

# GEORGIAN MEDICAL NEWS

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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 ВИНТИ 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 ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებში.

## WEBSITE

[www.geomednews.com](http://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](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. Корректур авторам не высылаются, 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.nlm.nih.gov/bsd/uniform_requirements.html)  
[http://www.icmje.org/urm\\_full.pdf](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 საყურადღებო!

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. რენტგენოგრაფიის ფოტოსურათები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგების 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 ფხიხლებში 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.

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## PREVENTION IN THE PARENTAL GENERATION OF EXPOSED RATS: CONSEQUENCES OF TOXIC EXPOSURE TO CHROMIUM AND GAMMA IRRADIATION IN AN EXPERIMENTAL MODEL

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### Abstract.

**Background:** Toxic environmental exposures, such as chromium and gamma irradiation, are known to have detrimental effects on health. These exposures can lead to genetic alterations, developmental defects, and increased susceptibility to diseases in subsequent generations. This study aims to assess the effectiveness of Nettle and Burdock oils in preventing the negative effects of chromium and gamma radiation in rats directly exposed to these toxicants, focusing on oxidative stress, hormonal disruption, and reproductive health.

**Methods:** An experimental study was conducted using 120 rats divided into 8 groups, administering Nettle and Burdock oils (0.5 mL/day) to investigate their potential protective effects against chromium and gamma radiation exposure. Parameters including blood and sperm analysis, oxidative stress markers, and reproductive health were assessed. Statistical analysis was performed using SPSS and Microsoft Excel.

**Results:** The analysis showed that exposure to chromium and gamma rays had a considerable adverse effect on hormonal and sperm parameters. Testosterone levels were significantly lower in the  $\gamma$  ( $2.6 \pm 0.179$ ) and Cr+6 +  $\gamma$  ( $2.5 \pm 0.200$ ) groups than in the control group ( $3.4 \pm 0.277$ ). Estradiol levels were also reduced in exposed groups compared to controls ( $\gamma$ :  $0.190 \pm 0.012$ ; Cr+6 +  $\gamma$ :  $0.175 \pm 0.009$  vs. control:  $0.206 \pm 0.008$ ). Additionally, sperm concentration was significantly lower in the Cr+6 +  $\gamma$  group ( $4.7 \pm 0.366$ ). Although the addition of Nettle and Burdock oils showed some improvement, the protective effects were minimal. No statistically significant differences were observed between treatment and exposed groups ( $p = 1.000$ ).

**Conclusion:** The study demonstrates that chromium (Cr+6) and gamma irradiation significantly impaired hormonal, sperm health, and oxidative stress markers in the parental generation of exposed rats. While the addition of Nettle and Burdock oils showed some improvement, their protective effects were minimal. Statistical analysis revealed no significant differences between treatment groups, indicating limited efficacy of these oils in mitigating toxic exposure effects.

**Key words.** Prevention, first generation, toxic exposure, chromium, gamma irradiation, Nettle oil, Burdock oil, oxidative stress, sperm health.

### Introduction.

The contamination of the environment and the exposure to lethal elements is a phenomenon of great concern all over the globe. Many activities such as industrial, agricultural and urban activities add hazardous substances into ecosystems. Among the most concerning toxic agents are chromium, which is heavy metal used in numerous industrial sectors, and gamma irradiation, which is a type of ionizing radiation. These two factors alone can single handedly deteriorate health condition, not only on the currently exposed individuals but to the future generations as well by inducing changes in the heritable genes which can affect population health over time [1,2]. Such exposures are largely found in industrialized regions as well as places where nuclear activities have taken place thus being worrisome for the local as well as international health care systems [3].

Public health in Ukraine, which suffers from both industrial pollution and the remains of the Chernobyl nuclear plant, is at peril. The aftermath of these events continues to affect the ecosystem negatively and, subsequently, the health of the populace [4]. Understanding the impacts of various toxic materials, specifically chromium and gamma radiation, is instrumental in crafting policies that would lessen their effects on both present and future citizens. Upon assessing these comprehensive impacts, it is vital to take steps towards effectively preventing health issues in Ukraine and improving the population's health indices [5,6].

The use of hexavalent Chromium (Cr VI) is noteworthy as it is a known carcinogenic which poses health risk for those in industries like steel making, electroplating, and tanning leather. Some issues that can arise from prolonged exposure include but are not limited to skin rashes, breathing challenges, kidney damage, and increasing the risks of cancer of the lungs and even the digestive system. In addition to these, altered genetic material in a chromosomal structure can lead to an array of chromium coupled issues along various Chromosomal Disorders which diverse familial conditions and impact descendants for generations [7].

Conversely, gamma irradiation is a type of exposure which can damage the cellular DNA due to ionizing radiation and is known to have far-reaching consequences. If one is exposed to these factors for too long or at too great an intensity, there

is a risk of incurring genetic mutations, cancer, heart disease, or other chronic complications. As noted above, gamma radiation is hazardous not only to the individual, but it also has the potential to cause mutations in the heritable DNA that can lead to developmental disorders, issues with reproduction, and disease for offspring of future generations [8,9].

The two forms of more gamma radiation and exposure to chromium are capable of producing oxidative damage, stress, inflammation, and injury which could potentially change the genetic structure of germ cells. These changes in genetically altered forms are potentially less harmful but pose concerns for the later generations and increase the chances for developing diseases or genetically guaranteed illnesses, creating a greater threat to population health over time [10,11].

From a societal and economic perspective, the consequences of toxic exposure are glaring. These consequences have the potential to affect both individual health as well as the economy on a national and global scale. The direct health costs like medical treatment, hospitalization, and rehabilitation are especially challenging for healthcare systems to deal with when caring for chronic disease as well as cancer and genetic disorders stemming from organic toxins. Indirect costs such as loss of productivity, untimely death, and additional costs that come with disabled people and their fiscal support put even more strain on the system [12].

For countries like Ukraine, where a substantial portion of the population resides in industrialized regions or areas affected by nuclear disasters, the socio-economic consequences are exceptionally severe. Populations exposed to elevated levels of chromium and gamma radiation experience chronic health conditions, placing considerable strain on already overburdened health care systems. These direct health-related costs may also diminish workforce productivity, thereby hinder economic growth and exacerbate social inequality. The combined impact of toxic exposure and socio-economic decline underscores the urgency of identifying and addressing this critical issue [13]. Although much study has been done on the direct impacts of radiation chromium and gamma irradiation on people, there are many gaps when it comes to potential repercussions for descendants. Most of the research has centered on the health outcomes of individuals, overlooking how these exposures impact their offspring's genetics [14]. Such absence of research on how these substances impact inheritance poses a problem in toxicological science.

Instead of developing new strategies to tackle the genetic impacts of environmental pollutants and ionizing radiation, current preventive strategies target restricting exposures or dealing with the health impacts of the toxins. This gap in research tinders many future generations without shields from the inherited effects of these environmental contaminants and ionizing radiation. Hence, it becomes vital to formulate methods of addressing these exposures for children so that their health will not be adversely affected in the future.

#### **Purpose of the Study.**

The primary aim of this study is to analyze how well preventive actions protect the parental generation of rats directly exposed to toxic substances such as chromium and gamma irradiation. The

research supportive activities for conceivably hereditary health issues include vitamin supplementation and antioxidant therapy aimed at mitigating genetic injury facilitating more favorable health results for the descendants of the exposed people. This approach is aimed at understanding the mechanisms of these preventive methodologies and will deepen the existing base within clinical toxicology and preventative medicine. In addition, the approach will assist in combating a profound lack of understanding regarding the preventative actions of transgenerational environmental exposures, especially in geographies prone to pollution.

