# GEORGIAN MEDICAL MEWS

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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 computer-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. მიკროფოტო-სურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სუ-რათის 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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# THE PROTECTIVE EFFECTS OF SELENIUM-ENRICHED HYDROPONIC RADISH ON PARACETAMOL-INDUCED LIVER DAMAGE IN RATS

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#### Abstract.

Paracetamol-induced liver toxicity in rats is a well-studied model for liver damage. The present study investigates the morphological changes in hepatic blood vessels and the protective effects of hydroponically grown Radish (Raphanus sativus L.) pretreatment in rats with paracetamol-induced liver damage. The results indicate significant alterations in vascular morphology and liver enzyme levels, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which serve as indicators of hepatotoxicity.

The findings suggest that hydroponic Radish may mitigate the adverse effects of paracetamol on liver health, highlighting the importance of dietary components in preventing micronutrient deficiencies and promoting overall health.

**Key words.** Paracetamol toxicity, liver, ALT, AST, hydroponic Radish.

#### Introduction.

The incidence of acute liver failure is roughly 10 per one million people annually in developed countries [1]. Paracetamol (Acetaminophen; N-acetyl-p-aminophenol) is widely used as an antipyretic and analgesic, and it produces acute liver damage if administrated in excess [2]. Paracetamol is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates [3]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when part of it is activated by hepatic cytochrome P–450 [4] to form the highly reactive metabolite N–acetyl–P–benzoquinone imine (NAPQI) [5]. NAPQI covalently binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts [6]. The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol, thus preventing covalent binding to liver proteins [7].

Paracetamol-induced liver toxicity in rats is a well-studied model for liver damage. In one study, rats were given paracetamol to induce hepatotoxicity, resulting in significant hepatic damage as indicated by elevated levels of serum markers [8]. Other studies have investigated the mechanism of toxicity in rats and mice, comparing protein adducts, mitochondrial dysfunction, and oxidative stress [9]. Several studies have also investigated potential treatments for paracetamol-induced liver damage in rats, including the administration of methanol extract of Muntingia calabura L. leaves and nerol [10]. Monitoring specific liver proteins in serum, such as ALT and AST, serves as biomarkers for drug-related liver toxicity. These biomarkers can significantly aid clinicians in identifying and avoiding

drug-induced liver failure by facilitating early detection and intervention [11].

The Radish (Raphanus sativus L.) is a representative root vegetable of the family Brassicaceae, which is extensively cultivated worldwide. Radishes are an agricultural product with excellent nutritional value owing to their rich moisture and fiber content, and high content of vitamins A and C. In addition, they have traditionally been known to have various benefits, such as digestion-promoting, stomach-protecting, anti-inflammatory, anticancer, antioxidant, and hemostatic benefits [12].

A study on the cancer preventive effect of Thai rat-tailed Radish (Raphanus sativus L. var. caudatus Alef) extract found that it prevented hepatocarcinogenesis in rats by blocking mutagenicity [13]. A study on the antioxidant effect of squeezed juice from black Radish (Raphanus sativus L. var. niger) in alimentary hyperlipidaemia in rats found that supplementation of a lipid-rich diet with black Radish juice resulted in significant improvement of biomarkers related to lipid peroxidation and antioxidant enzyme activities [14]. A study on the pharmacological evaluation of the anxiolytic-like effects of an aqueous extract of Raphanus sativus L. sprouts in mice found that the anxiolytic activity involves GABAA/BDZs site and serotonin 5-HT1A receptors [15].

Se deficiency is considered a serious problem [16]. The individual human Se intake ranges from 3 to  $7000\,\mu g$  Se/day worldwide, with most cases at the lower end of the range [17]. The World Health Organization (WHO) recommends that adults consume 55–70 micrograms ( $\mu g$ ) of selenium daily. This range accommodates different needs, particularly for women who are pregnant or lactating, who may require slightly higher amounts—60  $\mu g$  during pregnancy and 70  $\mu g$  while breastfeeding [18,19].

