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# Evaluation of the neuroprotective effects of Korean Ginseng root extract in an experimental model of multiple sclerosis

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ABSTRACT

Objective: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterised by demyelination. The aim of this study was to evaluate the neuroprotective effects of Korean ginseng root extract (KGE) using a cuprizone-induced demyelination model.

Materials and Methods: C57BL/6 mice were divided into control, demyelination and remyelination groups and each group was treated with KGE. Demyelination was induced with 0.2% cuprizone in the diet for four weeks. KGE (100 mg/kg) was administered by gavage during or after the cuprizone exposure. Body weight, food and water intake, and motor performance parameters were investigated. In addition, glutathione (GSH), glutathione-S-transferase (GST), superoxide dismutase (SOD) malondialdehyde (MDA), oligodendrocyte transcription factor-2 (OLIG2) and myelin basic protein (MBP) levels were measured in brain samples, while MBP and glial fibrillary acidic protein (GFAP) expression was assessed by immunohistochemistry and myelin status was examined using Luxol Fast Blue staining.

Results: Korean ginseng root extract prevented myelin loss, promoted remyelination, and improved motor performance. It reduced oxidative stress by increasing GSH, GST, and SOD levels while decreasing MDA. KGE also suppressed demyelination by reducing astrogliosis and restoring OLIG2 and MBP levels.

Conclusion: Korean ginseng root extract exhibits neuroprotective properties during demyelination and promotes remyelination, highlighting its therapeutic potential for MS.

Keywords: Rotarod, MBP, OLIG2, GFAP, LFB, Demyelination

## 1. INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS), characterized by inflammation and progressive demyelination [1]. These pathological processes lead to the development of lesions in both gray and white matter [2]. MS is known to be one of the most common causes of chronic neurological functional disability, typically appearing between the ages of 20 and 40, and occurring twice as frequently in women as in men [3]. About 2.5 million people globally are affected by this disease, with its incidence and prevalence increasing in recent years [4, 5]. The etiology of the disease is influenced by more than 100 genetic variants and a range of environmental factors. However, the triggering events differ between individuals, and the phenotypic consequences of these events have not been fully understood [1].

Modern MS therapies focus on targeting the immunological mechanisms underlying the disease, aiming to reduce neuroinflammation, slow progression, and lower the frequency of relapses. Current treatment options include oral medications such as dimethyl fumarate, teriflunomide and fingolimod; injectable agents such as interferon-β and glatiramer acetate and infusion therapies such as natalizumab and mitoxantrone [6]. However, the limited effects of current treatments on enhancing

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myelin regeneration and repairing neuronal damage highlight the increasing need for novel therapeutic approaches to facilitate remyelination [2, 7]. Therefore, therapeutic approaches designed to support myelin sheath repair by increasing the activation of oligodendrocyte precursor cells, which both support the antioxidant system and repair neuronal damage, have gained importance.

Experimental models are critically important for elucidating the pathophysiological mechanisms of the disease and evaluating potential treatment strategies. The cuprizone model is a frequently used experimental MS model that induces demyelination by causing oligodendrocyte loss and myelin damage in the CNS [8, 9]. According to the studies conducted by Hiremath et al., the most prominent demyelination was observed in the corpus callosum when the diets of 8-10 week old male C57BL/6 mice were supplemented with 0.2% cuprizone for four weeks [10]. Cuprizone induces oligodendrocyte degeneration through oxidative stress and mitochondrial dysfunction, thereby, presenting a pathology similar to the demyelination processes observed in MS. The characteristics of cuprizone makes it a validated model for studying the demyelination mechanisms and assessing potential therapeutic candidates [11].

Korean ginseng, belonging to the Araliaceae family, has been traditionally used as a medicinal preparation for over 2,000 years to treat various neurodegenerative diseases. The main active ingredients are called ginsenosides, which are triterpene glycosides [12, 13]. Korean ginseng and ginsenosides exhibit positive effects on inflammatory [14], cardiovascular [15], and neurodegenerative diseases, including Parkinson's [16], Alzheimer's and Huntington's diseases [12, 16]. Recent studies have shown that Korean ginseng root extract (KGE) exerts neuroprotective effects on Huntington-like symptoms by regulating the nuclear factor-kappa B signaling pathway and microglia activation. In addition, polysaccharide derived from Korean ginseng has been shown to ameliorate experimental autoimmune encephalomyelitis and promote the elevation of regulatory T cells [17,18]. Furthermore, two studies conducted parallel to ours indicated that KGE contributes to decreased demyelination, enhanced remyelination, and reduced inflammatory responses [19, 20]. These findings support the curative potential of KGE that may play a beneficial role in the treatment of autoimmune diseases like MS.

In this study, our goal was to comprehensively investigate the neuroprotective effects of KGE in a cuprizone-associated demyelination mouse model. Our study may provide evidence supporting the potential therapeutic application of KGE in the treatment of MS.

## 2. MATERIALS and METHODS

### *Experimental animals and conditions*

Eight-week-old (15–25 g) C57BL/6 male and female mice were used. The animals were obtained from the Experimental Animal Research Unit (DEHAL) of Medeniyet University and housed in accordance to the international ethical standards. Following a one-week adaptation period before the experiments, a 12-hour light/12-hour dark cycle, a constant temperature of 22 ± 2 °C, and appropriate humidity conditions were maintained. All animals were provided with ad libitum access to designated feed and drinking water. We confirm that we have reviewed the journal's ethical publication policies and that this report complies with these guidelines. This study received approval from the Marmara University, Experimental Animals Local Ethics Committee. (approval number: 23-2023mar, date. 9 May, 2023).

### *Cuprizone administration and experimental design*

A diet containing 0.2% (w/w) cuprizone was prepared according to widely accepted protocols in the literature for use in the experiment. Standard pellet rat feed was ground into a fine powder with a food processor, and 0.2% (w/w) cuprizone powder (Sigma-C9012) was uniformly added. The mixture was turned into a dough-like consistency with sterile distilled water, reformed into pellet shape, and stabilized by drying in an oven at low temperature <60 °C [21]. KGE was obtained from Casel İlaç Sanayi A.Ş. (Istanbul, Turkiye) and dissolved in distilled water.

To evaluate the potential effects of KGE during the demyelination and remyelination stages, a total of 52 C57BL/6 male and female mice were divided into six main experimental groups. Each group was further divided into male and female subgroups, creating a total of twelve subgroups. Three females and three males were in the control subgroups, while five females and five males were in the experimental subgroups.

In the experiment, the mice in the control group were given a standard diet for four weeks, while the Control+KGE group was given a standard diet with 100 mg/kg KGE (i.g) for the same period. To induce demyelination, the DM group was given a 0.2% cuprizone diet for four weeks, while the DM+KGE group received the same diet with 100 mg/kg KGE (i.g) treatment. During the remyelination stage, the RM group was switched to a standard diet for two weeks after a cuprizone diet for four weeks, while the RM+KGE group received an additional 100 mg/kg KGE (i.g) treatment during this period. We determined the dose of 100 mg/kg KGE based on the study by Zhu et al., which reported significant neuroprotective effects in a vascular dementia rat model using this concentration [22].

### *Rotarod Test*

Following the conclusion of the experiment, a rotarod apparatus was used to assess the motor coordination and locomotor activity of the mice. The rotarod test is widely used to quantitatively assess the effects of CNS injuries, neurodegenerative diseases, and drug treatments on motor functions. This apparatus consists of a rotating rod with a gradually increasing speed over a predefined time interval. Each mouse underwent a short training and adaptation session before the test and was then placed on a rotarod at a low speed. The speed of the rod was gradually increased from 4 to 40 rpm over a 5-minute period using an accelerating protocol. The time the mice remained on the rod before falling (latency to fall) was recorded as the primary performance measure using an automatic timer. Each animal was subjected to the test three times, and the average latency


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Marmara Med J 2025;38(2): 158-170

to fall from three independent trials was used for evaluation to minimize individual variation [23, 24].