#### **Literature review.**

Research on the effects of gamma radiation on certain species is quite extensive and has been performed on its transgenerational aspects too. One study was analyzing *Drosophila* embryos which experienced gamma radiation during an early developmental phase. It was noted that embryos are most vulnerable to radiation 30 minutes after the egg has been laid. The Low Dose Rate (LDR) radiation (50 and 97 mGy/h) resulted in the shortening of the eclosion periods while High Dose Rate (HDR) Radiation (23.4 to 495 Gy/h) resulted in increased embryotoxicity. While larvae from the irradiated embryos did not show any remarkable differences in the locomotor activity with respect to dose rates, they did display hypoactivity with 7 Gy doses. Furthermore, radiation-induced depigmentation (A5pig-) was observed in males and transmitted across up to 12 generations, highlighting the epigenetic inheritance of these effects. This study emphasizes that radiation-induced effects do not follow Mendelian inheritance and are influenced by both the dose and dose rate, providing insights into the long-term genetic impacts of ionizing radiation (15).

Chromium exposure is another environmental concern with significant biological effects. A study investigating the accumulation of chromium (Cr(VI)) in *Helianthus annuus* L. focused on its impact on photosynthesis, lipid peroxidation, and antioxidative responses. In a pot experiment with Cr(VI) concentrations of 15, 30, and 60 mg kg<sup>-1</sup> of soil, results showed that Cr(VI) accumulation was 2-3 times greater in the roots than in the shoots suggesting root as the main accumulation organ. Plant growth, stomatal activity, photosynthetic pigments, gas exchange, and PSII (Fv/Fm) efficiency were adversely affected by increased Cr(VI) concentrations. Moreover, Cr(VI) caused increased MDA and H<sub>2</sub>O<sub>2</sub> inducing lipid peroxidation. On the contrary, the plant showed its willingness to endure by strengthening its anti-oxidative defense responses through both enzymatic (SOD, APX, GR) and non-enzymatic (GSH, AsA) systems, thus proving his role of an efficient Cr(VI) accumulator with tolerance mechanisms towards Cr(VI) [16].

The effects of gamma ionizing radiation on aquatic organisms have also been widely studied. One investigation compared the impacts of gamma irradiation and tritium (beta ionizing radiation) on zebrafish embryos and larvae. Zebrafish larvae were exposed to gamma radiation at dose rates of  $3.3 \times 10^1$ ,  $1.34 \times 10^2$ , and  $1.24 \times 10^3$   $\mu$ Gy/h for 10 days. The study assessed various endpoints, including embryo-larval development, muscle tissue, genotoxicity, reactive oxygen species (ROS) production, and gene expression. The results revealed that

gamma radiation induced molecular changes, increased ROS production, and caused tissue damage, particularly in muscles. However, no significant effects on survival or hatching were observed, and DNA damage was not detected. The muscle damage identified was consistent with findings from tritium exposure at comparable dose rates, indicating that certain molecular responses to different types of ionizing radiation may be similar. This provides valuable insight into the effects of gamma radiation on aquatic organisms [17].

Further studies have examined the impact of gamma radiation on chromatin structure. Research involving zebrafish and Atlantic salmon demonstrated that direct exposure of zebrafish embryos to gamma radiation resulted in hyper-enrichment of H3K4me3 at specific gene loci (*hnf4a*, *gmnn*, *vegfab*). A comparable effect was observed in irradiated Atlantic salmon embryos. In adult zebrafish ovaries, irradiation during gametogenesis led to reduced H3K4me3 enrichment and decreased histone H3 levels. Notably, F1 embryos from irradiated parents exhibited hyper-methylation of H3K4me3, H3K9me3, and H3K27me3 at the same loci, while no such alterations were detected in F2 embryos. These findings suggest that gamma radiation induces locus-specific histone modifications that alter chromatin structure and are inheritable by the first generation but not transmitted further [18].

#### Objective.

To determine the effectiveness of preventive measures to prevent the negative effects of chromium and gamma radiation on the on the parental generation of rats exposed under experimental conditions.

#### Materials and Methods.

This experimental study examined the direct impact of preventive treatments on rats exposed to chromium (Cr+6) and gamma radiation. The study focused on evaluating how these interventions affected blood parameters, sperm quality, and oxidative stress in the exposed parental generation. In this study, the term “parental generation” refers to the animals directly exposed to toxicants and treatments. This study does not investigate effects on offspring of these animals.

#### Object:

The participating subjects in this experiment were rats (*Rattus norvegicus*), commonly used for toxicology and pharmacology research due to physiological similarities to humans. All animals studied belonged to the directly exposed parental generation. No breeding was performed to study offspring in this experiment.

#### Sampling:

The experiment utilized 120 rats in total, which were separated into 8 groups according to the treatment received. Each group consisted of 10-15 rats for adequate statistical power. The rats were kept in a controlled environment that included a 12-hour light/dark schedule, regulated room temperature, and ad libitum access to food and water as shown in Table 1.

#### Description of Chromium Dose, Irradiation Parameters, and Duration:

**Chromium Exposure:** Based on the previously documented protocols concerning chromium exposure, the rats belonging to the Cr+6 and Cr+6 +  $\gamma$  exposure groups received an oral dose of 180 mg/kg body weight per day of hexavalent chromium (Cr+6) for a continuous period of 30 days. This amount is recognized to cause observable toxic impacts in rats, though does not immediately kill them.

**Gamma Radiation Exposure:** The gamma and Cr+6 + gamma groups underwent gamma radiation from a Cobalt-60 source. The irradiation was done at a dose rate of 0.2 Gray per session. This amount of radiation simulates chronic environmental radiation exposure over time. The amount of time allotted for gonadal gamma radiation was done to guarantee sufficient biological changes so that both immediate and delayed effects could be recorded.

Although 0.2 Gy exceeds environmental levels, it's standard in rodent studies to induce measurable biological damage for evaluating protective treatments. Lower doses might cause undetectable changes. The chosen dose reflects sublethal experimental ranges, comparable to therapeutic or accidental exposures, ensuring clear assessment of nettle and burdock oil efficacy.

#### Preventive Measures:

**Nettle Oil:** The  $\gamma$  + Nettle Oil and Cr+6 +  $\gamma$  + Nettle Oil groups had preventative measures taken for their rats by administering nettle oil. Rats were given 0.5 mL of nettle oil daily throughout the course of the experiment. Nettle oil possesses antioxidant and anti-inflammatory properties, potentially assisting in mitigating the oxidative impacts of radiation and chromium exposure.

**Burdock Oil:** Likewise, the  $\gamma$  + Burdock Oil and Cr+6 +  $\gamma$  + Burdock Oil groups were treated with burdock oil, with a dosage of 0.5 mL per rat per day. Due to growing evidence of antioxidant properties, burdock oil was selected as a candidate to mitigate Cr+6 and gamma radiation toxicity.

**Table 1.** Overview of Experimental Groups and Exposure Conditions.

Group	Description
Control	No exposure to hexavalent chromium (Cr <sup>6+</sup> ) or gamma ( $\gamma$ ) radiation.
$\gamma$ Gamma Radiation	Exposed to $\gamma$ radiation only.
Cr <sup>6+</sup> + $\gamma$	Exposed to both Cr <sup>6+</sup> and $\gamma$ radiation.
$\gamma$ + Nettle Oil	Exposed to $\gamma$ radiation with nettle oil administration as a protective intervention.
$\gamma$ + Burdock Oil	Exposed to $\gamma$ radiation with burdock oil administration as a protective intervention.
Cr <sup>6+</sup> + $\gamma$ + Nettle Oil	Exposed to both Cr <sup>6+</sup> and $\gamma$ radiation, with nettle oil administration as a protective intervention.
Cr <sup>6+</sup> + $\gamma$ + Burdock Oil	Exposed to both Cr <sup>6+</sup> and $\gamma$ radiation, with burdock oil administration as a protective intervention.

### Justification:

The choice of nettle and burdock oils was based on their unique pharmacological profiles rather than general antioxidant capacity.

Nettle (*Urtica dioica*) is rich in flavonoids, lignans, and scopoletin, and is reported to exhibit anti-inflammatory, anti-androgenic, and hormone-modulating effects, making it relevant for reproductive and hormonal endpoints.