Selenium is an essential trace element that plays a critical role in various bodily functions, including DNA synthesis, reproduction, and protection against oxidative damage. It is primarily stored in muscle tissue and the thyroid gland, where it is involved in the metabolism of thyroid hormones [20].

The selenium level in most soils is generally less than 1 mg Se/kg soil; however, the selenium content in seleniferous soils can be as high as 4 to 100 mg Se/kg soil. The selenium content of plants in most soils is less than 1 mg/kg plant dry weight, whereas most plants grown in seleniferous soils show selenium levels in the range of 1 to 10 mg/kg plant dry weight. In the case of Se-hyperaccumulator plants, this can increase to between 1000 to 15,000 mg/kg [21].

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Several studies have investigated the hepatoprotective effects of Radish on the liver. The research suggests that Radish extract may have a hepatoprotective effect on liver damage induced by various factors such as carbon tetrachloride (CCl4), acetaminophen, and a fat-rich diet. The hepatoprotective activity of Radish extract was evaluated in vitro and in vivo, and the results indicated a reduction in oxidative stress and apoptosis, as well as a protective effect against liver injury. Additionally, black Radish extract was found to attenuate oxidative stress and exert hepatoprotective effects on carbon tetrachloride-induced hepatic injury. Furthermore, fermented black Radish was reported to exert hepatoprotective effects against acute and chronic liver injury. These findings suggest that Radish extract, particularly black Radish extract, may have potential hepatoprotective and antifibrotic effects on the liver in rat models [12].

#### Materials and Methods.

#### Animals, cultivation, and ethics approval:

Adult male albino rats (n=15) weighing 200±20 g were purchased from the experimental center of Orbeli Institute of Physiology NAS RA. The experiments were performed at the same time period of the day and during the light period of the light–dark cycle (09:00–18:00 h). The animals were maintained at 25±2 °C, 12 h light – dark cycle and lights on 07:00 – 19:00 h. Food and water ad libitum was provided to the animals. The experimental protocol was performed in accordance with the guidelines of the European Communities Council Directive (86/609/EEC) and was approved by the Ethics Committee of Yerevan State Medical University after Mkhitar Heratsi (Identification code N 4-2/18. Date: 15 November 2018).

Three groups of rats were established for this study:

- 1. **Paracetamol Group:** Rats in this group received a single toxic dose of paracetamol at 3000 mg/kg, administered on the 15th day of the experiment.
- 2. Radish (enriched in Se) Group: Rats in this group were administered hydroponic Radish enriched with selenium (1 g/kg, with selenium content of 1000 mg) daily for 14 consecutive days. On the 15th day, these rats also received a single toxic dose of paracetamol (3000 mg/kg).
- 3. **Control Group:** Rats in this group were maintained under standard conditions for 15 days, serving as a baseline for comparison.

After 16 days, biochemical assays for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, as well as histological examinations of the rat liver, were conducted to assess liver function.

# Cultivation.

Hydroponic Radish cultivation was conducted from 2021 to 2023 at the Institute of Hydroponics Problems, named after G.S. Davtyan (Figure 1). The institute is located at an altitude of approximately 850-900 meters above sea level, in a region characterized by a dry continental climate. The average annual air temperature in this area ranges from 11.0 to 11.8°C. The soil at the Institute is rich in phosphorus and potassium, with a humus content of approximately 1.5-2.5%. Plants grown in soil were irrigated with artesian water. In the hydroponic system,

#### Hydroponic Radish (Raphanus sativus L)



Figure 1. Cultivation of hydroponic Radish (Raphanus sativus L.).

plants were nourished with a nutrient solution recommended by Davtyan. The substrate for the hydroponic system consisted of a mixture of gravel and volcanic slag in a 3:1 ratio, with particle sizes ranging from 3 to 15 mm. Prior to planting, the substrate was disinfected with a 0.05% solution of KMnO<sub>4</sub> [22].

