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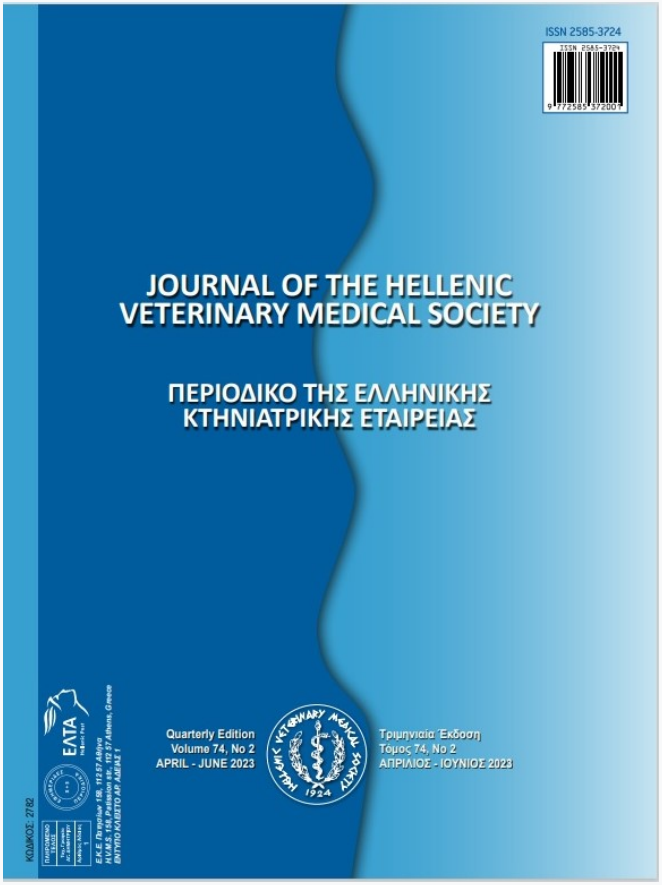

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Research article
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Kaempferol and Isorhamnetin Alleviate Lipopolysaccharide-Induced Anxiety and Depression-Like Behavioral in Balb/C Mice

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ABSTRACT: The etiology of anxiety and depression is linked to inflammation and oxidative stress. Isorhamnetin and Kaempferol are strong antioxidants with anti-inflammatory neuroprotective properties. This study, it was aimed to investigate the effect of Kaempferol and Isorhamnetin in Lipopolysaccharide (LPS)-induced anxiety and depression model in mice. Thirty Balb/C mice were divided into six groups of five mice each weighing 25-35 g. Kaempferol (50 mg/kg and 100 mg/kg) and Isorhamnetin (30 mg/kg and 60 mg/kg) were given orally to the treatment group, and the vehicle was given to the control and LPS groups for fourteen days, followed by intraperitoneal (0.83 mg/kg) LPS injection (control group excluding) on the fifteenth day. At 3 hours after the administration of LPS, each group was applied with the Elevated Plus-Maze (EPM) test, the Light/Dark test, and the Open Field test (OFT). At 24 hours after LPS administration, the Forced Swimming Test (FST) was applied and at 28 hours, the Tail Suspension Test (TST). After the behavioral test, the prefrontal cortex and hippocampus tissues of rats were harvested by cervical dislocation under high-dose anesthesia. From these tissues, malondialdehyde (MDA) levels, total oxidant status (TOS) and total antioxidant status (TAS) levels, tumor necrosis factor alpha (TNF-α), interleukin-1beta (IL-1β), and interleukin-6 (IL-6) levels, and brain-derived neurotrophic factor (BDNF) levels were measured. Kaempferol and Isorhamnetin ameliorated LPS-induced anxiety evaluated by OFT, Light/Dark test, and EPM, and LPS-induced depression evaluated by FST and TST. Kaempferol and Isorhamnetin alleviated LPS-induced increased oxidative stress in the prefrontal cortex and hippocampus by decreasing MDA and TOS levels and increasing TAS levels. In addition, it was observed that Kaempferol and Isorhamnetin regulated the increase in prefrontal and hippocampal inflammation caused by LPS by decreasing TNF-α, IL-1β, and IL-6 levels. Most importantly, LPS reduced prefrontal and hippocampal BDNF levels, but the treatment groups reversed it. These results show the possible therapeutic potential of Kaempferol and Isorhamnetin, along with the importance of oxidative stress, inflammation, and BDNF in the etiopathogenesis of anxiety and depression.

***Keywords*:** Anxiety, Depression, Oxidative Stress, Mice, Kaempferol

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INTRODUCTION

Anxiety and depression are commonly seen as psychiatric disorders throughout the world (Steimer, 2002). These two psychiatric disorders are related to biochemical, cognitive, behavioral, and psychological changes which are defined as negative emotional experiences in individuals (Steimer, 2002). Both depression and anxiety have negative effects on the life of the individual and cause a significant functional loss, and depression and anxiety disorders are frequently observed together (Brenes, 2007; Hussein et al., 2018). The etiopathogenesis of both disorders has not yet been clarified, and antidepressant drugs from the selective serotonin reuptake inhibitors (SSRI) group are the first choice in treatment. Drug therapy has been reported to be effective in approximately one-third of patients, including the prevention of recurrence (Xu et al., 2012; Zhao et al., 2016; Jamali-Raeufy et al., 2019).

