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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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

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

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

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

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

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

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

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

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

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

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

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

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

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

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

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

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

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

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

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

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

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

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

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

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

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

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

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

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

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

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

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

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

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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Содержание:

ROLE OF ANTIOXIDANT FOLIUM EXPOSURE ON OXIDATIVE SRESS IN A VALPROIC ACID-INDUCED ANIMAL MODEL OF AUTISM

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Abstract.

Introduction: Autism spectrum disorder (ASD) is associated with increased levels of oxidative stress and decreased antioxidant capacity. The present study aimed to investigate the effects of different types of herbal antioxidant supplement Folium (F) - F. Relax, F. P53, F. pX, and F. Immuno (BAO Health Resources Corporation, USA) on reducing the severity of oxidative stress in a rat model of ASD induced by prenatal administration of valproic acid (VPA).

Methods: The study was conducted on male outbred white rats. Rats in the VPA treated groups (group - VPA), at 2 months of age, received intraperitoneally (17 mg/kg, for 21 days) different types of Folium. At the end of treatment, measurements were taken in both the control and experimental behaviorally characterized groups, including systemic blood pressure, heart rate, as well as oxidative (d-ROM) and antioxidant (PAT) statuses. Statistical analysis was performed using SigmaStat statistical software. Data for all parameters were analyzed statistically by one- or two-way ANOVA followed by post hoc comparisons. Student's t-test was used to compare the mean values of two independent groups.

Results: The results of behavioral studies showed that prenatal VPA treatment reduced social exploration, impaired social novelty preference, decreased anxiety, and increased locomotor activity. High blood pressure [systolic (SBP) and diastolic (DBP)] was observed in rats of the VPA group, along with an increased heart rate. VPA rats also showed elevated levels of free radicals (d-ROMs) and a higher oxidative stress index (OSI), indicating the presence of oxidative stress. Notably, treatment with different types of Folium revealed that: (i) administration of all these supplements restored blood pressure (systolic SBP and diastolic DBP) to the normal range; regarding heart rate, only F. Immuno did not produce a decrease; (ii) d-ROMs levels and the OSI in the VPA group were not significantly different from those in the VPA + F. pX group.

Conclusion: Taken together, we provide evidence that prenatal administration of VPA to rats can induce ASD-like behavioral patterns accompanied by increased oxidative stress, as reflected by elevated levels of free radicals (d-ROMs) and a higher oxidative stress index (OSI). Based on the data obtained, it can be assumed that three of the four antioxidant supplements tested - F. Relax, F. P53, and F. Immuno, which showed positive effects on oxidative stress markers, may be suitable for use in individuals with ASD to alleviate oxidative stress and regulate blood pressure. However, to draw definitive conclusions, further research in collaboration with scientific research institutions and medical schools is required.

Key words. Folium, ASD, ROS, Oxidative stress, VPA.

Introduction.

A large number of autism spectrum disorders (ASD) are the result of the interaction of genetic, epigenetic, and environmental factors [1,2]. Clinical studies indicate that autism is approximately four times more common in males than in females, although the exact reasons for this sex difference remain unknown. There is a concept - "autistic brain", under which numerous brain structures involved in the pathogenesis of autism are considered [3]. At the same time, the view that autism is not limited to the involvement of certain, even numerous structures, but involves the whole body, is increasingly established. In particular, there are pronounced systemic changes in the body's immune and metabolic functions [4,5]. Autism can appear at any age, but in most cases, symptoms appear in the early stages of postnatal development - within the first three years of life. That is why it is considered a developmental disorder. Late detection of autism is associated with regression of normal brain development [6,7]. Individuals with autism primarily exhibit difficulties in communicating and building relationships with other individuals. They are also characterized by restricted interests, repetitive behavior, sensory disturbances, disturbances of the emotional sphere, gastrointestinal symptoms, seizures, and a range of other issues that prevent the normal integration of autistic individuals into society [8]. Because of this diversity, these conditions are not regarded as a single specific disease but are instead grouped under the term ASD.

Take in consideration that autism is a polyetiological disorder, and only experimental animal models allow us to fully study and gain new insights into its pathogenesis. One of the most widely used models of ASD is the experimental rodent model of fetal valproate syndrome (FVS), which involves prenatal administration of valproic acid (VPA) [9]. The offspring of such rats' exhibit behaviors that share key features with the main symptoms of autism, including deficits in social behavior, communication disorders, stereotypies, and anxiety [10,11]. This model is recognized by scientists and clinicians worldwide and is considered one of the most appropriate models of ASD to date [12]. VPA has also been adopted in the present study for the construction of an ASD rat model.

It is noteworthy that a key feature of many, if not all, neurological disorders is the generation of reactive oxygen species (ROS). Oxygen radicals have been shown to play a

fundamental role in the pathogenesis, progression, and severity of Alzheimer's, Parkinson's, autism, and Huntington's diseases [13-16]. An imbalance between the production of reactive oxygen/nitrogen species (ROS/RNS) and the body's ability to neutralize their harmful effects through antioxidant systems causes oxidative stress.

Oxidative stress results from the excessive production of free radicals when the cell's antioxidant defenses are unable to counterbalance this overproduction. Throughout evolution, organisms have developed protective systems that operate at various levels and through different mechanisms. These defenses can be classified as endogenous and include enzymes, antioxidants, and molecular mechanisms capable of repairing cellular damage. When vitamins and other antioxidants are obtained through food, they are classified as exogenous. It is known that individuals with ASD have lower levels of endogenous antioxidant molecules compared to healthy controls. For these reasons, the intake of exogenous antioxidants may help strengthen the body's defense system against endogenous reactive oxygen species (ROS). Exogenous antioxidants obtained through diet are also key factors in preventing or at least reducing oxidative stress in humans. These compounds may help protect cellular macromolecules from damage [17]. There is growing scientific consensus on the potential role of dietary agents, particularly antioxidant-rich foods, in reducing symptom severity and improving behavioral disorders in children with autism [18]. Exogenous antioxidants include ascorbic acid (vitamin C), which scavenges hydroxyl and superoxide radicals; tocopherols and tocotrienols (vitamin E), which help limit lipid peroxidation in cell membranes; and phenolic antioxidants such as stilbene derivatives (e.g., resveratrol), phenolic acids, flavonoids, as well as trace elements like selenium and zinc, among others [19].

