60 °C) to produce pure glucan (glucan-P3).

4. Determination of Carbohydrate Concentration:

As described by Dubois and his colleagues [6] the phenol-sulphuric acid method was carried out as mentioned in subsequent steps. From glucose stock solution (100 µg/ml) different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 g/ml) were prepared with a final volume of 1 ml. One milliliter of solvent and one milliliter of 5% phenol were mixed in a test tube. With vigorous mixing, 5 mL of strong sulfuric acid was added, and it was then allowed to cool at room temperature. It was allowed for the reaction mixture to cool to room temperature The blank contained 1 milliliter of distilled water, 1 milliliter of a 5% phenol solution, and 5 milliliters of H2SO4. Measurements were made of the absorbance at 490 nm. The glucose concentrations were plotted against the corresponding absorbance on a standard curve (Figure 1). Using a solution of glucan at a concentration of 1 mg/ml, the amount of carbohydrate in glucan was determined (glucan- p1, glucan- p2 and glucan-P3). and put through the same prior addition, then the absorbance at 490 nm was measured. The standard curve was used to calculate the carbohydrate concentration (Figure 1).

5. Determination of Protein Concentration

Bradford [7] described how to determine protein content as follows: In order to create a standard curve for bovine serum albumin, several concentrations of the BSA stock solution (0, 20, 40, 60, 80, and 100 µg/mL) were prepared. Coomassie Brilliant Blue G-250 dye were added to each concentration in a volume of 2.5 ml, stirred, and allowed to stand for two minutes at 37˚C. The blank was made up of 2.5 ml of the dye reagent and 0.1 ml of Tris-HCl solution and used to measure the absorbance at 595 nm. A standard curve was created by comparing the BSA concentrations to the matching bovine serum albumin absorbance. Glucan sample (0.1 ml of 100 g/ml Tris-HCl) underwent the identical procedures as the preceding steps (Figure 2).

6. Anticancer effect of β-glucan

Preparation of β- glucan Concentrations: β- Glucan (glucan-P3) stock was prepared by dissolving 200 mg of prepared extract

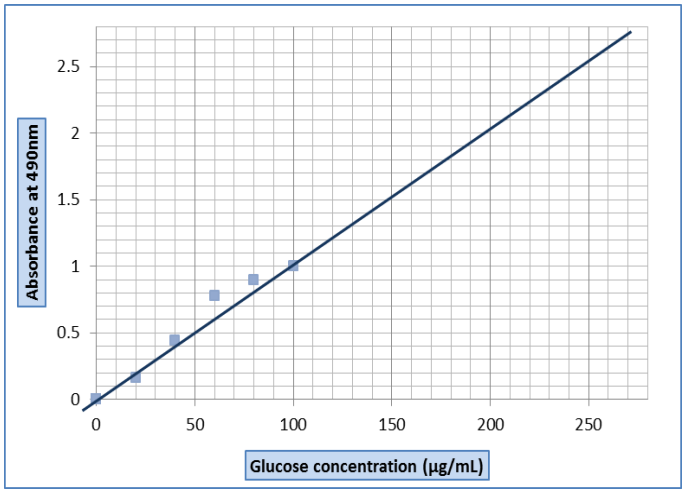

Figure 1. *Standard curve of glucose.*

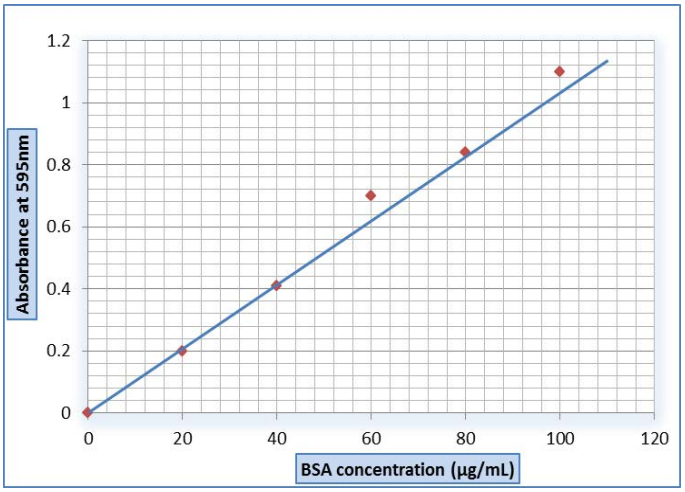

Figure 2. *Standard Curve of BSA.*

powder in 10 mL phosphate puffer saline and filtering through a sterile Millipore filter (0.22µm). Different concentrations were prepared starting from concentration (1000 µg/mL) ending with the concentration of (31.25 µg/mL) by using sterile serum free medium.