### *Biochemical and molecular analyses*

Under light ether anesthesia, brain tissues were collected from decapitated mice. Brain samples were homogenized in phosphate buffered saline (PBS) solution (pH 7.4) at 10% concentration. Homogenized tissues were then centrifuged at 4°C to obtain the supernatant, which was used for biochemical analyses. To preserve enzymatic activities, supernatants were stored in single-use aliquots and analyzed as soon as possible. All biochemical tests were performed following standard protocols, ensuring accuracy and reproducibility. Oligodendrocyte transcription factor-2 (OLIG2) levels were measured using the Mouse OLIG2 ELISA Kit (ODC-Lab, Turkiye). Myelin basic protein (MBP) levels were determined using the Mouse MBP ELISA Kit (Elabscience, E-EL-M0805). Oxidative stress was assessed by measuring malondialdehyde (MDA) levels, lipid peroxidation (LPO) [25], glutathione (GSH) [26] and glutathione-S-transferase (GST) [27] levels. Superoxide dismutase (SOD) [28] activities were evaluated as an indicator of free radical neutralization [29]. For biochemical analyses, tissues from multiple animals per group were pooled to obtain sufficient sample volume, and statistical analyses were performed on group-level data rather than individual animal data.

### *Histological evaluation*

Brain tissues were fixed in 4% paraformaldehyde (in 0.1 M PBS pH 7.4) and processed for light microscopic investigations. Tissues were embedded in paraffin and coronal brain sections (5 µm-thick) cut by rotary microtome (Leica RM2155, Germany) were stained with 0.1% luxol fast blue (LFB) and cresyl violet stain to determine myelin integrity. Finally, sections were evaluated under a photomicroscope (Olympus BX51, Tokyo, Japan) with a digital camera (Olympus DP72, Tokyo, Japan) attachment.

After light microscopic preparation, 5 µm thick coronal brain sections taken from paraffin-embedded brain tissue blocks on positively charged slides with the help of a microtome (Leica RM2125 RTS) were deparaffinized at 37°C for one night. After deparaffinization in xylene, 96% ethanol was applied to the sections, followed by suppression of endogenous peroxidase activity in the tissue using 3% hydrogen peroxide for 20 minutes. Brain tissue sections were protein-blocked (UltraTek Hrp Anti – Polyvalent, ScyTek). MBP (E-AB-70266, Elabscience, 1:10,000) and glial fibrillary acidic protein (GFAP) (clone GA5, MAB 360, Merck Millipore, 1/500) primary antibodies were then added to the sections and incubated at +4°C temperature for one night. At the end of the incubation period, sections were incubated in a biotinylated secondary antibody (UltraTek Hrp Anti-Polyvalent, ScyTek) for 20 minutes. After washing with PBS again, streptavidin peroxidase (UltraTek Hrp Anti-Polyvalent, ScyTek) was added and incubated for 20 minutes. Sections washed with PBS were treated with 3,3' – diaminobenzidine chromogen (Elabscience E-IR-R101). The sections were washed with distilled water, counterstained with Mayer Hematoxylin and covered with entellan, and the sections were finally evaluated under a computerized photo-light microscope (Olympus BX51, Tokyo, Japan) with a CCD camera (Olympus DP 72, Tokyo, Japan) attachment and photographs were taken. The MBP immunostaining was semi-quantitatively scored as 0: none; 1: mild staining; 2: moderate staining; 3:severe staining and average immunostaining scores for each group were statistically analyzed.

### Statistical Analyses

GraphPad Prism 9 (GraphPad Software, San Diego, USA) was used for all statistical analyses. Data were analyzed using one-way analysis of variance (One-Way ANOVA) and post-hoc Tukey's test was applied for multiple comparisons. All results are expressed as mean ± SEM, and statistical significance was set at $p < 0.05$. This methodology facilitated our statistical comparison of demyelination and remyelination processes and allowed us to quantitatively evaluate the therapeutic potential of KGE.

## 3. RESULTS

The data presented in Figure 1 demonstrate that cuprizone treatment caused significant weight loss in both sexes compared to the control group ($p < 0.01$ and $p < 0.01$), DM+KGE groups partially alleviated weight loss in male mice ($p < 0.05$), showing a more pronounced recovery trend than in female mice. During the RM phase, both male and female mice regained significant weight relative to the DM group ($p < 0.05$ and $p < 0.01$, individually), with female mice approaching body weights closer to the control group.

Figure 2A highlights food consumption results. The control group exhibited normal food consumption throughout the experiment. In contrast, the DM group showed a substantial decline in food consumption compared to the controls ($p < 0.001$). KGE administration in the DM+KGE group significantly increased food consumption ($p < 0.01$). Food consumption in the RM group partially approached to that of the controls but was not statistically significant and KGE supplementation did not significantly alter the food consumption. Figure 2B shows water intake results. While the control group maintained consistent water intake, the DM group exhibited a pronounced reduction in water intake ($p < 0.001$). Partial recovery in water intake was observed with KGE treatment in the DM+KGE group but these changes remained limited. KGE treatment demonstrated restorative effects, particularly in food consumption, with modest improvements in body weight.

In the DM group, GSH levels were dramatically lower compared to the control group ($p < 0.05$ in female mice, $p < 0.01$ in male mice, Figure 3A). The male DM+KGE groups had significantly increased GSH levels ($p < 0.05$), while the increase observed in female mice was not statistically significant. In RM+KGE groups, GSH levels approached to those in control groups, but no meaningful variation was noted. Similarly, GST levels in the DM groups pronouncedly decreased compared to the controls ($p < 0.001$, Figure 3B). KGE treatment significantly increased GST levels in DM+KGE groups ($p < 0.01$). In the RM group,


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GST levels in KGE-treated groups approached control values ($p < 0.01$ in males, $p < 0.001$ in females), indicating substantial recovery in GST activity. As seen in Figure 3C, DM caused a significant reduction in SOD activity relative to controls ($p < 0.001$). KGE treatment significantly increased SOD activity ($p < 0.01$). While, SOD levels in male in the RM group exhibited a notable improvement relative to the DM group ($p < 0.01$), the development in female did not reach statistically significant levels. On the other hand, MDA levels markedly increased relative to the controls ($p < 0.05$, Figure 3D). KGE treatment significantly reduced MDA levels in male mice ($p < 0.05$), while the reduction observed in female mice was not statistically significant. MDA levels in the RM group in KGE-treated male mice approached control levels ($p < 0.01$), but no statistically notable decrease was observed in female mice.

In the DM groups, MBP levels presented a significant reduction compared to the control group ($p < 0.001$, Figure 4A in both male and female mice). In female mice, KGE treatment partially prevented MBP loss compared to the DM group ($p < 0.01$), while in male mice, KGE treatment pronouncedly increased MBP levels ($p < 0.01$). In the RM group, female mice exhibited a notable increase in MBP levels compared to the DM group ($p < 0.001$), whereas no meaningful variation in MBP levels was observed between the DM and RM groups in male mice. However, an important difference in MBP levels was found between the RM and RM+KGE groups in male mice ($p < 0.05$). Overall, changes in MBP levels followed a similar pattern in both sexes, but MBP recovery was observed to occur more rapidly in female mice. As seen in Figure 4B, in female mice, a significant reduction in OLIG2 levels was detected in the DM group compared to the control group ($p < 0.05$), while a partial increase, though not statistically significant, was noted after KGE treatment. In the RM group, OLIG2 levels were very close to the control group. In male mice, the decrease in OLIG2 levels was more pronounced than in females ($p < 0.001$), and KGE treatment resulted in a significant improvement ($p < 0.05$). KGE treatment did not show a significant effect on OLIG2 levels in the RM+KGE groups in both genders.

As seen in Figure 5, cuprizone significantly worsened motor performance in both genders ($p < 0.001$). KGE treatment significantly improved impaired motor performance in the DM group ($p < 0.01$). Motor performance approached control values in the RM group ($p < 0.001$), but KGE showed a limited contribution in the RM+KGE group. There was no statistically significant difference in motor performance between genders, and the effects of KGE were similar in both genders.