Among all vitamins, vitamin C (VC) is considered one of the most extensively studied and potent antioxidants. It is found in various plant cell types, organelles, and the apoplast, as a major hydrophilic compound involved in reactive oxygen species (ROS) detoxification. Due to its structure, VC can effectively scavenge superoxide and hydroxyl radicals and reduce H_2O_2 in water through the ascorbate peroxidase reaction. Additionally, VC regenerates vitamin E (VE) from its radical form (tocopherol, TOH), thereby contributing to membrane protection [20]. Several recent studies have reported lower blood levels of VC in children with ASD compared to healthy controls [21,22]. It is the most important antioxidant in the lipid phase. VE is classified as a chain-breaking antioxidant because it can directly scavenge oxidative radicals, thus preventing lipid peroxidation caused by PUFA oxidation. Consistent with these findings, reduced blood levels of VE have also been reported in children with autism and are associated with ASD-like behaviors in these individuals [23].

To date, only a limited number of studies have investigated the potential benefits of dietary polyphenols in patients with ASD, some of which have aimed to alleviate ASD symptoms. Resveratrol, for example, has been shown to inhibit mitochondrial ROS generation in stimulated hippocampal astrocytes [24,25]. It is important to note that not all polyphenols can cross the blood–brain barrier and, for this reason, may have low bioavailability in the brain despite being efficiently absorbed from the intestinal lumen. However, they can still influence the composition of the gut microbiota, which plays a critical role in ASD and is often disrupted in individuals with the disorder, reflecting alterations in the microbiota–gut–brain axis [26]. Overall, current evidence suggests that polyphenols may be a class of natural compounds with potential benefits for alleviating ASD symptoms [27].

As previously mentioned, exogenous antioxidants include ascorbic acid (vitamin C), which scavenges hydroxyl and superoxide radicals; tocopherols and tocotrienols (vitamin E), which help limit lipid peroxidation in cell membranes; and phenolic antioxidants such as stilbene derivatives (e.g., resveratrol), phenolic acids, flavonoids, as well as trace elements like selenium, zinc, and others [19].

Present study aimed to investigate the effects of different types of the herbal antioxidant supplement Folium (Folium Relax, Folium P53, Folium pX, and Folium Immuno) on reducing the severity of oxidative stress in behaviorally characterized rat model of ASD. Folium is a plant-based nutraceutical product distributed by BAO Health Resources (Los Angeles, California, USA). It possesses powerful antioxidant properties. It should be note that while there is currently no cure for autism, such research is important to identify specific treatments and services that reduce symptoms and improve people's ability to function. We hypothesize that administration of different types of the Folium in an animal model of autism will restore oxidative stress markers to normal levels.

Folium possesses powerful antioxidant properties. Its spectrum of action is not attributed to a single ingredient; rather, its therapeutic effect results from the synergistic interaction of multiple components. It contains a natural blend of potent exogenous super antioxidants, primarily derived from its two main ingredients: pine bark extract and grape seed extract. These ingredients are common to all four types of Folium and are combined using a precise, high-tech process. In addition to the main ingredients, each type of Folium contains additional ingredients that differ from one another. A brief overview of the composition of this compound is provided below.

Folium Immuno: Extracts of Pine Parts (needles, bark, cones) and Grape Seed Extract. Other ingredients: Ginger Extract, Turmeric Extract, Green Tea Extract, White Pepper Extract, Mustard Extract, Pomegranate Extract (peel and seed), Microcrystalline Cellulose, Hypromellose, Magnesium Stearate, and Silicon Dioxide.

Folium Relax: Extracts of Pine Parts (needles, bark, cones) and Grape Seed Extract. Other ingredients: Folate, Ginkgo Biloba, Green Tea, Peony Root, Pomegranate Juice Powder, Pomegranate Pulp, Rhodiola Rosea, and Valerian Root.

Folium pX: is composed of all-natural fusions of powerful super-antioxidants derived from its two key ingredients: pine bark extract and grape seed extract. These are combined through a precise, high-tech process with other all-natural ingredients from plants and flowers, resulting in one of the most powerful and safest cleansing supplements available.

Folium p53: was created by combining the extract powders of Folium Immuno and Folium Relax.

Materials and Methods.

Animals:

Outbred albino rats were procured from the Laboratory Animal Division of the Ivane Beritashvili Center of Experimental Biomedicine. All experimental procedures were conducted in accordance with the European Communities Council Directive Guidelines for the care and use of Laboratory animals (2010/63/EU—European Commission) and approved by the animal care and use committee at the Ivane Beritashvili Center of Experimental Biomedicine. The rats were housed under standard laboratory conditions, with controlled temperature (22 ± 2°C), relative humidity (25 ± 5%), and a 12-hour light-dark cycle, and provided with ad libitum access to food and water. Measures were taken to minimize any discomfort to the animals and to reduce the total number of rats used.

The VPA rat model of autism:

For experimental purposes, male and female rats were allowed to mate overnight. The morning following mating, vaginal secretion was collected, and if spermatozoa were present, it was designated as day 0.5 of pregnancy. On the 12.5^{th} day of gestation, half of the pregnant rats received a single intraperitoneal injection of 500 mg/kg sodium valproate (Sigma-Aldrich) dissolved in 0.9% saline, while the other half (control group) was administered by saline alone. Females were left undisturbed to rear their offspring until postnatal day 20, at which point the male pups were weaned.

