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Research Article

Rutin ameliorates lipopolysaccharide-induced depressive-like behaviours in mice model via inhibition of neuroinflammation

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Keywords:

Rutin, Depression, Lipopolysaccharide, Flavonoids, Inflammation, Oxidative Stress

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ABSTRACT

Background: Depression is a common illness that affects millions of people in the world. Rutin is a flavonol, and flavonols stand out due to their antioxidant, anti-inflammatory, neuroprotective, and antidepressant effects. Inflammation is associated with oxidative stress and the brain is highly sensitive to changes in redox conditions. There is a paucity of data on the effects of rutin on lipopolysaccharide (LPS)-induced depression. This study aimed at investigating the protective roles of rutin on depressive-like behaviors in the LPS model of depression in mice, focusing on inflammation and oxidative stress. **Methods:** Thirty-six (36) Swiss Albino mice were randomly divided into six groups (n = 6). All treatments were administered intraperitoneally every day for 21 days, except LPS which was administered on the 21st day only; group I served as the normal control (Distilled water 10 ml/kg), group II received LPS 0.5 mg/kg only, group III received fluoxetine 20 mg/kg + LPS 0.5 mg/kg, group IV received rutin 25 mg/kg + LPS 0.5 mg/kg, group V received rutin 50 mg/kg + LPS 0.5 mg/kg, and group VI received rutin 100 mg/kg + LPS 0.5 mg/kg. Mice behaviors were evaluated through a tail suspension test (TST), followed by assessments of MDA, IL-6, TNF-α, and BDNF. **Results:** Rutin improved behavioral despair induced by LPS by significantly reducing immobility time in the TST. It also prevented the increase of inflammatory cytokine IL-6 in the hippocampus due to LPS. Moreso, rutin prevented an increase in brain-derived neurotrophic factor (BDNF) by LPS. **Conclusion:** These findings suggest the potentials of rutin to prevent LPS-induced depressive-like symptoms, by its anti-inflammatory activities through cytokine IL-6 in mice.

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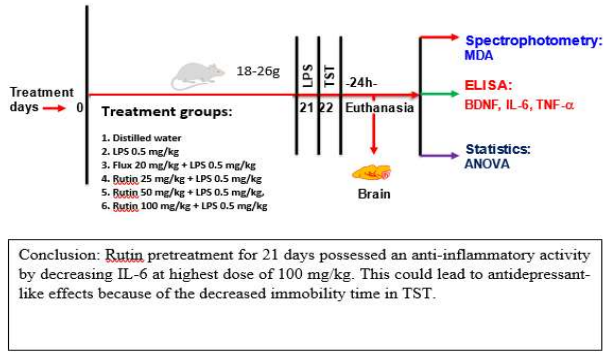


Graphical abstract

Introduction

Depression is a chronic and recurrent disorder associated with high morbidity and risk of suicide (Cordeiro *et al.,* 2019). It presents with a depressed mood, loss of interest or pleasure (anhedonia), decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, poor concentration, sadness, decreased locomotor activity, and increased sensitivity to pain (Liu *et al.,* 2016; Dabana and Gobir, *et al*., 2018). Major Depressive Disorder is the more severe persistent depression type. It's different from depression caused by a loss of a loved one, or a medical condition such as thyroid disorder in severity of symptoms (Hall-flavin, 2017).

Depression is a major cause of disability and a major contributor to the overall global burden of disease (WHO, 2020) with an estimated 3.8% of the population affected. It is the third leading cause of years lived with disability worldwide and has a lifetime prevalence of 14.6 % among people living in high-income countries (Cordeiro *et al.,* 2019). Also, approximately 280 million people in the world have depression (WHO, 2021). It may result in premature death, and major social and economic consequences (Marcia *et al.,* 2016; Muraro *et al.,* 2019). It is often associated with poor quality of life and increased utilization of healthcare resources (Ademola *et al.,* 2019). Over the past 25 years, the US prevalence of adolescent and adult depression indicators has increased (Hasin *et al.,* 2018). Suicide is the worst complication of depression, with an estimated 800, 000 people dying due to suicide (Moledina *et al.,* 2018; WHO, 2020).