Burdock (*Arctium lappa*) oil contains polyphenolic compounds, caffeic acid derivatives, and inulin, and has demonstrated organ-protective and free radical scavenging properties in prior models of chemical and radiation-induced toxicity.

These oils were selected over generic antioxidants (e.g., Vitamin C or E) because of their multifactorial activity and traditional use in reproductive and endocrine disorders.

However, their comparative efficacy in this context remains validated, which is a key objective of this study.

### Assessment Methods:

**Biochemical Indicators:** After 5- and 10-month periods of exposure, rats were euthanized for blood and sperm analysis. Biochemical indicators of interest were as follows:

**Testosterone and Oestradiol:** Concentrations of these hormones were determined by enzyme linked immunosorbent assay (ELISA), which is a sensitive method for detecting and quantifying soluble substances.

**Oxidative Stress Markers:** Additional measures of oxidative damage included Malondialdehyde (MDA), Catalase and Superoxide Dismutase (SOD). MDA levels were measured with the thiobarbituric acid reactive substances (TBARS) assay. Catalase and SOD activities were quantified with routine spectrophotometric techniques.

### Morphological Assessment:

**Sperm Concentration:** Sperm concentration within the selected sample was measured with a hemocytometer after diluting the sample for light concentration counting to measure the impact of Cr + 6 and gamma radiation on the reproductive parameters.

**Motility:** Sperm motility was checked by estimating the ratio of motile sperm while looking at the sample slide using standard sperm motility protocol with a microscope.

**Abnormal Spermatozoa:** The abnormal sperms percentage determined from the prepared sperm smear slides were visually classified under a microscope according to the observed morphological abnormalities.

### Analysis Tools:

Data were analyzed using SPSS version 26. Each parameter was expressed in terms of descriptive statistics (mean  $\pm$  SD). One-Way ANOVA was done to extract differences between groups. Further data processing and visualization, such as computing percentage change from control values and graphing results, were performed in Microsoft Excel. The criteria for statistical significance were set to  $p < 0.05$  for all tests.

### Ethical Considerations:

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and adhered to ethical standards for the use of animals in research. Efforts were made to minimize suffering by employing humane methods

of handling, exposure, and euthanasia in accordance with established guidelines.

### Results.

Table 2 presents a detailed analysis of the hormonal and cytokine responses under various treatment conditions. The experiment aimed to assess the effects of chromium (Cr+6), gamma irradiation ( $\gamma$ ), and herbal oils (Nettle and Burdock) on testosterone, Oestradiol, thyroid hormones (T3 and T4), and cytokines (IL-6, TNF- $\alpha$ , IL-10) in the parental generation of exposed rats over a five-month period.

Testosterone levels were highest in the control group ( $3.6 \pm 0.252$ ), and exposure to  $\gamma$  radiation or the combination of Cr+6 and  $\gamma$  irradiation significantly decreased testosterone levels. Although the  $\gamma$  + Nettle oil group and the  $\gamma$  + Burdock oil group showed slightly higher mean testosterone values compared to the Cr+6 +  $\gamma$  group, these differences were not statistically significant ( $p = 1.000$ ). The addition of Burdock oil to the Cr+6 +  $\gamma$  exposure appeared to partially mitigate the testosterone reduction, but this effect was not significant.

Oestradiol levels were generally lower in the exposed groups compared to the control group, with the lowest levels observed in the Cr+6 +  $\gamma$  group ( $0.160 \pm 0.006$ ). Although both Nettle and Burdock oils showed numerically higher mean oestradiol levels, these differences were not statistically significant, and no definitive protective effect can be concluded.

Thyroid hormones (T3 and T4) were also significantly affected by the exposure treatments. T3 levels were lowest in the Cr+6 +  $\gamma$  group ( $0.90 \pm 0.054$ ) and highest in the  $\gamma$  group ( $1.50 \pm 0.071$ ). The T3 levels were slightly restored in the groups treated with Nettle and Burdock oils, with the Burdock oil group showing a slightly better recovery but these changes were not statistically significant. T4 levels exhibited a similar trend, with the Cr+6 +  $\gamma$  group having the lowest T4 levels ( $26.0 \pm 1.441$ ). The control and  $\gamma$  + Burdock oil groups showed the highest T4 levels, indicating that Burdock oil may have a protective effect on thyroid function in the presence of toxic exposure, but no significant differences were found.

Cytokine responses were varied across the different exposure treatments. IL-6 levels were highest in the Cr+6 +  $\gamma$  group ( $43.0 \pm 9.869$ ), suggesting a pro-inflammatory response induced by the combined exposure. Although Nettle and Burdock oils showed numerically lower IL-6 levels, these differences were not significant. TNF- $\alpha$  levels were also elevated in the Cr+6 +  $\gamma$  group ( $80 \pm 10.94$ ), while both Nettle and Burdock oils did not significantly reduce TNF- $\alpha$  levels. IL-10, an anti-inflammatory cytokine, was highest in the control group ( $62 \pm 8.692$ ), with all exposure treatments reducing IL-10 levels. While the  $\gamma$  + Nettle oil and  $\gamma$  + Burdock oil groups had numerically higher IL-10 levels than the Cr+6 +  $\gamma$  group, these changes were not statistically significant.

Table 2 summarizes several biological parameters, including testosterone, oestradiol, thyroid hormones (T3 and T4), and the cytokines IL-6, TNF- $\alpha$ , and IL-10. Testosterone levels were measured across different groups, revealing that the control group had the highest average testosterone level ( $3.4 \pm 0.277$ ). Exposure to  $\gamma$  radiation alone led to a decrease in testosterone ( $2.6 \pm 0.179$ ), while the addition of Cr+6 with  $\gamma$  radiation resulted in a further decline ( $2.5 \pm 0.200$ ). Supplementation

**Table 2.** Hormonal and Cytokine Responses to Chromium ( $Cr^{6+}$ ), Gamma Irradiation ( $\gamma$ ), and Herbal Oil Treatments (Burdock and Nettle Oils) After 5 and 10 Months of Exposure.

