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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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PARTIAL PURIFICATION OF GLUTATHIONE PEROXIDASE ENZYME FROM WOMEN WITH BREAST CANCER

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Abstract.

The study included the purification of glutathione peroxidase enzyme (GPX) in the serum of women with breast cancer, which involved 60 samples of serum from women with breast cancer, and 30 samples from healthy individuals. The results of the study showed a significant decrease at a probability level of $p<0.0001$ for the activity of the GPX enzyme in the serum of women with breast cancer. Additionally, the GPX enzyme was purified from the serum of women with breast cancer through precipitation with ammonium sulfate and dialysis, and the use of DEAE-Cellulose ion exchange chromatography and gel filtration chromatography using Sephadex G-100, where a main protein band was separated, which was relied upon in determining the optimal conditions for the partially purified enzyme. The optimal conditions for the partially purified enzyme from the serum of women with breast cancer were determined and the highest activity was for the substrate concentration of 0.1 mM H_2O_2. The maximum speed Vmax was 3.125IU/L and the Michaelis-Menten constant Km was 0.0179 M using Lineweaver-Burk plot, the optimal pH was at 8.5, temperature at 37°C, and the highest activity time was at 5 minutes.

Key words. Breast Cancer, Glutathione Peroxidase Enzyme, Purification.

Introduction.

Breast cancer (BC) is the most common type of cancer among women. Some types are benign and can be treated with surgery [1]. Another type is metastatic cancer, which spreads to other organs such as the bones, brain, liver, and lungs [2]. Breast cancer forms when cells grow abnormally, rapidly dividing and multiplying to form masses of tissue known as tumors. Tumors may be invasive or non-invasive, usually starting in the lobules or milk ducts [3]. Some malignant tumors begin in the milk ducts, which deliver milk to the nipple, called ductal carcinoma, while others start in the milk-producing gland lobules, called lobular carcinoma [4]. The presence of a tumor in a patient can be detected by observing calcifications or masses in the breast [5].

At the onset of the disease, there are no symptoms by which breast cancer can be diagnosed, as the tumor is usually small initially. However, as the tumor grows, it can be felt and detected by its size [6]. The most common symptoms of breast cancer include changes in the texture and shape of the breast and nipple, and nipple discharge. Regular screening helps in the early detection of the disease. Doctors must perform several tests to determine whether the tumor is benign or malignant [7].

There are several factors that determine the treatment for breast cancer, such as the stage of the tumor, the patient's age, and hormone levels. Often, more than one treatment method can be used simultaneously. If the tumor is detected early, it can be surgically removed and treated with radiation to kill any remaining active cancer cells. If the tumor is large and has spread to the lymph nodes, chemotherapy, hormone therapy, and targeted therapy may be added [8,9].

Glutathione peroxidase (GPX) belongs to the oxidation-reduction enzymes E.C.1.11.1.9 and is an enzymatic antioxidant. It prevents oxidation in the cells of a living organism. The level of this enzyme decreases in cases of oxidative stress and also with aging due to a decline in its synthesis within the cells of the organism [10]. This enzyme is found in different parts of cellular organelles, especially in the mitochondria and cytoplasm of red blood cells, liver tissue cells, and sperm. It is one of the most important enzymatic antioxidant systems due to its ability to eliminate peroxides resulting from oxidation processes as well as free radicals. The molecular weight of glutathione peroxidase is about 44,000 daltons, and it contains selenium. This enzyme is classified as a selenoprotein or selenoenzyme [11]. The inclusion of selenium in the structure of glutathione peroxidase protects cellular components and biological membranes from oxidative damage. Its importance lies in protecting cells from oxidative stress. The biochemical activity of the enzyme is limited to the reduction of hydrogen peroxide to water and the reduction of lipid hydroperoxides to alcohols by reduced glutathione as a proton donor according to the following equation [12]:

$$2GSH + H_2O_2 \xrightarrow{GPX} GSSG + 2H_2O$$

Practically, the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) inside cells is a measure of cellular toxicity, as the level of the oxidized form is less than the reduced form in cells and tissues, thus the GSSG/GSH ratio is high. This ratio is maintained by a mechanism that works to reduce oxidized glutathione (GSSG) and convert it into reduced glutathione, regulated by the enzyme glutathione reductase (GRd) and the coenzyme Nicotinamide adenine dinucleotide phosphate - NADPH according to the following equation [13]:

$$GSSG + NADPH + H_2 \xrightarrow{GRD} 2GSH + NADP_2$$

Materials and Methods.

Study design: Sixty blood samples were collected from women diagnosed with breast cancer, ranging in age from 21 to 75 years, attending the Oncology Teaching Hospital/Medical City in Baghdad. Additionally, 30 blood samples were collected from non-affected women aged between 20 and 55 years as a control group, over the period from May to September 2023. The diagnosis was made by specialist doctors.

Sample Preparation: After withdrawing 5 ml of blood from the women attending the hospital using a 5 ml medical syringe, the blood samples were placed in dry and clean gel tubes and left for 15 minutes at room temperature. They were then centrifuged for 15 minutes at a speed of 3000 rpm for separation. The serum was collected using a micropipette, the residue discarded, and the serum samples were distributed into small quantities in Eppendorf tubes and stored at -20°C until testing.

Glutathione Peroxidase Estimation: The activity of the glutathione peroxidase enzyme (GPX) was estimated according to the method followed by researcher Rotrouck [14].

Enzyme Purification: Precipitation by Ammonium Sulfate: Serum proteins were precipitated using graded concentrations of ammonium sulfate until reaching 60% saturation. The mixture was stirred with a magnetic stirrer at 4°C for 60 minutes, then left in the refrigerator for 24 hours, after which the precipitate was separated from the filtrate using a refrigerated centrifuge at 8000 rpm for 30 minutes. The precipitate was then dissolved in the smallest amount of 0.1M Tris-HCl buffer, pH 7.2, and the GPX activity and protein concentration were estimated.

Dialysis: Dialysis, one of the oldest methods used in protein purification, aimed to remove the remaining ammonium sulfate added to the protein precipitate by placing the protein in a dialysis bag and immersing it in 0.1M Tris-HCl buffer, pH 7.2. The buffer solution was changed periodically over 24 hours at 4°C. Enzyme activity and protein were measured.

Ion Exchange Chromatography [15,16]: Solutions preparation of 0.25 N Sodium Hydroxide was prepared by dissolving 5g of sodium hydroxide in 500 ml of distilled water. 0.25 N Hydrochloric Acid was prepared by adding 10.4 ml of 12 N concentrated hydrochloric acid to 400 ml of distilled water, then completing the volume to 500 ml with distilled water. 200 ml sodium chloride solution was prepared by dissolving 11.7 g of sodium chloride in 1000 ml of 0.1 M Tris-HCl buffer, pH 7.2, from which a series of solutions containing graded concentrations of sodium chloride starting from 50 mM to 200 were prepared. 0.1 M Tris-HCl at pH= 7.2 was prepared by dissolving 15.76 g of Tris-HCl in 900 ml of distilled water and adjusting the pH to 7.2, then completing the volume to 1000 ml with distilled water.

The ion exchange column was prepared according to the method of Whitaker and Bernard, using 20 g of DEAE-Cellulose powder suspended in 1000 ml of distilled water and left to settle. The supernatant was discarded, and this step was repeated several times until the supernatant was clear. DEAE-Cellulose was activated with 0.25 N HCl for 30 minutes, then filtered through a Buchner funnel containing Whatman No.1 filter paper and washed twice with distilled water. After that, DEAE-Cellulose was activated with 0.25 N NaOH, and the filtering and washing processes were repeated twice. The activated DEAE-Cellulose was calibrated with 0.1 M Tris buffer pH 7.2 and packed into a column with dimensions of 3 × 18 cm.