Experimental groups:

Part of the VPA-treated rats at 2 months of age received intraperitoneally (17 mg/kg, for 21 days) different types of Folium - Folium Relax, Folium P53, Folium pX, Folium Immuno. Subsequently, the 3-month-old male offspring from both the saline and VPA-exposed dams were randomly assigned to one of six experimental groups: (1) Contr – Control group with prenatal saline injection; (2) VPA – Prenatally VPAinjected group; (3) VPA+F. pX – Prenatally VPA-injected group with administration of Folium pX; (4) VPA+F. Immuno – Prenatally VPA-injected group with administration of Folium Immuno; (5) VPA+F. P53 - Prenatally VPA-injected group with administration of Folium P53; (6) VPA+F. Relax - Prenatally VPA-injected group with administration of Folium Relax.

Behavioral study: apparatus and procedures:

Three chamber social interaction test: The three-chamber social approach task was used to assess the level of sociability. This task was adapted from Kim et al. [28,29]. The social approach apparatus consists of a rectangular, three-chambered box, with each chamber measuring 60 cm (length) × 40 cm (width) × 40 cm (height). The dividing walls are made of clear Plexiglas and contain small openings (15 cm wide × 10 cm high) that allow access between the chambers. The center chamber serves as the starting location. The task consists of three sessions. In the three-chamber assay, sociability and social preference were measured. In the first session, the subject was placed in the middle compartment and habituated for 10 minutes. After habituation, a stranger animal (Stranger 1 - of the same age and strain, with no prior contact with the subject) was randomly introduced inside a wire cage in either the left or right compartment (stranger zone 1), while the other wire cage remained empty (empty zone) for the 10-minute sociability test. Time spent in the stranger zone 1 and in the empty zone, also sniffing time of a stranger 1 animal and the empty cage was measured. Social preference test was conducted for another 10 min directly after the termination of the sociability test. Another stranger animal (a stranger 2) was introduced in the wire cage of the opposite compartment (stranger zone 2) and same parameters were measured as with the previous session to find a preference of the subject animals to the novel over the familiar animal in the wire cage.

Elevated plus-maze: Anxiety-related behavior was evaluated using the elevated plus-maze test, which was adapted from Holmes et al. [30] and Yang et al. [31]. The maze consists of two open arms (50 × 10 cm), two closed arms (50 × 10 × 30 cm), and a central area (10 × 10 cm). Rats were placed in the central area and allowed to explore the maze freely for 5 minutes. To eliminate odor traces, the apparatus was cleaned with a 70% ethanol solution and then dried with a cloth after each test. Over the subsequent 5-minute test period, the following parameters were recorded: the number of entries into the open and closed arms, and the time spent in both the open and closed arms. Based on these measurements, the following variables were calculated: the ratio of time spent in the open arms relative to the total time spent in both the open and enclosed arms, and the total number of entries into both the open and enclosed arms. An entry was defined as the rat entering one of the arms with all four paws. For each rat, the number of entries and time spent in the open and closed arms of the elevated plus-maze, were scored offline from video recordings using stopwatches. Scoring was performed by an individual who was blinded to the treatments and did not participate in the animal testing.

Oxidative stress assessment:

For the global assessment of oxidative stress in the body, we used the FRAS5 (H&D) photometric analytical system, which is designed to evaluate overall oxidative stress in biological systems. This system allows for the determination of pro-oxidant status in biological samples (heparinized blood plasma) using the rapid d-ROMs test, and antioxidant status using the PAT test. The principle involves measuring the absorbance of a sample solution using a monochromatic light beam in a cuvette. After obtaining the absorbance value, the instrument automatically converts it to the appropriate measurement units using its builtin software [32]. The rapid d-ROMs photometric test allows for the assessment of pro-oxidant status in a biological sample by measuring the concentration of hydroperoxides (ROOH). The test is based on the principle of the Fenton reaction. By mixing the biological sample with an acidic buffer (reagent R1), transition metal ions (such as iron or copper) decompose hydroperoxides, generating new radicals, including hydroperoxyl (ROO*) and alkoxyl (RO*) radicals. By adding a chromogen (N, N-diethylparaphenylenediamine, reagent R2) to the sample—which can donate an electron and changes color upon oxidation by free radicals—it becomes possible to determine the amount of hydroperoxides in the biological sample through photometric reading using the FRAS5 analytical device. The d-ROMs test

results are expressed in Carratelli Units, where 1 CARR U is equivalent to 0.08 mg/100 ml H_2O_2 [33].

The PAT test assesses the antioxidant capacity of plasma by measuring its iron recovery capacity. Recovery of iron ions at low pH results in a color change, which is also evaluated photometrically using the integrated analytical device FRAS5. PAT test results are expressed in Cornelli Units, where 1 Cor=1.4 µmol/L of ascorbic acid. During the PAT test, a small amount of plasma (10 µl) is added to a colored solution obtained by mixing a source of iron ions (reagent R2 – FeCl3 ferric chloride) with a chromogen (reagent R1 – a chromogenic mixture containing thiocyanate). After 1 minute of reading at 370C, the solution will change color and the intensity of this chromatic change will be directly proportional to the ability of the plasma to reduce ferrous ions to ferrous ions. By photometrically evaluating the intensity of bleaching, the amount of reduced iron ions can be adequately estimated, allowing effective measurement of antioxidant capacity [34].