Depletion of monoamines (e.g., serotonin, epinephrine, and dopamine) has been suggested as a central mechanism of depression (Beatrice *et al.,* 2016). The inhibition of monoamine oxidases (MAOs) in the brain gives vital antidepressant and behavioral advantages (Ramsay and Albreht, 2018). Factors associated with depression, according to a study, are lack of education, being unmarried, and being unemployed (Ademola *et al.,* 2019). The inflammatory response system is driven by pro-inflammatory cytokines that are produced by macrophages, T cells, and Natural Killer cells in response to immune activation (Ng *et al.,* 2018). A meta-analysis by Dowlati *et al.* (2010) have shown that elevations of interleukin IL-6 and tumor necrosis factor TNF-α in peripheral blood are reliable biomarkers of depression. Cytokines such as TNF-*α* and IL-6, are pro-inflammatory (responsible for initiating, propagating, and maintaining inflammation) (Zhu *et al.,* 2018).

Lipopolysaccharides (LPS) are the major outer membrane constituent of Gram-negative bacteria, and initiate the production of pro-inflammatory cytokines such as induced nitric oxide synthase (iNOS), interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α), as well as immune mediators such as Nitric oxide (NO), in various cell types (Park *et al.,* 2017). Studies have shown that the increased pro-inflammatory cytokines can trigger oxidative stress, a pathological event that could be found in depressed patients, thereby promoting depression onset or progression Valvassori *et al.,* 2018). Moreover, increased translocation of Gram-negative bacteria in depression is associated with higher levels of immune activation and oxidative stress. LPS is composed of lipid A, core sugars, and O-antigen repeats. Lipid A is known to be responsible for the toxic effects of infections with Gram-negative bacteria (Wang, X and Quinn, 2010). Administration of LPS activates the expression of proinflammatory cytokines in the brain, and the occurrence of sicknesses such as decreased motor activity and appetite, and also social withdrawal. These sicknesses are followed by a phase of depressive-like behavior which can be improved by antidepressant treatment Dang *et al.,* 2019). Examples of LPS-induced models of depression include the study of Li *et al*. (2015) and Domingues *et al*. (2018) where a single administration of LPS (0.5 and 0.83 mg/kg via I. P) leads to increased immobility time in the tail suspension test, reduced sucrose preference, neuroinflammation, and oxidative stress. Peripheral administration of LPS increases the extracellular concentration of noradrenaline, serotonin, and dopamine in the brain (Liu *et al.,* 2016).

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol among flavonoids found in many plants, including citrus fruits, buckwheat, and Asparagus (Al-dhabi *et al.,* 2015). The first isolation of rutin was in early 1900s, it has in vitro and in vivo activities. Besides, it shows antioxidant activity and lack of toxicity, as a result, more than 130 pharmaceutical preparations contain rutin as an active principle (Sancineto *et al.,* 2021). The findings of Jia *et al.* (2022) suggest that flavonoids might improve symptoms of depression and anxiety.

The limits of pharmacotherapy and pharmacological classification based on serotonin, norepinephrine, and dopamine (monoamines) imply that the 'monoamine hypothesis' is insufficient in explaining the etiology of depression (Lee *et al.,* 2018). Several plant derivatives have been used as anti-inflammatory agents (Kheiry *et al.,* 2019). The development of improved therapeutics for depression is important due to an increasing disease burden, the high lifetime prevalence of this disabling condition, coupled with limitations in existing medications such as numerous side effects (Domingues *et al.,* 2018; Shen *et al.,* 2018). The available antidepressants in clinics, like selective monoamine oxidase inhibitors and serotonin reuptake inhibitors, prevent depressive symptoms majorly by correcting the monoamine dysfunction in the brain (Zhang *et al.,* 2016; Qiu *et al.,* 2020). Thus, given its ability to attenuate inflammation and oxidative stress which are implicated in depression, we investigated whether rutin would also be effective in treating depressive-like symptoms.

Materials and Methods

Materials

Drugs and Reagents:

Lipopolysaccharide (LPS) from *Escherichia coli*, strain 055: B5, and rutin trihydrate (cat#R8170) were purchased from Solarbio Life Sciences, China. Fluoxetine was purchased from Bristol Laboratories Ltd., Hertfordshire, the UK with batch number: 8775 and product license number: PL11311/0047. All drugs were freshly prepared and diluted in saline solution. BDNF (cat NO. AD20097MO), IL-6 (cat No. AD20303MO), and TNF alpha (cat No. AD20506MO) ELISA kits were purchased from Biotuva Life sciences, the UK. MDA (cat No. YX-C-A401) ELISA kits were purchased from Shanghai Coon Koon Biotech Co., Ltd. Shanghai, China.

