ORIGINAL ARTICLE

Eastern Journal of Medicine
East J Med 29(4): 524-530, 2024
DOI: 10.5505/ejm.2024.85353

Esculetin improves chemotherapy-induced impaired anxiety-depression-like behaviors and learning activity

Filiz Taşpınar*, Mehmet Öz

Department of Physiology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye

ABSTRACT

This study aimed to assess the neuroprotective effectiveness of esculetin in preventing cognitive and emotional impairments caused by chemotherapy.

In order to cause cognitive impairment in mice, a single intraperitoneal dosage of methotrexate (Mtx) at a concentration of 40 mg/kg was administered. Esculetin (Elt) was administered orally at a daily dose of 40 mg/kg one week before the Mtx injection. The locomotor activity was assessed using the Pole (PT) and open field (OF) tests. Anxiety-like behaviors were evaluated using the open field and elevated plus maze (EPM) tests. Depression-like behaviors were assessed using the tail suspension test (TST). Learning and memory activity was evaluated using the novel object recognition test (NOR).

Mtx did not affect the locomotor activities of mice, but anxiety-like behaviors were found to be increased in the OF and EPM tests. It also increased the duration of immobility in the TST, causing a negative discrimination index in the NOR. Elt alleviated anxiety and depression-like behaviors generated by Mtx and enhanced cognitive function related to learning and memory. Elt significantly increased the time spent in the center zone in the OF test, the total time spent in the open arms in the EPM, and the immobility time in the TST. Moreover, it improved the discrimination index in NOR, which had been reduced by Mtx.

Administering a single dose of Mtx at 40 mg/kg resulted in increased anxiety and depression-like behaviors, impaired learning and memory functions. Besides that, supplementing with Elt significantly mitigated the neurological impairments caused by Mtx.

Keywords: methotrexate, esculetin, anxiety, depression, learning

Introduction

Adjuvant chemotherapy is frequently used in malignant diseases and improves the survival rates of individuals suffering from these diseases. However, the observation of both short- and long-term complications of chemotherapy, primarily cognitive and mood disorders, has led to increased interest in the scientific community to improve the quality of life of individuals suffering from these diseases (1,2). Methotrexate (MTX), a folate analog, is a cytotoxic chemotherapeutic agent widely used in childhood acute lymphoblastic leukemia, breast cancer, and other malignant diseases (3,4). Cognitive impairment following the use of MTX and/or other chemotherapeutic drugs is referred to in the literature as chemotherapy-induced cognitive impairment or chemobrain (5,6). Therefore, experimental animal studies have been conducted for a better understanding of the exact mechanism of this phenomenon. A recent study in our laboratory revealed that a single dose of Mtx in mice causes mood disorders such as anxiety and depression and that neuroinflammation may play a role in this physiopathology (7). In our other study, we reported that Mtx causes behavioral disturbances in rats and that inflammation, oxidative stress, and disruption of brain-derived neurotrophic factor expression and cholinergic pathway homeostasis play a role in this complication. (8). Some reports have shown that Mtx increases lipid peroxidation and suppresses antioxidant enzyme activity in the prefrontal cortex and hippocampus, and this has been associated with cognitive impairment (9,10). Taken together, considering that oxidative stress and antioxidant defense systems play an essential role in Mtx-induced cognitive impairment, the use of agents with solid antioxidant potential may be useful in treating or effectively managing this disorder.

*Corresponding Author: Filiz Taşpınar, Aksaray University, Faculty of Medicine, Department of Physiology Aksaray, Türkıye
E-mail: ftaspinartr@gmail.com, Phone: 0 (507) 118 31 88
ORCID ID: Filiz Taşpınar: 0000-0003-3780-4021, **Mehmet Öz:** 0000-0003-4167-2623
Received: 21.05.2024, **Accepted:** 28.09.2024