The concentrated enzyme from the previous steps was added to the ion exchange column. The flow rate was regulated to be 1ml/min for the washed and filtered samples, collected at 3ml per fraction. A 0.1M Tris solution, pH= 7.2, was prepared for washing the enzymes, and the enzyme was eluted with the same solution using graded concentrations of NaCl ranging from 50 to 200 mM. The active fractions were collected for measuring enzyme activity and protein. The enzyme was concentrated by filling a semi-permeable membrane (dialysis bag) and covering it with sucrose for 24 hours in a dialysis to use in later steps.

Gel Filtration Chromatography [17]: 0.1 M Tris solution, pH 7.2 was prepared by dissolving 15.76g of Tris-HCl in 900 ml of distilled water, adjusting the pH to 7.2, and then completing the volume to 1000 ml with distilled water. 500 mM sodium chloride was prepared by dissolving 29.25 g of sodium chloride in 1000 ml of 0.1M Tris-HCl pH 7.2.

Sephadex G-100 gel was prepared according to the instructions of Pharmacia Fine Chemical Company. The gel was suspended in 0.1 M Tris HCl buffer pH 7.2 as in the degassing and was poured into a glass column with dimensions of 2×40 cm. The column was equilibrated by adding enough 0.1 M Tris-HCl buffer pH 7.2, and the pH of the effluent was adjusted to 7.2 at a flow rate of 5ml/3min. After preparing the column, the concentrated enzyme solution obtained from the ion exchange chromatography effluent was gradually added to the column surface, and the sequential filtration was done using 0.1 M Tris-HCl buffer at pH 7.2 at a flow rate of 5ml/3min. The peak fractions with enzyme activity were collected and their volumes were determined.

The protein concentrations in the peak fractions were measured to determine the specific activity of the enzyme. The collected peak fractions were concentrated by filling a semi-permeable membrane and covering it with sucrose in a dialysis process and passed over the same column under the same conditions for further purification. The enzyme activity and protein concentration in the purified fraction were measured; the purified enzyme was stored in freezing conditions for characterization processes.

Glutathione Peroxidase Enzyme Kinetics: The Effect of Substrate Concentration: This effect was studied using different concentrations of H_2O_2 on GPX enzyme activity. The used concentrations were 0.01, 0.02, 0.04, 0.06, 0.1, 0.2 millimolar to find the optimal substrate concentration for GPX activity. Then, enzyme activities were measured, and the relationship between reaction rate and substrate concentration was plotted to determine the optimal substrate concentration where the reaction rate reaches its maximum (Vmax).

Determination of Michaelis-Menten Constant (Km): The Km value for GPX was determined using the Lineweaver-Burk plot, which relates the inverse values of both the reaction rate and substrate concentration (1/v vs. 1/[s]).

Determination of the Optimal pH: The effect of the buffer pH (10 mM Tris–HCl pH 7.2) on GPX reaction speed was studied using solutions with different pH values: 2.5, 4.5, 6.5, 8.5, 10.5, 12.5, in the presence of the substrate H_2O_2 at a concentration of 0.1 mM and a temperature of 37°C. Enzyme activities were measured and the optimal pH was determined by plotting the relationship between reaction speed and pH.

The Effect of Temperature: GPX activity was measured at different temperatures: 7, 17, 27, 37, 47, 57 degrees Celsius with a buffer solution at pH=8.5, and a concentration of 0.1 mM of H_2O_2. Then, the relationship between the reaction rate and

temperature was plotted to determine the optimal temperature for the reaction.

The Effect of Time on Enzyme Activity: The effect of incubation time on GPX activity was studied using a concentration of 0.1 mM of H_2O_2 with a buffer solution at pH=8.5, for 5, 10, 20, and 30 minutes, and a temperature below 37°C. After that, the relationship between enzyme activity and time was plotted to determine the effect of incubation time on the enzyme reaction rate.