Experimental Animals:

Thirty-six (36) *Swiss Albino* mice, aged 6-8 weeks, weighing 18 to 26 g were purchased from the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University (A.B.U) Zaria-Nigeria. They were housed in cages containing 2-4 mice per cage with water and food *ad libitum*. They were kept at room temperature and a normal 12 h light/dark cycles were followed. All efforts were made to minimize animals' suffering and to reduce the number of animals used. Ethical approval was obtained on 20th December 2021 from Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC), Ahmadu Bello University, Zaria-Nigeria, with approval No. ABUCAUC/2021/153.

Animal groupings:

The study comprised the following groups where each group comprised six mice (n = 6) (Figure 1)

Group I: Animals served as the normal control [distilled water (DW) 10 ml/kg]

Group II: Animals received LPS 0.5 mg/kg only

Group III: Animals received fluoxetine 20 mg/kg + LPS 0.5mg/kg

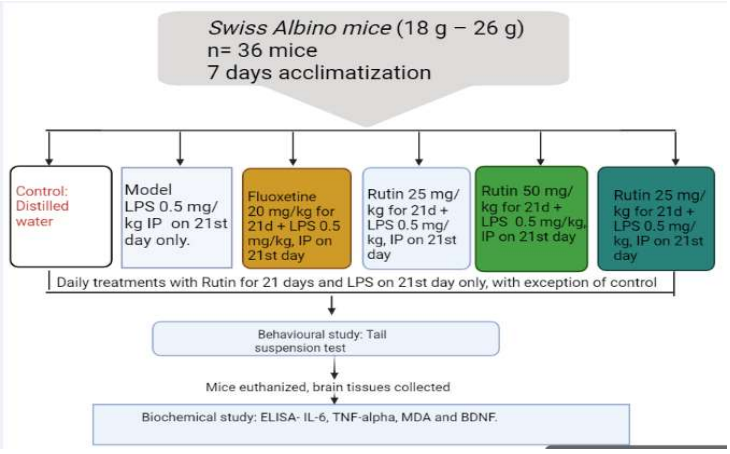
Group IV: Animals received rutin 25 mg/kg + LPS 0.5 mg/kg

Group V: Animals received rutin 50 mg/kg + LPS 0.5 mg/kg

Group VI: Animals received rutin 100 mg/kg + LPS 0.5 mg/kg

Experimental Design

Figure 1: Flow chart of the experiment



Induction of Depressive-like behaviour

Lipopolysaccharide was dissolved in sterile isotonic saline solution and administered intraperitoneally (i.p.) to all the treated groups i.e., excluding the normal control group at a dose of 0.5 mg/kg 30 min after the last drug administration (Li *et al.,* 2015)

Behavioural Assessments
Twenty-four (24) h after the administration of LPS in the morning, animals were subjected to a tail suspension behavioral test for one day (O'Connor *et al.,* 2009).

Tail Suspension Test (TST)
This is a test for behavioral despair. The apparatus consists of a three-walled rectangular compartment (55 height X 15 width X 11.5 cm depth). There were six such identical compartments in the apparatus and six mice were tested at a time. The mice were suspended in the middle of this compartment and the width and depth are sufficiently sized so that the mouse cannot make contact with the walls. Mice were brought into the neurobehavioral laboratory 1 h prior to testing. Seventeen centimetres (17 cm) of adhesive tape was cut and marked at 2 cm on both ends in order to hang the tail on the suspension bar. The tail of each mouse was tight using the 2 cm from one end of the tape, leaving approximately 1mm from the tip of the tail, while the other 2cm end was suspended on the suspension bar (1 cm. height x 1 cm width x 60 cm. length). The compartments were wiped thoroughly after each session with 70 % ethanol to prevent olfactory cues. A climbing stopper was used to prevent the animal from tail climbing during the experiment. The whole period of immobility time was recorded manually using a stopwatch. The duration of each of the tests was 6 min and immobility time was measured during the last 4 min. Mice were considered immobile only when they hung passively and were completely motionless.