Esculetin (Elt), a coumarin derivative, is found in some plants used in traditional medicine and has an extra hydroxyl group, which inhibits reactive oxygen species more effectively in pathological conditions (11). Elt has been shown to be effective in preventing pathologies such as cardiomyocyte hypoxia/reoxygenation injury (12), non-alcoholic fatty liver syndrome in diabetic mice (13), aluminum chloride-induced reproductive toxicity (14), lipopolysaccharide/D-galactosamine-induced acute liver failure (15), the ovarian ischemia-reperfusion model (11), and lupus nephritis (16). A recent study reported that Elt suppressed pentylenetetrazole-induced seizures and cognitive impairment, as well as penicillin-induced epileptic activity, and that prevention of neuroinflammation was important in this effect (17). Elt also ameliorated lipopolysaccharide-induced depressive-like behaviors by preventing neuroinflammation (18,19), prevented brain ischemia-reperfusion-induced cognitive impairment in mice (20,21), showed neuroprotective efficacy in a mouse model of Parkinson's disease (22), and ameliorated restraint stress-induced cognitive impairment in mice 11 months of age (23). These investigations showed that Elt's anti-oxidant and anti-inflammatory properties mediate its neuroprotective effects. We hypothesized that reducing oxidative stress and inflammation could ameliorate Mtx-induced cognitive and mood impairment. This study was therefore carried out to test whether Elt could ameliorate the chemobrain phenomenon induced by Mtx.

Material and Methods

Study Design and Group Formation: This research was performed on 8–10-week-old male mice seized from the Aksaray University Experimental Animal Research and Application Center. The Experimental Animal Unit was approved by the relevant ministries and provided standardized conditions for laboratory animals (temperature, lighting, ventilation, etc.). Mice had unlimited access to routine mouse chow and tap water. At the beginning of the study, all mice were randomly grouped and placed in cages of similar size. One week was waited for acclimatization, and our study started after obtaining permission from the local ethics committee. The project was carried out on a total of 28 mice, with seven mice in each group, and the formation of the groups and the procedures performed are as follows:

The first group (CON) consisted of control mice, which received no treatment but received the same volume of the drug solvent and the same injection route as the other groups.

The second group (MTX) was treated with methotrexate (Metoart Con, Kocak Farma Drug and Chemical Industry A.Ş., Istanbul, Turkey). On the seventh day of the study, 40 mg/kg Mtx was injected intraperitoneally (i.p.) as a single dose and 1 hour after vehicular administration (24).

Mice in the third group (MTXELT) was administered both Elt and Mtx. Elt [Esculetin (sc-200486) Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was administered at 40 mg/kg for seven days. One hour after Elt administration on the seventh day, 40 mg/kg Mtx was injected (19).

Mice in the fourth group (ELT) received only Elt treatment, and the same volume of saline was injected instead of Mtx. All injections were performed by the same investigator at the same time of day.

Behavioral Tests: The same researcher performed behavioral tests that started 24 hours after the Mtx injection and lasted for two days. The mice were brought into the test room one hour before the procedure and allowed to acclimate to the environment. Maximum effort was made to avoid external factors (noise, odor, lighting, etc.) affecting the behavioral tests. The testing arena was wiped off using a 70% alcohol solution after ever mouse trial.

Pole Test: The test is designed to evaluate the mouse's ability to grip a pole and sink into the bottom of the cage in order to evaluate its motor coordination. A 55 cm long, 1 cm in diameter wooden pole was employed for this purpose, and it was positioned vertically inside a cage. The mouse was placed at the top of this pole with its head facing upwards. The total time from the mouse was placed on the pole until it came down was recorded. This test was repeated four times at 5-minute intervals, and the mean times per mouse were compared between the groups (25).

Open Field Test: The open field test (OF) was used to evaluate locomotor activity and analyze anxiety-like behaviors in experimental animals. An empty 40 x 40 x 40 cm water-resistant black wood arena was used for this purpose. The floor of the arena was marked with lines in 16 equal squares. The four squares located in the central zone were labeled as "center," while the remaining squares were referred to as "periphery." During the experiment, mice were introduced into the central zone of the OF arena and given 5 minutes to explore the arena. Their motions were captured on

camera simultaneously. The number of times they crossed each line was used for locomotor activity. Total time spent in the center area and rearing were used as anxiety behaviors (26).