Fluorescent determination of Se mass concentration in air-dried material: The air-dried material is pre-treated to obtain selenite ions (SeO<sub>3</sub>). Ignition is conducted in an alkaline environment to prevent the loss of volatile selenium forms. Selenium mass concentration is then determined using a fluorescent detection technique. This method involves the interaction of selenite ions with the reagent 2,3-diaminonaphthalene in an acidic environment, forming a compound known as 4,5-benzopyrazoselenol. This compound is extracted with hexane and exhibits yellow-red fluorescence. The fluorescence intensity is measured using a fluorimeter. To ensure accuracy, hydroselenide ions and selenium from organic compounds are converted into selenite ions by treating them with a mixture of nitric and hydrochloric acids. The measurement range for this method is 0.1 to 5.00  $\mu$ g/L.

# Measurement of ALT/AST levels:

Liver injury was evaluated by measuring biomarkers, such as serum ALT and AST levels, using commercial assay kits [23].

# Morphological analysis of rat liver using a calcium adenosine triphosphate non-injection histological and angiological method:

The evaluation of hepatic microvasculature was conducted using a non-injection histological and angiological method that employs calcium adenosine triphosphate (Ca-ATP), as developed by Chilingaryan et al. [23]. This method involves the selective precipitation of phosphorus cleaved from ATP by calcium ions, which forms black lead sulfide, enabling threedimensional visualization of the microvascular network in thick tissue sections. The technique effectively differentiates components of the microvascular bed, such as arterioles, capillaries, and venules. In the experiment on albino rats, liver tissue was fixed in 5% neutral formalin for 24 hours, and 90 µm thick sections were incubated in a mixture containing ammonia, calcium chloride, ATP, and distilled water. Incubation lasted 30 minutes to 20 hours, after which sections were treated with a lead mixture, then immersed in ammonium acetate and sodium sulfide solutions. Following washes with distilled water, the

sections were mounted in Canadian balsam for permanent slide preparation. Morphological analysis was performed using a light optical microscope.

# Methodology for quantification of capillary network morphometry based on the open-source software Fiji (Image J)

This protocol is instrumental in identifying pathological changes or pharmacological effects within the vascular anatomy. An accurate morphological characterization of the liver is crucial for understanding its functions and its vulnerability to paracetamol toxicity. Our morphometric analysis, facilitated by ImageJ, enabled the visualization and quantification of individual liver capillaries.

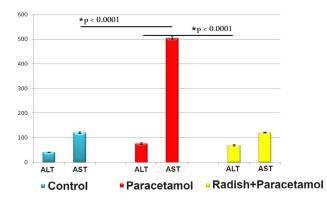
#### **Statistics:**

The significance of the differences observed among multiple groups was evaluated using a one-way analysis of variance (ANOVA). Post hoc individual comparisons were made using Tukey's honestly significantly different test. A value of p < 0.05 was considered statistically significant. Data were analysed using GraphPad Prism version 8.0 software (Graphpad Software Inc).

#### Results.

Liver damage induced by a Paracetamol overdose can alter the activities of serum liver enzymes, such as ALT and AST, which serve as indicators of hepatotoxicity [24]. Therefore, plasma ALT and AST levels were measured to assess the protective effect of hydroponically grown Radish (Raphanus sativus L.) pretreatment in rats with Paracetamol-induced liver damage.

The concentrations of ALT and AST in the three groups were analyzed, revealing significant differences among them, as presented in Figure 2. The significant elevation in ALT levels in both the Radish + Paracetamol (69.20  $\pm$  2.59 U/L) and Paracetamol (75.60  $\pm$  2.96 U/L) groups, compared to the Control group (40.00  $\pm$  1.48 U/L), strongly indicates liver damage. ALT, a sensitive marker of liver cell injury, showed a 73% increase in the Radish + Paracetamol group and an 89% increase in the Paracetamol group compared to the Control. A significant difference in ALT levels between the groups was found (F(2,27) = 191.6277, p < 0.0001). The most significant finding is the dramatic increase in AST levels specifically in the Paracetamol group (503.30  $\pm$  5.77 U/L) compared to the



**Figure 2.** Comparative biochemical assay results for ALT and AST in the control, paracetamol, and hydroponic Radish-treated groups.