Statistical Analysis: The study results were statistically analyzed using the SPSS statistical program employing ANOVA test, and the arithmetic means of the parameters were compared using Duncan's multiple range test at a significance level of ($P < 0.05$), and non-significant at ($P > 0.05$). The results were expressed in percentages and the mean ± standard deviation was found. Microsoft Excel was also used to create graphs, charts, and tables.

Results.

The study included 60 cases of women diagnosed with breast cancer. Additionally, it encompassed 30 samples from the control group, serving as a comparative group, with ages ranging from 21 to 79 years.

The activity of glutathione peroxidase enzyme was measured in the serum of women with breast cancer and those who were healthy. The results showed that the mean ± standard deviation for the enzyme activity in women with breast cancer was 2.033 ± 0.472 IU/L, and the mean ± standard deviation for the control group was 12.777 ± 2.550 IU/L. Upon conducting the statistical comparison, significant differences in the level of glutathione peroxidase enzyme were found between women with breast cancer and the control group, with a probability level of $P < 0.0001$, indicating that the activity level of glutathione peroxidase enzyme is decreased in the blood of women with breast cancer.

Proteins are typically concentrated in the initial stages of enzyme purification by removing a large proportion of water to achieve a degree of purity. Salts such as ammonium sulfate are often used for this purpose due to their good solubility in water. Protein precipitation by salts occurs as a result of the neutralization of protein charges by the salt, leading to reduced protein solubility and precipitation, a process known as "salting out". Therefore, a process of separation and partial purification of glutathione peroxidase enzyme from the serum of women with breast cancer was carried out in several stages. In the initial purification steps, the GPX enzyme was precipitated using ammonium sulfate to 60% saturation, leading to a decrease in the specific activity of partial purification of GPX in the crude sample to 0.058 U/mg after the first purification step. The results (Table 2) also indicate a reduction in purification fractions from 1 to 0.907 after precipitation. The excess salt was removed during the dialysis process using 0.1 M Tris-HCl pH 7.2. Dialysis led to an increase in the specific activity of partial purification of the GPX enzyme to 0.091 U/mg, with a 1.42-fold increase in the degree of enzyme purification at this stage, an enzyme yield of 51.7%, and then the ion exchange chromatography technique was used to further purify the enzyme. The protein solution resulting from the dialysis process was passed through a separation column containing the ion exchanger (DEAE-Cellulose), and the side profile of the partially purified enzyme indicated the appearance of an enzyme peak (Figure 1A and Table 2), with a specific activity of 0.37 U/mg, a 5.7-fold purification, and an enzyme yield of 75.8%. This peak represents the enzyme isoform that serves as a source of the enzyme for further purification steps.

This technique was utilized for the purification of glutathione peroxidase enzyme after the stages of ammonium sulfate precipitation and separation of its isoforms by ion exchange using a DEAE-Cellulose column. It was then partially purified using gel filtration chromatography with a column containing Sephadex G100. The specific activity of the GPX enzyme reached 0.243 U/mg, with a purity degree of 3.80, and an enzyme yield of 15% (Table 2 and Figure 1B).

Factors Affecting the Rate of Enzymatic Reaction.

Effect of Substrate Concentration: The activity of the glutathione peroxidase enzyme was measured with varying concentrations of the substrate H_2O_2 (0.01, 0.02, 0.04, 0.06, 0.1, 0.2 millimolar). It was found that the maximum activity of GPX was obtained using 0.1 millimolar of H_2O_2 (Figure 2). It was observed that the rate of the enzymatic reaction increases directly with the increase in substrate concentration until it reaches a constant rate, beyond which no further increase in the rate of enzymatic reaction occurs with an increase in substrate