One of the main features of the FRAS system is the integration of the values of the d-ROMs test and the PAT test into one value - the Oxidative Stress Index (OSI), which provides a simple and convenient but comprehensive picture of the oxidative stress status of the biological system. The value of the OSI index shows the deviation of the oxidative balance from the normal state (zero value); It is the perfect balance between the pro-oxidant and antioxidant components of the oxidative balance. The OSI index increases proportionally at any level of oxidative imbalance; its increase can be caused by an increase in pro-oxidant species (highlighted by the results of the d-ROMs test), or by a decrease in antioxidant protection (highlighted by the results of the PAT test). The higher the OSI index, the greater the deviation from the normal state. The OSI index does not replace d-ROM and PAT test results but complements and enhances their value [35].

Arterial Blood Pressure:

Blood pressure was discretely measured by the "bloodless" method using the «Neurobotics Sistola» device, with a small cuff placed on the animal's tail, which was warmed to 37°C. The device's software displayed the data on a computer monitor, recording systolic and diastolic blood pressure as well as heart rate.

Statistical analysis:

Statistical analysis was performed using SigmaStat statistical software. All the data were expressed as a mean ± standard error of the mean (SEM). Differences were considered significant when $p < 0.05$. Data for all behavioral parameters as well as changes in markers of oxidative stress were statistically analyzed by one- or two-way ANOVA followed by post hoc comparisons. Student's t-test was used to compare the mean values of two independent groups.

Results.

Elevated plus-maze:

The anxiety level in rats of all groups was assessed in the elevated plus-maze. Two-way ANOVA showed significant effect of group ($F_{1,21}$ = 9.601; P <0.006), significant effect of arms (open, closed) ($F_{1,21}$ = 463.557; P <0.001) and significant interaction between groups and arms ($F_{1,21}$ = 28.562; P <0.001). Post hoc (Holm-Sidak method) analyses revealed significant differences between the time spent in open and closed arms in control and VPA-treated rats (control: t = 19.931; P <0.000; VPA: t = 10.958; P <0.000). There was a significant difference in time spent in the closed arm between control and VPA-treated rats (t = 5.970; P <0.000) and no significant difference in the open arm (t = 1.588; P = 0.130; Figure 1A). After the results were obtained, to more accurately identify differences in anxiety between groups the following variables were calculated: the ratio of time spent in the open arms relative to the total time spent in both the open and enclosed arms, and the total number of entries into both the open and enclosed arms. Statistical analysis (Student's t-test) revealed significant differences between groups in both cases: the ratio of time spent in open arms was higher in the VPA group compared with the control group (t = -2.447, P = 0.037; Figure 1B) and the total number of entries was higher in the VPA group compared with the control group (t = -3.694; P = 0.002; Figure 1C).

Social interaction task:

Sociability: After a habituation session to check social behavior, two types of social behavior tests were performed using a three-chamber apparatus. In the second session, we assessed sociability by measuring time spent in the chambers, as well as the sniffing time of stranger1 rat or an empty wire cage. Statistical analysis (t-test) showed that control rats spent significantly more time in the chamber with the stranger 1compared with the empty chamber (t = -3.582; P = 0.007). In contrast VPA-treated rats spent significantly less time in the chamber with the stranger 1 rat compared with empty chamber (t = 7.059; P = <0.001; Figure 2A). Statistical analysis showed that control rats in contrast to VPA-treated rats spent significantly more time sniffing the stranger 1 (control: t = 2.591; P = 0.032; VPA: t = -0.132; P = 0.899; Figure 2B).

Social preference. In the social preference phase, with the introduction of an unfamiliar (stranger 2) rat into another wire cage, statistical analysis (t-test) revealed that control rats spent significantly more time in chamber containing stranger 2 than in the chamber with stranger 1 (t = -2.734; P = 0.026). Differences between time spent in chambers was not significant in VPA treated rats (t = 0.0358; P = 0.972; Figure 2C). Statistical analysis revealed the same results comparing sniffing times spent with stranger 1 and stranger 2 in control (t = -2.576 P = 0.033) and VPA treated rats (t = -0.748; P = 0.476; Figure 2D).

Systolic blood pressure:

One-way ANOVA showed a significant effect of group ($F_{5,35}$ = 90.947; P < 0.001). Post hoc (Tukey Test) analyses revealed significant differences between VPA and Control, VPA and VPA+F. Relax, VPA and VPA+F. pX, VPA and VPA+F. P53, VPA and VPA+F. Immuno groups (P < 0.001, in all cases). There were significant differences between Control and VPA+F. Relax (P = 0.001), Control and VPA+F. Immuno (P = 0.010) groups, and no significant differences between Control and VPA+F. pX (P = 0.550) and Control and VPA+F. P53 (P = 0.575) groups (Figure 3A).