Elevated Plus Maze Test: An additional test used to assess anxiety-like behaviors was the elevated plus maze (EPM) test. A specifically manufactured black wood device, measuring 35 cm in length and 5 cm in width and with two open and two closed arms, was employed. It was positioned 50 cm above the field. The mice were settled at the intersection of the arms and agreed to explore the maze freely for 5 minutes. The number of times the mice entered the open arm and the total time spent in the open arms were recorded and compared across the groups (27).

Tail Suspension Test: Depression-like behaviors were assessed by a tail suspension test (TST). For this purpose, a maze made of black wood with a height of 50 cm and divided into 20 cm intervals, in which four mice were tested simultaneously, was used. The mice were attached by their tails to the top of the maze with the help of a tape, and a video was recorded for 6 minutes. The immobilization time—the amount of time the mice stayed motionless during the final 4 minutes of the video recording—was measured and compared between the groups (28).

Novel Object Recognition Test (NOR): The novel object recognition test (NOR) was used to assess mice's learning and memory activity. This test used an open-field arena and was performed one day after OF. The OF was considered the familiarization phase of this test. The next day, two equivalent objects were fixed to the floor of the open field arena (with double-sided tape), and all mice freely explored these two identical objects for 5 minutes. After 1.5 hours, one of the objects was replaced with a novel object, and the mice were allowed to search the arena for 5 minutes. The total time the mice were interested in the novel object and the usual object was recorded. Touching the objects and coming within 2 cm of the objects with their noses were considered of interest. The discrimination index was calculated from these times and compared between the groups. Discrimination index = (time of interest in the novel object - time of interest in the familiar object) / (time of interest in the novel object + time of interest in the familiar object) (29).

Statistics: This study, planned as an experimental animal study, employed the resource equation method, and the minimum and maximum values for the number of subjects (n) were determined using the notation [20/4+1 > n > 10/4+1]. Accordingly, the number of subjects per group was calculated to be between 16 and 24. To account for potential dropouts, a total of 28 subjects were used in the study, with 7 subjects assigned to each group (30). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test of normality. IBM SPSS Statistics for Windows, Version 26.0. was used to evaluate the data obtained in the research. Differences between the means of four independent groups were evaluated by the one-way analysis of variance technique, and Tukey's HSD multiple comparison tests were used to determine the groups that created the difference. Mean differences were indicated using indices placed on descriptive statistics, presented as mean ± SD. For results where $p<0.05$ was considered statistically significant, groups sharing the same letter index showed no significant difference, while those with different letter indices were statistically different ($p<0.05$). And the post hoc power for the primary outcome variable, "center", was calculated as 0.952.

Results

General toxicity and body weight changes: During the 9-day study, general health checks of the mice were performed every day, and there was no loss in all groups. The weights of the mice were recorded at the beginning and the end of the study. At the beginning of the study, the weights of the mice were similar, and there was no important difference between the groups (Fig. 1A, $p > 0.05$). According to the weight changes measured at the end of the study, no difference was observed between the MTX, MTXELT, and ELT groups, but the difference between the CON group and the MTXELT group was statistically significant (Fig. 1B, p= 0,011).

Mtx had no effect on the locomotor activity of mice.: The locomotor activity of the mice was assessed by the number of crossings in the OF and the time it took to descend from the wooden bar in the pole test. According to the one-way ANOVA test, there was no significant disparity between all groups in the time to descend in PT or by the number of crossings in the OF test (Fig. 1C-D, $p > 0.05$). The fact that neither MTX treatment as a single dose of 40 mg/kg nor Elt changed locomotor activity in mice in our study is an essential result in terms of showing that the data obtained in behavioral tests were not related to locomotor weakness but were realized by the effect of the drugs administered in the study.