Cell Culture and Maintenance: Brain cancer cells (ANGM) was graciously provided by the Iraqi center for cancer and medical genetics research (ICCMGR), Mustansiriyah University. In RPMI-1640 (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum cells were maintained and cultivated. In tissue culture flasks cells were seeded and allowed to reach an 80–90% confluent monolayer (24 to 48 h). Cultures were kept at 37°C in a humid atmosphere using a CO2 incubator. Cells were harvested using mild trypsinization (50 mg trypsin mL^{-1}) [8].

MTT cytotoxicity assay: Using the 3-(4,5-Dimethylthyiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the cytotoxic effect of β-glucan on ANGM was calculated. In 96-well plates, cells were grown and incubated until they reached 80% confluence. 200 µL of various β -glucan concentrations (31.25, 62.5, 125, 250, 500 and 1000 µL/mL) were added to the corresponding wells containing the cells after the medium was discarded. Cells that had not been treated were used as the negative control in wells. 10 µL of MTT was applied to each well after 24 hours. Plates were incubated for an additional 4 hours at 37°C and 5% CO2. After carefully removing the media, 100 L of dimethyl sulfoxide was added to each well, and each was then given 5 minutes to incubate. A microplate reader for an ELISA was used to quantify absorbance at 540 nm [8]. The following formula was used to compute the inhibition percentage: (%) IR (%) = (ODc−ODt) / OD Control ×100

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

Statistical Analysis: Data were presented as means with standard deviations (SD) and evaluated using GraphPad Prism's one-way analysis of variance (ANOVA) and Dunn's test (Graph Pad Software Inc.). A statistically significant difference between groups was defined as $p< 0.05$.

Results.

Isolation and Purification of β- Glucan: Ion exchange chromatography and gel filtration were employed in two steps

to purify β-glucan from date fruits after it had been isolated using water extraction:

1. Water extraction method.

The method of water extraction was used to extract glucan-P1. Yield of the glucan-P1 fraction was 62 g.

2. Ion-exchange chromatography.

A DEAE cellulose-52 column was used to purify glucan-P1. The elution curve for glu-can-P1 by the DEAE cellulose-52 column is shown in Figure (3). Elution peaks were observed in the tubes numbered 18–48, 59–74, and 94–105. Less protein impurity was found; it only appeared in the tubes 65–81 with a weak elution peak. The main sugar-containing fractions that were present in 18–48 tubes were combined to produce glcan–P2, and the yield was 7.25g.

3. Gel filtration chromatography.

The glucan-P2 recovered via ion exchange chromatography was further purified using a Sephadex G-100 column. The results are shown in Figure 4. The eluted fractions (18 to 42 tubes) showed a single, symmetrical peak on the Sephadex G-100 column. A pure glucan was produced after this component's collected solution was dried by oven at 60˚ C to obtain glucan-P3, a light-yellow refined product. The yield was 3.84g.

Table (1) shows the residual protein content, Carbohydrate concentration and the recovery yield of β- glucan at each step of purification.

Cytotoxic effect of β-glucan extract on Brain cell line.

The effect of different concentrations of β- glucan (31.25, 62.5, 125, 250, 500, and 1000 µg/ ml) on tumor cell line after (24, 48 and 72) hours of exposure in Figure 5. The results revealed significant cytotoxic effect at levels ($P<0.05$) for all concentration. β- glucan had highest inhibitory growth on ANGM at the highest dose (1000 µg/ml) for 48 hours of exposure. In addition, the morphology of ANGM cells was changed after treatment with β-glucan at higher concentration (1000 µg/ml) and cell density and adherent capacity de-creased (Figure 6).