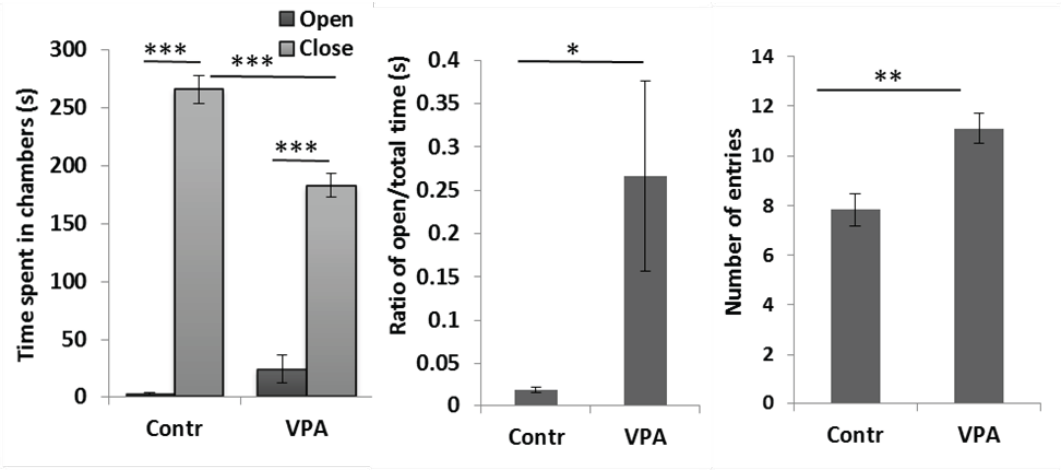

Figure 1. Effects of VPA on locomotor activity and anxiety level assessed and elevated plus-maze task. *(A) Histograms show the time spent in open and closed arms in the elevated plus-maze apparatus by control and VPA-treated rats; (B) Ratio of time spent in open arms to total time spent in both open and closed arms; (C) Number of entries in both (open, closed) arms. Note: the results showed that prenatal exposure of rats to VPA reduced anxiety and increased locomotor activity assessed in elevated plus-maze tasks. Data are presented as mean ± SEM; *** P < 0.001, **P < 0.01, * P < 0.05.*

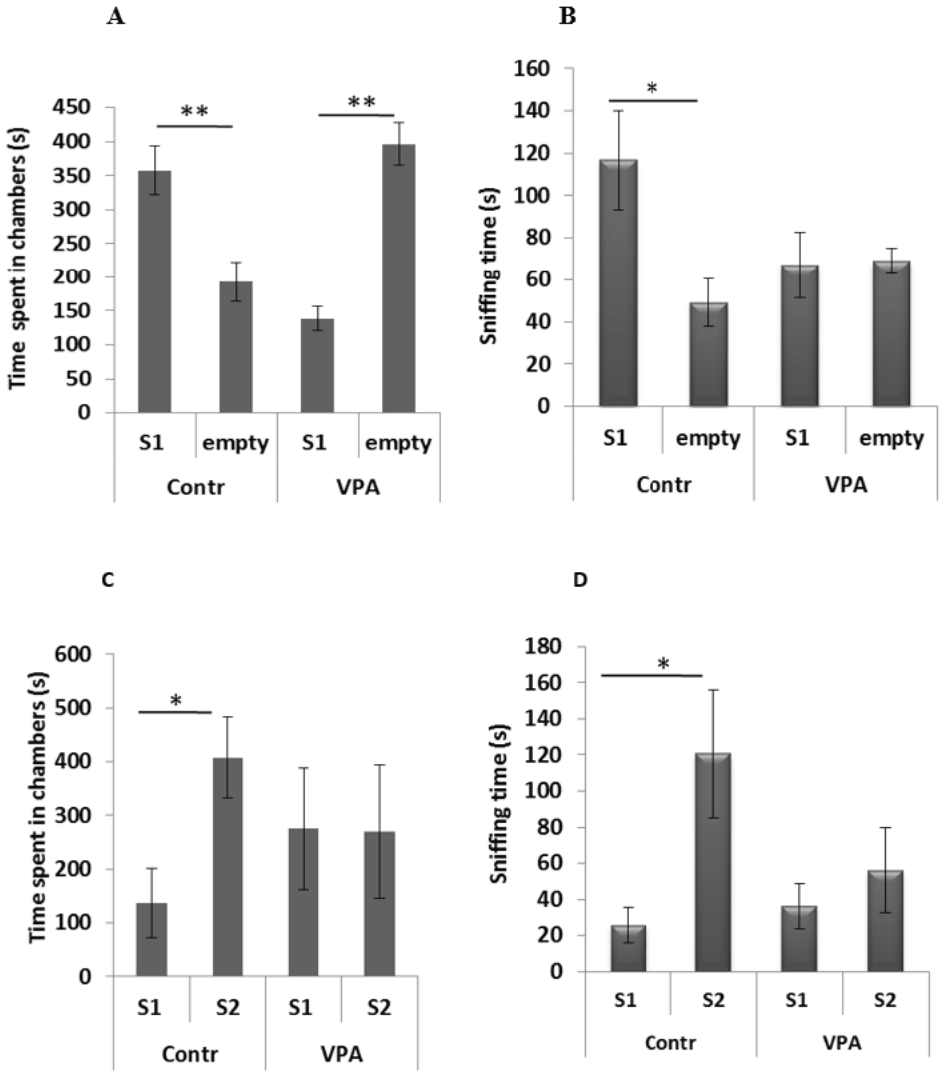

Figure 2. Sociability phase: histograms showing *(A) the time spent in chambers and (B) sniffing time of stranger1 or empty wire cage by control and VPA-treated rats. Social preference phase: histograms showing (C) the time spent in chambers and (D) sniffing time of stranger 1 or stranger 2 by control and VPA-treated rats. Note: the results of behavioral studies showed that prenatal VPA treatment reduces social exploration, impairs social novelty preference in the three-chamber task. Data are presented as mean ± SEM; *P <0.05, ** P < 0.01.*