The efficacy of flavonoids, which affect cytokine, neurotrophin, and oxidative system, has been investigated in the treatment of depression and anxiety (Mesfin et al., 2014; Saki et al., 2014; Sulakhiya et al., 2016). Cytokines play a role in the modulation of various neurological functions (Mormède et al., 2004; O'Connor et al., 2009). The brain can rapidly create new synapses in the functions necessary for learning and adaptation or eliminate synapses. BDNF is one of the molecular factors required for healthy neuroplasticity. BDNF is the neurotrophin enabling growth and differentiation of neurons, synapse formation, and the survival of existing neurons (Cheng et al., 2018). Free radicals are highly reactive chemical species that emerge during normal metabolic processes (Chi et al., 2016). Compared to other organs, the central nervous system is more sensitive to the toxic effects of reactive oxygen species.

Flavonoids have a diphenyl propane structure and are secondary metabolites of a plant group (Ko et al., 2020). Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a natural flavonoid widely found in various fruits and vegetables (tea, apples, strawberries, green beans, broccoli, spinach). Isorhamnetin (30-O-methyl-quercetin) is a flavonoid found in various plant-based food products such as apples and grapes, and herbal drugs such as sea buckthorn and *Ginkgo biloba* (Rammal et al., 2008; Zhang et al., 2012; Jangra et al., 2014). In vivo studies have shown that both of these flavonoids have several antioxidants, anti-inflammatory, and neuroprotective biological activities (Brenes, 2007; Xu et al., 2012; Zhao et al., 2016; Hussein et al., 2018; Jamali-Raeufy et al., 2019).

There are various therapeutic regimes for anxiety and depression. However, the treatment process is negatively affected by the low efficacy of the drugs, delayed therapeutic effects, various side effects, and poor drug compliance (Mesfin et al., 2014; Sulakhiya et al., 2016). Therefore, it is important that the etiopathogenesis of these disorders is investigated and that more effective new compounds with a low side-effect profile are developed (Saki et al., 2014). The aim of this study was to investigate the protective effect against depression and anxiety of Kaempferol and Isorhamnetin, which are flavonoids with neuroprotective, anti-inflammatory, and antioxidant properties, in a lipopolysaccharide-induced model in mice through animal behavior tests and biochemical analyses.

MATERIALS AND METHODS

Chemical reactants and drugs

Kaempferol and Isorhamnetin were purchased from Aktin Chemicals, Inc. (Chengdu, China) and LPS from Sigma Chemical Company (St. Louis, MO, USA). Trichloroacetic acid (TCA), thiobarbituric acid (TBA) and Hydrochloric acid (HCl) were obtained from Merck Chemicals (Darmstadt, Germany). These chemicals were used instead of a kit for MDA analysis. The commercial ELISA kits used in the measurements of TOS, TAS, TNF-α, IL-1β, and IL-6 levels, and BDNF, were purchased from Shanghai Sunred Biological Technology Co., Ltd. (Shanghai, China). All the chemical reactants were of analytical purity.

Animals and study groups

This study was conducted in the Experimental Animals Laboratory of Sivas Cumhuriyet University Medical Faculty in compliance with the guidelines for the Care and Use of Laboratory Animals. The study protocol was approved by the Animal Ethics Committee of Sivas Cumhuriyet University (decision no: 65202830-050.04.04-172). The study sample comprised thirty male mice (Balb/C), each weighing 25-35 g. The mice were kept under standard animal laboratory conditions of a 12-hour light/dark cycle, at 24 ± 2°C, 35-60% humidity, and food and water *ad libitum*. Following an adaptation period of one week, the mice were randomly divided into 6 groups (n=5/group): Control, LPS, LPS+Kaempferol50, LPS+Kaempferol100, LPS+Isorhamnetin30, and LPS+Isorhamnetin60 groups.

The control group received 10% Dimethyl sulfoxide (DMSO) by oral gavage for 14 days and followed by intraperitoneal (i.p.) saline on day fifteen. The LPS group received 10% DMSO by oral gavage (dose volume 5 ml/kg bw) for 14 days, then a single i.p. injection (dose volume 10 µl/g bw) of 0.83 mg/kg LPS on the 15th day (Mormède et al., 2004; O'Connor et al., 2009). Kaempferol was administered to the LPS+-Kaempferol50 and LPS+Kaempferol100 groups for 14 days by oral gavage (dose volume 5 ml/kg bw) at doses of 50 mg/kg and 100 mg/kg, respectively, followed by a single i.p. injection of LPS at a dose of 0.83 mg/kg on the 15th day (Cheng et al., 2018). The LPS+Isorhamnetin30 and LPS+Isorhamnetin60 groups received Isorhamnetin by oral gavage (dose volume 5 ml/kg bw) at a dose of 30 mg/kg and 60 mg/kg, respectively, for 14 days, then a single i.p. injection of LPS at a dose of 0.83 mg/kg on the 15th day (Chi et al., 2016) (Figure 1).

The LPS was dissolved in saline. The animals were taken to the laboratory room where the behavioral tests were to be performed 30 mins before the behavioral tests for adaptation to the environment. The laboratory room was illuminated with dim red lights and the behavioral tests were conducted in this environment.

