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Protective activities of some bioactive components from *Salvia officinalis* extracts and essential oil in acetaminophen-induced model of acute liver and kidney injury in mice

Seyed Ali Sobhanian*✉, Abbas Ahmadi, Mehrdad Roghani***, Parinaz Hassanlo**, Shima Khamoushi****

**Department of Medicinal Chemistry, TeMS.C., Islamic Azad University, Tehran, Iran*
***Department of Chemistry, Ka.C., Islamic Azad University, Karaj, Iran*
****Neurophysiology Research Center, Shahed University, Tehran, Iran*

Abstract. *Drugs and toxic compounds-induced liver and kidney injuries are mostly responsible for hepatic and renal dysfunction. Many treatments have recently been proposed for these injuries, like chemicals and traditional medicines. Thus, looking for new classes of natural and safe compounds is a global demand. The genus Salvia from the Lamiaceae family are well-studied and vastly used in traditional medicine. The plant contains a variety of secondary metabolites, like terpenoids, which have shown many pharmacological activities. Based on the various reported pharmacological effectiveness of Salvia officinalis L. (sage, I), the protective effects of some extracts and essential oil of this plant by the number of polar and nonpolar organic as well as aqueous solvents on the liver and kidney injury in acetaminophen-induced mice investigated in this research. The results showed that chloroformic and carbon tetrachloride extracts of Salvia officinalis had the best effects for protecting against kidney injury, while for liver, chloroformic, ethanolic, and hydroethanolic extracts also had such effects. It can be concluded that extraction of Salvia officinalis with polar and nonpolar organic and aqueous solvents could separate various bioactive components which appropriately and remarkably improve some serum markers following acetaminophen-induced liver and kidney damage in mice.*

Keywords: *liver and kidney injuries, acetaminophen-induced model, traditional medicine, Salvia officinalis (sage), polar and nonpolar extracts*

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ФИЗИКО-ХИМИЧЕСКАЯ БИОЛОГИЯ

Научная статья
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Защитное действие некоторых биоактивных компонентов из экстрактов и эфирного масла шалфея лекарственного при остром поражении печени и почек у мышей, вызванном ацетаминофеном

С.А. Собханян*✉, А. Ахмади, М. Рогани***, П. Хассанло**, Ш. Хамуши****

**Исламский университет Азад, филиал в г. Тегеране, Тегеран, Иран*
***Исламский университет Азад, филиал в г. Карадже, Карадж, Иран*
****Центр нейрофизиологических исследований, Университет Шахед, Тегеран, Иран*

Аннотация. *Повреждения печени и почек, вызванные лекарственными препаратами и токсичными веществами, являются основной причиной печеночной и почечной дисфункции. В последнее время предложено множество способов лечения такого рода поражений – как с помощью химических препаратов, так и при помощи методов традиционной медицины. В связи с этим поиск новых классов природных и безопасных соединений для данных целей является настоятельной потребностью. Род Salvia семейства яснотковых (Lamiaceae) хорошо изучен и широко используется в традиционной медицине. Растение содержит множество вторичных метаболитов, таких как терпеноиды, которые обладают разнообразной фармакологической активностью. На основании многочисленных данных о фармакологической эффективности Salvia officinalis L. (шалфея лекарственного, l) в данном исследовании изучено защитное действие некоторых экстрактов и эфирного масла этого растения при поражении печени и почек у мышей, подвергшихся воздействию ацетаминофена. Результаты показали, что наилучшее защитное действие при поражении почек оказывают хлороформенный и четыреххлористоуглеродный экстракты шалфея лекарственного, при поражении печени эффективны хлороформенный, спиртовой и водно-спиртовой экстракты шалфея лекарственного. Можно сделать вывод, что экстракция шалфея лекарственного полярными и неполярными органическими и водными растворителями позволяет выделить различные биоактивные компоненты, которые значительно улучшают некоторые сывороточные маркеры повреждения печени и почек, вызванного ацетаминофеном у мышей.*

Ключевые слова: *повреждения печени и почек, ацетаминофен, традиционная медицина, Salvia officinalis (шалфей), полярные и неполярные экстракты*

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INTRODUCTION

As vital metabolic organs, the liver and kidneys are vulnerable to oxidative stress, which seriously impacts overall health. In recent years, studies have illustrated that oxidative stress can cause liver damage and kidney fibrosis. Thus, it is crucial to look for safe and natural antioxidants to protect the normal function of tissues and organs like the liver and kidneys [1–3].

In general, toxic compounds cause damage to the liver and kidneys as the target organs. These organs actively detoxify and handle endogenous and exogenous chemicals [4, 5].

Today, there is much interest in traditional medicines and herbal-based treatments all over the world. Thus, numerous experimental and clinical studies are being practiced on medicinal plants and there is a requirement for updating and integrating the findings [6].

Salvia officinalis (Lamiaceae family, sage, l) is known as a medicinal and aromatic plant for its richness of natural active substances [7]. Because of its antioxidant [8] and

anti-inflammatory [9] properties, sage extracts exhibit lots of beneficial health effects like phytoestrogenic [10], neuroprotective [11], anti-microbial [12], and anticancer [13] activities. More importantly, the plant has been vastly used in the treatment of most gastrointestinal diseases such as diarrhea and dyspepsia [14].

In recent years, lots of studies have been practiced to document the traditional usages of *S. officinalis* and to find novel biological effects of the plant. This research has revealed a range of pharmacological activities such as anti-nociceptive, antimutagenic, anti-dementia, hypoglycemic, and hypolipidemic effects [6].

This plant is an inexhaustible reservoir of chemical compounds like terpenes, alkaloids, carbohydrates, and phenolic compounds [15].

It has protective effects against liver and kidney injuries caused by high-fat diets, which have been common among most people in recent years. Therefore, it seems that using medicinal plants with lipid-lowering effects to improve liver and kidney function is highly requested [16].

In this work, according to the mentioned pharmacological activities of this plant, the therapeutic effects of different polarities' extracts and essential oil of *S. officinalis* with organic and aqueous solvents studied on liver and kidney damages caused by acetaminophen and the results were compared to the control and other groups in mice.