Table 1. *Glutathione Peroxidase Enzyme Activity in Women with Breast Cancer.*

Activity (mean±SD)	NO.	GPX (IU/L)	P value
Patients	60	0.47 ±2.03	< 0.0001
Control	30	12.8± 2.55	

Table 2. *Purification steps of GPX enzyme in patients with breast cancer.*

Purification Steps	Volume (ml)	Activity (IU/ml)	Total Activity (IU/ml)	Protein Conc. (mg/ml)	Specific Activity (U/mg)	Recovery Yield %	Fold of Purification	Total protein
Crude	10	3.9	39	61	0.0639	100	1	610
Precipitation	9	3.03	30.3	52	0.058	76.9	0.907	468
Dialysis	8	2.02	20.2	22	0.091	51.7	1.42	176
Ion exchange (DAEA-Cellulose)	5	2.96	29.6	8	0.37	75.8	5.7	40
Gel Filtration	5	2.73	27.3	3	0.243	70	3.80	15

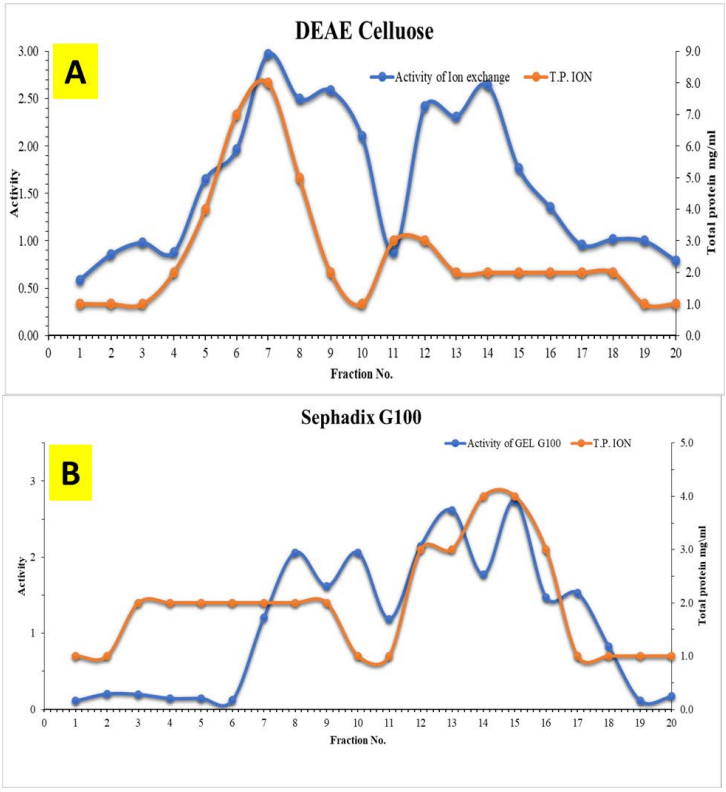

Figure 1. *Partial Purification of GPX Enzyme from the Serum of Breast Cancer Patients by (A) Ion Exchange Using DEAE-Cellulose A-50. (B) Gel Filtration Using Sephadex G100.*

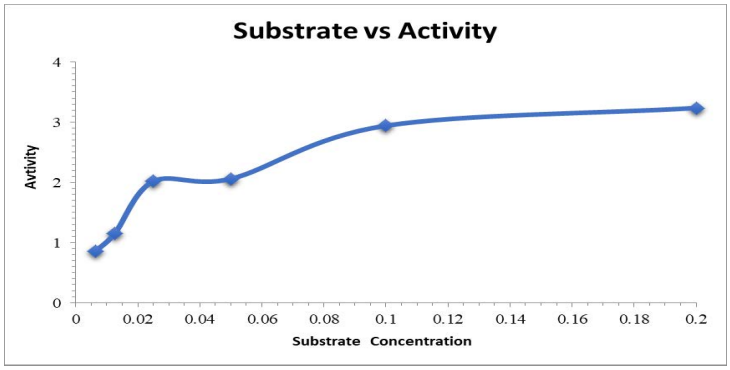

Figure 2. *Effect of different concentrations of the substrate H2O2 on the activity of GPX enzyme.*