As seen in Figure 6, LFB staining in the experimental groups revealed a decrease in the myelinated areas of the corpus


***Figure 1.*** *The effects of cuprizone administration and KGE treatment on body weight in female, and male mice. Bars represent the mean ± SEM. Sample sizes per group: Control and Control+KGE groups (n=3 per sex); DM, DM+KGE, RM and RM+KGE groups (n=5 per sex). *: p<0.05, **: p<0.01: vs control groups. +: p<0.05: vs DM group. λ : p<0.05, λλ: p<0.01: vs DM group. KGE, Korean Ginseng Root Extract.*

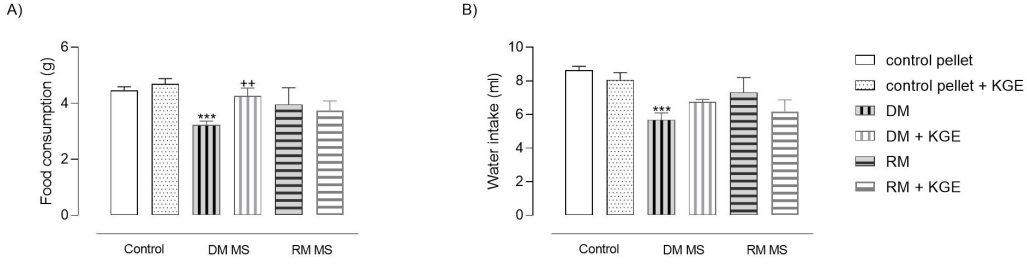


***Figure 2.*** *The effects of cuprizone administration and KGE treatment on A) food consumption, and B) water intake in mice. Bars represent the mean ± SEM. Sample sizes per group: Control and Control+KGE groups (n=3 per sex, total n=6); DM, DM+KGE, RM and RM+KGE groups (n=5 per sex, total n=10 per group). ***: p<0.001: vs control groups. ++: p<0.01: vs DM group. KGE, Korean Ginseng Root Extract.*


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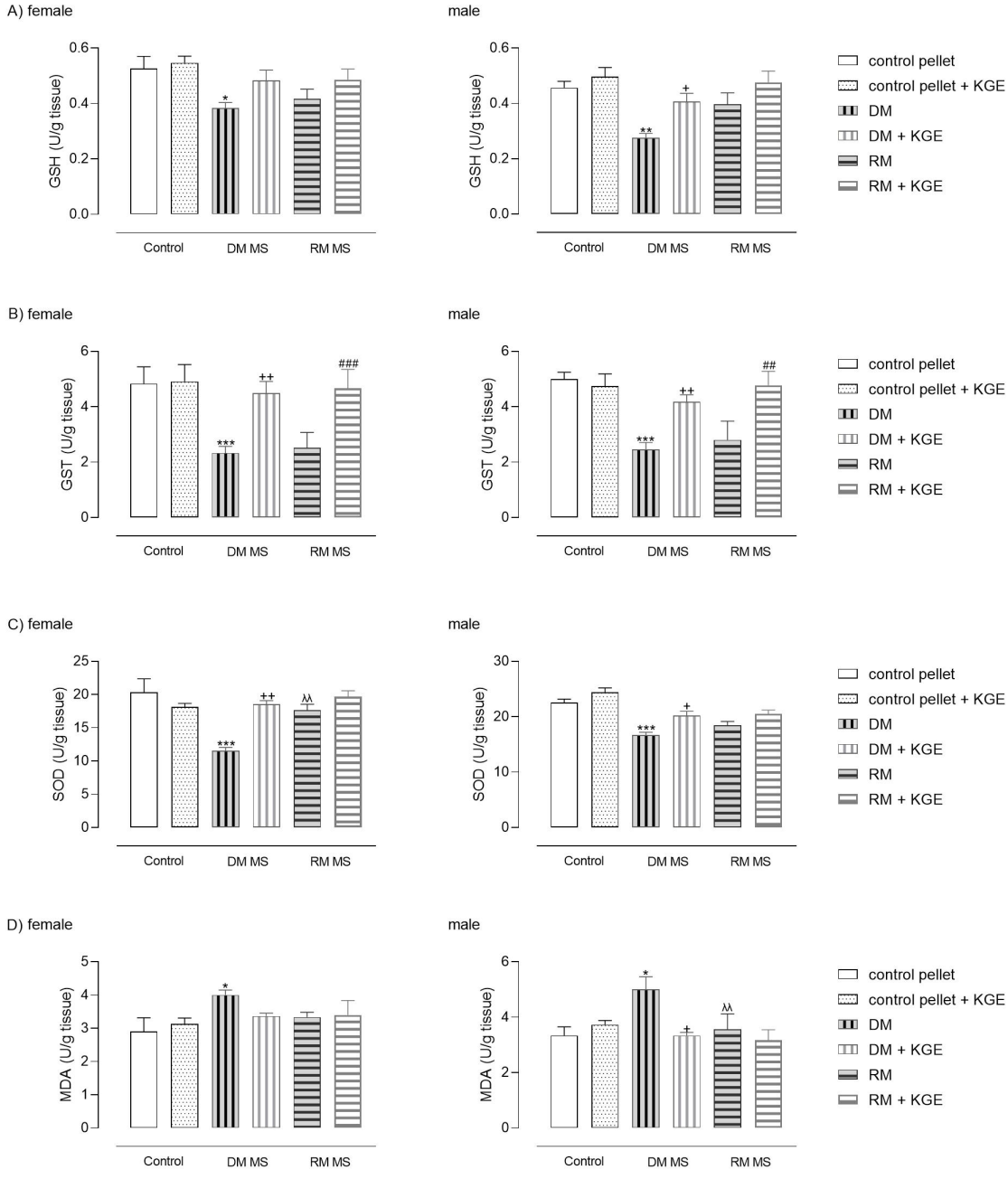


***Figure 3.*** *Effects of cuprizone administration and KGE treatment on antioxidant enzyme activities: A) GSH, B) GST, C) SOD, and D) MDA levels in brain tissue. Bars represent the mean ± SEM from pooled samples (each sample represents tissues from 3 animals per sex in the Control and Control+KGE groups, and 5 animals per sex in DM, DM+KGE, RM and RM+KGE groups). * : p<0.05, ***: p<0.001: vs control groups. + : p<0.05, ++: p<0.01: vs DM group. λλ: p<0.01: vs DM group. GSH, Glutathione; GST, Glutathione S-transferase; SOD, Superoxide Dismutase; MDA, Malondialdehyde; KGE, Korean Ginseng Root Extract.*


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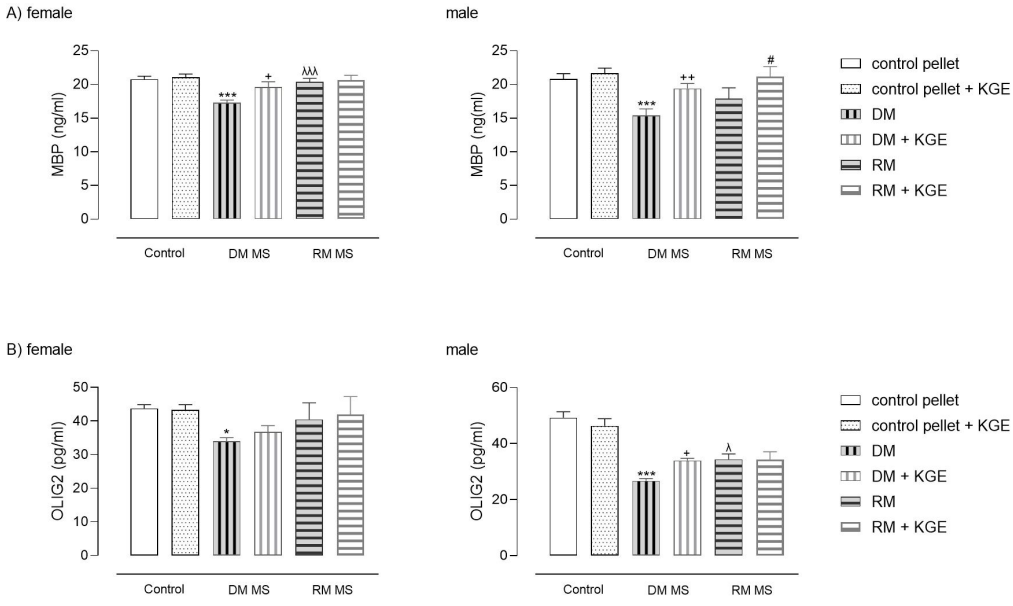