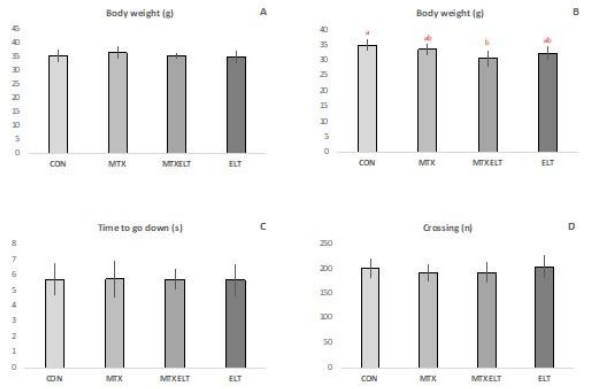

Fig. 1. Effects of Mtx and Elt on body weight and locomotor activity of mice. (A) Body weights before Mtx administration, (B) Body weights at the end of the study, (C) Time to descend from the pole to the cage in the pole test, (D) Number of crossings in open field test. The data are expressed as the means ± SD. There was no difference between the averages expressed with the same letter index, while the group averages expressed with different letter indices were statistically different (one-way ANOVA followed by Tukey's post hoc test).

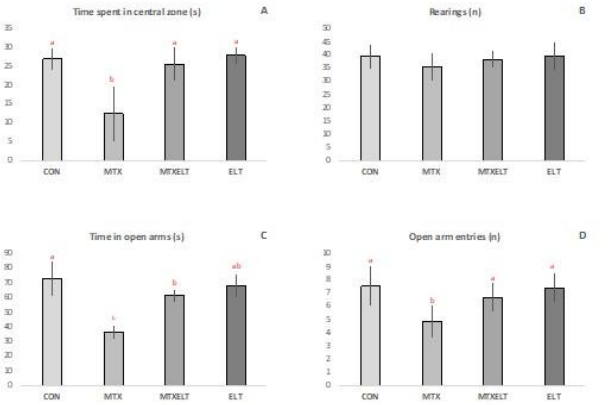

Fig. 2. Effect of esculetin on Mtx-induced impaired anxiety-like behaviors on the open field data:, the time spent in central zone (A), rearings numbers (B) and on the elevated plus maze data:, the time spent in open arms (C), the number of open arms entries in male mice. Data were expressed as mean ± SD. There was no difference between the averages expressed with the same letter index, while the group averages expressed with different letter indices were statistically different (one-way ANOVA followed by Tukey's post hoc test).

The impact of Esculetin on anxiety and depression-like behaviors in mice treated with Mtx: OF and EPM tests were used to evaluate the anxiety-like behavior of mice. The OF test assessed the time spent in the central zone and the frequency of rearings. In the EPM test, the total time spent in the open arms and the frequency of entry into the open arms were analyzed. It was found that mice receiving Mtx spent less time in the central zone in the OF test compared to control mice (Fig 2A, p= 0.001). Elt treatment ameliorated this Mtx-induced reduction (Fig. 2A, p= 0.001). There was no statistical difference between the CON, MTXELT, and ELT groups in terms of total time spent in the central zone (Fig. 2A, p > 0.05). There was no substantial disparity in the number of rearings between all groups in the OF test (Fig. 2B, p > 0.05). The EPM test revealed a considerable reduction in the duration of time spent in the open arms due to Mtx medication (Fig. 2C, p= 0.001). ELT treatment ameliorated this Mtx-induced reduction (Fig. 2C, p= 0.001) but was lower than in control mice (Fig. 2C, p= 0,001). The number of open-arm entries in the EPM test was significantly lower after Mtx treatment compared to all other groups (Fig. 2D, p= 0,002). Elt treatment ameliorated this Mtx-induced decrease (Fig. 2D, p= 0.002) and brought it to the level of control mice.