Open Field Test (OFT)
The open field test was conducted to evaluate spontaneous locomotor activities of the experimental mice. The apparatus consists of floor space with the dimension of 40 cm x 40 cm and 30 cm in height. The floor space is divided into 16 squares equally. At the beginning of the test, mice were left for 1 h in the animal behavior room for habituation. Then, the mice were placed individually at the center of the open field arena and were observed for 5 min for their locomotor activity (number of lines crossed) as described by Yusha'u *et al.* (2021). A mouse is considered to have crossed a line when all of its four paws are within one square.

Blood collection
24 h after behavioural tests, mice were euthanized by anesthesia (ketamine 60 mg/kg). Blood samples were drawn by cardiac puncture and collected in plain tubes. The serum was obtained by centrifugation of blood at 489 x g for 10 min and was used for the assessment of MDA concentrations.

Harvest of Hippocampus (HC)
The whole brain was harvested and placed on a cold plate, followed by rapid isolation of the HC. The tissues were then homogenized in cold PBS. The homogenates were centrifuged at 489 x g for 10 min (Domingues *et al.,* 2018) at 4 °C. The supernatants were aliquoted and stored at -80 ^{0}C for later use. The samples were used for BDNF and inflammatory markers assessments

Assessment of brain biomarkers of depression, inflammation, and oxidative stress markers:
The assay for BDNF, IL-6, TNF α, and MDA was conducted using their respective ELISA kits according to the manufacturer's instructions and quantified by a microplate reader at 630 nm.

Statistical Analysis
Data were expressed as Mean ± SEM. All analyses were carried out using a One-Way analysis of variance (ANOVA), followed by Tukey's post - hoc test for multiple comparisons using GraphPad Prism version 9. Values with $P < 0.05$ were considered statistically significant.

Results and Discussion
The findings from this study have shown the antidepressant-like effects of rutin. It has also shown that its twenty-one days of pretreatment on the LPS model of depression in mice are via inhibition of proinflammatory responses in the hippocampus of mice. The Hippocampus, which is part of the limbic system is a structure of the brain that is considered to be tightly associated with mood and behavior regulation (Lucassen *et al.,* 2014). Rutin improved behavioral despair induced by LPS by significantly reducing immobility time in the TST. It also prevented the increase of inflammatory cytokine IL-6 in the hippocampus due to LPS. Moreso, rutin prevented an increase in BDNF by the model. The antidepressant potentials of rutin in other models have been highlighted by previous studies (Al-dhabi *et al.,* 2015; Yusha'u *et al.,* 2017; Anjomshoa *et al.,* 2020).

Effects of Pre-treatment with Rutin on behavioral despair in LPS-induced mice exposed to tail suspension test

There were significant differences in immobility time in the TST between distilled water (DW) and the treated groups as indicated by one-way ANOVA {F (5, 30) = 7.442, P = 0.0001}. Turkey post-hoc test showed a significant increase in immobility time between DW (59.3 ± 11.5) and model (LPS) (127.7 ± 5.3, P = 0.00), DW and rutin 50 mg/kg + LPS (101.8 ± 10.5, P = 0.027). There was also a significant decrease in immobility time between the model (LPS) and Flux + LPS (74.3 ± 7.6, P = 0.003), rutin 25 mg/kg + LPS (70.5 ± 11.0$^{\#}$, P = 0.01) and rutin 100 mg/kg + LPS (85.2 ± 6.8, P = 0.027) (Figure 2).

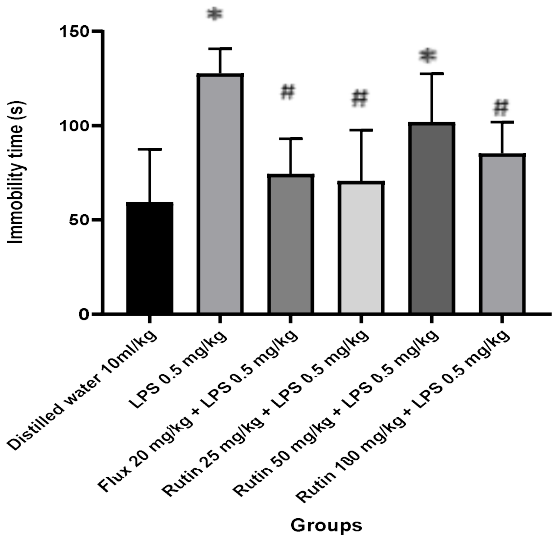


Figure 2: The antidepressant-like effects of Rutin on mice with LPS-induced depression in TST. Results presented as mean ± SEM, $P \geq 0.05$ considered not significant (n = 6) using one-way ANOVA, DW: Distilled water, LPS: Lipopolysaccharide, Flux: Fluoxetine, TST: Tail suspension test