Behavioral Analysis

At 3 hours after the administration of LPS, each group was applied with the Elevated Plus-Maze (EPM) test, the Light/Dark test, and the Open Field test (OFT). At 24 hours after LPS administration, the Forced Swimming Test (FST) was applied and at 28 hours, the Tail Suspension Test (TST). All the behavioral tests were conducted using behavioral platforms made specifically for each test and were recorded in a computer video recording tracking system and analyzed in Ethovision XT© software (Noldus Information Technologies, Wageningen, Holland). Moreover, to avoid bias, all behavioral tests were performed by experienced researchers unaware of the groups, using a blind technique. To minimize the potential outcomes of the behavioral evaluations on the biochemical parameters, the neurochemical and behavioral evaluations were made in distinct groups. The behavioral test and experiment design were adapted according to the previously described (Sulakhiya et al., 2016).

Open field test

This test is used to evaluate anxiety-like behavior and locomotion. The open field apparatus was made of a grey polyvinyl chloride plastic panel 40 cm high by 40 cm wide and 40 cm deep. Peripheral and central areas were formed. The middle area was defined as a 10 x 10 cm square. At the start of the test, each mouse was placed in the middle area and a video recording of 5 minutes was made. The time spent in the middle area was evaluated on the video recordings (Parrella et al., 2022).

Light/Dark test

This test is used to evaluate anxiety-like behavior. Mice have an innate tendency to avoid light areas and unfamiliar surroundings. An apparatus was created for this purpose consisting of two sections of light and darkness. The time that the mice spent in the light and dark sections were measured throughout the test period of 5 minutes. Each mouse was placed in a box measuring 40 x 40 x 40 cm in a dark box measuring 40 x 40 x 20 cm, with a 6 x 6 cm door connecting the light

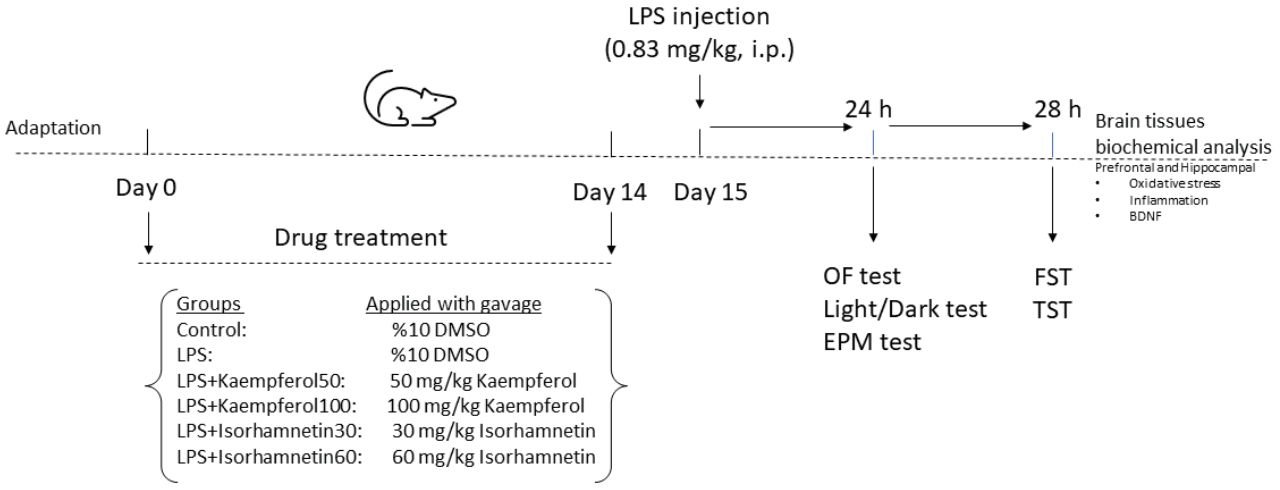

Figure 1: Experimental design

and dark sections. The times spent in the light and dark areas were recorded for evaluation (Peng et al., 2019).

Elevated Plus-Maze test

This test is used to evaluate anxiety-like behavior. There are a total of four arms, two open opposite each other and two closed on the opposite sides. These arms are separated from each other by a central region in the middle. In this test apparatus, the open and closed arms are 35 cm long and 5 cm wide, the middle area is 5 x 5cm and it is elevated from the ground at 50 cm. It is an elevated test apparatus. The elevation and open arms are perceived by the mice as unfamiliar areas. Each mouse was placed in the middle area and the time spent in the open and closed arms was evaluated throughout 5 minutes (Sulakhiya et al., 2016; Peng et al., 2019).

Forced Swimming test

This test is used to evaluate depression-like behavior. Water at a temperature of 25°C was placed at a depth of 20 cm in a glass cylinder 25 cm in depth and 10 cm in diameter. Each mouse was placed in the water and left to swim for 6 minutes. The period of complete lack of movement was measured in the last four minutes of the six minutes (Sulakhiya et al., 2016).

Tail Suspension test

This is a mouse behavioral test used in the screening of potential antidepressant drugs and evaluation of other manipulations expected to be affected by behavior related to depression. Each mouse is suspended by the tail which is taped so that it cannot escape or grasp nearby surfaces. Throughout the test period of 6 mins, the time spent mobile and immobile is measured (Sulakhiya et al., 2016).