MATERIAL AND METHODS

Ethanol, carbon tetrachloride, chloroform as well as other chemicals were obtained from Merck (Darmstadt, Germany) as well as Sigma-Aldrich chemical companies (USA).

Gas chromatographic analysis was conducted on an Agilent 7890N chromatograph paired with a mass-spectrometer 5975C, MODE EI. A capillary column, HP-5MS (30 m × 0.25 mm i.d.; the film thickness 0.25 μm) was applied. Its column operating conditions were as follows: 60 °C for 2 min and then 7 °C/min to 280 °C. The carrier gas was helium at the rate of 3 ml/min. Samples (1 μl) of diluted essential oils were manually injected.

The parts of extracts were identified through their retention time, retention indices which are relevant to C_6-C_{35} *n*-alkanes computer matching with the Wiley / National Institute of Standards and Technology library, and also by comparing their mass spectra with the genuine samples or with available data in the literature. The proportion of composition of the identified compounds was evaluated from the gas chromatography peak area with no correction factor and calculated relatively.

Leaves of *S. officinalis* are obtained from the local market in Tehran and authenticated in the herbarium laboratory of the agriculture department of our university (Voucher Numbers: 5997). This part of the sage was protected from sunlight. Then air-dried in the shade, powdered well, then extracted by maceration with the mentioned solvents with various polarities (70% ethanol-water, 96% ethanol-water, chloroform, carbon tetrachloride and aqueous) separately for 72 hours (100 g of the powdered plant macerated in 500 ml solvent). The mixtures were filtered and concentrated to the yield extracts applied for gas chromatography – mass spectrometry (GC – MS) analysis and protective effects investigation on acetaminophen-induced liver and kidney damages in mice.

Male NMRI mice (n = 56) weighing 19 to 24 g (Pasteur's Institute in Tehran, Iran) were kept in an air-conditioned animal houseroom at 22±2 °C and provided with a standard diet and tap water. The procedures were in compliance with National Institutes of Health guidelines for the care and use of research animals.

Mice were divided into controls, acetaminophen, acetaminophen + chloroform, acetaminophen + carbon tetrachloride, acetaminophen + hydroalcoholic 70%, acetaminophen + hydroalcoholic 96% as well as acetaminophen + essential oil groups. These extracts (200 mg/kg) are dissolved in the cold physiologic saline and fed daily by gavage to mice one week before acetaminophen injection (300 mg/kg, i.p.) for liver and kidney toxicity.

Liver and kidney homogenates were prepared based on a published report [17].

The homogenate protein content was determined according to a published report[1]. Then, liver & kidney serum markers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutathione (GSH), catalase (CAT), malondialdehyde (MDA), blood urea nitrogen (BUN), and creatinine were analyzed by standardized procedures using commercial kits (Pars Azmoon Company, Tehran, Iran, and Kia Zist, Hamedan, Iran), following the instrument manufacturer's protocol.

All the data were illustrated as means ±*SEM*. Statistical analysis was conducted with the aid of Graph Pad Prism

Quantitative analysis of some phytochemicals in hydroethanolic 96%, hydroethanolic 70%, chloroform, carbon tetrachloride extracts, and essential oil of *Salvia officinalis* identified by gas chromatography – mass spectrometry, %

Составы водно-спиртового 96%-го и 70%-го, хлороформенного, четыреххлористоуглеродного экстрактов и эфирного масла шалфея лекарственного, идентифицированные методом газовой хроматографии – масс-спектрометрии, %

Chemical family	Hydroethanolic 96%	Hydroethanolic 70%	Chloroform	Carbon tetrachloride	Essential oil
Monoterpenes & sesquiterpens	26.71	20.85	42.93	40.73	66.78
Alcohols	14.54	–	14.11	20.67	5.94
Alkaloids	4.45	–	3.54	3.34	–
Vitamins	9.78	–	5.81	7.40	–
Phenols	–	4.32	–	–	–
Imidazoles	–	9.37	–	–	–
Others	44.52	65.46	33.61	27.86	27.28

[1]Becker J.M., Caldwell G.A., Zachgo E.A. Biotechnology: a laboratory course. San Diego: Academic Press, 1996. 283 p.

Software version 8.0 and one-way analysis of variance (ANOVA), followed by a coupled Tukey post-hoc statistical test. The Kolmogorov-Smirnov test was used for verification of normal distribution. A statistical *p*-value under 0.05 was considered significant.

RESULTS AND DISCUSSION

The desired titled extracts and essential oil were made from the maceration of *Salvia officinalis* L (sage, I) in the stated organic and aqueous solvents. GC-MS results for each extract and essential oil were summarized in Table.

As it was shown, in *S. officinalis* extracts, monoterpenes and sesquiterpens, alcohols, alkaloids, vitamins and phenols with many pharmacological activities [18–25] were more abundant compared with other compounds extracted by solvents.

Mortality (death rate), morbidity (known as any abnormal condition or behavior), irritability (a condition of aggressiveness or raised response to handling), and other relevant abnormal states were witnessed in the experimental animals. However, the motor coordination index (measured by Rota-rod apparatus, Harvard, UK) did not indicate any significant differences among the treated animals.

Figure 1 shows the results of serum BUN in different groups. As it can be seen, in the acetaminophen group, a clear and significant increase in BUN was obtained compared with the control group ($p < 0.001$). A similar significant rise in BUN was obtained in acetaminophen groups treated with 96% ethanol, carbon tetrachloride, chloroform extracts, and essential oil compared with the control group. On the other hand, a significant decrease in BUN was obtained in the acetaminophen groups treated with 70% ethanol ($p < 0.01$), 96% ethanol ($p < 0.01$), and chloroform ($p < 0.05$) extracts compared with the acetaminophen group. Also, the reduction of BUN in the two acetaminophen groups treated with carbon tetrachloride extract, or essential oil was not significant compared with the acetaminophen group.

Figure 2 shows the results of serum creatinine levels in various groups. As can be seen, in the acetaminophen group, a statistically significant increase in serum creatinine was obtained compared with the control group ($p < 0.01$). A similar significant rise in creatinine was obtained in the acetaminophen group treated with carbon tetrachloride extract compared with the control group ($p < 0.05$). On the other hand, a significant decrease in creatinine was obtained in the acetaminophen groups treated with 70% ethanol ($p < 0.01$), 96% ethanol ($p < 0.05$), and chloroform ($p < 0.05$) extracts compared with the acetaminophen group. Additionally, in the two acetaminophen groups treated with carbon tetrachloride extract and essential oil, the decrease in serum creatinine level was not significant compared with the acetaminophen group.