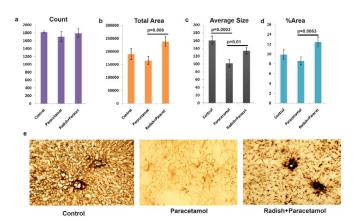


Figure 3. Histopathological evaluation scores of vascular alterations in control, paracetamol, and hydroponic Radish-treated rats (a-d). Microscopic images of the liver for control and experimental groups (e). The magnification levels are as follows: ×100 (Control); ×100 (Radish+Paracetamol); ×400 (Paracetamol).

Control ( $120.00 \pm 3.16$  U/L) and Radish + Paracetamol ( $120.90 \pm 1.15$  U/L) groups. Unlike ALT, which is primarily a liver enzyme, AST is found in multiple tissues, including the liver, heart, and muscles. Therefore, the sharply elevated AST levels in the Paracetamol group suggest potential damage to these organs beyond the liver, a serious concern that warrants further investigation to pinpoint the exact tissue affected. The relatively stable AST levels in the Control and Radish + Paracetamol groups indicate minimal damage to tissues containing AST in these groups. A significant difference in AST levels between the groups was revealed (F(2,27) = 13,602.2139, p < 0.0001) (Figure 2).

# Effect of hydroponic Radish (Raphanus sativus L.) on liver vascular changes in paracetamol-induced liver-damaged rats:

Next, we evaluated the morphological changes in hepatic blood vessels across three experimental groups: Control, Paracetamol, and Radish + Paracetamol (Figure 3a-e). In the Control group, the observed count of hepatic vessels was  $1823 \pm 24.87$ , with a total area of  $189,966 \pm 21,058 \, \mu m^2$  and an average vessel size of  $160.46 \pm 11.45 \ \mu m^2$ . The percentage area occupied by hepatic vessels was  $9.89 \pm 1.1\%$  (Figure 3). These baseline measurements served as a reference for assessing the effects of treatments in the other groups. In the Paracetamol group, the count of hepatic vessels was  $1705 \pm 130$ , with a total area of  $165,320 \pm 15,787$  $\mu$ m<sup>2</sup> (Figure 3a). The average vessel size was  $101.6 \pm 9.65 \mu$ m<sup>2</sup>, and the percentage area occupied by the vessels was  $8.61 \pm$ 0.82% (Figure 3a-d). The average vessel size in the Paracetamol group was significantly smaller compared to the Control group (F = 227.1, p = 0.0003), indicating that paracetamol may lead to a reduction in vessel size, potentially affecting vascular integrity or remodelling. Moreover, the total area and percentage area of hepatic vessels in the Paracetamol group were significantly different from those in the Radish + Paracetamol group (F = 227.1, p = 0.006). The Radish + Paracetamol group exhibited a count of  $1794 \pm 110$  hepatic vessels, with a total area of 239,054  $\pm$  19,555  $\mu$ m<sup>2</sup> and an average vessel size of 133.5  $\pm$  7.9  $\mu$ m<sup>2</sup>. The percentage area occupied by hepatic vessels was 12.45  $\pm$ 1.02% (Figure 3 a-e). The results indicated a significant increase

in average vessel size compared to the Paracetamol group (F = 227.1, p = 0.01). Additionally, significant differences were observed in total area (F = 227.1, p = 0.006) and percentage area (F = 227.1, p = 0.0063) when compared to the Paracetamol group (Figure 3 a-e).