Analysis of Prefrontal and Hippocampal Oxidative Stress, Inflammation, and BDNF levels

After completion of the behavioral tests, the animals were anaesthetized with xylazine (10mg/kg, i.p., Xylazine Bio 2%, Bioveta, Czech Republic) and ketamine (60 mg/kg, i.p., Alfamine, Egevet, Turkey), blood samples were taken then euthanasia was applied with the cervical dislocation method. The prefrontal and hippocampal regions were isolated from the brain. An amount of tissue was weighed, and then placed in phosphate-buffered saline (pH:7.4) buffer solution, to which three 2mm metal beads were added. The tissues were then double cycle homogenized in a bead beater homogenizer [HT 24 bead beating homogenizer (OPS Diagnostics)]. A portion of the homogenates was separated for MDA analysis. The other portion was centrifuged for 10 mins at +4°C at 4000 rpm. The supernatants collected were stored at -80°C until analysis. The levels of TOS, TAS, BDNF, and proinflammatory cytokines (TNF-α, IL-1β and IL-6) were measured with commercial ELISA kits (Sunred Biological Technology, Shanghai, China). Briefly, serum samples, biotinylated antibodies, and Streptavidin-HRP were added sequentially to the plate coated with canine-specific antibodies and allowed to incubate for one hour. Then, the washing process was performed, the substrate solutions were added, and the reaction was started, respectively. The reaction was stopped by adding a stop solution and reading in an ELISA plate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, USA) at 450 nm within 10 minutes. Analyzes were performed in duplicate from all samples. The lipid peroxidization level (MDA) from the homogenate was determined spectrophotometrically (Ohkawa et al., 1979). The total protein amount from the supernatant was measured with an autoanalyzer.

Statistical analysis

Data obtained in the study were analyzed statistically using SPSS 23.0 software. Conformity of the behavioral test data to normal distribution was assessed with the Kolmogorov-Smirnov test. In all the groups, the data for each behavioral test and all the biochemical data were determined to show normal distribution. Statistically significant differences between groups were evaluated with One-way Variance analysis (ANOVA). The post-hoc Tukey HSD test was used in the comparisons of sub-groups in respect of the total time in the central area in the OFT, the time spent in the light and dark areas in the Light-Dark Test, the total time spent in the open and closed arms of the EPM test, the total periods of immobility in the FST and TST, and all the biochemical analysis data. Results were expressed as mean ± standard deviation (SD) values. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Behavioral Results

Effect of Kaempferol and Isorhamnetin on OFT

In the OFT, the time spent in the middle area was statistically significantly shorter in the LPS group than in the control group ($p<0.05$). The time spent in

the middle area was statistically significantly longer in the LPS+Kaempferol50 and LPS+Isorhamnetin30 groups than in the LPS group ($p<0.05$) (Table 1).

Effect of Kaempferol and Isorhamnetin on Light-Dark Test

In the Light-Dark Test, the time spent in the light area was statistically significantly shorter in the LPS group than in the control group ($p<0.05$). The time spent in the light area was statistically significantly longer in the LPS+Kaempferol50, LPS+Kaempferol100, and LPS+Isorhamnetin30 groups than in the LPS group ($p<0.05$) (Table 2). The time spent in the dark area was statistically significantly longer in the LPS group than in the control group ($p<0.05$). The time spent in the dark area was statistically significantly shorter in the LPS+Kaempferol50, LPS+Kaempferol100, and LPS+Isorhamnetin30 groups than in the LPS group ($p<0.05$) (Table 2).

Effect of Kaempferol and Isorhamnetin on EPM Test

In the EPM test, the time spent in the open arms was statistically significantly shorter in the LPS group than in the control group ($p<0.05$). The time spent in the open arms was statistically significantly longer in the treatment groups than in the LPS group ($p<0.05$) (Table 3). The time spent in the closed arms was statistically significantly longer in the LPS group than in the control group ($p<0.05$). The time spent in the closed arms was statistically significantly shorter in the LPS+Kaempferol50, LPS+Isorhamnetin30, and LPS+Isorhamnetin60 groups than in the LPS group ($p<0.05$) (Table 3).

Effect of Kaempferol and Isorhamnetin on FST

In the FST, the duration of total immobility was evaluated. The duration of total immobility was statistically significantly longer in the LPS group than in the control group ($p<0.05$), and statistically significantly shorter in the LPS+Kaempferol50, and LPS+Isorhamnetin30 groups than in the LPS group ($p<0.05$) (Table 4).

Effect of Kaempferol and Isorhamnetin on TST

In the TST, the duration of total immobility was evaluated. The duration of total immobility was statistically significantly longer in the LPS group than in the control group ($p<0.05$), and statistically significantly shorter in all the treatment groups than in the LPS group ($p<0.05$) (Table 5).