Parameter	Group	5 months (Mean $\pm$ SD)	10 months (Mean $\pm$ SD)
Testosterone (ng/mL)	Control	3.6 $\pm$ 0.252	3.4 $\pm$ 0.277
	$\gamma$	2.7 $\pm$ 0.173	2.6 $\pm$ 0.179
	$Cr^{6+} + \gamma$	2.1 $\pm$ 0.110	2.5 $\pm$ 0.200
	$\gamma$ + Nettle oil	3.0 $\pm$ 0.166	3.2 $\pm$ 0.254
	$\gamma$ + Burdock oil	3.3 $\pm$ 0.270	3.1 $\pm$ 0.312
	$Cr^{6+} + \gamma$ Nettle oil	2.5 $\pm$ 0.141	3.0 $\pm$ 0.192
	$Cr^{6+} + \gamma$ Burdock oil	3.1 $\pm$ 0.141	3.2 $\pm$ 0.145
Oestradiol (pg/mL)	Control	0.216 $\pm$ 0.007	0.206 $\pm$ 0.008
	$\gamma$	0.192 $\pm$ 0.009	0.190 $\pm$ 0.012
	$Cr^{6+} + \gamma$	0.160 $\pm$ 0.006	0.175 $\pm$ 0.009
	$\gamma$ + Nettle oil	0.210 $\pm$ 0.008	0.200 $\pm$ 0.012
	$\gamma$ + Burdock oil	0.220 $\pm$ 0.011	0.210 $\pm$ 0.014
	$Cr^{6+} + \gamma$ Nettle oil	0.180 $\pm$ 0.008	0.183 $\pm$ 0.011
	$Cr^{6+} + \gamma$ Burdock oil	0.200 $\pm$ 0.011	0.191 $\pm$ 0.012
T3 (ng/mL)	Control	1.20 $\pm$ 0.0675	1.33 $\pm$ 0.055
	$\gamma$	1.50 $\pm$ 0.071	1.69 $\pm$ 0.075
	$Cr^{6+} + \gamma$	0.90 $\pm$ 0.054	0.91 $\pm$ 0.046
	$\gamma$ + Nettle oil	1.33 $\pm$ 0.039	1.35 $\pm$ 0.059
	$\gamma$ + Burdock oil	1.17 $\pm$ 0.073	1.33 $\pm$ 0.065
	$Cr^{6+} + \gamma$ Nettle oil	1.24 $\pm$ 0.042	1.30 $\pm$ 0.070
	$Cr^{6+} + \gamma$ Burdock oil	1.27 $\pm$ 0.047	1.25 $\pm$ 0.090
T4 ( $\mu$ g/dL)	Control	33.3 $\pm$ 1.753	26.0 $\pm$ 2.107
	$\gamma$	39.0 $\pm$ 1.372	33.0 $\pm$ 2.409
	$Cr^{6+} + \gamma$	26.0 $\pm$ 1.441	19.0 $\pm$ 1.982
	$\gamma$ + Nettle oil	36.0 $\pm$ 1.652	28.5 $\pm$ 1.424
	$\gamma$ + Burdock oil	34.0 $\pm$ 1.308	28.0 $\pm$ 2.087
	$Cr^{6+} + \gamma$ Nettle oil	30.0 $\pm$ 1.585	25.0 $\pm$ 1.488
	$Cr^{6+} + \gamma$ Burdock oil	36.0 $\pm$ 1.382	29.0 $\pm$ 1.6147
IL 6 (pg/mL)	Control	30.0 $\pm$ 4.761	32.0 $\pm$ 1.604
	$\gamma$	38.1 $\pm$ 6.866	46.0 $\pm$ 2.726
	$Cr^{6+} + \gamma$	43.0 $\pm$ 9.869	50.0 $\pm$ 3.655
	$\gamma$ + Nettle oil	32.0 $\pm$ 6.653	33.7 $\pm$ 5.131
	$\gamma$ + Burdock oil	34.0 $\pm$ 5.754	32.3 $\pm$ 2.552
	$Cr^{6+} + \gamma$ Nettle oil	38.0 $\pm$ 7.883	38.0 $\pm$ 2.422
	$Cr^{6+} + \gamma$ Burdock oil	39.0 $\pm$ 9.832	37.0 $\pm$ 3.071
TNF- $\alpha$ (pg/mL)	Control	57 $\pm$ 7.134	30.6 $\pm$ 0.744
	$\gamma$	66 $\pm$ 8.628	37.2 $\pm$ 1.827
	$Cr^{6+} + \gamma$	80 $\pm$ 10.94	45.0 $\pm$ 2.639
	$\gamma$ + Nettle oil	52 $\pm$ 6.580	30.5 $\pm$ 1.642
	$\gamma$ + Burdock oil	54 $\pm$ 7.379	31.4 $\pm$ 0.775
	$Cr^{6+} + \gamma$ Nettle oil	61 $\pm$ 7.690	31.0 $\pm$ 1.547
	$Cr^{6+} + \gamma$ Burdock oil	63 $\pm$ 8.524	32.0 $\pm$ 1.553
IL 10 (pg/mL)	Control	62 $\pm$ 8.692	74.0 $\pm$ 2.051
	$\gamma$	66 $\pm$ 9.672	65.0 $\pm$ 2.055
	$Cr^{6+} + \gamma$	49 $\pm$ 7.424	60.0 $\pm$ 2.765
	$\gamma$ + Nettle oil	71 $\pm$ 8.363	67.0 $\pm$ 2.592
	$\gamma$ + Burdock oil	72 $\pm$ 10.382	70.0 $\pm$ 2.493
	$Cr^{6+} + \gamma$ Nettle oil	57 $\pm$ 7.823	68.0 $\pm$ 3.157
	$Cr^{6+} + \gamma$ Burdock oil	56 $\pm$ 8.433	66.6 $\pm$ 3.168

**Note:** T3 – Triiodothyronine; T4 – Thyroxine; IL-6 – Interleukin-6; TNF- $\alpha$  – Tumor Necrosis Factor-alpha; IL 10 – Interleukin-10;  $Cr^{6+}$  – Hexavalent Chromium;  $\gamma$  – Gamma Irradiation;  $\pm$  – Standard Deviation (SD); data presented as Mean  $\pm$  SD

with Nettle or Burdock oil alongside  $\gamma$  and Cr+6 resulted in numerically higher testosterone levels, but these differences were not statistically significant.

Similar to testosterone, oestradiol levels were found to be lower in the exposed groups compared to the control. The control group had a mean oestradiol of  $0.206 \pm 0.008$ , while  $\gamma$  exposure resulted in a slight decrease to  $0.190 \pm 0.012$ . The combination of Cr+6 with  $\gamma$  resulted in the lowest oestradiol levels at  $0.175 \pm 0.009$ . Supplementation with Nettle oil or Burdock oil resulted in slightly higher oestradiol levels in some groups, but no significant differences were observed.

The levels of thyroid hormones T3 and T4 exhibited distinct responses to exposure. T3 levels were generally lower in the exposed groups, particularly in the Cr+6 +  $\gamma$  group, which had the lowest level of  $0.91 \pm 0.046$ , compared to the control group's  $1.33 \pm 0.055$ . Although supplementation with Nettle or Burdock oil was associated with slightly higher T3 levels, these differences were not statistically significant. T4 levels showed a similar trend, with the control group having  $26.0 \pm 2.107$  and the Cr+6 +  $\gamma$  group having the lowest at  $19.0 \pm 1.982$ . Supplementation with Nettle or Burdock oil did not result in statistically significant changes in T4 levels compared to the exposed groups.

The results showed that exposure to  $\gamma$  radiation and Cr+6 significantly increased pro-inflammatory cytokines. IL-6 levels in the  $\gamma$  exposure group were elevated to  $46.0 \pm 2.726$  compared to the control group's  $32.0 \pm 1.604$ , and Cr+6 exposure further increased IL-6 levels to  $50.0 \pm 3.655$ . While supplementation with Nettle or Burdock oil resulted in lower mean IL-6 levels, these differences were not statistically significant. For TNF- $\alpha$ , the levels in the  $\gamma$  and Cr+6 exposed groups were also higher ( $37.2 \pm 1.827$  and  $45.0 \pm 2.639$ , respectively) than in the control group ( $30.6 \pm 0.744$ ), and the oils did not produce significant reductions. IL-10 levels, on the other hand, were slightly reduced in the exposed groups compared to the control, but supplementation with oils did not significantly restore IL-10 levels.

Table 3 presents combined results across several key parameters, including the spermatogenic index, sperm motility, percentage of abnormal sperm, malondialdehyde levels, and the activities of catalase and superoxide dismutase (SOD).

Sperm Concentration was observed to be highest in the control group ( $7.2 \pm 0.410$ ), followed by the  $\gamma$  and Cr+6 +  $\gamma$  groups, which had concentrations of  $6.0 \pm 0.503$  and  $4.4 \pm 0.467$ , respectively. Although the addition of Nettle oil or Burdock oil to  $\gamma$  irradiation resulted in slightly higher mean sperm concentrations, these differences were not statistically significant. Similarly, sperm concentration remained low in the Cr+6 +  $\gamma$  combination with Nettle oil and Burdock oil at  $5.7 \pm 0.278$  and  $6.3 \pm 0.520$ .

Motility was significantly reduced in the  $\gamma$  group ( $45 \pm 3.373$ ) and the Cr+6 +  $\gamma$  group ( $33 \pm 2.792$ ), with both showing a decrease compared to the control ( $75 \pm 5.583$ ). While the addition of Nettle oil and Burdock oil to  $\gamma$  exposure was associated with numerically higher motility, these differences were not statistically significant. The Cr+6 +  $\gamma$  + Nettle oil and Cr+6 +  $\gamma$  + Burdock oil groups showed motility values of

$45 \pm 3.253$  and  $54 \pm 4.592$ , respectively, but these were not significantly different from the Cr+6 +  $\gamma$  group.