***Figure 4.*** *Effects of cuprizone administration and KGE treatment on A) MBP and B) OLIG2 levels in brain tissue. Bars represent the mean ± SEM from pooled samples (each sample represents tissues from 3 animals per sex in the Control and Control+KGE groups, and 5 animals per sex in DM, DM+KGE, RM and RM+KGE groups). ***: p<0.001: vs control groups. ++: p<0.01: vs DM group. λ: p<0.05, λλ: p<0.01: vs DM group. MBP, Myelin Basic Protein; OLIG2, Oligodendrocyte Transcription Factor 2; KGE, Korean Ginseng Root Extract.*

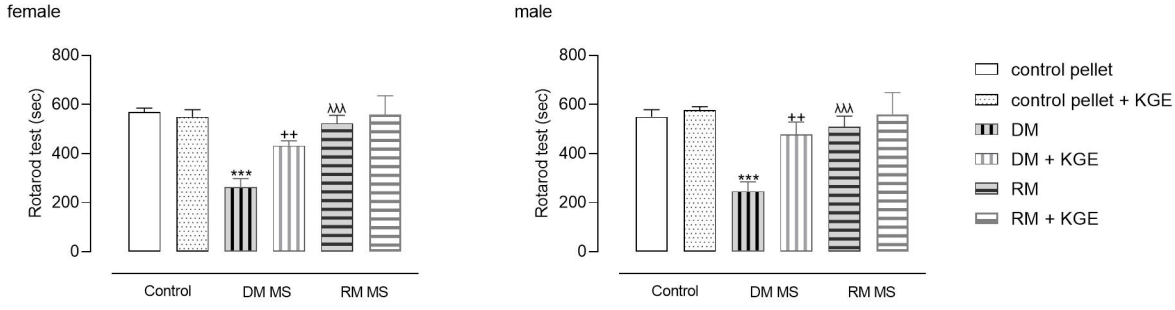


***Figure 5.*** *Effects of cuprizone administration and KGE treatment on Rotarod performance. Bars represent the mean ± SEM. Sample sizes per group: Control and Control+KGE groups (n=3 per sex); DM, DM+KGE, RM and RM+KGE groups (n=5 per sex). ***: p<0.001: vs control groups. ++: p<0.01: vs DM group. λλλ: p<0.001: vs DM group KGE, Korean Ginseng Root Extract.*

callosum in the DM and RM groups compared to the controls (Figure 6 A, B, C, D). On the other hand, KGE administration slightly increased myelinated areas in the corpus callosum of mice DM+KGE and RM+KGE groups showed slightly increased myelinated areas in the corpus callosum compared to the DM and RM groups (Figure 6 E, F).

In the corpus callosum region of cuprizone administered mice immunostained with MBP (DM group, Figure 7C), MBP-positive immunostaining were observed to be decreased when compared to that of the control group (Figure 7A). On the other hand, an increase in the immunostaining with MBP was observed in the DM+KGE (Figure 7D) and RM+KGE (Figure 7F) groups when compared to the DM and RM (Figure 7E) groups, respectively.

As presented in Figure 8, MBP semiquantitative scoring revealed that there was a considerable reduction in the DM group (p <


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Marmara Med J 2025;38(2): 158-170

0.001), while KGE treatment notably increased the MBP staining in the DM+KGE and RM+KGE groups ($p < 0.01$).

Compared to the control groups (Figure 9A, B), an increase in GFAP expression was observed in the hippocampal dentate gyrus regions of the mice in the DM group (Figure 9C). In the DM+KGE (Figure 9D) and RM+KGE groups (Figure 9F), a decrease in GFAP immunoexpression was identified relative to the DM group (Figure 9C) and RM (Figure 9E) groups, respectively.

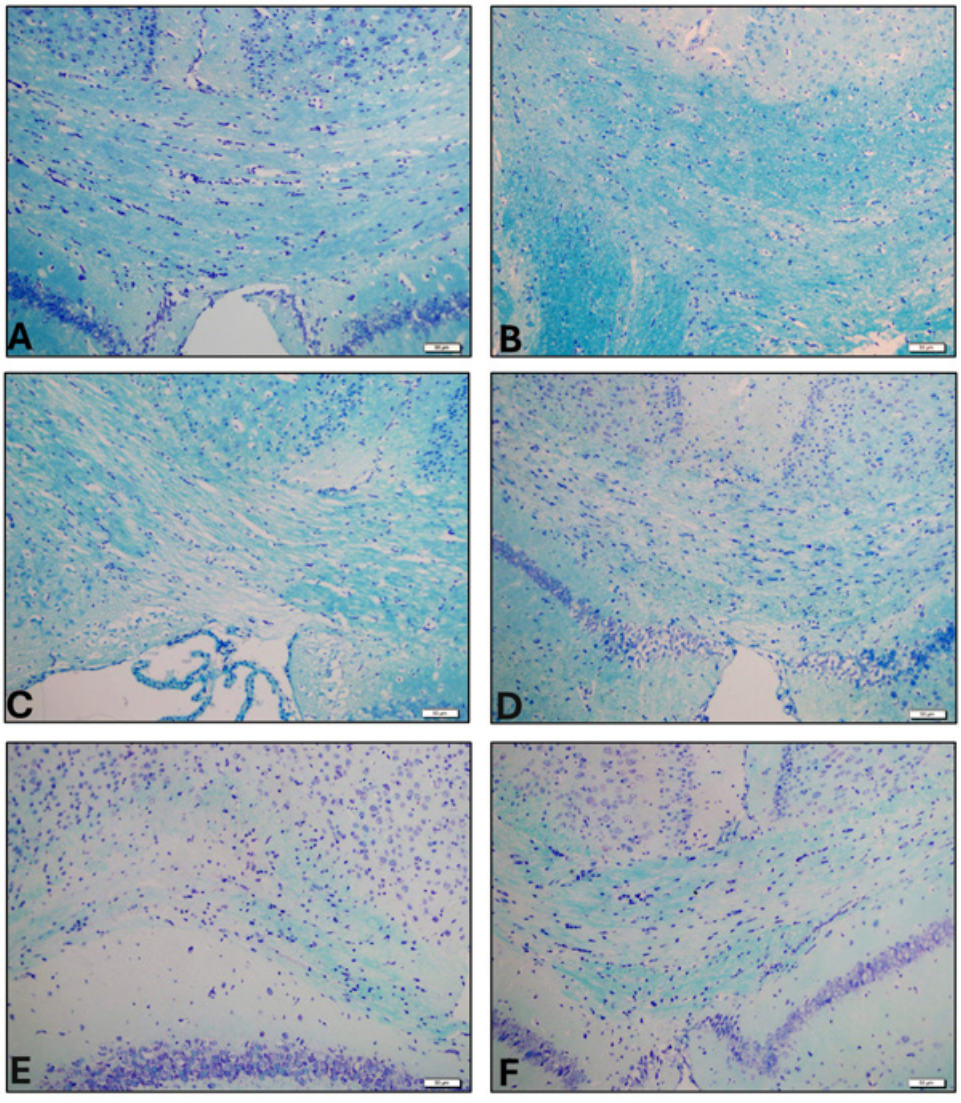


***Figure 6.*** *Representative photomicrographs of LFB staining in the corpus callosum region of experimental animals. A) Control pellet, B) Control pellet + KGE, C) DM, D) DM+KGE, E) RM and F) RM+KGE. Scale bars = 50 µm. Images are representative of Control and Control+KGE groups (n=3 per sex, total n=6); DM, DM+KGE, RM, and RM+KGE groups (n=5 per sex, total n=10 per group). LFB, Luxol Fast Blue; KGE, Korean Ginseng Root Extract.*