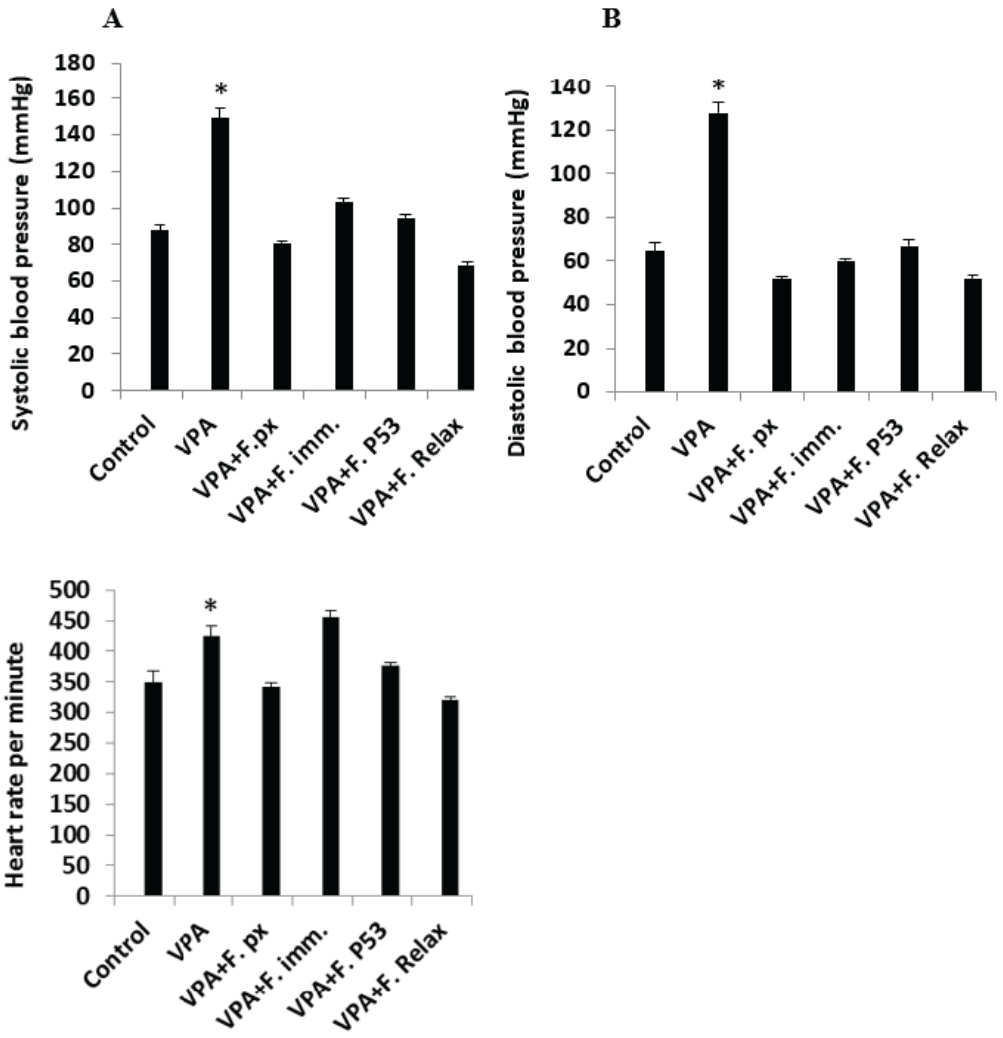

Figure 3. Systemic arterial pressure: *A – systolic blood pressure; * P < 0.001 - VPA vs all groups. B – diastolic blood pressure; * P < 0.001 - VPA vs all groups. C – heart rate; * P < 0.001 - VPA vs Control, VPA vs VPA+F. Relax, VPA vs VPA+F. pX groups. Note: a 21-day intraperitoneal injection of all four supplements led to the return of blood pressure (systolic SBP, diastolic DBP) to normal range; as for the heart rate, only injection of F. Immuno did not show its decrease. Data are presented as mean ± SEM.*

Diastolic blood pressure:

One-way ANOVA showed a significant effect of group ($F_{5,35}$ = 102.766; P < 0.001). Post hoc (Tukey Test) analyses revealed significant differences between VPA and Control, VPA and VPA+F. Relax, VPA and VPA+F. pX, VPA and VPA+F. P53, VPA and VPA+F. Immuno groups (P <0.001, in all cases). There were significant differences between VPA+F. P and VPA+F. px (P = 0.008), VPA+F. P and VPA+F. Relax (P = 0.010) groups, and no significant differences between Control and VPA+F. P53 (P = 0.992) and Control and F. Immuno (P = 0.850) groups (Figure 3B).

Heart rate:

One-way ANOVA showed a significant effect of group ($F_{5,35}$ = 20.257; P < 0.001). Post hoc (Tukey Test) analyses revealed significant differences between Control and VPA and Control and VPA+F. Immuno groups, as well as between VPA vs. VPA+Folium pX and VPA vs. VPA+Folium Relax (P < 0.001, in all cases). Statistical analyses revealed no significant differences between Control and VPA+F. P53 (P = 0.588), Control and VPA+F. Relax (P = 0.446) and Control and VPA+F. pX (P = 0.993) groups (Figure 3C).

D-ROMs levels (U. CARR): One-way ANOVA showed a significant effect of group ($F_{5,31}$ = 11.951; P < 0.001). Post hoc (Tukey Test) analyses revealed significant differences between VPA and Control (p < 0.001), VPA and VPA+F. Relax (P = 0.001), VPA and VPA+F. Immuno (P = 0.003) and VPA and VPA+F. P53 (P = 0.023) groups and no significant differences between VPA and VPA+F. pX (P = 0.940) groups. There were significant differences between the Control and VPA+F. pX (P < 0.001) groups and no significant differences between the Control and VPA+F. P53 (P = 0.846) and Control and VPA+F. Immuno (P = 1.000) groups (Figure 4A).

PAT levels (U.COR):

One-way ANOVA showed a significant effect of group ($F_{5,31}$ = 3.569; P = 0.014). Post hoc (Tukey Test) analyses revealed significant differences between VPA and Control groups (p = 0.019). There were no significant differences between any other groups (p > 0.05, in all cases; Figure 4B).