The percentage of abnormal spermatozoa was elevated in the  $\gamma$  group ( $9.0 \pm 0.453$ ) and the Cr+6 +  $\gamma$  group ( $15.6 \pm 0.933$ ), indicating that exposure to these toxic agents significantly impacted sperm morphology. Although Nettle oil and Burdock oil were associated with lower mean abnormalities in some groups, these reductions were not statistically significant.

The malondialdehyde (MDA) levels, a marker of oxidative stress, were significantly increased in the  $\gamma$  ( $9.85 \pm 0.110$ ) and Cr+6 +  $\gamma$  ( $12.15 \pm 0.210$ ) groups, compared to the control ( $8.62 \pm 0.120$ ). The addition of Nettle oil and Burdock oil resulted in numerically lower MDA levels in some groups, but these differences were not statistically significant.

Catalase activity was lower in the Cr+6 +  $\gamma$  group ( $2.70 \pm 0.226$ ) compared to the control ( $3.32 \pm 0.150$ ), indicating that the combined toxic exposure compromised antioxidant defense. Although the addition of Nettle oil and Burdock oil resulted in slightly higher catalase activity in some groups, the differences were not significant. In the Cr+6 +  $\gamma$  + Nettle oil and Cr+6 +  $\gamma$  + Burdock oil groups, catalase activity remained relatively low, with values of  $3.31 \pm 0.154$  and  $3.03 \pm 0.097$ , respectively.

Superoxide Dismutase (SOD) activity, a key antioxidant enzyme, was notably higher in the  $\gamma$  group ( $86 \pm 2.93$ ) and the Cr+6 +  $\gamma$  group ( $60 \pm 2.10$ ) compared to the control ( $72 \pm 2.57$ ), indicating a compensatory response to oxidative stress. Although the addition of Nettle oil and Burdock oil was associated with numerically higher SOD activity in some groups, these changes were not statistically significant. However, the Cr+6 +  $\gamma$  + Nettle oil and Cr+6 +  $\gamma$  + Burdock oil groups ( $70 \pm 2.42$  and  $66 \pm 1.73$ ) exhibited reduced SOD activity, which may be due to the complex interactions of toxic exposure to the oils.

Table 3 presents the experiment measured parameters such as sperm concentration, motility, abnormal spermatozoa count, malondialdehyde (MDA), catalase, and superoxide dismutase (SOD) levels across different treatment groups. Regarding sperm concentration, the control group showed the highest mean value ( $6.3 \pm 0.366$  million sperm/mL), with the group exposed to both chromium and gamma irradiation (Cr+6 +  $\gamma$ ) showing the lowest concentration ( $4.7 \pm 0.366$  million sperm/mL). The addition of Nettle oil or Burdock oil to the Cr+6 +  $\gamma$  exposure resulted in slight improvements in sperm concentration, with values of  $5.5 \pm 0.382$  and  $5.7 \pm 0.419$  million sperm/mL, respectively. However, all experimental groups showed lower sperm concentrations compared to the control group.

Motility followed a similar trend. The control group exhibited the highest sperm motility at  $72.0 \pm 5.654\%$ , while the Cr+6 +  $\gamma$  group had significantly reduced motility ( $51.0 \pm 3.443\%$ ). The addition of Nettle oil and Burdock oil to the treatment groups led to some recovery in motility, with the  $\gamma$  + Nettle oil and  $\gamma$  + Burdock oil groups showing motilities of  $69.0 \pm 4.755\%$  and  $65.0 \pm 3.636\%$ , respectively. The Cr+6 +  $\gamma$  Nettle oil and Cr+6 +  $\gamma$  Burdock oil groups also exhibited improvements in motility ( $64.0 \pm 3.817\%$  and  $68.4 \pm 3.651\%$ , respectively) but were still lower than the control group.

The abnormal spermatozoa count, indicating sperm morphology issues, showed a noticeable increase in the exposed groups.

**Table 3.** Sperm Health and Oxidative Stress Markers in the Parental Generation of Exposed Rats After Chromium ( $Cr^{6+}$ ) and Gamma Irradiation with or without Nettle and Burdock Oil Supplementation After 5 and 10 Months.

Parameter	Group	5 months (Mean $\pm$ SD)	10 months (Mean $\pm$ SD)
Sperm concentration	Control	7.2 $\pm$ 0.410	6.3 $\pm$ 0.366
	$\gamma$	6.0 $\pm$ 0.503	5.3 $\pm$ 0.428
	$Cr^{6+} + \gamma$	4.4 $\pm$ 0.467	4.7 $\pm$ 0.366
	$\gamma$ + Nettle oil	6.6 $\pm$ 0.480	5.8 $\pm$ 0.365
	$\gamma$ + Burdock oil	7.1 $\pm$ 0.460	6.0 $\pm$ 0.423
	$Cr^{6+} + \gamma$ Nettle oil	5.7 $\pm$ 0.278	5.5 $\pm$ 0.382
	$Cr^{6+} + \gamma$ Burdock oil	6.3 $\pm$ 0.520	5.7 $\pm$ 0.419
Motility (%)	Control	75 $\pm$ 5.583	72.0 $\pm$ 5.654
	$\gamma$	45 $\pm$ 3.373	62.0 $\pm$ 3.423
	$Cr^{6+} + \gamma$	33 $\pm$ 2.792	51.0 $\pm$ 3.443
	$\gamma$ + Nettle oil	57 $\pm$ 2.954	69.0 $\pm$ 4.755
	$\gamma$ + Burdock oil	63 $\pm$ 3.932	65.0 $\pm$ 3.636
	$Cr^{6+} + \gamma$ Nettle oil	45 $\pm$ 3.253	64.0 $\pm$ 3.817
	$Cr^{6+} + \gamma$ Burdock oil	54 $\pm$ 4.592	68.4 $\pm$ 3.651
Abnormal Spermatozoa (%)	Control	6.2 $\pm$ 0.175	5.3 $\pm$ 0.237
	$\gamma$	9.0 $\pm$ 0.453	6.3 $\pm$ 0.234
	$Cr^{6+} + \gamma$	15.6 $\pm$ 0.933	7.7 $\pm$ 0.274
	$\gamma$ + Nettle oil	7.5 $\pm$ 0.652	5.7 $\pm$ 0.325
	$\gamma$ + Burdock oil	8.0 $\pm$ 0.661	5.6 $\pm$ 0.370
	$Cr^{6+} + \gamma$ Nettle oil	12.0 $\pm$ 1.134	6.2 $\pm$ 0.231
	$Cr^{6+} + \gamma$ Burdock oil	10.0 $\pm$ 0.813	5.9 $\pm$ 0.232
MDA (nmol/mL)	Control	8.62 $\pm$ 0.120	8.54 $\pm$ 0.216
	$\gamma$	9.85 $\pm$ 0.110	9.85 $\pm$ 0.365
	$Cr^{6+} + \gamma$	12.15 $\pm$ 0.210	10.85 $\pm$ 0.312
	$\gamma$ + Nettle oil	8.71 $\pm$ 0.250	8.86 $\pm$ 0.289
	$\gamma$ + Burdock oil	8.74 $\pm$ 0.130	8.13 $\pm$ 0.195
	$Cr^{6+} + \gamma$ Nettle oil	8.81 $\pm$ 0.115	8.94 $\pm$ 0.313
	$Cr^{6+} + \gamma$ Burdock oil	8.92 $\pm$ 0.047	8.90 $\pm$ 0.355
Catalase (U/mg protein)	Control	3.32 $\pm$ 0.150	5.13 $\pm$ 0.305
	$\gamma$	3.72 $\pm$ 0.220	4.25 $\pm$ 0.305
	$Cr^{6+} + \gamma$	2.70 $\pm$ 0.226	3.81 $\pm$ 0.199
	$\gamma$ + Nettle oil	3.80 $\pm$ 0.377	5.31 $\pm$ 0.294
	$\gamma$ + Burdock oil	4.00 $\pm$ 0.311	4.90 $\pm$ 0.272
	$Cr^{6+} + \gamma$ Nettle oil	3.31 $\pm$ 0.154	4.99 $\pm$ 0.246
	$Cr^{6+} + \gamma$ Burdock oil	3.03 $\pm$ 0.097	5.09 $\pm$ 0.263
SOD (U/mg protein)	Control	72 $\pm$ 2.57	75.0 $\pm$ 4.712
	$\gamma$	86 $\pm$ 2.93	57.6 $\pm$ 2.110
	$Cr^{6+} + \gamma$	60 $\pm$ 2.10	52.0 $\pm$ 2.100
	$\gamma$ + Nettle oil	90 $\pm$ 6.90	73.3 $\pm$ 3.430
	$\gamma$ + Burdock oil	95 $\pm$ 3.78	76.0 $\pm$ 2.044
	$Cr^{6+} + \gamma$ Nettle oil	70 $\pm$ 2.42	69.0 $\pm$ 2.482
	$Cr^{6+} + \gamma$ Burdock oil	66 $\pm$ 1.73	68.5 $\pm$ 2.070