Figure 3 shows the results related to the tissue level of MDA as an index of lipid peroxidation in kidney tissue in different groups. As can be seen, in the acetaminophen group, a strong and significant increase in MDA was observed compared with the control group ($p < 0.001$). A similar significant rise in MDA was obtained in acetaminophen groups treated with essential oil or extracts to a lesser extent. Also, there was a significant fall in MDA in the acetaminophen groups treated with 70% ethanol

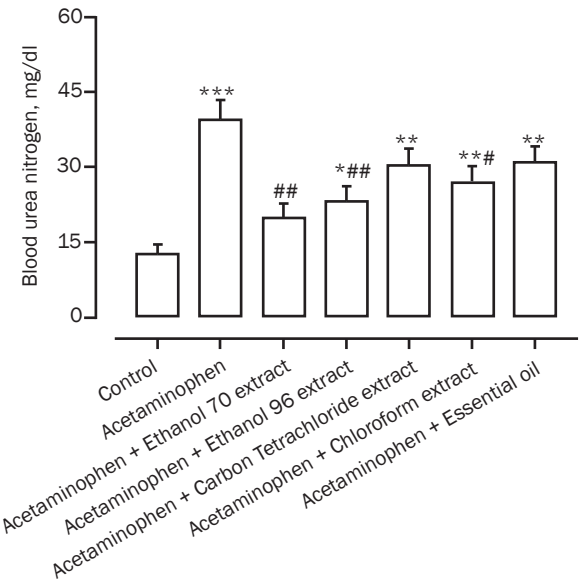

Fig. 1. Serum blood urea nitrogen level in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 1. Уровень азота мочевины сыворотки крови в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

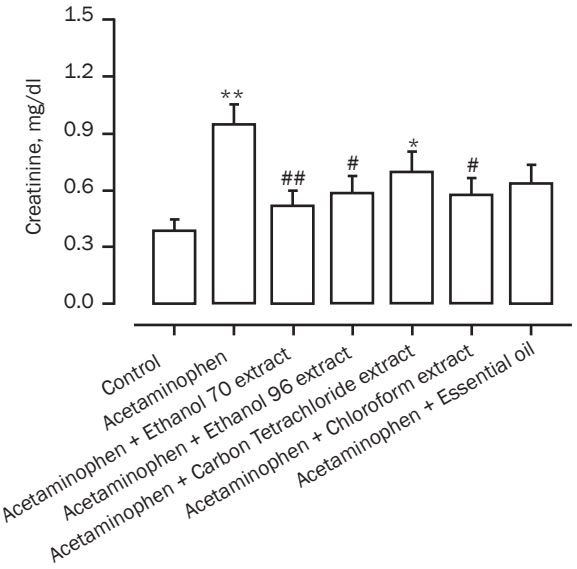

Fig. 2. Serum creatinine level in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 2. Уровень креатинина сыворотки крови в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

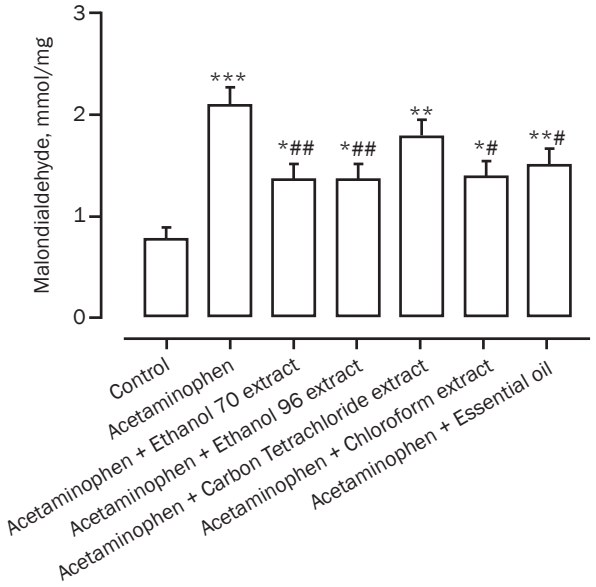

Fig. 3. Renal malondialdehyde levels in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 3. Уровень малонового диальдегида в почках в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

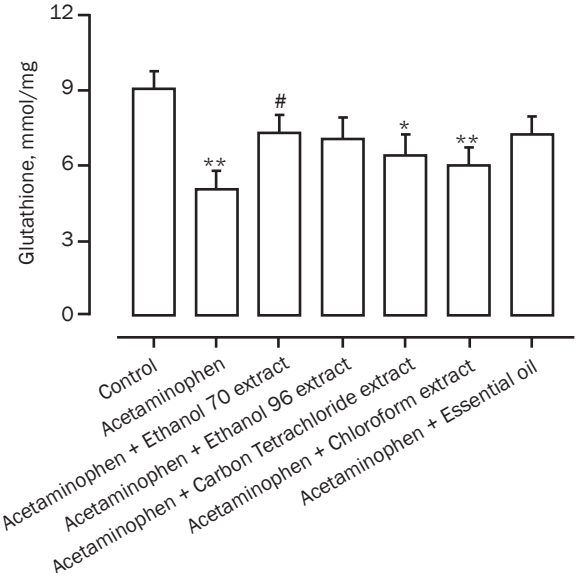

Fig. 4. Renal glutathione levels in different groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 4. Уровень глутатиона в почках в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

($p < 0.01$), 96% ethanol ($p < 0.01$), chloroform ($p < 0.05$), and essential oil ($p < 0.05$) extracts compared with the acetaminophen group.

Figure 4 shows the results of GSH in various groups. By measuring the renal level of GSH in different groups, it was found that this parameter shows a significant fall in the acetaminophen group compared with the control group ($p < 0.01$). A similar significant decrease of GSH was obtained in two acetaminophen groups treated with carbon tetrachloride ($p < 0.05$) and chloroform ($p < 0.01$) extracts compared with the control group. Such a significant reduction was not obtained in other treated acetaminophen groups. Also, a significant fall in the renal level of GSH was observed only in the acetaminophen group treated with 70% ethanol extract ($p < 0.05$).