Table 1. Open Field test results

	The time spent in the middle area (sec)
Control	82.2 ± 7.3
LPS	46.0 ± 6.5*
LPS+Kaempferol50	70.4 ± 7.9#
LPS+Kaempferol100	53.8 ± 4.7
LPS+Isorhamnetin30	73.0 ± 9.3#
LPS+Isorhamnetin60	49.8 ± 8.7

Data are presented as the mean ± SD
ANOVA, $p<0.05$
*$p<0.05$ versus Control
#$p<0.05$ versus LPS

Table 2. Light/Dark test results

	The time spent in the light area (sec)	The time spent in the dark area (sec)
Control	130.4 ± 11.9	169.6 ± 11.9
LPS	52.4 ± 7.3*	247.6 ± 7.3*
LPS+Kaempferol50	86.4 ± 12.7#	213.6 ± 12.7#
LPS+Kaempferol100	73.4 ± 9.2#	226.6 ± 9.2#
LPS+Isorhamnetin30	76.4 ± 7.5#	223.6 ± 7.5#
LPS+Isorhamnetin60	64.8 ± 8.7	235.2 ± 8.7

Data are presented as the mean ± SD
ANOVA, $p<0.05$
*$p<0.05$ versus Control
#$p<0.05$ versus LPS

Table 3. Elevated Plus-Maze test results

	The time spent in the open arms (sec)	The time spent in the closed arms (sec)
Control	73.0 ± 8.8	231.4 ± 10
LPS	20.8 ± 4.3*	284.6 ± 12.1*
LPS+Kaempferol50	54.4 ± 10.6#	269.4 ± 14.6
LPS+Kaempferol100	71.8 ± 7.8#	242.0 ± 11.2#
LPS+Isorhamnetin30	50.4 ± 8.8#	257.2 ± 15.1#
LPS+Isorhamnetin60	72.0 ± 8.5#	231.6 ± 7.9#

Data are presented as the mean ± SD
ANOVA, $p<0.05$
*$p<0.05$ versus Control
#$p<0.05$ versus LPS

Table 4. Forced Swimming test results

	Immobility (sec)
Control	58.8 ± 8.6
LPS	87.8 ± 6.2*
LPS+Kaempferol50	77.0 ± 8.3
LPS+Kaempferol100	61.8 ± 7.5#
LPS+Isorhamnetin30	75.6 ± 9.8
LPS+Isorhamnetin60	61.4 ± 8.7#

Data are presented as the mean ± SD
ANOVA, $p<0.05$
*$p<0.05$ versus Control
#$p<0.05$ versus LPS

Table 5. Tail Suspension test results

	Immobility (sec)
Control	130.6 ± 15.7
LPS	223.2 ± 22.2*
LPS+Kaempferol50	178.4 ± 8.6#
LPS+Kaempferol100	153.0 ± 9.3#
LPS+Isorhamnetin30	143.0 ± 7.8#
LPS+Isorhamnetin60	123.8 ± 10.6#

Data are presented as the mean ± SD
ANOVA, $p<0.05$
*$p<0.05$ versus Control
#$p<0.05$ versus LPS

Biochemical Analysis Results

Effect of Kaempferol and Isorhamnetin on Prefrontal and Hippocampal Oxidative Stress

In the evaluation of the MDA level in the prefrontal and hippocampal regions, the MDA level was statistically significantly higher in the LPS group than in the other groups ($p<0.05$) (Figure2b). The prefrontal and hippocampal MDA levels in the treatment groups were statistically significantly lower than those of the LPS group ($p<0.05$). The TOS values in the hippocampal area were statistically significantly lower in the control group than in the LPS group ($p<0.05$). Except for the LPS+Kaempferol50 group, the values of the LPS group were statistically significantly higher than in the other groups where flavonoid was used ($p<0.05$). The TOS values in the prefrontal area were statistically significantly higher in the LPS group than in the other groups ($p<0.05$) (Figure2a).

The TAS values in the hippocampal area were statistically significantly lower in the LPS group than in the other groups ($p<0.05$). In the evaluation of the TAS values in the prefrontal area, no statistically significant difference was determined between the control group and the LPS group ($p>0.05$). The values in the LPS group were statistically significantly lower than in the LPS+Kaempferol100, and LPS+Isorhamnetin60 groups ($p<0.05$) (Figure2c).

Effect of Kaempferol and Isorhamnetin on Prefrontal and Hippocampal Inflammation

TNF-α, IL-6, and IL-1β values in the prefrontal and hippocampal areas were determined to be statistically significantly higher in the LPS group than in the control group ($p<0.05$). However, Kaempferol and Isorhamnetin statistically decreased these proinflammatory cytokines in both brain tissue ($p<0.05$) (Figure 3a, 3b, 3c).

Effect of Kaempferol and Isorhamnetin on Prefrontal and Hippocampal BDNF levels

The BDNF level in the hippocampal area was statistically significantly lower in the LPS group than in the control group ($p<0.05$). However, both treatment groups statistically increased the hippocampal level of BDNF ($p<0.05$). On the other hand, BDNF level in the prefrontal area was statistically significantly lower in the LPS group than in the control, LPS+Kaempferol100, LPS+Isorhamnetin30, and LPS+Isorhamnetin60 groups ($p<0.05$) (Figure4).