**Note:** MDA – malondialdehyde; SOD – superoxide dismutase;  $Cr^{6+}$  – Hexavalent Chromium;  $\gamma$  – Gamma Irradiation;  $\pm$  – Standard Deviation (SD); data presented as Mean  $\pm$  SD.



The Cr+6 +  $\gamma$  group had the highest abnormal spermatozoa percentage ( $7.7 \pm 0.274\%$ ), while the control group had the lowest ( $5.3 \pm 0.237\%$ ). Nettle oil and Burdock oil treatments appeared to have minimal effect on reducing abnormalities, as the values remained similar to the  $\gamma$  and Cr+6 +  $\gamma$  groups.

In terms of oxidative stress markers, malondialdehyde (MDA) levels, which indicate lipid peroxidation, were elevated in the exposed groups compared to the control. The Cr+6 +  $\gamma$  group had the highest MDA level ( $10.85 \pm 0.312$ ), while the control group had a lower value ( $8.54 \pm 0.216$ ). The oil treatments did not significantly reduce MDA levels, but the group treated with Burdock oil ( $\gamma$  + Burdock oil) showed the lowest MDA level ( $8.13 \pm 0.195$ ), suggesting some protective effect.

The catalase enzyme activity, which helps mitigate oxidative stress, was significantly reduced in the exposed groups. The control group had the highest catalase activity ( $5.13 \pm 0.305$ ), whereas the Cr+6 +  $\gamma$  group exhibited the lowest activity ( $3.81 \pm 0.199$ ). The addition of Nettle oil and Burdock oil slightly improved catalase levels in the treatment groups, but none reached the control group's level.

Finally, superoxide dismutase (SOD) activity, another key antioxidant enzyme, was reduced in all exposed groups, with the Cr+6 +  $\gamma$  group showing the lowest SOD activity ( $52.0 \pm 2.100\%$ ). The addition of Nettle oil and Burdock oil had a somewhat beneficial effect on SOD activity, with the  $\gamma$  + Nettle oil group ( $73.3 \pm 3.430\%$ ) and the  $\gamma$  + Burdock oil group ( $76.0 \pm 2.044\%$ ) showing higher values compared to the Cr+6 +  $\gamma$  group, though still lower than the control group ( $75.0 \pm 4.712\%$ ).

Table S1 present the ANOVA results for hormonal and cytokine measurements indicate no significant difference between the groups, as shown by the p-value of 1.000. This confirms that none of the observed numerical differences reached statistical significance. The lack of significance could be due to the complexity of the interactions or the experimental conditions not being sufficient to generate a measurable effect across all groups.

Table S2 illustrates the ANOVA results for the hormonal and cytokine data, indicating that the differences between the groups were not statistically significant ( $F = 0.014$ ,  $p = 1.000$ ). This suggests that, despite some variations in hormone and cytokine levels across the different exposure and treatment groups, the effects of  $\gamma$  radiation, Cr+6 exposure, and oil supplementation were not statistically distinguishable in terms of overall variance.

In conclusion, the study highlights that while exposure to chromium and gamma radiation influenced various hormonal and inflammatory markers, the addition of Nettle or Burdock oils did not result in statistically significant improvements in these parameters. The lack of significant differences as indicated by the ANOVA suggests that the oils did not produce meaningful protective effects against the detrimental impact of chromium and gamma radiation exposure on these biological markers.

Table S3 presents the ANOVA results, which showed no significant differences between the groups for sperm health and oxidative stress markers ( $F = 0.066$ ,  $p = 0.999$ ), indicating that the treatments, including Nettle oil and Burdock oil, did not result in statistically significant improvements or adverse effects compared to toxic exposures alone.

In summary, the study found that exposure to  $\gamma$  irradiation and Cr+6 significantly impaired sperm health, as reflected in reduced sperm concentration, motility, and increased abnormalities. While the addition of Nettle oil and Burdock oil helped to mitigate some of these effects, the overall results were not significantly different from the toxic exposure groups.

Table S4 shows the ANOVA analysis indicated that there were no significant differences between the groups in terms of sperm health and oxidative stress markers, with a p-value of 1.000, suggesting that the treatment conditions did not lead to statistically significant variations across the groups.

This result implies that the protective effects of Nettle oil and Burdock oil were not strong enough to counteract the effects of chromium and gamma irradiation within the 10-month period of this study.

### Discussion.

The results of the current study indicate reveal that gamma irradiation and chromium (Cr+6) exposure markedly lowered testosterone levels at 5 months, with only numerical trends toward recovery in the oil-treated groups (Nettle and Burdock oils), though these changes were not statistically significant. In the 10-month evaluations, oil-treated group testosterone concentrations also showed non-significant numerical increases, but still remained lower than controls. Nevertheless, the 10-month data showed a greater decline in testosterone levels in the exposed groups which suggest that these toxic exposures become increasingly worse with time, corroborating earlier findings that demonstrated exposure to hexavalent chromium decreases testosterone levels. A 2024 study conducted in mice found testicular injury and hormonal derangement associated with Cr (VI) could be attributed to altered lipid metabolism of the testes. In particular, the AMPK/SREBP1 pathway, whose activation led to autophagy and disrupted lipophagy, caused lower testosterone secretion through increased capture and destruction of cellular components. In this study, doses of Cr (VI) ranging from 75 to 125 mg/kg were administered for 30 days [19].

Burdock, also called *Arctium lappa*, is a plant with well-known application in Chinese medicine for different health problems. Burdock has shown possible therapeutic functions owing to its bioactive components such as polyphenolic antioxidants, flavonoids, and fructo-oligosaccharides. These compounds are effective in attenuating toxicity of the liver, oxidative stress, diabetes, hypolipidemia and even Alzheimer's disease, indicating the broad pharmacological and nutraceutical scope of burdock [20]. In particular, the oil extracted from the root of the burdock plant is suggested to have antioxidant properties which protect the body from oxidative stress resulting from exposure to gamma radiation and Cr+6. One study noted decreased indicators of protection from antioxidants after simultaneous influence of gamma radiation and potassium dichromate. In contrast, prior dosing with oil from the root of burdock significantly improved antioxidant protection in the tissues in question, indicating its use as a protective agent against oxidative harm resulting from these toxic exposures [21]. However, in the current study, such protective effects were not statistically significant.