Figure 5 shows the results related to the kidney level of CAT enzyme in different groups. By measuring the level of CAT enzyme activity in the kidney, it was found that in the acetaminophen group, there was a significant decrease in this parameter compared with the control group ($p < 0.01$). This significant decrease in CAT enzyme activity was obtained in two acetaminophen groups treated with carbon tetrachloride extract, or essential oil compared to the control group ($p < 0.01$). Such a significant fall in CAT enzyme activity was not observed in other groups compared with the control. Also, a significant increase in the renal activity of CAT enzyme was observed in the acetaminophen groups treated with 70% ethanol ($p < 0.05$), 96% ethanol ($p < 0.05$), and chloroform ($p < 0.05$) extracts compared with the acetaminophen group. Such a statistically significant rise was not observed in the activity of CAT enzyme in other treated groups.

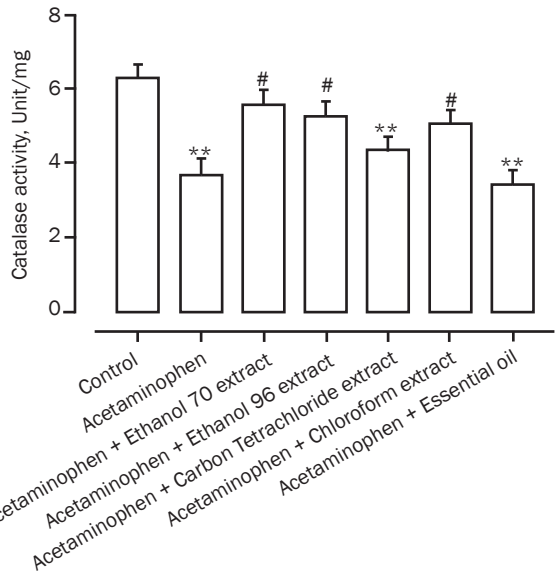

Fig. 5. Renal catalase enzyme activity in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 5. Активность почечной каталазы в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

Figure 6 shows the results of ALT enzyme activity in serum in different groups. As can be seen, in the acetaminophen group, there was a strong and significant rise ($p < 0.001$) compared with the control group. The same increase was obtained to a lesser extent in the acetaminophen groups treated with 70% ethanol, 96% ethanol, carbon tetrachloride, chloroform extracts, and essential oil compared with the control group. Also, in the three acetaminophen groups treated with 70% ethanol ($p < 0.01$), 96% ethanol ($p < 0.05$), and chloroform ($p < 0.05$) extracts, a significant fall in ALT activity was obtained compared with the acetaminophen group. Although the same reduction was present in the groups treated with carbon tetrachloride extract and essential oil, this reduction was not statistically significant compared with the acetaminophen group.

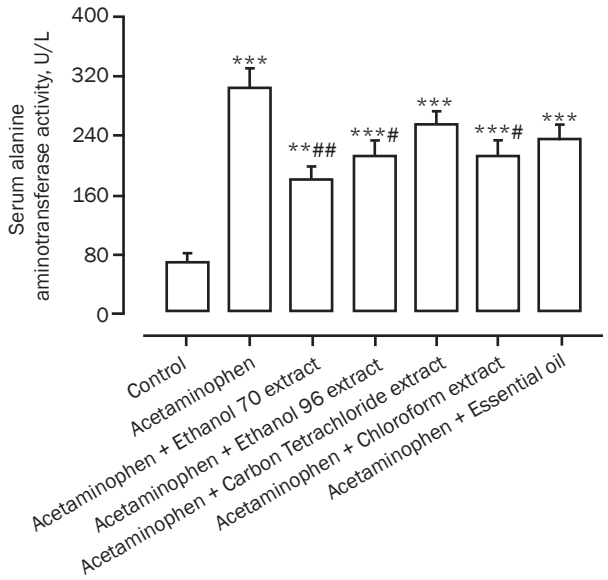

Fig. 6. Serum alanine aminotransferase activity in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 6. Активность сывороточной аланинаминотрансферазы в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

Figure 7 shows the results of AST activity in serum in different groups. As it can be seen, in the acetaminophen group, a significant increase in the activity of the enzyme was obtained compared with the control group ($p < 0.001$). The same significant rise was obtained to a lesser extent in the acetaminophen groups treated with 70% ethanol, 96% ethanol, carbon tetrachloride, chloroform extracts, and essential oil compared with the control group. On the other hand, in the acetaminophen groups treated with 70% ethanol ($p < 0.01$), 96% ethanol ($p < 0.01$), carbon tetrachloride ($p < 0.05$), and chloroform ($p < 0.01$) extracts, there was a significant fall in the activity of this enzyme compared with the acetaminophen group. Also, in the acetaminophen group treated with essential oil, although a fall in enzyme activity was observed compared with the acetaminophen group, this decrease was not statistically significant.

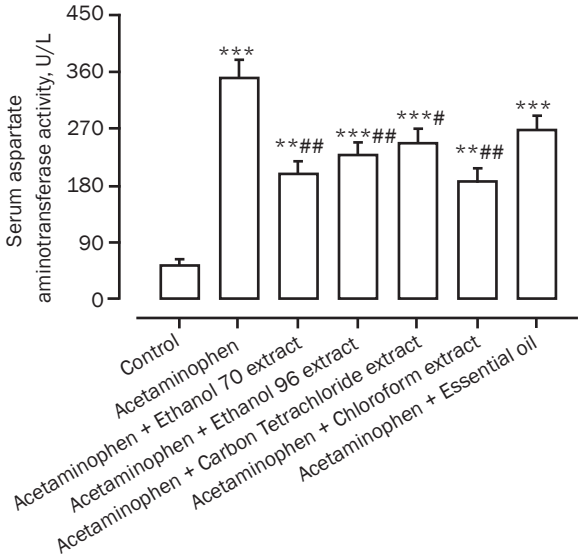

Fig. 7. Serum aspartate aminotransferase activity in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 7. Активность сывороточной аспартатаминотрансферазы в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