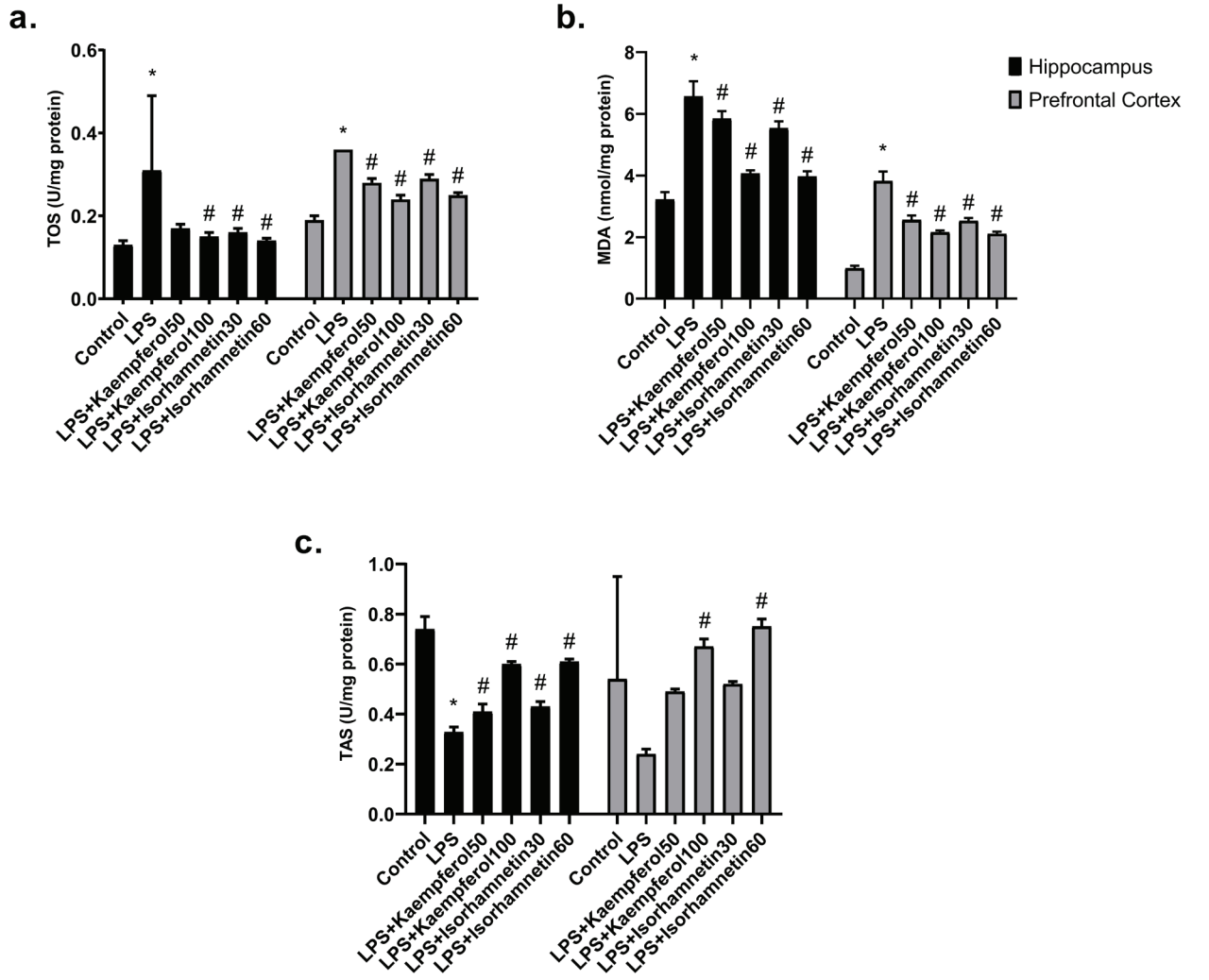

Figure 2: The effect of Kaempferol and Isorhamnetin on oxidative stress in the prefrontal cortex and hippocampus. The changes of TOS (a), MDA (b), and TAS (c)levels. *p<0.05 compared to Control group, #p<0.05 compared to LPS group using one way ANOVA post hoc Tukey test