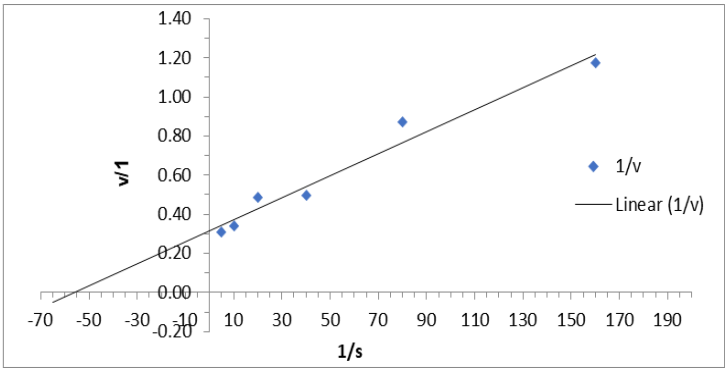

Figure 3. *Lineweaver-Burk plot for the purified GPX enzyme from the serum of breast cancer patients.*

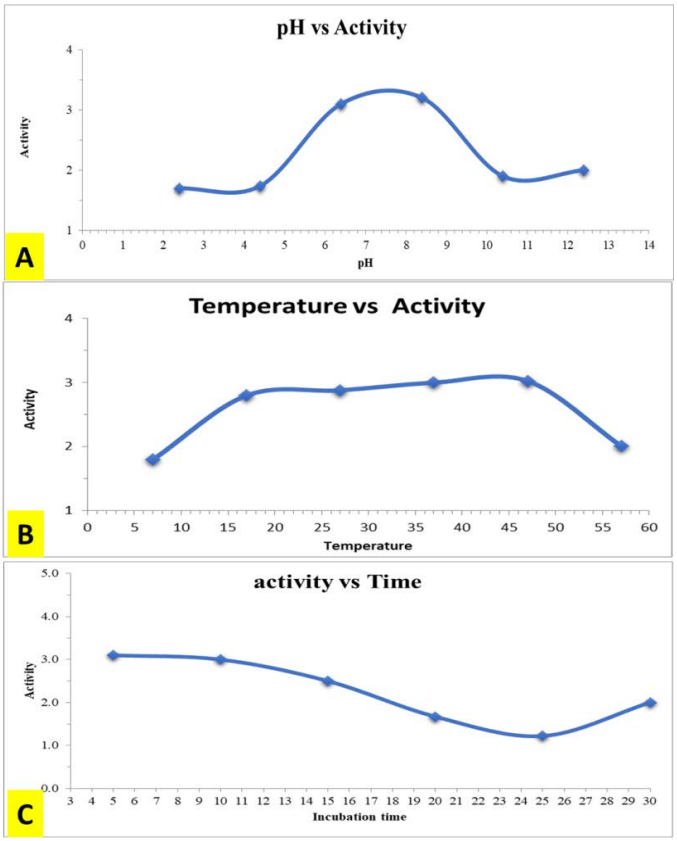

Figure 4. *Effect of pH, temperature, and time on the activity of GPX Enzyme.*

concentration. This maximum rate at the highest substrate concentration is termed the maximum velocity (Vmax) of the enzyme. Using the Lineweaver-Burk plot, the Vmax was found to be 3.125 IU/L, and the Michaelis-Menten constant (Km) was determined to be 0.0179 M (Figure 3).

Effect of pH on enzyme activity: A study was conducted to understand the varying effects of pH on the activity of the GPX enzyme. The activity increased after pH =4 and decreased after pH = 10. The results showed that the maximum activity of the enzyme was at pH=8.5 (Figure 4A).