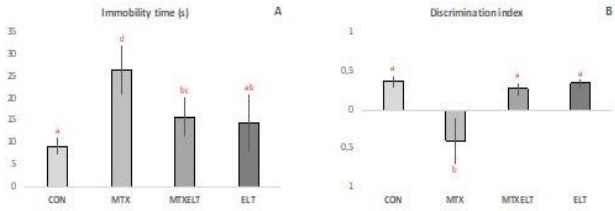

Fig. 3. Effect of esculetin on Mtx-induced impaired depression-like behaviors and learning and memory activities. Immobility time (A) in tail suspension test and discrimination index (B) in novel object recognition test. Data were expressed as mean ± SD. There was no difference between the averages expressed with the same letter index, while the group averages expressed with different letter indices were statistically different (one-way ANOVA followed by Tukey's post hoc test).

Mtx-induced impaired cognitive activity treated with Esculetin: To identify Mtx-induced alterations in learning and memory activity, a NOR test was employed. This study examines whether mice inherently like unfamiliar environments over ones they are acquainted with. It is anticipated that a mouse exposed to the new object after encoding the familiar object will recall the former and spend more time examining the latter. In our study, Mice treated with Mtx exhibited reduced curiosity towards the unfamiliar object and spent less time interacting with it compared to mice that were not treated; as a result, the discrimination index was much lower (Fig. 3B, p= 0.001). Elt supplementation restored the Mtx-induced decreased discrimination index

closer to the control group and ameliorated the impaired learning-memory activity. The discrimination index of CON, MTXELT, and ELT groups was close to each other, and no statistically significant difference was found (Fig. 3B, $p > 0.05$).

Discussion

This study evaluated the protective effect of Elt on chemotherapy-induced cognitive and mood impairment in mice. Mtx treatment at a single dose of 40 mg/kg increased anxiety and depression-like behaviors and impaired learning and memory activity in mice. Mtx did not affect locomotor activity in the OF and PT tests. It decreased the total time spent in the central zone in the OF test, the total time spent in the open arms, and the number of entries into the open arms in the EPM test. Furthermore, it reduced the discrimination index in the NOR test. Elt was administered orally at 40 mg/kg daily, starting one week before the Mtx injection. Elt has a preventive impact on the Mtx-caused impaired anxiety and depression-like behaviors and learning and memory activity.

Our results from behavioral experiments indicated that Mtx treatment increased anxiety and depression-like behaviors while impairing learning-memory activity in mice. The findings are consistent with previous experimental studies in our laboratory (7,8). In our previous studies, we focused more on the mood disorders of Mtx, whereas here we also evaluated learning and memory functions, in which MTX group mice exhibited a negative discrimination index in the NOR test, indicating that the mice were unable to discriminate between novel and usual objects in the final phase of the NOR test. Chemotherapy-induced cognitive impairment is closely associated with the disruption of the equilibrium between the production and scavenging of reactive oxygen species, as well as the occurrence of neuroinflammation (8–10). indicated Another recent study found that Mtx raised MDA levels in brain tissue while decreasing SOD, glutathione (GSH), and glutathione peroxidase activity, as well as increasing inflammatory markers. The authors reported that these Mtx-induced changes were associated with impaired cognitive function as assessed by the Nor and Morris water maze (31). On the other hand, Mtx causes damage to organs other than the brain. Experimental studies have shown that Mtx treatment causes oxidative stress and inflammation in the liver (32,33), kidney (33), oral mucosal tissue (34), ovarium and uterus (35), lung (36), small intestine (37), and heart (38). The indirect contribution of systemic inflammation to neuroinflammation and ultimately to cognitive and mood disorders is an important issue that should not be overlooked, as pro-inflammatory cytokines such as TNF-alpha produced in systemic tissues by Mtx or any other agents can not only cause damage to the tissues in which they are produced but can also enter the brain via the bloodstream and cause neuroinflammation(39). Therefore, targeting oxidative stress and inflammatory processes, which play an essential role in the physiapathogenesis of Mtx-related behavioral disorders, would be the most reasonable approach to treating and/or controlling these disorders.