Concerning the oestradiol levels, the results of the oestradiol

**Table S1.** ANOVA Results for Hormonal and Cytokine Levels by Treatment Groups After 5 Months of Exposure.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62.290	6	10.382	.013	1.000
Within Groups	32704.869	42	778.687		
Total	32767.159	48			

**Note:** *df* – Degrees of freedom; *F* – *F*-statistic from ANOVA test; *Sig.* – Significance value (0.05).

**Table S2.** ANOVA Results for Hormonal and Cytokine Levels by Exposure and Treatment Groups After 10 Months.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51.281	6	8.547	.014	1.000
Within Groups	26584.747	42	632.970		
Total	26636.028	48			

**Note:** *df* – Degrees of freedom; *F* – *F*-statistic from ANOVA test; *Sig.* – Significance value (0.05).

**Table S3.** ANOVA Analysis of Sperm Quality and Oxidative Stress Markers After 5 Months of Exposure.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	399.834	6	66.639	.066	.999
Within Groups	35314.325	35	1008.981		
Total	35714.159	41			

**Note:** *df* – Degrees of freedom; *F* – *F*-statistic from ANOVA test; *Sig.* – Significance value (0.05).

**Table S4.** ANOVA Evaluation of Sperm Health and Oxidative Stress Markers in the Parental Generation of Rats After 10 Months of Chromium and Gamma Irradiation Exposure.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	220.306	6	36.718	.038	1.000
Within Groups	33725.140	35	963.575		
Total	33945.446	41			

**Note:** *df* – Degrees of freedom; *F* – *F*-statistic from ANOVA test; *Sig.* – Significance value (0.05).

assay within the current study demonstrated that gamma irradiation and chromium (Cr+6) exposure lowered oestradiol levels at the 5-month mark. The exposed groups showed low oestradiol levels compared with the control, with only minor, non-significant numerical changes among the Nettle and Burdock oil treated groups. At the 10-month mark, the oil treatments did not produce significant improvements in oestradiol levels, which were still lower than those of the control group. Nonetheless, the  $\gamma$  + Burdock oil group showed some numerical increase. These results indicate that while the oils might offer minimal protective trends against hormonal imbalance, the changes were not statistically significant, and the oils were not sufficient to overcome the effects of these toxic exposures over time. This illustrates the difficulty faced in dealing with the enduring impact of environmental toxins on hormones. To support these results, one study looked at the effect of estradiol and some of its metabolites on the metalloestrogen Cr (VI) using an in vitro model. The researchers observed that pre-incubation with estradiol (E2) and its metabolites had a synergistic effect of Cr (VI), especially at very low concentrations of estrogens. Consequently, it appears that insufficient levels of estrogens may potentiate the toxic impacts of Cr (VI), making it plausible that the oils provide some degree of protection, but their ability to mitigate chronic hormonal imbalances is considerably restricted [22]. Another study examined the combination of toxic chromium (VI) and estrogen, analyzing its impact on breast cancer cell lines and assessing if estrogen provided any sort of

protective mechanism. The study determined that Cr(VI) had the most debilitating impact on MCF-7 and MDA-MB-175- VII cells, while 17 $\beta$ -E2 had the least impact. During the combined exposure with metalloestrogens and estrogens, 17 $\beta$ -E2 showed some level of protection against the cytotoxicity from Cr(VI). This evidence supports the idea that estrogen, especially 17 $\beta$ -E2, protects against some damaging effects of Cr(VI), but the overall effect remains uncertain in a long-term treatment paradigm [23].

The current study shows that both T3 and T4 levels were significantly lower in the Cr+6 +  $\gamma$  group at both 5 months and 10 months. At 5 months, there were minor numerical increases with the oils, with Burdock oil showing slightly better values compared to Nettle oil, but these differences were not statistically significant. In the 10-month period, Burdock oil again demonstrated numerically higher T3 and T4 levels compared to Nettle oil, though neither treatment fully restored thyroid function to control levels, and the changes were not statistically significant. These findings suggest that prolonged exposure leads to sustained disruption in thyroid hormone levels, with minimal benefit from the oils. This aligns with established mechanisms of chromium toxicity, where chronic exposure results in persistent hormonal imbalances. A similar study corroborated these findings, revealing that the persistent suppression of T3 and T4 levels in Cr+6-exposed groups is linked to chromium's ability to induce oxidative stress and structural damage to thyroid follicles, disrupting hormone synthesis

[24,25]. Animal studies have shown that Cr (VI) exposure reduces follicular size, increases interstitial spaces, and alters thyroglobulin processing, all of which mirror the sustained hormone disruption observed in this study. A study confirmed that chromium exposure disrupts thyroid homeostasis through inflammatory cytokine dysregulation and antioxidant depletion, supporting the minimal recovery of T3 and T4 levels seen even after 10 months of treatment [26]. The effectiveness of burdock oil in this study is likely due to its high iodine content (2.3-3.8 mg per 100g), which assists in the production of thyroxine and may mitigate iodine deficiency due to chromium. In addition, burdock root contains zinc and flavonoids that improve enzyme activity necessary for conversion of T4 to T3. These factors might partially explain the numerical trends observed in thyroid hormone levels with burdock oil treatment, although the effects were not statistically significant [27]. Nettle oil, however, was less effective, which is at odds with its common use for thyroid support as the literature has shown. Although nettle does contain important nutrients like iron and B vitamins that support thyroid function, it does not have any hormone-modifying iodine or thymoquinone [28]. A study conducted on nettle supplementation showed improvement in iron levels, but no significant changes in TSH or T4 levels were noted, which aligns with the lesser recovery of thyroid hormones observed in this study [29].

The current study found that the groups which had been exposed showed significant pro-inflammatory responses, which included raised IL-6, TNF- $\alpha$  levels, and lowered IL-10 levels, both at the 5 month and 10-month marks. Treatment with oils at 10 months showed only minimal, non-significant decreases in IL-6 and TNF- $\alpha$ . The data collected in 10 months was on par with the observed trends at 5 months, showing that the oils had no statistically significant impact on the modulation of inflammatory cytokines over time. These results are indicative that while the oils might aid in some short-term situations, the lack of statistical significance suggests their ability to alleviate chronic inflammation is inadequate, especially regarding long-term toxic exposure. More research has shown the benefit of helping mineral supplementation in controlling inflammatory responses, such as boron, which is known to reduce oxidative stress and restore antioxidant enzymes (SOD, CAT) in chromium/irradiation exposed mice [30]. Likewise, some irradiation-modified compounds, such as gamma-irradiated sericin (I-sericin), have been shown to decrease TNF- $\alpha$  while promoting immune cell proliferation, demonstrating that inflammation and its regulation can be counter-intuitive [31]. Still, the oils tested in this study were unable to meet expectations, likely due to the lack of significant changes and possibly differing composition of the oils or delivery methods used [32]. The lack of significant improvement in cytokine levels at 10 months is consistent with findings in irradiated NK-92 cells, where cytokine secretion (e.g., IFN $\gamma$ ) remained stable for 48 hours post-irradiation, but effector molecules like perforin declined by day 3. This suggests that chronic exposure may overwhelm any transient anti-inflammatory effects, highlighting the need for higher-dose or combinatorial therapies to address sustained inflammatory responses [33,34]. Radiation-induced DNA damage is known

to trigger NF- $\kappa$ B pathways, which upregulate IL-6 and TNF- $\alpha$  in intestinal epithelia, further supporting the idea that prolonged exposure to gamma radiation and chromium disrupts immune system regulation [35].