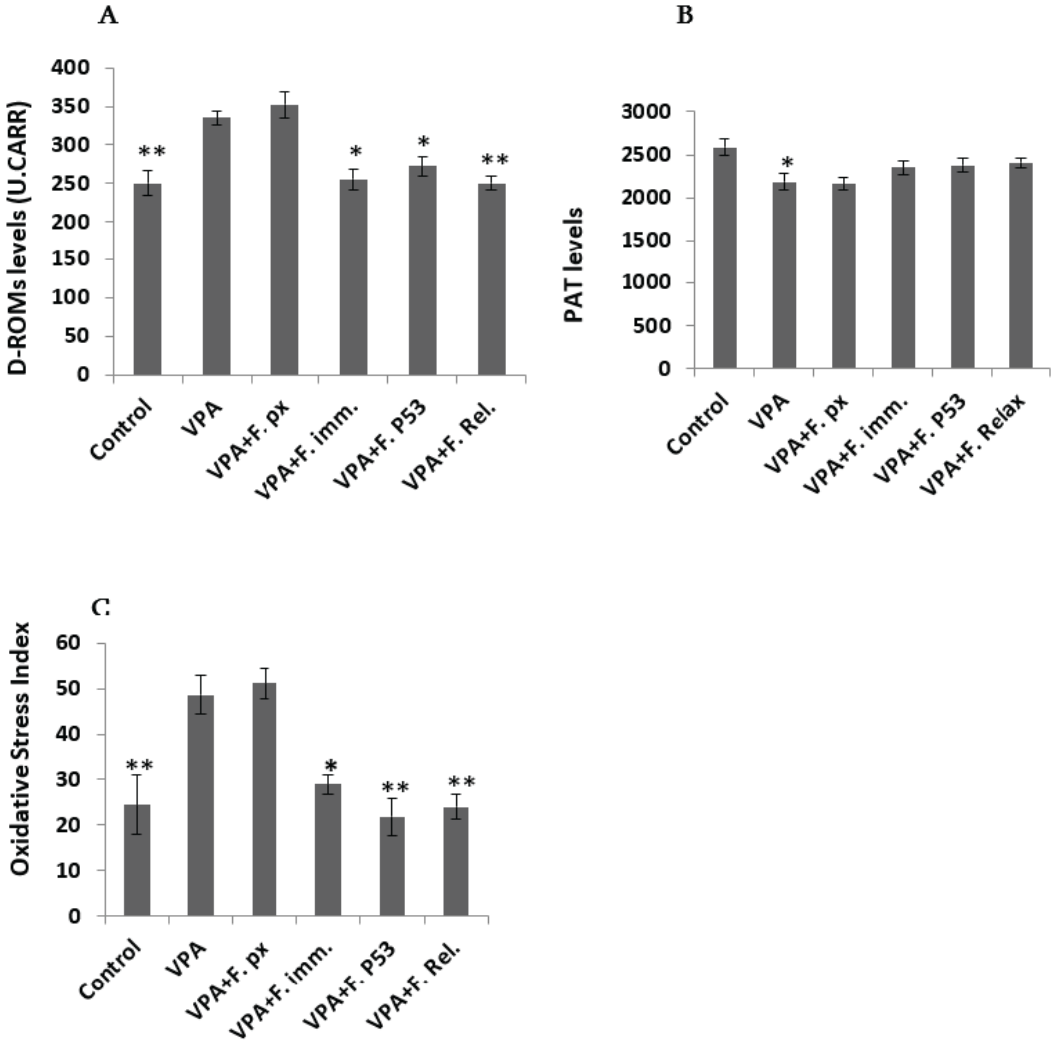

***Figure 4. Oxidative and antioxidative activity: A** - amount of free radicals (* P < 0.01 - vs VPA; ** P < 0.001 - vs VPA); **B** - amount of antioxidants (* P < 0.02 - VPA vs Control); **C** - oxidative stress index (*P < 0.01 - VPA vs VPA+F. Immuno, **P < 0.01 - VPA vs Control, VPA+F. P53 and VPA+F. Relax). Note: a 21-day intraperitoneal injection of various antioxidant supplements Folium (F. Relax, F. P53, F. pX, F. Immuno) causes different effects; in contrast of other supplements, Folium pX not showed positive effects. Data are presented as mean ± SEM.*

Oxidative stress index (OSI):

One-way ANOVA showed a significant effect of group ($F_{5,31}$ = 9.528; P <0.001). Post hoc (Tukey Test) analyses revealed significant differences between VPA and Control (P = 0.003), VPA and VPA+F. P53 (P = 0.002), VPA vs VPA+F. Immuno (P < 0.01) and VPA and VPA+F. Relax (P = 0.004) groups. There were no significant differences between any other groups (p > 0.05, in all cases; Figure 4C).

Discussion.

The present study is the first to test the effects of different types of herbal antioxidant supplement Folium (Folium Relax, Folium P53, Folium pX, and Folium Immuno) on reducing the severity of oxidative stress in a behaviorally characterized rat model of ASD. The results indicate that prenatal administration of VPA to rats can induce ASD-like behavioral patterns accompanied by an increase in the severity of oxidative stress.

The results of behavioral studies showed that prenatal VPA treatment reduces social exploration, impairs social novelty preference in the three-chamber task, and reduces anxiety while increasing locomotor activity in the elevated plusmaze test. It should be noted that the elevated plus-maze is a behavioral method used to assess anxiety-like behavior. In our study, a reduced anxiety level in VPA-exposed rats compared with control rats was observed in the elevated plus-maze test. Our results are consistent with those of some other studies [36,37]; however, they differ from the findings of other reports [38,39]. Such discrepancies may have several causes, including differences in animal strain, age, and other methodological factors, such as sample size. In the present study, the presence of behavioral changes characteristic of the valproate model of ASD was confirmed in experimental rats, which were then used in further experiments.