Elt is a chemically simple coumarin derivative with an additional hydroxyl group found in some plants (such as Artemisia scoparia, Artemisia capillaries, Ceratostigma willmottianum and Citrus limonia) used in traditional medicine (40). Elt has been shown to prevent neuroinflammation-induced mood disorders in mice by decreasing hippocampal lipid peroxidation, increasing antioxidant activity, and regulating cytokine homeostasis (18). Furthermore, Elt decreased the increased inactivity time observed in the forced swim test (FST) to acute restraint stress in 11-month-old mice and reversed the impaired contextual memory in the passive avoidance test, suggesting that this effect of Elt was mediated by the prevention of acute stress-induced oxidative stress (23). In the middle cerebral artery occlusion mouse model, Elt ameliorated neurological deficits by reducing infarct volume (20), while in the transient bilateral cerebral artery occlusion mouse model, Elt improved cognitive impairment by regulating hippocampal oxidative stress and preventing inflammation (21). In a study evaluating the effect of Elt on LPS-induced depressive behavior in mice, Elt reduced LPS-induced inactivity time in the TST and FST but did not alter locomotor activity in the OF test. A reduction in the amount of LPS-induced inflammatory cytokines was effective in this effect of Elt (19). Considering these studies, it is understood that the results obtained are consistent with our study and that oxidative stress and neuroinflammation play an essential role in the neuroprotective effect of Elt. In our study, Elt supplementation reduced Mtx-induced anxiety and depressive behaviors and improved impaired learning and memory activity. According to our findings, we do not know whether oxidative stress and/or neuroinflammation mediate the

mechanism of action of Elt, which constitutes the main limitation of our study. However, the first indication of the neuroprotective effect of Elt on Mtx-induced cognitive and mood impairment could encourage further studies in this area, ultimately conduce to the understanding of the phenomenon of chemotherapy-induced cognitive impairment.

This study was carried out to determine whether Elt has a protective effect against Mtx-caused anxiety and depression-like behaviors, as well as impaired learning activity. The results showed that a single dose of Mtx administered at 40 mg/kg increased anxiety and depression-like behaviors and impaired learning and memory functions, as measured by the NOR test. However, Elt supplementation effectively ameliorated these Mtx-induced neurological deficits.