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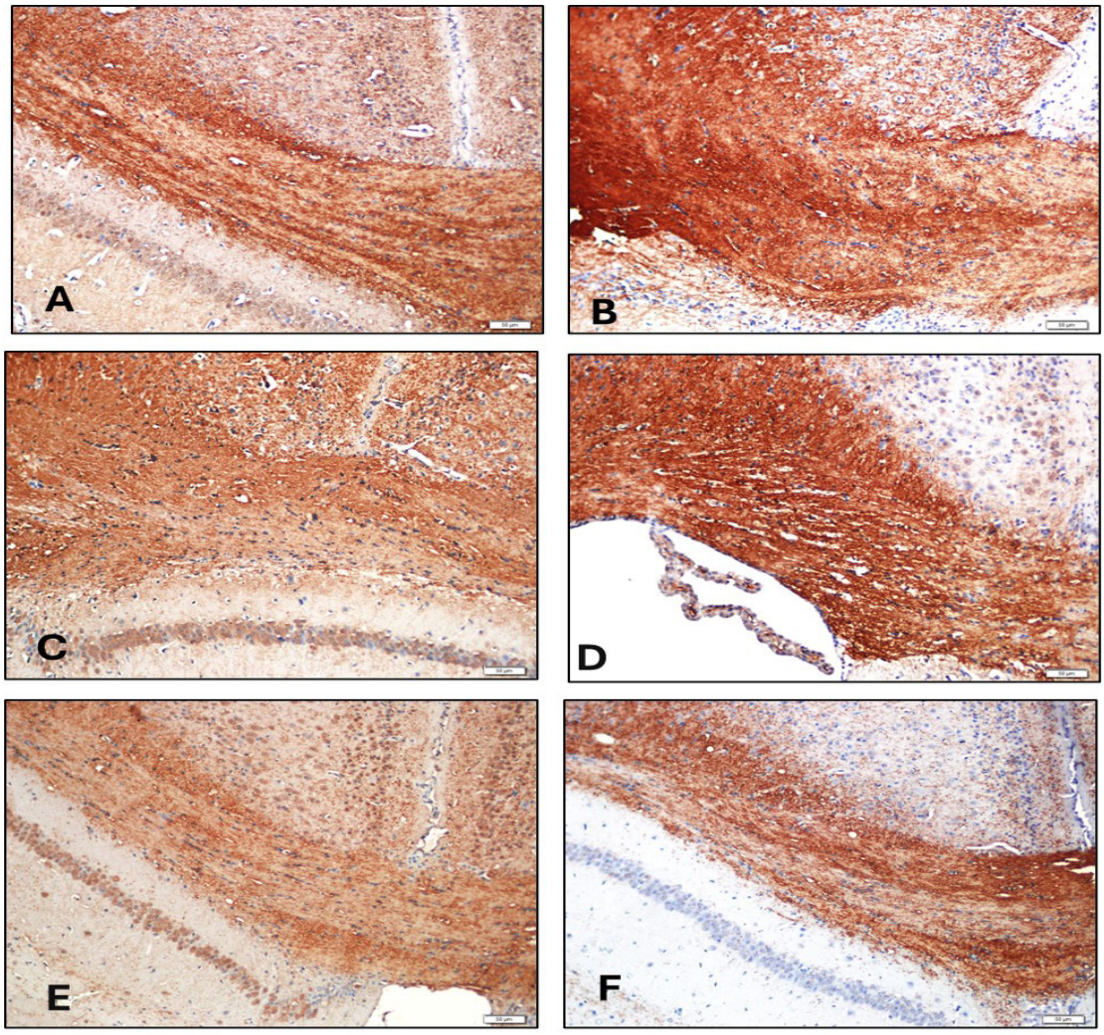


***Figure 7.*** *Representative photomicrographs of MBP immunohistochemistry in the corpus callosum regionA) Control pellet, B) Control pellet + KGE, C) DM, D) DM+KGE, E) RM and F) RM+KGE. Images are representative of Control and Control+KGE groups (n=3 per sex, total n=6); DM, DM+KGE, RM, and RM+KGE groups (n=5 per sex, total n=10 per group). Scale bar = 20 µm. MBP, Myelin Basic Protein; KGE, Korean Ginseng Root Extract.*

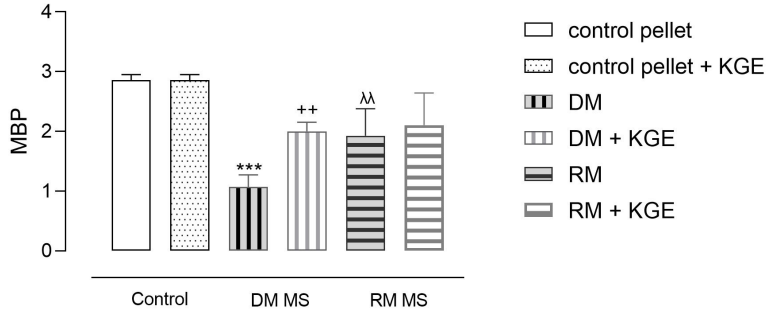


***Figure 8.*** *Immunohistochemistry scoring analysis of MBP immunoreactive area levels in the corpus callosum. Bars represent the mean ± SEM. Sample sizes per group: Control and Control+KGE groups (n=3 per sex, total n=6); DM, DM+KGE, and RM groups (n=5 per sex, total n=10 per group). ***: p<0.001: vs control groups. ++: p<0.01: vs DM group. λλ: p<0.001: vs DM group. MBP, Myelin Basic Protein; KGE, Korean Ginseng Root Extract.*


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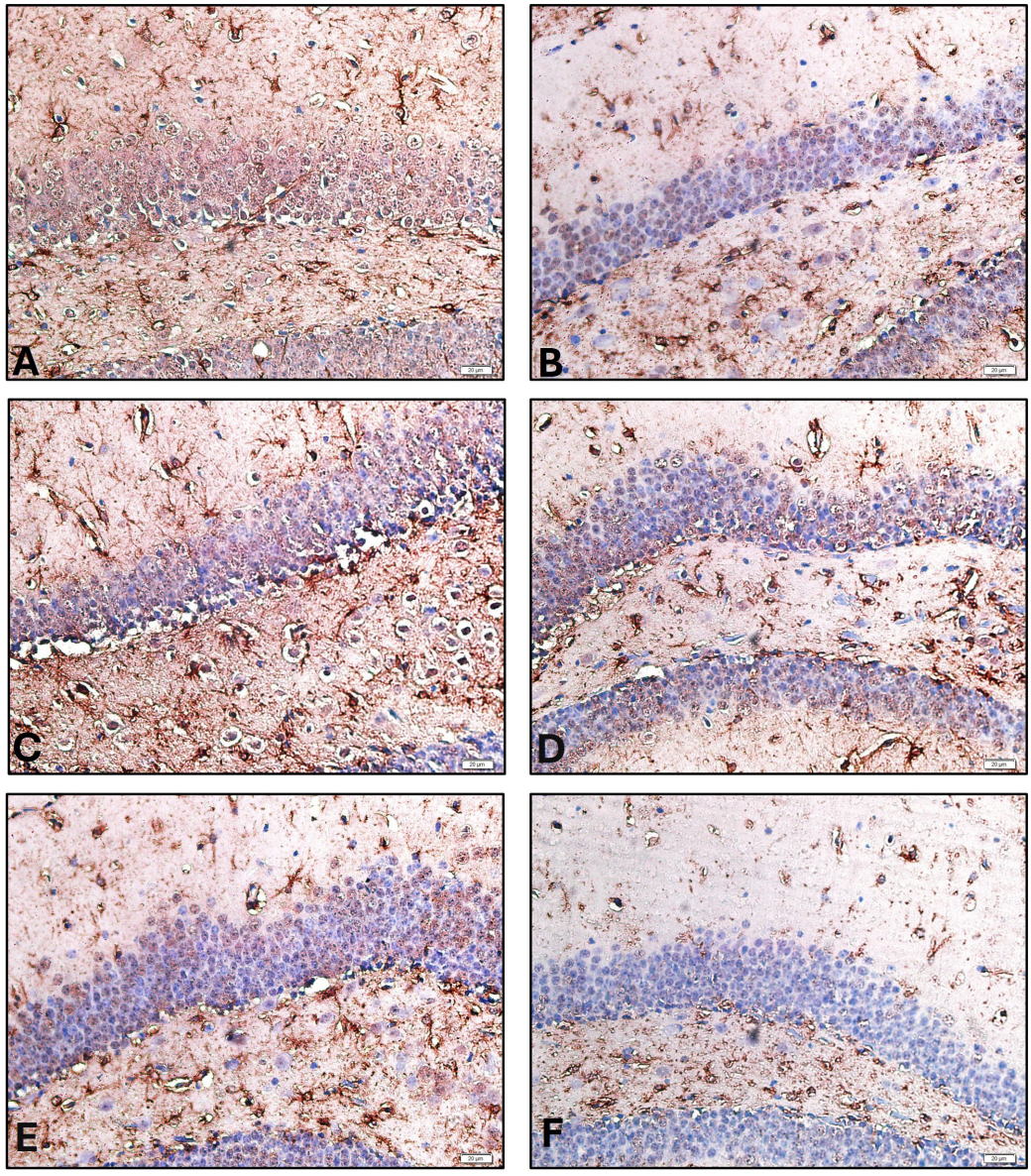