The results of the present study showed that in rats with autism spectrum disorder (VPA model), levels of free radicals (d-ROMs) and the oxidative stress index (OSI) were increased, indicating the presence of oxidative stress. Notably, treatment with Folium Relax, Folium P53, Folium pX, and Folium Immuno demonstrated the following: (a) Injection of all these supplements led to the restoration of blood pressure (systolic

SBP, diastolic DBP) to the normal range; regarding heart rate, only the injection of Folium Immuno did not result in a decrease. (b) There was no statistically significant difference in heart rate between the VPA group and the VPA+Folium P53 or VPA+Folium Immuno groups. (c) The d-ROMs level showed no significant difference between the VPA group and the VPA+Folium pX group. (d) The OSI in the VPA group was not significantly different from that in the VPA+Folium pX group. It is noteworthy that Folium pX showed fewer positive effects compared to the other substances, and, in general, the observed differences warrant special attention. It can be assumed that the varying effects of these substances are due to Folium being a multi-component supplement with different ingredient compositions. Accordingly, its therapeutic effects likely result from the synergistic interaction of multiple components.

A brief overview of the composition of the root and other substances contained in the folium are provided below.

Pine bark extract powder (from pine bark and cones) contains a unique combination of procyanidins, bioflavonoids, and phenolic acids that combat oxidative stress and free radical damage. These compounds help protect immune cells from oxidative damage, supporting their optimal function. The proanthocyanidins in pine bark extract powder enhance the body's antioxidant defences by increasing the production of enzymes such as superoxide dismutase and glutathione peroxidase, which provide cellular antioxidant protection.

Grape seed extract is a powerful antioxidant. The exogenous antioxidants it contains—such as procyanidins, resveratrol, and vitamins E, A, and C—help eliminate free radicals from the body and enhance immune function.

Grape seed oil is rich in vitamins E, A, C, B1, B2, B3, B6, B9, B12—and minerals such as potassium, sodium, calcium, and iron. It possesses anti-inflammatory, regenerative, antithrombotic, hypolipidemic, and anticarcinogenic properties. The resulting hydrophilic extract powder, characterized by its content of phenolic compounds, flavan-3-ols, and leucoanthocyanidins, is rich in biologically active substances.

Curcuminoids (polyphenolic compounds) are powerful antioxidants that neutralize free radicals and strengthen the body's antioxidant defense system.

Gingerol inhibits the formation of nitric oxide, which in turn prevents the formation of the harmful free radical peroxynitrite in the body.

Green tea is a powerful antioxidant due to its rich content of polyphenolic compounds, particularly epigallocatechin-3gallate (EGCG). The combination of caffeine and L-theanine in green tea is believed to synergistically enhance mood and cognitive performance.

Pomegranate is a powerful antioxidant. This is due to the plant substances contained in its composition: polyphenols (ellagic acid) and tannins - punicalagin and punicalin. However, the spectrum of action of pomegranate is not associated with just one ingredient, so the healing effect of pomegranate is due to the synergistic interaction of many ingredients.

Mustard seeds are a rich source of fiber, selenium, magnesium, manganese, and other nutrients. They also contain beneficial plant compounds such as glucosinolates, isothiocyanates, sinigrin, and carotenoids, which offer important therapeutic properties. Glucosinolates are known for their antioxidant and anti-inflammatory effects.

Valerian root contains essential oils, the primary component of which is a complex ester of borneol and isovaleric acid, along with free valeric acid.

Ginkgo biloba, containing flavone glycosides and terpene lactones, helps reduce the permeability of blood vessel walls and prevents platelet aggregation. By regulating metabolism, these compounds protect cells and tissues from damage caused by oxygen deprivation. Ginkgo biloba also improves memory due to its strong antioxidant properties, protects cell membrane lipids from oxidative stress, and exerts a neuroprotective effect.

White peony root extract contains active compounds such as paeoniflorin, paeonol, and polysaccharides. Studies have shown that paeoniflorin in white peony extract powder can significantly inhibit the production of inflammatory cytokines, chemokines, and other pro-inflammatory mediators, thereby helping to reduce inflammation. It also has analgesic properties.

As we can see, the spectrum of action of Folium is not limited to a single ingredient. Its main components - pine bark and grape seed extracts - are common to all four types of Folium. The differences lie in the additional ingredients each formulation contains. It is believed that the healing effects of Folium result from the synergistic interaction of its various components. However, the manufacturer does not provide a detailed list of these additional ingredients, stating only that they are all-natural plant- and flower-based substances. Therefore, the relatively modest antioxidant properties of Folium pX may be attributed to this limited transparency or variability in its composition.

Conclusion and Future Directions.

Taken together, we provided evidence that prenatal administration of VPA to rats can induce ASD-like behavioral patterns, accompanied by increased oxidative stress, as evidenced by higher levels of free radicals (d-ROMs) and the oxidative stress index (OSI), indicating the presence of oxidative stress. The results of the experiments showed that treatment with Folium Relax, Folium P53, Folium pX, and Folium Immuno produced noticeably different effects—mostly positive in some cases, and no effect in others. We assumed that the varying effects of these substances result from differences in the composition of the various types of folium. Their effects are not due to a single ingredient; instead, the therapeutic benefits come from the synergistic interaction of numerous components, including all the substances mentioned above. In recent years, various nutritional interventions have been developed for individuals with ASD to improve their quality of life. Innovative treatments targeting ASD symptoms and behavioral abnormalities may benefit from antioxidant-based interventions, either as dietary supplements or through food. Positive effects can be achieved by providing specific antioxidants or by consuming foods rich in bioactive nutritional compounds, which may exert additive or synergistic effects. It is worth noting that we conducted preliminary experiments to evaluate the effectiveness of these supplements in improving metabolic imbalances, such as total cholesterol, HDL-C, LDL-C, and triglyceride levels, and obtained promising results. Accordingly, future research in

this area is planned. Finally, we suggest that further research is needed to elucidate the underlying causes of the identified discrepancies and to draw conclusions about the positive effects of different types of folium on oxidative processes. This study highlights the need for further research at scientific research institutions and medical schools.

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