Author Contributions: Conceptualization, M.O.; investigation, M.O., and F.T.; methodology, M.O. and F.T.; validation, M.O., and F.T.; writing—original draft, F.T.; writing—review & editing, M.O. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cheung YT, Krull KR. Neurocognitive outcomes in long-term survivors of childhood acute lymphoblastic leukemia treated on contemporary treatment protocols: A systematic review. Neurosci Biobehav Rev. 2015;53:108-120.
2. Christie LA, Acharya MM, Parihar VK, Nguyen A, Martirosian V, Limoli CL. Impaired cognitive function and hippocampal neurogenesis following cancer chemotherapy. Clin Cancer Res. 2012;18(7):1954-1965.
3. Pui CH, Yang JJ, Hunger SP, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol. 2015;33(27):2938-2948.
4. Yang V, Gouveia MJ, Santos J, et al. Breast cancer: insights in disease and influence of drug methotrexate. RSC Med Chem. 2020;11(6):646-664.
5. Turrina S, Gibelli F, De Leo D. Chemotherapy-induced cognitive impairment from the forensic medicine perspective: A review of the updated literature. J Forensic Leg Med. 2020;76:102070.
6. Gaman AM, Uzoni A, Popa-Wagner A, Andrei A, Petcu EB. The Role of Oxidative Stress in Etiopathogenesis of Chemotherapy Induced Cognitive Impairment (CICI)-"Chemobrain". Aging Dis. 2016;7(3):307-317.
7. Oz M, Akaras N. Effect of a TNF-Alpha Inhibitor on Anxiety and Depression-Like Behaviors in a Mouse Chemobrain Model. New Trends in Medicine Sciences. 2023;4(2):58-65.
8. Aslanlar DA, Vişneci EF, Oz M, Nurullahoglu Atalik KE. N-acetylcysteine ameliorates chemotherapy-induced impaired anxiety and depression-like behaviors by regulating inflammation, oxidative and cholinergic status, and BDNF release. Behavioural brain research. 2024;458:114740.
9. Sritawan N, Suwannakot K, Naewla S, et al. Effect of metformin treatment on memory and hippocampal neurogenesis decline correlated with oxidative stress induced by methotrexate in rats. Biomed Pharmacother. 2021;144:112280.
10. Welbat JU, Naewla S, Pannangrong W, Sirichoat A, Aranarochana A, Wigmore P. Neuroprotective effects of hesperidin against methotrexate-induced changes in neurogenesis and oxidative stress in the adult rat. Biochem Pharmacol. 2020;178:114083.
11. Güvenç M, Yüksel M, Kutlu T, Etyemez M, Gökçek İ, Cellat M. Protective effects of esculetin against ovary ischemia-reperfusion injury model in rats. J Biochem Mol Toxicol. 2024;38(1):e23528.
12. He Y, Li C, Ma Q, Chen S. Esculetin inhibits oxidative stress and apoptosis in H9c2 cardiomyocytes following hypoxia/reoxygenation injury. Biochem Biophys Res Commun. 2018;501(1):139-144.
13. Choi RY, Ham JR, Lee MK. Esculetin prevents non-alcoholic fatty liver in diabetic mice fed high-fat diet. Chem Biol Interact. 2016;260:13-21.
14. Türk E, Ozan Tekeli I, Özkan H, et al. The protective effect of esculetin against aluminium chloride-induced reproductive toxicity in rats. Andrologia. 2021;53(2):e13930.
15. Mohamadi-Zarch SM, Baluchnejadmojarad T, Nourabadi D, Ramazi S, Nazari-Serenjeh M, Roghani M. Esculetin Alleviates Acute Liver Failure following Lipopolysaccharide/D-Galactosamine in Male C57BL/6 Mice. Iran J Med Sci. 2021;46(5):373-382.
16. Zhang Y, Li Z, Wu H, Wang J, Zhang S. Esculetin alleviates murine lupus nephritis by inhibiting complement activation and enhancing Nrf2 signaling pathway. J Ethnopharmacol. 2022;288:115004.
17. Danis A, Baranoglu Kilinc Y, Torun IE, Hanci F, Kilinc E, Ankarali H. Esculetin alleviates pentylenetetrazole-induced seizures, cognitive impairment and pro-inflammatory cytokines and suppresses penicillin-induced epileptiform activity in rats. Life Sci. 2023;313:121300.