***Figure 9.*** *Representative photomicrographs of GFAP immunohistochemistry in the hippocampal dentate gyrus region. A) Control pellet, B) Control pellet + KGE, C) DM, D) DM+KGE, E) RM and F) RM+KGE. Images are representative of Control and Control+KGE groups (n=3 per sex, total n=6); DM, DM+KGE, RM, and RM+KGE groups (n=5 per sex, total n=10 per group). Scale bar = 20 µm. GFAP, Glial Fibrillary Acidic Protein; KGE, Korean Ginseng Extract.*

## 4. DISCUSSION

In this study, we assessed the potential neuroprotective effects and sex-specific differences of KGE during cuprizone-induced DM and subsequent RM phases in C57BL/6 mice. The results showed that KGE has the ability to reverse DM processes and promote RM processes. This is consistent with the established literature on the antioxidant and neuroprotective effects of KGE, and our study further strengthens its role in neuroprotection mechanisms. In addition to the toxic effects of cuprizone on CNS demyelination, it is known to cause significant impairments in energy and appetite mechanisms [8]. As illustrated in Figure 1, administration of cuprizone in the DM group led to weight loss in both male and female mice. However, weight loss was more pronounced in females, indicating that the toxic effects of cuprizone on the CNS may impair energy metabolism more severely in females. This situation is consistent with literature that cuprizone disrupts energy metabolism and appetite


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Marmara Med J 2025;38(2): 158-170

mechanisms. [10, 30]. KGE treatment significantly increased body weight in the female DM group, approaching control levels. Furthermore, it showed that KGE reduces the cachectic effects of cuprizone and promotes weight gain. However, in the male DM group, KGE did not lead to significant improvement and body weight did not approach control levels. This indicates that the effects of KGE on energy metabolism may differ between the sexes. In the RM group, body weight almost returned to control levels through natural RM processes, and when KGE was added in the RM+KGE group the change in body weight was minimal. In short, the toxic effects of cuprizone on the CNS disrupted energy and appetite mechanisms, causing weight loss, and this weight loss was more substantial in female mice.

As seen in Figure 2A, the toxic effects of cuprizone in the DM group resulted in a significant reduction in food consumption. This is consistent with literature suggesting that cuprizone suppresses appetite mechanisms and creates a cachectic state [30]. In the RM group, although, food consumption levels did not approach control levels, mice tended to gain weight. When food consumption data were compared, there was a two-week difference in the six-week follow-up period of the RM group compared to the four-week follow-up period of the DM and control groups. We expected a similar increase in weight gain to be accompanied by a parallel increase in food consumption. However, we did not observe a corresponding increase in food consumption. This suggests that the RM phase compensates for the cachectic state caused by cuprizone toxicity. Furthermore, we found that KGE did not significantly affect body weight or food consumption in the RM+KGE groups. In addition, water intake parameters shown in Figure 2B also decreased in parallel with food consumption and did not show a statistically significant increase with the addition of KGE. These changes in water intake were consistent with food consumption and reflected the general health status of the mice. In short, the cachectic state induced by cuprizone led to decreased food and water intake and weight loss, while the RM process partially attenuated these effects. KGE administration contributed minimally to the improvement of metabolic parameters.

Cuprizone is known to trigger the DM process in the CNS by triggering oxidative stress, neuroinflammation, and imbalances in energy metabolism [31]. GSH plays a critical role in maintaining cellular redox balance against the harmful effects of ROS. Disruption of this balance by cuprizone is known to lead to oligodendrocyte apoptosis and damage to the myelin sheath [32]. As shown in Figure 3A, GSH levels were significantly reduced in the DM group cpmpared to the control group. KGE treatment brought GSH levels close to control levels in the DM+KGE group. This effect may be related to the ginsenoside content of KGE, which increases GSH levels through the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway [33]. This increase highlights the protective role of KGE. We observed a more significant decrease in GSH levels in male DM groups compared to females. In male groups with KGE treatment, GSH levels reached levels close to control levels and these changes were not statistically significant in females. These findings suggest that female mice may be more resistant to cuprizone-induced oxidative stress and that KGE may have a more limited effect in females. GST is an important enzyme responsible for detoxifying toxic intermediates of ROS. As we show in Figure 3B, GST levels were considerably reduced in the DM group, while KGE treatment reversed this decrease. The increase in GST levels with KGE treatment is associated with the ability of ginsenosides to support antioxidant defense systems and improve cellular stress response. These findings suggest that KGE contributes to the restoration of detoxification capacity during the remyelination process. These data are consistent with the literature showing that ginsenosides promote the expression of detoxification enzymes such as GST by activating the Nrf2 signaling pathway [34]. We also observed that GST levels were suppressed in mice of both sexes and that KGE treatment alleviated these adverse effects. SOD is an antioxidant enzyme that plays an important role in protecting against oxidative stress by converting superoxide anions into nontoxic molecules [35]. As we show in Figure 3C, SOD activity in the DM group was significantly reduced compared to the control. Although, the RM process provided a significant recovery of SOD activity, KGE treatment further enhanced this recovery. This effect of KGE can be explained by its ability to restore ROS balance, thus supporting the efficient use of energy and structural components by oligodendrocytes [36, 37]. SOD activity was generally similar between the male and female groups. However, in the female RM group, SOD levels were close to control values even in the absence of KGE treatment. This finding suggests a more effective RM process and reduced oxidative stress load in the RM group. MDA, a by product of lipid peroxidation, is used as a biomarker to assess LPO levels. As shown in Figure 3D, DM group had increased LPO levels, while the RM process partially reduced this increase. KGE treatment reduced LPO levels in the DM+KGE and RM+KGE groups, alleviating oxidative damage. It is known that cuprizone disrupts the structural integrity of the cell membrane, causing formation of lipid radicals, leading to myelin sheath destruction, and our findings are parallel to the literature [32]. We also observed that the increase in LPO levels induced by cuprizone in male mice was more pronounced compared to female mice, and the suppressive effect of KGE on this increase was stronger. We can explain the differences based on female sex by the fact that female sex hormones, such as estrogen, play a protective role against oxidative stress by supporting antioxidant defense mechanisms [38, 39].