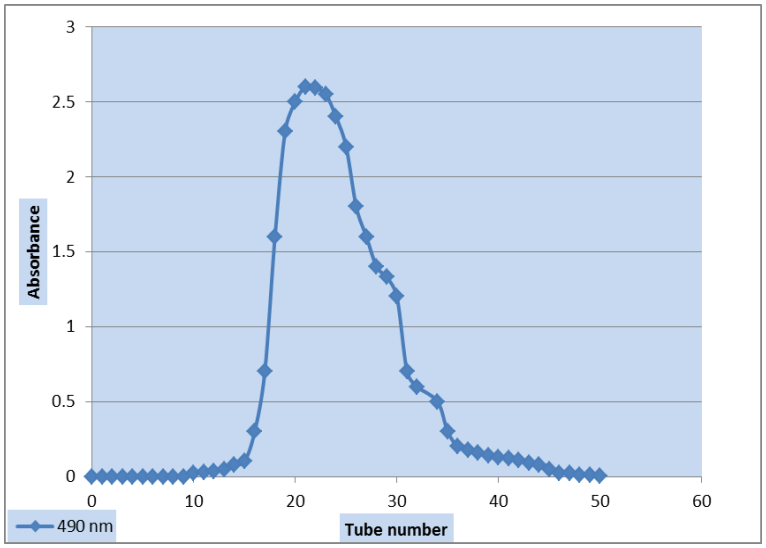

Figure 4. *Glucan-P3 elution curve by Sephadex G-100 column chromatography.*

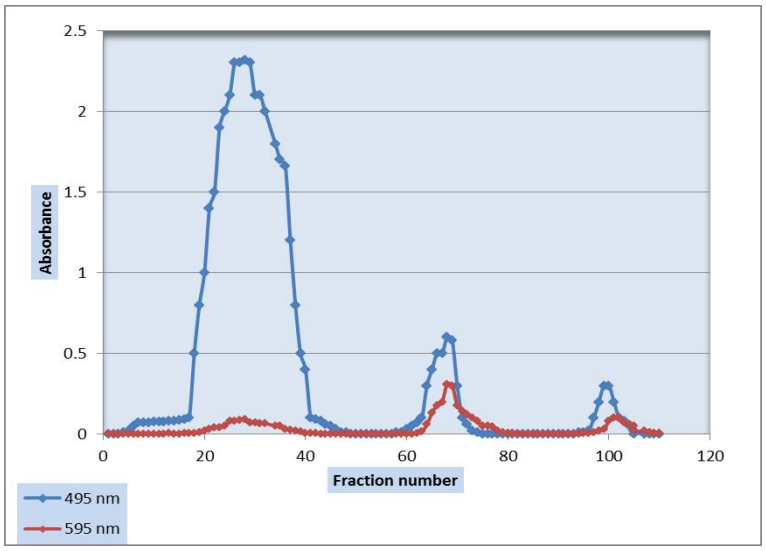

Figure 3. *Gradient elution profile of β–glucan extracted from Phoenix dactylifera using a 2.5 x 10 cm ion exchange column, 100 ml/hr flow rate, and 5 ml fraction.*

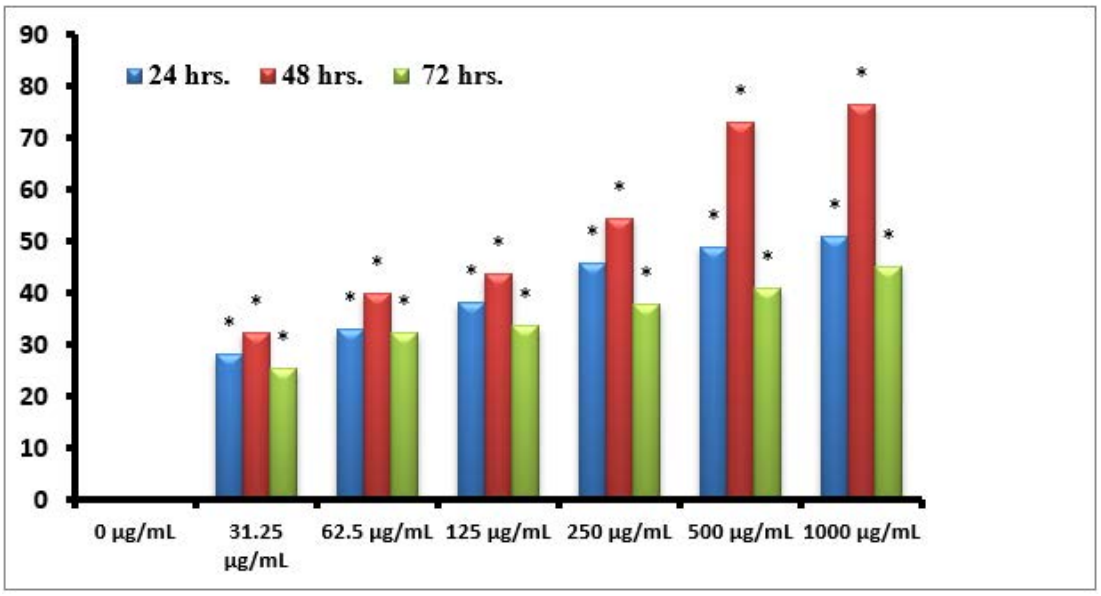

Figure 5. *The inhibitory effects of β-glucan extract of Phoenix dactylifera on ANGM cell line growth during three periods of exposure.*