18. Sulakhiya K, Keshavlal GP, Bezbaruah BB, et al. Lipopolysaccharide induced anxiety- and depressive-like behaviour in mice are prevented by chronic pre-treatment of esculetin. Neurosci Lett. 2016;611:106-111.
19. Zhu L, Nang C, Luo F, et al. Esculetin attenuates lipopolysaccharide (LPS)-induced neuroinflammatory processes and depressive-like behavior in mice. Physiol Behav. 2016;163:184-192.
20. Wang C, Pei A, Chen J, et al. A natural coumarin derivative esculetin offers neuroprotection on cerebral ischemia/reperfusion injury in mice. J Neurochem. 2012;121(6):1007-1013.
21. Xu B, Zhu L, Chu J, et al. Esculetin improves cognitive impairments induced by transient cerebral ischaemia and reperfusion in mice via regulation of mitochondrial fragmentation and mitophagy. Behavioural brain research. 2019;372:112007.
22. Subramaniam SR, Ellis EM. Neuroprotective effects of umbelliferone and esculetin in a mouse model of Parkinson's disease. J Neurosci Res. 2013;91(3):453-461.
23. Martín-Aragón S, Villar Á, Benedí J. Age-dependent effects of esculetin on mood-related behavior and cognition from stressed mice are associated with restoring brain antioxidant status. Prog Neuropsychopharmacol Biol Psychiatry. 2016;65:1-16.
24. Yang M, Kim JS, Kim J, et al. Acute treatment with methotrexate induces hippocampal dysfunction in a mouse model of breast cancer. Brain Res Bull. 2012;89(1-2):50-56.
25. Dockman RL, Carpenter JM, Diaz AN, Benbow RA, Filipov NM. Sex differences in behavior, response to LPS, and glucose homeostasis in middle-aged mice. Behavioural brain research. 2022;418:113628.
26. Wang JY, Zhang Y, Chen Y, et al. Mechanisms underlying antidepressant effect of transcutaneous auricular vagus nerve stimulation on CUMS model rats based on hippocampal α7nAchR/NF-κB signal pathway. J Neuroinflammation. 2021;18(1):291.
27. Fathalizade F, Baghani M, Khakpai F, Fazli-Tabaei S, Zarrindast MR. GABA-ergic agents modulated the effects of histamine on the behaviour of male mice in the elevated plus maze test. Exp Physiol. 2022;107(3):233-242.
28. Zhang B, Wang PP, Hu KL, et al. Antidepressant-Like Effect and Mechanism of Action of Honokiol on the Mouse Lipopolysaccharide (LPS) Depression Model. Molecules. 2019;24(11).
29. Schlunk F, Fischer P, Princen HMG, et al. No effects of PCSK9-inhibitor treatment on spatial learning, locomotor activity, and novel object recognition in mice. Behavioural brain research. 2021;396:112875.
30. IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp
31. Khedr LH, Rahmo RM, Eldemerdash OM, et al. Implication of M2 macrophage on NLRP3 inflammasome signaling in mediating the neuroprotective effect of Canagliflozin against methotrexate-induced cognitive impairment. Int Immunopharmacol. 2024;130:111709.
32. Dogra A, Gupta D, Bag S, et al. Glabridin ameliorates methotrexate-induced liver injury via attenuation of oxidative stress, inflammation, and apoptosis. Life Sci. 2021;278:119583.
33. Türk E, Güvenç M, Cellat M, et al. Zingerone protects liver and kidney tissues by preventing oxidative stress, inflammation, and apoptosis in methotrexate-treated rats. Drug Chem Toxicol. 2022;45(3):1054-1065.
34. Bayramoglu Z, Mokhtare B, Mendil AS, et al. Effect of taxifolin on methotrexate-induced oxidative and inflammatory oral mucositis in rats: biochemical and histopathological evaluation. J Appl Oral Sci. 2022;30:e20220115.
35. Kiremitli T, Kiremitli S, Akselim B, et al. Protective effect of Coenzyme Q10 on oxidative ovarian and uterine damage induced by methotrexate in rats. Hum Exp Toxicol. 2021;40(9):1537-1544.
36. Zaki SM, Hussein GHA, Khalil HMA, Abd Algaleel WA. Febuxostat ameliorates methotrexate-induced lung damage. Folia Morphol (Warsz). 2021;80(2):392-402.
37. Natarajan K, Abraham P, Kota R, Selvakumar D. Aminoguanidine pretreatment prevents methotrexate-induced small intestinal injury in the rat by attenuating nitrosative stress and restoring the activities of vital mitochondrial enzymes. J Basic Clin Physiol Pharmacol. 2017;28(3):239-247.
38. Aboubakr M, Farag A, Elfadadny A, Alkafafy M, Soliman A, Elbadawy M. Antioxidant and anti-apoptotic potency of allicin and lycopene against methotrexate-induced cardiac injury in rats. Environ Sci Pollut Res Int. 2023;30(38):88724-88733.
39. Pan W, Kastin AJ. TNFalpha transport across the blood-brain barrier is abolished in receptor knockout mice. Exp Neurol. 2002;174(2):193-200.
40. Sim MO, Lee HI, Ham JR, Seo K ll, Lee MK. Long-term supplementation of esculetin ameliorates hepatosteatosis and insulin resistance partly by activating AdipoR2-AMPK pathway in diet-induced obese mice. J Funct Foods. 2015;15:160-171.