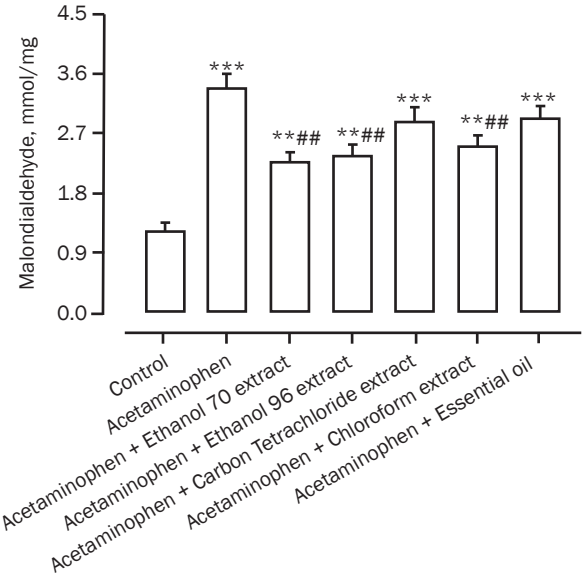

Fig. 8. Liver malondialdehyde levels in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 8. Уровень малонового диальдегида в печени в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

Figure 8 shows the level of MDA in liver tissue as an index of lipid peroxidation and oxidative stress in different groups. As it is shown in the acetaminophen group, there is a strong and significant rise in MDA compared with the control group ($p < 0.001$). This significant increase was observed in the acetaminophen groups treated with 70% ethanol, 96% ethanol, carbon tetrachloride, chloroform, and essential oil extracts compared with the control group. Also, in the acetaminophen groups treated with 70% ethanol ($p < 0.01$), 96% ethanol ($p < 0.01$), and chloroform ($p < 0.01$) extracts, a statistically significant fall in MDA was obtained compared with the acetaminophen group. In addition, the reduction of MDA in two acetaminophen groups treated with carbon tetrachloride extract ($p < 0.05$) as well as essential oil ($p < 0.05$) was not significant.

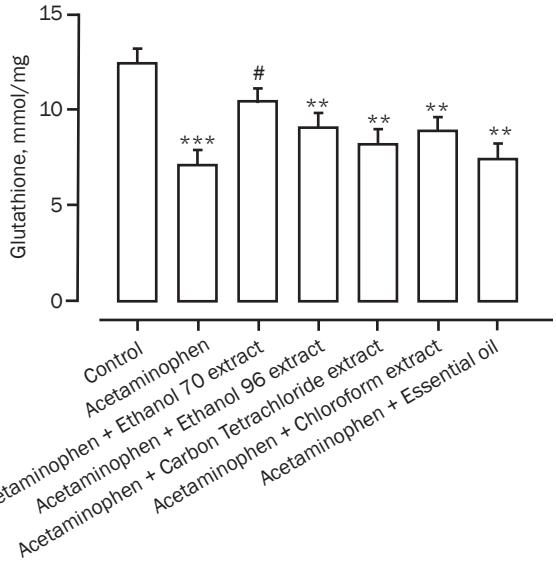

Fig. 9. Liver glutathione levels in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 9. Уровень глутатиона в печени в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

Figure 9 shows the results of the amount of GSH in liver tissue homogenate in different groups. As can be seen, in the acetaminophen group, a strong and significant fall of this parameter was obtained compared with the control group ($p < 0.001$). Also, such a significant decrease was observed in acetaminophen groups treated with 96% ethanol ($p < 0.01$), carbon tetrachloride ($p < 0.01$), chloroform ($p < 0.01$) extracts, and essential oil ($p < 0.01$) as compared with the control group. On the other hand, a significant increase in the liver level of GSH was obtained only in the acetaminophen group treated with 70% ethanol extract compared with the acetaminophen group ($p < 0.05$), and such a significant rise was not obtained in other treated acetaminophen groups.

Figure 10 shows the results of CAT enzyme activity in liver tissue homogenate in different groups. As can be seen, in the acetaminophen group, a significant decrease in the activity of the enzyme was obtained compared with the control group ($p < 0.01$). The same significant decrease in CAT enzyme activity was observed in acetaminophen groups treated with carbon tetrachloride ($p < 0.01$), chloroform ($p < 0.05$) extracts, and essential oil ($p < 0.01$) compared with the control group. On the other hand, a significant increase in CAT enzyme activity was obtained only in the two acetaminophen groups treated with 70% ($p < 0.05$) and 96% ethanol extracts ($p < 0.05$) compared with the acetaminophen group, and this increase in enzyme activity in other treated acetaminophen groups was not statistically significant.

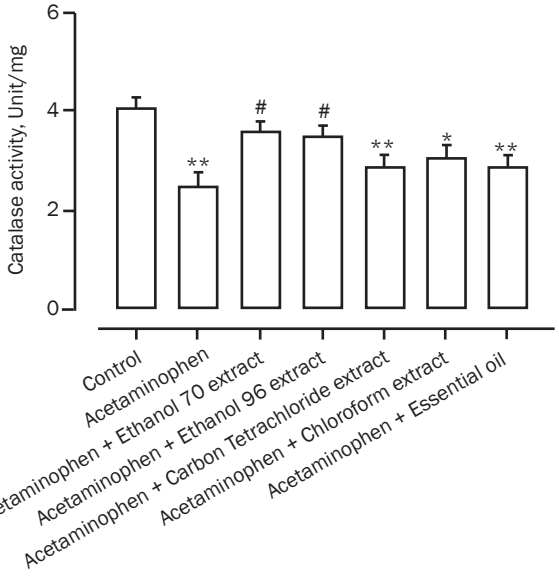

Fig. 10. Catalase enzyme activity in liver tissue homogenate in various groups: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ (compared with the control group); # – $p < 0.05$, ## – $p < 0.01$ (compared with the acetaminophen group)

Рис. 10. Активность каталазы в печени в различных группах: * – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$ (по сравнению с контрольной группой); # – $p < 0,05$, ## – $p < 0,01$ (по сравнению с группой, принимавшей ацетаминофен)

Kidney is the most vital part of the body which malfunctions and leads to serious illness and even death. Two main and important functions of the kidney include the elimination of toxins and waste materials, as well as the control of water levels, mineral and chemical substances in the body, and electrolytes such as sodium and potassium [26].