Effect of temperature on enzyme activity: The effect of varying temperatures was tested to determine the optimal temperature for enzyme activity at 7, 17, 27, 37, 47, 57 degrees Celsius. The optimal temperature was found to be 37°C. The results showed an increase in GPX enzyme activity with the rise in temperature up to the permissible limit, followed by a decrease in enzyme activity (Figure 4B).

Effect of time on glutathione peroxidase enzyme activity: Figure (4C) shows the dependence of GPX enzyme activity on time at 5, 10, 20, 25, 30 minutes. A 5-minute incubation period was adopted as the standard throughout the work because it relates to the linear region of the curve and provides reliable absorption values at 37 degrees Celsius, being considered the standard incubation time for the procedure as it is associated with a linear portion of the curve and yields reliable absorption values. This may be connected to the appropriate temperature for the enzyme; as the time increases, the bonds between amino acids may break down due to the effect of heat.

Discussion.

Many studies have indicated an increase in glutathione peroxidase levels in patients with breast cancer, including the findings of Hmood et al. which contrasts with the current study results that recorded a significant decrease in the enzyme's activity level in the group of patients with breast cancer compared to the control group [18]. The decrease in glutathione peroxidase, which enhances oxidative stress and tissue damage through the release of free radicals, may be attributed to the enzyme being consumed in reactions that protect the cell by removing the harmful compound. Glutathione peroxidase enzyme is the third line of defence against free radicals after superoxide dismutase and catalase in terms of enzymatic antioxidants [19].

Additionally, a deficiency in selenium, which is a fundamental component of the GPX enzyme and is present in the enzyme's active site as selenocysteine, may contribute to this decrease. Glutathione peroxidase consists of four identical subunits, each containing a selenium atom at its active site [20]. Selenium represents the prosthetic group of the enzyme, replacing sulphur in cysteine to form the active part of the enzyme that reduces organic peroxides and hydrogen peroxide to produce alcohol and water. It converts selenenic acid after its oxidation and two molecules of glutathione from the reduced form (GSH) to the oxidized form (GSSG) [21]. Flohé (2005) pointed out that selenium deficiency reduces the gene expression of glutathione peroxidase, especially the GPx1 isoform [22], which is associated with breast cancer, considering selenium as an essential element on which the activity of the glutathione peroxidase enzyme depends [23]. Furthermore, the decrease in the activity level of glutathione peroxidase is also due to the increased formation of hydrogen peroxide within the body, which is an oxidizing agent removed by the GPX enzyme, forming glutathione reductase. In this process, glutathione peroxidase acts as an electron donor, no significant changes in glutathione peroxidase levels in patients with breast cancer compared to the control group, which also contradicts the findings of our study, which recorded a decrease in enzyme activity in the group of patients with breast cancer compared to the control group [24,25].

Conclusion.

The research focused on the isolation and purification of the glutathione peroxidase (GPX) enzyme from the blood serum of women diagnosed with breast cancer. The process utilized 60 blood serum samples from affected women and 30 from healthy controls. Findings indicated a statistically significant reduction in GPX activity in the serum of the cancer-afflicted group, with a probability level of $P<0.0001$. The purification process of GPX from these patients' serum was conducted using a combination of ammonium sulfate precipitation, dialysis, DEAE-Cellulose ion exchange chromatography, and gel filtration with Sephadex G-100, leading to the isolation of a key protein fraction. This fraction facilitated the establishment of the optimal conditions for the enzyme in its partially purified form. The study established that the best activity of the partially purified enzyme was achieved with a substrate concentration of 0.1 mM H_2O_2, with a maximal velocity (Vmax) of 3.125IU/L and a Michaelis-Menten constant (Km) of 0.0179 M, as determined by the Lineweaver-Burk plot. The enzyme exhibited peak activity at

a pH of 8.5, a temperature of 37°C, and at an incubation time of 5 minutes.

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