The current study found that sperm concentration and motility were reduced in the 5-month exposure groups subjected to chromium and gamma irradiation, with only minor, non-significant trends toward improvement observed in the oil-treated groups. In the 10-month results, sperm concentration and motility remained lower in the exposed groups, but the oils showed non-significant numerical increases, especially in the  $\gamma$  + Burdock oil group. However, the 10-month data indicated a prolonged effect on sperm health, with none of the treatment groups reaching control levels. Furthermore, abnormal spermatozoa were more prevalent in the exposed groups at both time points, and the oils had minimal, non-significant impact in reducing these abnormalities. These findings highlight the long-term detrimental effects of chromium and gamma radiation on sperm health, with limited evidence of any significant improvements offered by the oil treatments. These results are consistent with other studies examining radiation-induced spermatogenic damage. The significant suppression of sperm concentration and motility observed at 5 months aligns with radiation thresholds reported in human studies. For instance, single doses greater than 2 Gy result in permanent azoospermia, while fractionated doses, such as a total of 2.5 Gy, lead to delayed recovery or permanent sterility due to germ cell apoptosis [36]. The persistence of damage at 10 months in this study matches the “reverse fractionation effect,” where repeated low-dose exposures amplify damage through unrepaired DNA breaks in spermatogonia. This effect suggests that long-term damage to sperm health may be due to the cumulative impact of sustained exposures. A study on mice revealed some recovery of fertility function 11 weeks post-6.4 Gy irradiation. However, the data in humans indicates that spermatogenesis recovery is slower owing to longer spermatogenic cycles (74 days). This is consistent with the findings of prolonged spermatogenic damage in the current study [37]. The incomplete recovery at ten months is certainly lacking when compared to rodent models which demonstrate spermatogonial stem cell (SSC) repopulation in a matter of weeks [38,39]. This may be due to species specific radiosensitivity or the summed consequences of chromium co-exposure which could reduce the regenerative potential of SSCs and subsequently retard or even stop the full recovery of spermatogenesis that this study set out to investigate. Other studies have shown Burdock oil offer some protective benefits, especially in the context of radiation and chromium damage to sperm. A study demonstrated that Burdock oil improved sperm viability by 32% in diabetic mice post exposure to gamma radiation and hexavalent chromium which aligns with the partial recovery of sperm health seen in this study. Here, Burdock oil was shown to improve sperm concentration and motility but in the current study, such improvements were not statistically significant [40].

The current study revealed that MDA levels were appreciably elevated in groups exposed to chromium and gamma irradiation at 5 months, with only minor, non-significant decreases noted

in the oil-treated groups. MDA levels continued being elevated in the exposed groups at 10 months, and the oils had negligible statistically significant effects on oxidative stress reduction. Catalase activity was reduced at both time points, showing non-significant numerical improvement in the oil-treated groups, though it did not reach control levels. SOD activity showed numerical increases in the 10-month oil-treated groups compared to the 5-month data, indicating a slight antioxidative effect from Nettle and Burdock oils. However, these changes were not statistically significant. These findings are consistent with existing literature on oxidative stress and the effects of chromium and gamma radiation. Gamma radiation and hexavalent chromium Cr (VI) are known to induce oxidative stress by generating reactive oxygen species (ROS), leading to cellular damage, DNA mutations, and impaired sperm health. This aligns with the increased oxidative stress markers (e.g., MDA) observed in the current study, reinforcing the detrimental effects of these exposures on reproductive health [41,42]. Another study suggests that natural extracts like Curcuma xanthorrhiza Xorb may reduce MDA concentrations, although the reductions are not always significant. This finding mirrors the current study, where Burdock and Nettle oils showed slight reductions in MDA levels, but these effects were not statistically significant. It highlights the potential of natural extracts in reducing oxidative stress, although their effectiveness may vary depending on exposure and treatment conditions [43]. Additionally, a study demonstrated that Nettle oil reduced oxidative stress caused by ionizing radiation, exhibiting antioxidant activity by scavenging free radicals. Pretreatment with Nettle oil before irradiation significantly decreased the levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), improving lipid profiles in rats with radiation-induced damage to the heart and lungs. These findings support the current study, where Nettle oil appeared to have some beneficial effects on oxidative stress markers, such as MDA and SOD, although the effects were not significant enough to counteract the damage caused by chromium and gamma irradiation exposure. While there were observable trends suggesting that Nettle and Burdock oils might mitigate some of the toxic effects of Cr+6 and gamma irradiation, the quantitative effects were not statistically significant when tested with ANOVA ( $p = 1.000$ ). The oils showed minimal, non-significant improvements in sperm health and oxidative stress markers, but they did not fully counteract the toxic effects of these environmental pollutants. The lack of significant differences indicates that, although the oils may offer some protective trends, they are not potent enough to substantially reverse the biological damage caused by these toxicants.

## Conclusion.

The primary goal of this study was to assess the hormonal, cytokine, sperm health, and oxidative stress responses in the parental generation of rats directly exposed to chromium (Cr+6) and gamma ( $\gamma$ ) irradiation, with and without supplementation with Nettle and Burdock oils. The experimental design aimed to understand the impact of these exposures and the potential protective role of herbal oils over a 5- and 10-month period.

While the study revealed observable trends in some of the biological markers, it failed to demonstrate statistically significant differences in most of the outcomes, as indicated by the ANOVA results.

**Hormonal and Cytokine Responses:** The hormonal levels, including testosterone, oestradiol, thyroid hormones (T3 and T4), and cytokines (IL-6, TNF- $\alpha$ , IL-10), showed trends consistent with expectations of disrupted endocrine functions following exposure to Cr+6 and  $\gamma$  irradiation. Testosterone, oestradiol, T3, and T4 levels were generally reduced in exposed groups, with some recovery seen in groups supplemented with Nettle or Burdock oils. However, these differences were not statistically significant. For cytokines, a pro-inflammatory response was induced by Cr+6 +  $\gamma$  exposure, but the oils did not significantly attenuate this response.

**Sperm Health and Oxidative Stress:** The sperm health parameters, including concentration, motility, and abnormal spermatozoa, were significantly compromised in the Cr+6 +  $\gamma$  groups, with slight improvements observed in the Nettle and Burdock oil treatment groups. Similarly, oxidative stress markers, such as MDA, catalase, and SOD, indicated heightened oxidative damage due to exposure, but the oils had minimal effects on reversing these changes. The addition of oils did not significantly reduce MDA levels or improve antioxidant activity beyond the levels seen in the toxic exposure groups.

**Quantitative Indicators:** In terms of sperm concentration, the control group had the highest value of 7.2 million sperm/mL, while the Cr+6 +  $\gamma$  group had the lowest at 4.4 million sperm/mL. Motility was significantly reduced in the  $\gamma$  and Cr+6 +  $\gamma$  groups (45% and 33%, respectively), and abnormalities were highest in the Cr+6 +  $\gamma$  group (15.6%). Oxidative stress markers like MDA were notably higher in the  $\gamma$  and Cr+6 +  $\gamma$  groups, with values of  $9.85 \pm 0.110$  and  $12.15 \pm 0.210$ , respectively, compared to the control's  $8.62 \pm 0.120$ .

**ANOVA Results:** Despite observable trends, the ANOVA analyses showed no statistically significant differences ( $p = 1.000$ ), indicating that the experimental treatments, including Nettle and Burdock oils, did not produce measurable effects in mitigating the adverse impacts of chromium and gamma irradiation exposure.

## Practical Recommendations for Prevention.

Based on the findings, it is recommended that further studies explore more potent protective agents or different formulations that may have stronger effects in counteracting the oxidative stress and hormonal disruptions caused by Cr+6 and  $\gamma$  exposure. Additionally, regular monitoring of hormonal and oxidative stress biomarkers could be implemented in environments where individuals are exposed to such toxic agents to identify early signs of biological disruption.

## Suggestions for Further Research.

Future research could focus on increasing the sample size and refining experimental conditions to achieve more robust results. It would be beneficial to examine alternative protective agents, such as other herbal oils or antioxidant-rich compounds, and explore their effects in combination with a wider range of toxicants. Additionally, long-term studies assessing cumulative exposure and its impact on reproductive health and disease

susceptibility could provide deeper insights into preventive strategies.

### Conflicts of Interest.

The authors declare no competing interests.

### Declaration.

We have not used any AI tools or technologies to prepare this manuscript.

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### Ethics Approval and Consent to Participate.

The study was approved by the Local Bioethics Committee of the West Kazakhstan Medical University named after Marat Ospanov, in accordance with GCP, WHO, and ICH-GCP ethical standards (Protocol № 8, Approval number: № 8.10, Session date: 20.10.2022, Approval date: 21.11.2022). The ethics review was conducted under full certification. No human participants were involved in the research. All procedures involving animals were in accordance with institutional guidelines and relevant ethical regulations.

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