The level of kidney function is determined by measuring blood urea and creatinine because these two substances are naturally excreted from the kidneys. Therefore, an increase in blood urea and creatinine indicates a decrease in kidney function, which can be very harmful. In acute kidney injury, early diagnosis is essential for successful treatment [27].

Old biomarkers for diagnosing kidney damage are non-specific and can be detected only after at least 24 hours after the onset of kidney damage. Therefore, the need for new biomarkers that are non-invasive, quick and easy,

and have high sensitivity and diagnostic specificity is very much felt [28].

According to the outcomes of GC-MS data (see Table), different extracts and essential oil of sage are rich in monoterpene compounds, which have been mentioned in several articles for their antioxidant and oxidative stress-reducing properties [29]. By comparing the results for different extracts, it was found that carbon tetrachloride and chloroform solvents showed better performance in extracting monoterpene compounds compared with 70 and 96% of ethanol solvents. Also, sage essential oil has the highest extraction rate of these compounds compared with other solvents.

Also, the results of pharmacology tests showed that carbon tetrachloride and chloroform extracts performed better than hydroalcoholic extract and essential oil for the treatment of kidney damage caused by acetaminophen in laboratory mice.

The liver is also known as another important organ due to its vital role in the body. The use of medicinal plants in the treatment of liver diseases is of particular importance [30]. Liver damage caused by drugs is one of the main causes of death in the world and is a serious concern. Acetaminophen is known as a widely used drug that is safe in therapeutic doses, but in high doses, it causes disruption of mitochondrial function and causes liver necrosis [1].

The toxic metabolite *N*-acetyl-*P*-benzoquinine imine, which is created through cytochrome P450, reduces GSH reserves in the liver, and the secondary toxic metabolite binds to protein and causes liver necrosis and can lead to oxidative stress and mitochondrial damage. To evaluate liver damage, blood tests and measurement of liver biomarkers are the priority for identifying and monitoring liver damage. ALT and AST enzyme activities are analyzed as indicators of liver cell damage and ALP enzyme activity is analyzed to evaluate cholestatic damage [31, 32]. Therefore, in the current study, important biomarkers in the evaluation of liver damage were analyzed.

In the present study, liver enzymes in the blood of laboratory mice increased after receiving a toxic dose of acetaminophen. Also, the level of MDA, which is an index of lipid peroxidation and indicates oxidative stress in the body, increased with acetaminophen poisoning in the blood. The amount of CAT enzyme is also one of the first and most important antioxidant enzymes that plays a role in the direct reduction of active oxygen metabolites and indicates liver disease. An increase in nitrogen and reactive oxygen species is caused by excessive consumption of acetaminophen and causes oxidative activity and, as a result, cell necrosis in the liver tissue [33].

With the administration of sage leaves extracts, liver markers have decreased. The results of pharmacology tests illustrated that extracts of 70% ethanol, 96% ethanol, chloroform, and essential oil had the greatest protective effects in the model of acute liver damage induced by acetaminophen in laboratory mice because it is rich in biological compounds which have therapeutic activities in liver diseases.

CONCLUSIONS

Since the solvent plays an important role in the extraction and separation of active medicinal substances in plants, in this research, the extraction procedure was performed by different solvents in terms of polarity. It can be concluded that chloroformic and carbon tetrachloride extracts of *S. officinalis* had the best effects for protecting against kidney injury, while for liver injury, chloroformic, ethanolic, and hydroethanolic extracts had the best. Although the underlying mechanism remains unclear.

REFERENCES

1. Qin J., Ma Y., Wang C., Li H., Zou Z., Zhang Y., et al. Effects of carnosine combined with Lactobacillus on the antioxidant capacity of liver and kidney in normal or stressed mice. *Food Bioscience*. 2024;59:103904. DOI: 10.1016/j.fbio.2024.103904.

2. Zheng X., Li S., Wang K., Wang Z., Li J., Yang Q., et al. Comparing the pharmacological effects of the prepared folium of *Epimedium brevicornu* Maxim. and *Epimedium sagittatum* Maxim. on kidney-Yang deficiency syndrome and liver injury complications. *Fitoterapia*. 2024;176:106006. DOI: 10.1016/j.fitote.2024.106006.

3. Li D.-D., Li W.-J., Kong S.-Z., Li S.-D., Guo J.-Q., Guo M.-H., et al. Protective effects of collagen polypeptide from tilapia skin against injuries to the liver and kidneys of mice induced by D-galactose. *Biomedicine & Pharmacotherapy*. 2019;117:109204. DOI: 10.1016/j.biopha.2019.109204.

4. Jaffri J.M., Mohamed S., Ahmad I.N., Mustapha N.M., Manap Y.A., Rohimi N. Effects of catechin-rich oil palm leaf extract on normal and hypertensive rats' kidney and liver. *Food Chemistry*. 2011;128(2):433-441. DOI: 10.1016/j.foodchem.2011.03.050.

5. Lafhal K., Sabir E., Elkhiat A., Hammoud M., Makbal R., Ezoubeiri A., et al. Rosa *damascena* Mill attenuated the liver and kidneys injuries in copper-overloaded mice. *Molecular Genetics and Metabolism*. 2021;132(2):S61. DOI: 10.1016/j.ymgme.2020.12.137.

6. Ghorbani A., Esmaeilizadeh M. Pharmacological properties of *Salvia officinalis* and its components. *Journal of Traditional and Complementary Medicine*. 2017;7(4):433-440. DOI: 10.1016/j.jtcme.2016.12.014.

7. Walker J.B., Sytsma K.J., Treutlein J., Wink M. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *American Journal of Botany*. 2004;91(7):1115-1125. DOI: 10.3732/ajb.91.7.1115.

8. Rasmy N.M., Hassan A.A., Foda M.I., El-Moghazy M.M. Assessment of the antioxidant activity of sage (*Salvia officinalis* L.) extracts on the shelf life of mayonnaise. *World Journal of Dairy & Food Sciences*. 2012;7(1):28-40.