MBP is an essential protein synthesized by oligodendrocytes in the CNS and plays a critical role in maintaining the structural integrity of the myelin sheath [40]. As we show in Figure 4A, cuprizone triggered a significant reduction in MBP levels in both male and female mice, consistent with the literature [41-43]. This decrease is associated with perturbations in oligodendrocyte metabolism, ROS accumulation, and activation of neuroinflammatory processes. This process disrupts the structural integrity of the myelin sheath in the CNS, resulting in MS-like neurological dysfunction. In the RM group, MBP levels were significantly reduced in female mice, approaching control levels, whereas the observed increase in MBP levels in male mice was not statistically significant. This suggests that male


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Marmara Med J 2025;38(2): 158-170

mice may be more resistant to remyelination. Furthermore, KGE treatment significantly restored MBP levels to near control levels only in the male DM+ KGE group. MBP levels in female mice approached control levels even without KGE treatment. This finding suggests that female sex hormones such as estrogen may exert protective effects on the CNS through their antioxidant and anti-inflammatory properties [38, 39]. OLIG2 serves a key role in oligodendrocyte differentiation and myelin repair in the CNS. In our study, as seen in Figure 4B, cuprizone treatment caused a significant reduce in OLIG2 levels in both genders. This decrease suggests that cuprizone suppresses oligodendrocyte differentiation and increases myelin loss in the CNS. KGE treatment increased OLIG2 levels in the DM+KGE groups. This increase was significant in male mice, but not in female mice. The natural remyelination process was more pronounced in female mice. In addition, KGE addition did not cause a significant difference in the remyelination period in mice of both genders. Finally, KGE treatment demonstrated a more pronounced increase in OLIG2 levels in male mice.

The Rotarod test is an important method to evaluate motor coordination and neurotransmission. As we show in Figure 5, cuprizone application significantly reduced motor performance due to demyelination, reinforcing the idea in the literature that myelin loss disrupts nerve conduction and leads to serious impairments in motor skills [8]. In the DM+KGE group, KGE treatment significantly improved cuprizone-induced motor impairments and caused a significant increase in Rotarod performance. We observed that in the RM group, natural remyelination processes brought motor performance closer to control levels, and that KGE had a limited contribution to motor performance in the RM+KGE group. Motor performance results were similar in both genders, suggesting that the therapeutic potential of KGE in improving motor functions is not affected by gender.

In Figure 6, we showed that KGE administration slightly increased myelinated areas in the corpus callosum in the DM+KGE and RM+KGE groups compared to the DM and RM groups. Our findings are in line with previous studies showing that KGE attenuated demyelination and promoted remyelination in the cuprizone-induced demyelination model. Lee et al. [19] and Kwon et al. [20] showed that Korean red ginseng reduced demyelination and improved remyelination by LFB staining.

As we shown in Figure 7, the effects of cuprizone administration on the CNS were assessed by immunohistochemical analysis. MBP staining in the corpus callosum revealed a significant decrease in myelin density in the DM group. This finding confirms the toxic effects of cuprizone on the CNS by triggering oligodendrocyte damage and myelin destruction [8]. However, a significant increase in MBP-positive fiber density was detected in the KGE-treated DM+KGE and RM+KGE groups. As seen in Figure 8, these findings were also confirmed by MBP scoring and scoring analyses showed findings parallel to the immunohistochemical staining results. The antioxidant and neuroprotective properties of ginsenosides contained in KGE may have supported myelin repair and accelerated the remyelination process.

As we shown in Figure 9, GFAP staining in the hippocampal dentate gyrus region showed that astrocyte activity increased and GFAP expression was significantly reduced in the DM group relative to the control group. This increase indicates that astrogliosis is triggered during the demyelination process and neuroinflammation in the CNS becomes prominent. The decrease in GFAP expression in DM+KGE and RM+KGE groups suggests that KGE suppresses neuroinflammatory processes and restores the homeostatic balance of the CNS. These effects are consistent with the literature suggesting that ginsenosides contribute to structural and functional recovery of the CNS by regulating astrocyte activity. Similarly, a study in a vascular dementia model showed that KGE treatment reduced GFAP-immunoreactive cells in the hippocampal region. This finding supports the role of KGE in modulating astrocyte responses and neuroinflammation [22].Consistently, our study demonstrated that cuprizone induces demyelination, oxidative stress, and motor dysfunction in the CNS, whereas KGE exerts protective and restorative effects against these processes. Notably, these effects appear to be influenced by gender differences. We also observed that KGE improves neuronal functions by promoting remyelination and suppresses neuroinflammation by reducing astrocytosis. Furthermore, we observed that KGE produces similar responses between genders, but recovery is generally faster in female mice. The more pronounced effects of KGE in female mice may be related to the effects of estrogen in increasing antioxidant defenses and suppressing neuroinflammation, or it may also be due to differences in food consumption.

Consistent with our findings, studies have shown that Korean red ginseng promotes RM and improves oligodendrocyte functions in a cuprizone-induced DM model [19, 20]. Similarly, it has been reported in the literature that resveratrol supports remyelination with its antioxidant and anti-inflammatory effects [44]. Curcumin suppresses oxidative stress while supporting oligodendrocyte functions [45], and melatonin reduces DM as a strong antioxidant [46]. The results in our study suggest that KGE may act through similar mechanisms as these agents.

This study has certain limitations. First, the long-term effects of KGE after RM are unknown. Furthermore, while the neuroprotective effects of KGE have been observed, the molecular mechanisms have not been investigated in detail. Although, we noted gender-based differences, the underlying mechanisms remain unclear. Addressing these limitations will help define the therapeutic potential of KGE for demyelinating diseases. These findings indicate that KGE may serve as a potential therapeutic agent and contribute to the advancement of new treatment strategies for demyelinating diseases including MS. In addition, more comprehensive studies are needed in the future to understand the gender-specific mechanisms of KGE.

## Compliance with Ethical Standards

**Ethical Approval**: Ethcial approval for this study was obtained from Marmara University, School of Medicine Experimental Animal Local Ethics Committee (date: 9 May, 2023, approval number: 23-2023mar)


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**Author contributions:** M O D: Conducted the experiments, analysed the results, G S: Wrote the protocol, TT A and G G S: Performed the biochemical analyses, D A and H U: Contributed to the histological anayses, M Z G: helped the preparation of the manuscript. All authors approved the final version of the manuscript.

## REFERENCES

[1] Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol 2015;15:545-58. doi:10.1038/nri3871.

[2] Charabati M, Wheeler MA, Weiner HL, Quintana FJ. Multiple sclerosis: Neuroimmune crosstalk and therapeutic targeting. Cell 2023;186:1309-27. doi:10.1016/j.cell.2023.03.008.

[3] Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol 2010;9:520-32. doi:10.1016/s1474-4422(10)70064-8.

[4] Walton C. Rising prevalence of multiple sclerosis worldwide: insights from the Atlas of MS, third edition. Mult Scler 2020;26:1816-9. doi:10.1177/135.245.8520970841.

[5] Sorensen PS. The apparently milder course of multiple sclerosis: changes in the diagnostic criteria, therapy and natural history. Brain 2020;143:2296-308. doi:10.1093/brain/awaa145.

[6] Mallucci G, Peruzzotti-Jametti L, Bernstock JD, Pluchino S. The role of immune cells, glia and neurons in white and gray matter pathology in multiple sclerosis. Prog Neurobiol 2015;127-8: doi:10.1016/j.pneurobio.2015.02.003.

[7] Franklin RJM, Ffrench-Constant C. Regenerating CNS myelin – from mechanisms to experimental medicines. Nat Rev Neurosci 2017;18:753-69.

[8] Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 2001;11:107-16.

[9] Carlton WW. Response of mice to the chelating agents sodium diethyldithiocarbamate, alpha-benzoinoxime, and biscyclohexanone oxaldihydrazone. Toxicol Appl Pharmacol 1966;8:512-21.

[10] Hiremath MM, Saito Y, Knapp GW, Ting JP, Suzuki K, Matsushima GK. Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. J Neuroimmunol 1998;92:38-49.

[11] Zirngibl M, Assinck P, Sizov A, Caprariello AV, Plemel JR. Oligodendrocyte death and myelin loss in the cuprizone model: an updated overview of the intrinsic and extrinsic causes of cuprizone demyelination. Mol Neurodegener 2022;17:34.

[12] Cho IH. Effects of Panax ginseng in neurodegenerative diseases. J Ginseng Res 2012;36:342-53. doi:10.5142/jgr.2012.36.4.342.