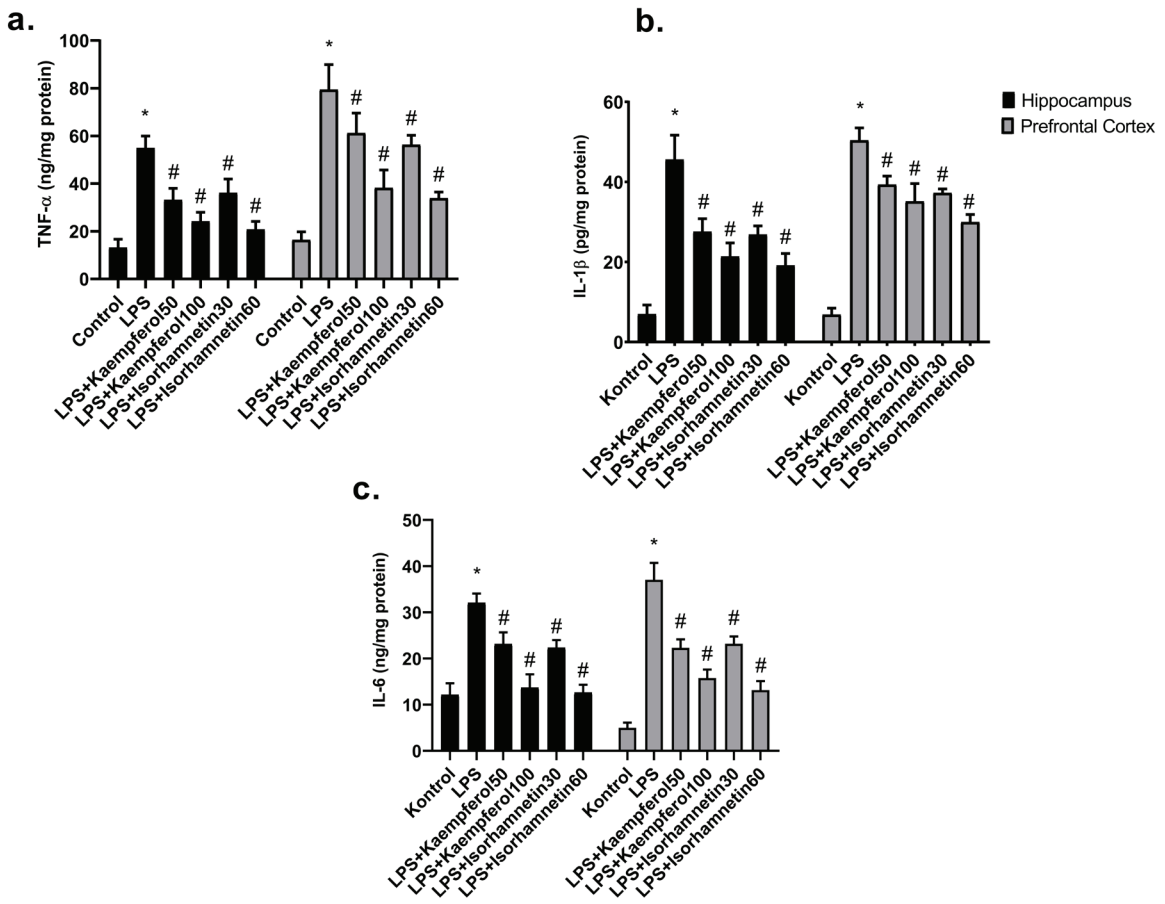

Figure 3: The effect of Kaempferol and Isorhamnetin on inflammation in the prefrontal cortex and hippocampus. The changes of TNF-α (a), IL-1β (b), and IL-6 (c) levels. *p<0.05 compared to Control group, #p<0.05 compared to LPS group using one way ANOVA post hoc Tukey test

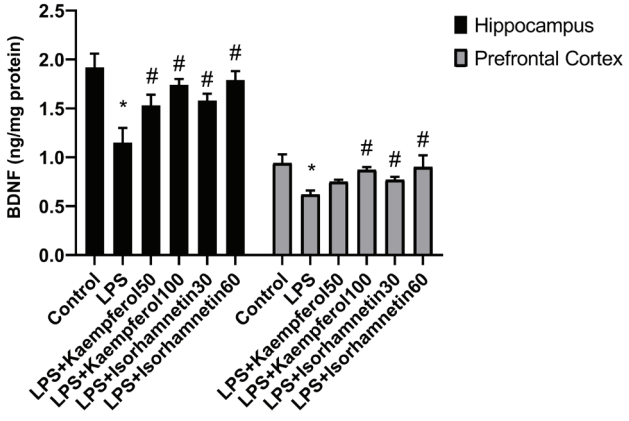

Figure 4: The effect of Kaempferol and Isorhamnetin on BDNF levels in the prefrontal cortex and hippocampus. *p<0.05 compared to Control group, #p<0.05 compared to LPS group using one way ANOVA post hoc Tukey test

DISCUSSION

The results of this study demonstrated that Kaempferol and Isorhamnetin, which have antioxidant, anti-inflammatory, and neuroprotective properties, improved the LPS-related behavioral changes in an LPS-induced experimental depression and anxiety model. These dose-dependent anxiolytic and antidepressant-like effects were determined to have occurred

by decreasing the increase in oxidative stress and inflammation and increasing the decreased BDNF in the hippocampus and prefrontal cortex caused by LPS.

Various behavioral tests are used to evaluate anxiety and depression in rodents. The tests in common use, the OFT, Light-Dark test, and EPM test are used to determine anxiety level, and the TST and the FST to determine the level of depression (Rammal et al., 2008; Mesfin et al., 2014; Sulakhiya et al., 2016). As there is a high frequency of anxiety and depression seen together and usually the same group of drugs are used in treatment, animal experimental apparatus for both anxiety and depression were used in this study. As a result of the tests applied, anxiety and depression-like behaviors were determined in the mice that were administered LPS, which was a result consistent with the findings of several studies (Jangra et al., 2014; Sulakhiya et al., 2014).

It is known that flavonoids obtained from several natural plants show an antidepressant and anxiolytic-like effect in experimental animal depression and anxiety models (Zhang et al., 2012; Ko et al., 2020). Although there are a few studies related to the anxiolytic and antidepressant effects of Kaempferol, there are insufficient studies of Isorhamnetin. In the current study, Kaempferol and Isorhamnetin were determined to have reduced anxiety and depression-like behaviors. This finding demonstrates that Kaempferol and Isorhamnetin could have a potential healing effect on anxiety and depression symptoms.