9. Fawzi M., Kadhem Z., Farhan S. Anti-inflammatory effect of sage (*Salvia officinalis*) extracts on oral health. *Iraqi Dental Journal*. 2017;39(1):1-6. DOI: 10.26477/idj.v39i1.111.

10. Monsefi M., Abedian M., Azarbahram Z., Ashraf M.J. *Salvia officinalis* induces alveolar bud growing in adult female rat mammary glands. *Avicenna Journal of Phytomedicine*. 2015;5(6):560-567. DOI: 10.22038/ajp.2015.4638.

11. Akhondzadeh S., Noroozian M., Mohammadi M., Ohadinia S., Jamshidi A.H., Khani M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double-blind, randomized and placebo-controlled trial. *Journal of Clinical Pharmacy and Therapeutics*. 2003;28:53-59. DOI: 10.1046/j.1365-2710.2003.00463.x.

12. Stanojević D., Čomić L., Stefanovic O., Solujić-Sukdolak S. *In vitro* synergistic antibacterial activity of *Salvia officinalis* L. and some preservatives. *Archives of Biological Sciences*. 2010;62(1):175-183. DOI: 10.2298/ABS1001167S.

13. Jedinák A., Mučková M., Košťálová D., Maliar T., Mašterová I. Antiprotease and antimetastatic activity of ursolic acid isolated from *Salvia officinalis*. *Zeitschrift für Naturforschung C*. 2014;61(11-12):777-782. DOI: 10.1515/znc-2006-11-1203.

14. Jedidi S., Rtibi K., Selmi S., Aloui F., Selmi H., Wannes D., et al. Phytochemical/antioxidant properties and individual/synergistic actions of *Salvia officinalis* L. aqueous extract and loperamide on gastrointestinal altering motor function. *Journal of Medicinal Food*. 2019;22(12):1235-1245. DOI: 10.1089/jmf.2019.0051.

15. Jedidi S., Aloui F., Rtibi K., Sammari H., Selmi H., Rejeb A., et al. Individual and synergistic protective properties of *Salvia officinalis* decoction extract and sulfasalazine against ethanol-induced gastric and small bowel injuries. RSC Advances. 2020;10(59):35998-36013. DOI: 10.1039/D0RA03265D.

16. Koubaa-Ghorbel F., Chaâbane M., Turki M., Makni-Ayadi F., El Feki A. The protective effects of *Salvia officinalis* essential oil compared to simvastatin against hyperlipidemia, liver, and kidney injuries in mice submitted to a high-fat diet. *Journal of Food Biochemistry*. 2020;44(4):e13160. DOI: 10.1111/jfbc.13160.

17. Amraoui W., Adjabi N., Bououza F., Boumendjel M., Taibi F., Boumendjel A., et al. Modulatory role of selenium and vitamin E, natural antioxidants, against bisphenol A-induced oxidative stress in Wistar albinos rats. *Toxicological Research*. 2018;34:231-239. DOI: 10.5487/tr.2018.34.3.231.

18. Chandorkar N., Tambe S., Amin P., Madankar C. A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus *Eucalyptus*. *Phytomedicine Plus*. 2021;1(4):100089. DOI: 10.1016/j.phyplu.2021.100089.

19. Zámboriné Németh É., Nguyen H.T. Thujone, a widely debated volatile compound: what do we know about it? *Phytochemistry Reviews*. 2020;19:405-423. DOI: 10.1007/s11101-020-09671-y.

20. Chebbac K., Moussaoui A.E.L, Bourhia M., Salamatullah A.M., Alzahrani A., Guemmouh R. Chemical analysis and antioxidant and antimicrobial activity of essential oils from *Artemisia negrei* L. against drug-resistant microbes. *Evidence-Based Complementary and Alternative Medicine*. 2021:5902851. DOI: 10.1155/2021/5902851.

21. Dew S.E., Wardlaw S.A., Ong D.E. Effects of pharmacological retinoids on several vitamin A-metabolizing enzymes. *Cancer Research*. 1993;53(13):2965-2969.

22. Trevizan L.N.F., do Nascimento K.F., Santos J.A., Liete Kassuya C.A, Lima Cardoso C.A., do Carmo Vieira M., et al. Anti-inflammatory, antioxidant, and anti-*Mycobacterium tuberculosis* activity of viridiflorol: the major constituent of *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Radlk. *Journal of Ethnopharmacology*. 2016;192:510-515. DOI: 10.1016/j.jep.2016.08.053.

23. Du Q., Xin H., Peng C. Pharmacology and phytochemistry of the *Nitraria* genus (review). *Molecular Medicine Reports*. 2015;11(1):11-20. DOI: 10.3892/mmr.2014.2677.

24. Jeong J.B., Hong S.C., Jeong H.J., Koo J.S. Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation, and acetylation of histone H3. *Archives of Pharmacal Research*. 2011;34:2109-2116. DOI: 10.1007/s12272-011-1214-9.

25. Verma A., Joshi S., Singh D. Imidazole: having versatile biological activities. *Journal of Chemistry*. 2013;2013:329412. DOI: 10.1155/2013/329412.

26. Morovvati H., Armand N. Investigate the effect of alcoholic extract of bulbs of narcissus (*Narcissus tazetta* L.) on indexes of renal function. *Feyz, Journal of Kashan University of Medical Sciences*. 2020;24(1):56-62. (In Arabic).

27. Hou G., Surhio M.M., Ye H., Gao X., Ye Z., Li J., et al. Protective effects of a Lachnum polysaccharide against liver and kidney injury induced by lead exposure in mice. *International Journal of Biological Macromolecules*. 2019;124:716-723. DOI: 10.1016/j.ijbiomac.2018.11.133.

28. Mohamadi Yarijani Z., Najafi H. Kidney injury in COVID-19 patients, drug development and their renal complications: review study. *Biomedicine & Pharmacotherapy*. 2021;142:111966. DOI: 10.1016/j.biopha.2021.111966.

29. Sheng M.-Y., Peng D.-W., Peng H.-M., Zhang Y.-L., Xiao L., Zhang M.-R., et al. Effective substances and molecular mechanisms guided by network pharmacology: an example study of Scrophulariae Radix treatment of hyperthyroidism and thyroid hormone-induced liver and kidney injuries. *Journal of Ethnopharmacology*. 2024;326:117965. DOI: 10.1016/j.jep.2024.117965.