#### Discussion.

Hepatotoxicity in rats can be induced by various factors, including the administration of certain substances. For example, a study investigating the hepatotoxicity induced by the aqueous extract of Polygoni Multiflori Radix in rats found that serum transaminase levels (ALT and AST) increased significantly in the group treated with PMR, suggesting hepatotoxicity related to the inhibition of CYP1A2 or CYP2E1 activity [25]. Another study highlighted significant species differences in hepatotoxic phenotypes between rats and mice, underscoring the importance of choosing the most suitable animal model to assess druginduced hepatotoxicity [26]. The precise molecular mechanism underlying paracetamol-induced liver injury incompletely understood [11]. Under therapeutic conditions, approximately 60%-90% of paracetamol is metabolized in the liver through glucuronidation and sulfation, while a smaller proportion (approximately 5%-15%) is metabolized via the cytochrome P450 pathway [27]. Walker et al. (1983) conducted a scanning electron microscopic examination of the livers of mice treated with acetaminophen, revealing significant changes in liver morphology. They observed endocytic vacuolation at the lateral and sinusoidal edges of centrilobular hepatocytes, while the periportal regions remained unaffected [28]. These findings emphasize the critical impact of paracetamol on liver morphology and function, highlighting the need for further investigation into the mechanisms underlying paracetamolinduced hepatotoxicity and potential therapeutic interventions.

The present study evaluated the morphological changes in hepatic blood vessels and the protective effects of hydroponically grown radish pretreatment in rats with paracetamol-induced liver damage. Paracetamol is widely recognized for its hepatotoxic potential, particularly in overdose situations, leading to significant alterations in liver enzyme activities, notably ALT and AST levels. These enzymes are critical indicators of hepatotoxicity, with elevated levels reflecting liver injury. The relatively stable AST levels observed in the Control and Radish + Paracetamol groups suggest minimal tissue damage in these groups. Morphological analysis of hepatic blood vessels revealed significant differences among the experimental groups. The Radish + Paracetamol group exhibited a significant increase in average vessel size compared to the Paracetamol group, indicating that the protective effects of selenium-rich radish may mitigate the adverse impacts of paracetamol on vascular morphology. This observation aligns with existing literature, which suggests that antioxidants found in radish may help protect against oxidative stress and inflammation associated with liver injury [29]. Radish extracts have been shown to significantly decrease levels of malondialdehyde, a marker of lipid peroxidation that increases during oxidative stress, suggesting that radish may restore the balance of oxidative stress in the liver, thereby protecting against cellular damage caused by paracetamol [12]. Additionally, studies have demonstrated that radish extracts reduce inflammatory cell infiltration in liver tissues affected by paracetamol. This reduction in inflammation correlates with improved histological outcomes, indicating that radish may counteract the inflammatory response triggered by paracetamol toxicity. Furthermore, radish extracts have been shown to enhance the expression of antioxidative factors such as Nrf2 and HO-1, which are crucial in defending against oxidative damage, and to influence apoptosis-related proteins, promoting cell survival in the context of paracetamol-induced liver injury [12,29].

The deficiency of Se in food chains and its subsequent impact on human health is largely attributed to low Se content in soils [30]. This deficiency can lead to significant health issues, as selenium plays a crucial role in various biological functions [31]. Many regions suffer from Se-deficient soils, resulting in low Se concentrations in crops and, consequently, in human diets [32]. In our research, we propose that selenium-enriched radish demonstrates protective effects against liver toxicity. In our previous research, we demonstrated the effectiveness of selenium-rich hydropnic Stevia in enhancing various health benefits, including its potential role in managing diabetes [33].

#### Conclusion.

The findings of our study underscore the importance of dietary components, such as hydroponic Radish, in potentially counteracting the adverse effects of pharmacological agents like paracetamol on hepatic vascular health. Given the significant role of paracetamol in acute liver failure and its associated mortality rates, further investigations are warranted to explore the mechanisms by which radish exerts its protective effects and its potential clinical applications in preventing paracetamolinduced liver damage.

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