For many years the hippocampus has been known to be associated with the regulation of learning and memory abilities, and it has an important role in mood regulation (Gould et al., 1992; Cameron and Gould, 1994). The findings in the hippocampus have also been reported to provide a gateway to the prefrontal cortex, which is another important brain region associated with stress and stress-related behaviors, and is important for working memory, executive function, and self-regulatory behaviors, and which responds to stress (McEwen and Morrison, 2013; McEwen et al., 2016).These two regions of the brain are areas affected by many psychiatric diseases, especially anxiety and depression (Liu et al., 2017). In this study, it was determined that MDA and TOS levels increased and TAS levels decreased in the hippocampus and prefrontal areas after the application of LPS. This result shows an increase in oxidative stress in the hippocampal and prefrontal areas due to LPS.

Previous studies have reported that oxidative stress plays an important role in the physiopathology of anxiety and depression (Bouayed et al., 2009; Grases et al., 2014). It has also been reported that while there is an increase in lipid peroxidization in anxiety and depression, there is a decrease in DNA damage, glutathione and antioxidant enzyme activity (Bouayed et al., 2009; Grases et al., 2014). In a mouse model of chronic social defeat stress (CSDS), Kaempferol increased the levels of SOD, GPx, CAT, and GST which were reduced in the prefrontal cortex, and decreased the MDA level (Gao et al., 2019). In a diabetic rat model, Isorhamnetin was shown to reduce the increased oxidative stress in the brain (Jamali-Raeufy et al., 2019).In addition, Isorhamnetin has been reported to suppress the increasing LPS-induced oxidative stress in BV2 microglia cell line (Xu et al., 2012). In the current study, Kaempferol and Isorhamnetin were determined to have reduced the oxidative stress formed in the hippocampus and prefrontal cortex with LPS. The use of Kaempferol and Isorhamnetin together can be evaluated as a promising approach to provide protection against anxiety and depression by suppressing oxidative stress.

There are studies in the literature showing that proinflammatory cytokines play an important role in the physiopathology of mood disorders (Sulakhiya et al., 2016; J. Zhao et al., 2016). In experimental studies to investigate the inflammatory mechanism of anxiety and depression, lipopolysaccharide (bacterial endotoxin) is one of the most widely used substances (O'Connor et al., 2009; Mesfin et al., 2014; Sulakhiya et al., 2016). It has been reported that anxiety and depression-like behaviors could be formed by the administration of LPS causing the expression of proinflammatory cytokines, such as TNF-α and IL-1β in the periphery and in the brain in experimental animals (Jangra et al., 2014; Sulakhiya et al., 2014). It has been reported high concentrations of proinflammatory cytokines, such as TNF-α and IL-1β in the serum or plasma of depressive patients (Hannestad et al., 2011).

By triggering an increase in oxidative stress and proinflammatory cytokines, LPS may also cause activation of the hypothalamus-hypophyseal-adrenal axis, consequently causing the expression of corticotropin-releasing hormone (CRH) related to the behavioral response such as anxiety and depression in animals (Sah et al., 2011). In the current study, LPS was determined to have increased the levels of

proinflammatory cytokines (TNF-α, IL-1β, and IL-6) in the hippocampus and prefrontal cortex. In an LPS-induced neuroinflammation mouse model study by Cheng *et al*, it was reported that Kaempferol decreased levels of IL-1β, IL-6, TNF-α, MCP-1, and COX-2 in brain tissue (Cheng et al., 2018).

In an experimental diabetic rat model, Isorhamnetin reduced increasing TNF-α levels in the brain (Xu et al., 2012).In another study of ischemic brain damage in mice, isorhamnetin reduced the increasing IL-1β, IL-6, and TNF-α levels in the ipsilateral cortex (Zhao et al., 2016). In the current study, Kaempferol and Isorhamnetin were observed to reduce the increasing proinflammatory cytokine levels due to LPS. When these results are examined, inflammation is significant in the pathophysiology of anxiety and depression.

Neurotrophins are proteins strongly associated with the survival, development and helping with the function of neurons. BDNF is one of the neurotropic factors found most abundantly in the central nervous system. Studies have shown the importance of BDNF on the pathogenesis of depression and anxiety (Chen et al., 2006; Kalueff et al., 2006). Clinical studies have also reported that BDNF concentration is reduced in the blood of depressive patients, but returns to normal levels with antidepressant treatment (Karege et al., 2002; Shimizu et al., 2003; Brunoni et al., 2008). There are also studies in literature showing that LPS application suppresses hippocampal BDNF production and that this shows a negative correlation with depression (Satomura et al., 2011; Jangra et al., 2014; Lin and Wang, 2014). In the current study, LPS was determined to have reduced BDNF levels in the hippocampus and prefrontal cortex. Kaempferol and Isorhamnetin were determined to have increased the reduced BDNF levels. These results demonstrate that neurotropic factors such as Kaempferol and Isorhamnetin, which increase the molecules of BDNF, could play a key role in improving anxiety and depression symptoms.

The fact that the additive effects of Kaempferol and Isorhamnetin have not been investigated is a limitation of this study. In addition, the fact that molecular techniques were not used in this study could be a limitation of the study.

CONCLUSIONS

The results of this study not only showed the importance of oxidative stress, inflammation, and plasticity in the etiopathogenesis of anxiety and depression, but also the efficacy of Kaempferol and Isorhamnetin in protecting against these two disorders could occur by increasing the antioxidative and anti-inflammatory effects and neuronal plasticity. Nevertheless, there is a need for further studies to show by which effect mechanism this protective effect is provided and that it could be a potential therapeutic agent.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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