30. Hao R., Ge J., Ren Y., Song X., Jiang Y., Sun-Waterhouse D., et al. Caffeic acid phenethyl ester mitigates cadmium-induced hepatotoxicity in mice: Role of miR-182-5p/TLR4 axis. *Ecotoxicology and Environmental Safety*. 2021;207:111578. DOI: 10.1016/j.ecoenv.2020.111578.

31. Aithal G.P., Watkins P.B., Andrade R.J., Larrey D., Molokhia M., Takikawa H., et al. Case definition and phenotype standardization in drug-induced liver injury. *Clinical Pharmacology & Therapeutics*. 2011;89(6):806-815. DOI: 10.1038/clpt.2011.58.

32. Saito C., Zwingmann C., Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and *N*-acetylcysteine. *Hepatology*. 2010;51(1):246-254. DOI: 10.1002/hep.23267.

33. He X., Peng X., Zhang S., Yang T., Huo J., Zhang Y. Hepatoprotective effect of diammonium glycyrrhizinate and neuroprotective effect of piperazine ferulate on AmB-induced liver and kidney injury by suppressing apoptosis *in vitro* and *in vivo*. *Toxicon*. 2024;246:107795. DOI: 10.1016/j.toxicon.2024.107795.

INFORMATION ABOUT THE AUTHOR

Seyed Ali Sobhanian,
Dr. Sci. (Pharmacy), Associate Professor,
Department of Medicinal Chemistry, TeMS.C.,
Islamic Azad University,
Zargandeh, Dr. Shariati St., Tehran,
1949635881, Iran,
✉ s.alisobhanian@gmail.com
https://orcid.org/0009-0004-5348-080X

Abbas Ahmadi,
Dr. Sci. (Chemistry), Professor,
Department of Chemistry, Ka.C.,
Islamic Azad University,
Amir al-Momenin Complex, Esteghlal Blvd., Karaj,
3149968111, Iran,
abbasahmadi3957@gmail.com
https://orcid.org/0000-0002-4813-2876

Mehrdad Roghani,
Dr. Sci. (Physiology), Professor,
Neurophysiology Research Center,
Shahed University,
1471, North Kargar Ave., Tehran,
3319118651, Iran,
mehjour@yahoo.com
https://orcid.org/0000-0002-9209-8484

Parinaz Hassanlo,
M. Sc., Assistant,
Department of Chemistry, Ka.C.,
Islamic Azad University,
Amir al-Momenin Complex, Esteghlal Blvd., Karaj,
3149968111, Iran,
Hassanloparinaz@gmail.com
https://orcid.org/0009-0004-9648-1482

Shima Khamoushi,
M. Sc., Assistant,
Department of Chemistry, Ka.C.,
Islamic Azad University,
Amir al-Momenin Complex, Esteghlal Blvd., Karaj,
3149968111, Iran,
shima.khamoushi@tutamail.com
https://orcid.org/0009-0005-5877-6906

ИНФОРМАЦИЯ ОБ АВТОРАХ

Собханян Сайед Али,
д.фарм.н., доцент,
Исламский университет Азад,
филиал медицинских наук в г. Тегеране,
1936893813, г. Тегеран, ул. доктора Шариати,
Зарганде, Иран,
✉ s.alisobhanian@gmail.com
https://orcid.org/0009-0004-5348-080X

Ахмади Аббас,
д.х.н., профессор,
Исламский университет Азад,
филиал в г. Карадже, химический факультет,
3149968111, г. Карадж, Бульвар Эстеглаль,
Комплекс Амир аль-Моменин, Иран,
abbasahmadi3957@gmail.com
https://orcid.org/0000-0002-4813-2876

Рогани Мехрдад,
д.м.н., профессор,
Центр нейрофизиологических исследований,
Университет Шахед,
3319118651, г. Тегеран, пр. Северный
Каргар, 1471, Иран,
mehjour@yahoo.com
https://orcid.org/0000-0002-9209-8484

Хассанло Париназ,
магистр, ассистент,
Исламский университет Азад,
филиал в г. Карадже, химический факультет,
3149968111, г. Карадж, Бульвар Эстеглаль,
Комплекс Амир аль-Моменин, Иран,
Hassanloparinaz@gmail.com
https://orcid.org/0009-0004-9648-1482

Хамуши Шима,
магистр, ассистент,
Исламский университет Азад,
филиал в г. Карадже, химический факультет,
3149968111, г. Карадж, Бульвар Эстеглаль,
Комплекс Амир аль-Моменин, Иран,
shima.khamoushi@tutamail.com
https://orcid.org/0009-0005-5877-6906

Contribution of the authors

Seyed Ali Sobhanian – conceptualization, methodology, investigation, project administration, supervision, validation, visualization, writing – original draft, editing.
Abbas Ahmadi – conceptualization, methodology, investigation, supervision, validation, visualization, writing – original draft, editing.
Mehrdad Roghani – conceptualization, methodology, formal analysis, investigation, software, supervision, validation, visualization, writing – original draft, editing.
Parinaz Hassanlo – data curation, formal analysis, investigation, resources, software.
Shima Khamoushi – data curation, formal analysis, investigation, resources, software.

Вклад авторов

С.А. Собханян – разработка концепции, разработка методологии, проведение исследования, административное руководство исследовательским проектом, научное руководство, валидация результатов, визуализация, написание черновика рукописи, редактирование рукописи.
А. Ахмади – разработка концепции, разработка методологии, проведение исследования, научное руководство, валидация результатов, визуализация, написание черновика рукописи, редактирование рукописи.
М. Рогани – разработка концепции, разработка методологии, формальный анализ, проведение исследования, разработка программного обеспечения, научное руководство, валидация результатов, визуализация, написание черновика рукописи, редактирование рукописи.
П. Хассанло – курирование данных, формальный анализ, проведение исследования, предоставление ресурсов, разработка программного обеспечения.
Ш. Хамуши – курирование данных, формальный анализ, проведение исследования, предоставление ресурсов, разработка программного обеспечения.

Conflict of interest

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