[13] Shibata S, Fujita M, Itokawa H, Tanaka O, Ishii T. Studies on the constituents of Japanese and Chinese crude drugs. XI. Panaxadiol, a sapogenin of ginseng roots. Chem Pharm Bull (Tokyo) 1963;11:759-61.

[14] Kaneko H, Nakanishi K. Proof of the mysterious efficacy of ginseng: basic and clinical trials: clinical effects of medical ginseng, Korean red ginseng: specifically, its anti-stress action for prevention of disease. J Pharmacol Sci 2004;95:158-62.

[15] Karmazyn M, Moey M, Gan XT. Therapeutic potential of ginseng in the management of cardiovascular disorders. Drugs 2011;71:1989-2008.

[16] Kim HJ, Kim P, Shin CY. A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in the central nervous system. J Ginseng Res 2013;37:8-29.

[17] Jang M, Lee MJ, Kim CS, Cho IH. Korean red ginseng extract attenuates 3-nitropropionic acid-induced Huntington's-like symptoms. Evid Based Complement Alternat Med 2013;2013:237207.

[18] Hwang I, Ahn G, Park E, Ha D, Song JY, Jee Y. An acidic polysaccharide of Panax ginseng ameliorates experimental autoimmune encephalomyelitis and induces regulatory T cells. Immunol Lett 2011;138:169-78.

[19] Lee MJ, Choi JH, Kwon TW, et al. Korean red ginseng extract ameliorates demyelination by inhibiting infiltration and activation of immune cells in cuprizone-administrated mice. J Ginseng Res 2023;47:672-80. doi:10.1016/j.jgr.2023.05.001.

[20] Kwon OW, Kim D, Koh E, Yang HJ. Korean Red Ginseng and Rb1 facilitate remyelination after cuprizone diet-induced demyelination. J Ginseng Res 2023;47:319-28. doi:10.1016/j.jgr.2023.09.005.

[21] Hochstrasser T, Exner GL, Nyamoya S, et al. Cuprizone-containing pellets are less potent to ınduce consistent demyelination in the corpus callosum of C57BL/6 mice. J Mol Neurosci 2017;61:617-24. https://doi.org/10.1007/s12031.017.0903-3

[22] Zhu JD, Wang JJ, Zhang XH, Yu Y, Kang ZS. Panax ginseng extract attenuates neuronal injury and cognitive deficits in rats with vascular dementia induced by chronic cerebral hypoperfusion. Neural Regen Res 2018;13:664-72. doi:10.4103/1673-5374.230292.

[23] Lubrich C, Giesler P, Kipp M, et al. Motor behavioral deficits in the cuprizone model: validity of the rotarod test paradigm. Int J Mol Sci 2022;23:11342. https://doi.org/10.3390/ijms231911342

[24] Madadi S, Pasbakhsh P, Tahmasebi F, Mortezaee K, Khanehzad M, Boroujeni FB, et al. Astrocyte ablation induced by La-aminoadipate (L-AAA) potentiates remyelination in a cuprizone demyelinating mouse model. Metab Brain Dis 2019;34:593-603. https://doi.org/10.1007/s11011.019.0385-9

[25] Ledwozyw A, Michalak J, Stepień A, et al. The relationship between plasma triglycerides, cholesterol, total lipids and

lipid peroxidation products during human atherosclerosis. Clin Chim Acta 1986;155:275-83. doi:10.1016/0009-8981(86)90247-0

[26] Beutler E. Red cell metabolism: A manual of biochemical methods. Orlando, FL: Grune & Stratton, 1984.

[27] Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. Methods Enzymol 1981;77:398-405. doi: 10.1016/S0076-6879(81)77053-8

[28] Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte SOD activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol 1986; 82: 512-20.

[29] Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003;57(3-4):145-55. doi:10.1016/s0753-3322(03)00043-x.

[30] Stidworthy MF, Genoud S, Suter U, Mantei N, Franklin RJM. Quantifying the early stages of remyelination following cuprizone-induced demyelination. Brain Pathol 2003;13:329-39.

[31] Torkildsen O, Brunborg LA, Myhr KM, Bø L. The cuprizone model for demyelination. Acta Neurol Scand Suppl. 2008;188:72-6. doi:10.1111/j.1600-0404.2008.01036.x.

[32] Zhang L, Xu S, Huang Q, Xu H. N-acetylcysteine attenuates the cuprizone-induced behavioral changes and oligodendrocyte loss in male C57BL/7 mice via its anti-inflammation actions. J Neurosci Res 2018;96:803-16.

[33] Yang Q, Lin J, Zhang H, et al. Ginsenoside compound k regulates amyloid β via the Nrf2/keap1 signaling pathway in mice with scopolamine hydrobromide-ınduced memory ımpairments. J Mol Neurosci 2019;67:62-71. doi: 10.1007/s12031.018.1210-3

[34] Ashrafizadeh M, Ahmadi Z, Yaribeygi H, Sathyapalan T, Jamialahmadi T, Sahebkar A. The effects of ginsenosides on the Nrf2 signaling pathway. Adv Exp Med Biol 2021;1328:307-22. doi:10.1007/978-3-030-73234-9_20.

[35] Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim) 2018;12:88-93.

[36] Kim JH, Yi YS, Kim MY, Cho JY. Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. J Ginseng Res 2013;37:386-94.

[37] Nguyen CT, Kim EH. Neuroprotective effects of ginseng on neurological disorders. J Ginseng Res 2021;45:362-71.

[38] Cagnacci A, Gazzo I, Stigliani S, et al. Oxidative stress: The role of estrogen and progesterone. J Clin Med 2023;12:7304. doi:10.3390/jcm12237304

[39] Stepniak J, Karbownik-Lewinska M. 17β-estradiol prevents experimentally-induced oxidative damage to membrane lipids and nuclear DNA in porcine ovary. Syst Biol Reprod Med 2016;62:17-21. doi:10.3109/19396.368.2015.1101510

[40] Galiano MR, Andrieux A, Deloulme JC, Bosc C, Schweitzer A, Job D, et al. Myelin basic protein functions as a microtubule stabilizing protein in differentiated oligodendrocytes. J Neurosci Res 2006;84:534-41. doi:10.1002/jnr.20960.

[41] Naeem AG, El-Naga RN, Michel HE. Nebivolol elicits a neuroprotective effect in the cuprizone model of multiple sclerosis in mice: emphasis on M1/M2 polarization and inhibition of NLRP3 inflammasome activation. Inflammopharmacology. 2022;30:2197-209. doi:10.1007/s10787.022.01045-4.

[42] Taylor LC, Gilmore W, Ting JP, Matsushima GK. Cuprizone intoxication induces cell intrinsic alterations in oligodendrocyte progenitor cells that precede demyelination. Glia. 2009;57:882-901.

[43] Hibbits N, Pannu R, Wu TJ, Armstrong RC. Cuprizone demyelination of the corpus callosum in mice correlates with altered social interaction and impaired bilateral sensorimotor coordination. ASN Neuro 2009;1:e00013. doi:10.1042/AN20090032

[44] Ghaiad N, Nooh M M, El-Sawalhi M M, Shaheen A A, El-Sherbiny NM. Resveratrol promotes remyelination in cuprizone-induced demyelination model of multiple sclerosis: Modulation of SIRT1, NRF2, and NF-κB signaling pathways. J Neuroimmunol 2017;305, 3-10. doi: 10.1016/j.jneuroim.2017.01.003

[45] Motavaf M, Sadeghizadeh M, Babashah S, Zare L, Javan M. Protective effects of a nano-formulation of curcumin against cuprizone-induced demyelination in the mouse corpus callosum. Iran J Pharm Res 2020;19:310-20. doi:10.22037/ijpr.2020.112.952.14033.

[46] Abo Taleb H A, Alghamdi B S. Neuroprotective effects of melatonin during demyelination and remyelination stages in a mouse model of multiple sclerosis. J Mol Neurosci 2020;70:386-402. doi: 10.1007/s12031.019.01425-6