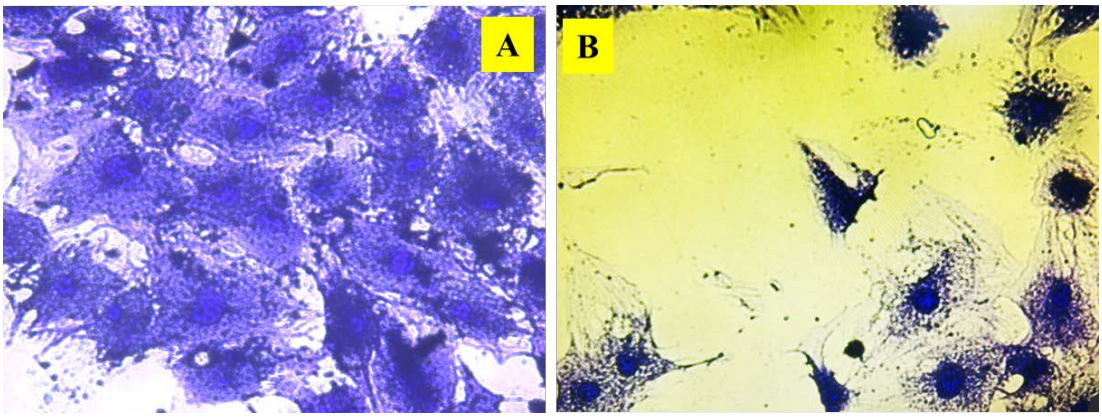

Figure 6. *Morphology of the human brain cancer cell line, A: control cells under inverted microscope, B: β-glucan -treated cells under inverted microscope (1000 µg/ml, at 48 hrs).*

Table 1. *Purification of β- glucan from date fruit.*

Purification step	**Protein Concentration (mg/ml)**	**Carbohydrate concentration (mg/ml)**	**β- Glucan yield (%- wt/ wt)**
Water extraction (glucan-p1)	2.8 ±0.15	90 ±4.37	36 ±1.42
DEAE chromatography (glucan-p2)	0.9 ±0.08	120 ±7.03	19 ±0.86
Gel Filtration chromatography (glucan-p3)	0.04 ±0.007	210 ±11.57	5 ±0.26
LSD	0.661 *	43.72 *	5.77 *

Discussion.

In the present result, we successfully isolated and purified β- glucan. The yield of extraction and purified β-glucan in the current investigation was 3.072%. Additionally, the waste residue from the extraction and purification process typically does not include poisonous and dangerous materials and may therefore be recycled. Additionally, the purified β-glucan's physicochemical characteristics were examined in light of their potential use in food and beverage applications. In comparison to alternative purification methods, the DEAE chromatography method was chosen because of its high sensitivity, and minimal sample loss [8]. The components of β-glucan extracted from date palm were determined using chromatography, which provided an effective approach for identifying the β-glucan [9].

β-Glucan had a concentration-dependent cytotoxic effect on ANGM cells. It significantly suppressed ANGM cell reproduction. The optical densities (OD) for the stained cell lines after treatment with various concentrations of the extracted β-glucan for 24, 48, and 72 hours showed that there were differences of (OD) between the concentrations, with the high concentration giving low value of OD, indicating maximum response, and the low concentration giving high value of OD, indicating minimum response in proportion to high percentage of viable cells. Previous studies have shown that β-glucans can

modify biological responses and produce anti-tumor effects both *in vitro* and *in vivo* [10]. In agreement with our findings, Zhang, and his colleagues, [11] reported that water-soluble β-glucan from *Poria cocos* inhibited cancer cell viability. The anticancer activity of β-glucans may be related to their ability to control inflammation through immune stimulatory patterns [12], and they may also have an effect on the regulation of gut hormones [13]. The bulk of anti-cancer medications work by putting tumor cells under oxidative stress, which is believed to be the cause of the majority of macromolecular changes in the cell. Among other macro-molecules, reactive oxygen species can damage DNA, membrane lipids, and proteins [14,15].

Conclusion.

In the present study, water soluble β-glucan was successfully isolated from *Phoenix dactylifera* by water extraction and further fractionated by DEAE-52 cellulose and Sephadex G-100 chromatography, then characterized by HPLC. The obtained β-glucan exhibited a significant antitumor activity against Brain cell line (ANGM) in concentration dependent manner.

Author Contributions.

All authors contributed to the study conception and design. Mate-rial preparation, data collection and analysis were performed by Hiba Mohammed Al-Khuzaay, Yasir Hussein Al-Juraisy and Ali Hussein Alwan. The first draft of the manuscript was written by Hiba Mohammed Al-Khuzaay and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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