*: Significant compared to DW, #: Significant compared to LPS

The tail suspension test (TST) is a well-characterized test for assessing depression-like and antidepressant-like activity (Cryan *et al.,* 2003). Our findings showed a decrease in immobility time in the TST after pretreatment with rutin 25, 50, and 100 mg/kg, this indicate an antidepressant-like effect of rutin because increase immobility is a symptom of depressive-like behaviour. For the lower dose of rutin (25 mg/kg), we observed a similar effect to that of fluoxetine, which is a standard antidepressant drug. In the present study, LPS induced a significant increase in the immobility time of TST in comparison with normal albino mice, and rutin was able to inhibit that effect. Remus and Dantzer (2016) stated that in the LPS-induced model of depression, sickness behaviors typically resolve within 24 h of LPS injection, when motor activity is back to normal, treated animals show increased immobility in TST. Our result here coincides with that of Yusha'u and Colleagues (2017) who reported that administration of the rutin supplement for sixteen days produced a significant reduction of immobility time in the open-space forced swim mouse model (at 30, 60, and 120 mg/kg). According to Seligman's theory of helplessness, if the animal is exposed to constant stressful situations and has no way to escape, it gradually loses hope of escape (Maier and Seligman, 1976).

Effects of Pre-treatment with Hispidulin on Locomotor activities in LPS-induced mice exposed to Open field test

There were no significant differences in locomotor activity in the OFT as indicated by one-way ANOVA between DW (59.8 ± 5.9) and LPS 0.5 mg/kg, Flux 20 mg/kg, rutin 25 mg/kg, rutin 50 mg/kg, and rutin 100 mg/kg (76.3 ± 3.0, 52.7 ± 8.9, 52.2 ± 5.6, 59.0 ± 8.7, 58.3 ± 7.3) {F (5, 30) = 1.625, P = 0.184} (Table 3.1).

Table 1: Effects of Pre-treatment with Hispidulin on Locomotor activities in LPS-induced mice exposed to Open field test

Groups	Frequency of Line Crossing
DW	59.8 ± 5.9
LPS 0.5 mg/kg	76.3 ± 3.0
Flux 20 mg/kg + LPS 0.5 mg/kg	52.7 ± 8.9
Rutin 25 mg/kg + LPS 0.5 mg/kg	52.2 ± 5.6
Rutin 50 mg/kg + LPS 0.5 mg/kg	59.0 ± 8.7
Rutin 100 mg/kg + LPS 0.5 mg/kg	58.3 ± 7.3

The findings of this study revealed that rutin 25, 50, and 100 mg/kg has no significant effect on line crossing (locomotor activity) in OFT. This is in agreement with studies conducted by Machado *et al*. (2008) which showed that acute treatment with rutin isolated from ethanolic extract of *Schinus molle* did not have a significant effect on locomotor activity in mice. However, the study of Yusha'u *et al*. (2017) revealed that chronic administration of rutin significantly increased locomotor activity in OFT. Also, Fouda *et al*. (2022) found rutin (80 mg/kg) to have an increased activity and antidepressant effect in OFT. Our findings

depict that rutin is not a psychostimulant and might possess antidepressant-like activity, following the previous report of Cryan *et al.* (2005) who reported that psychotonics are ineffective antidepressants.

Effects of Pre-treatment with Rutin on Malondialdehyde concentration in LPS-induced mice

The result of the effects of rutin on lipid peroxidation using one-way ANOVA indicated no significant difference in the concentration of the MDA in LPS-induced mice between DW (130.7 ± 16.1) and LPS 0.5 mg/kg, Flux 20 mg/kg, rutin 25 mg/kg, rutin 50 mg/kg, and rutin 100 mg/kg (92.3 ± 20.6, 121.9 ± 15.4, 136.7 ± 17.0, 121.8 ± 10.6, 136.8 ± 16.0) [F (5, 30) = 1.052, P = 0.418] (Figure 3)

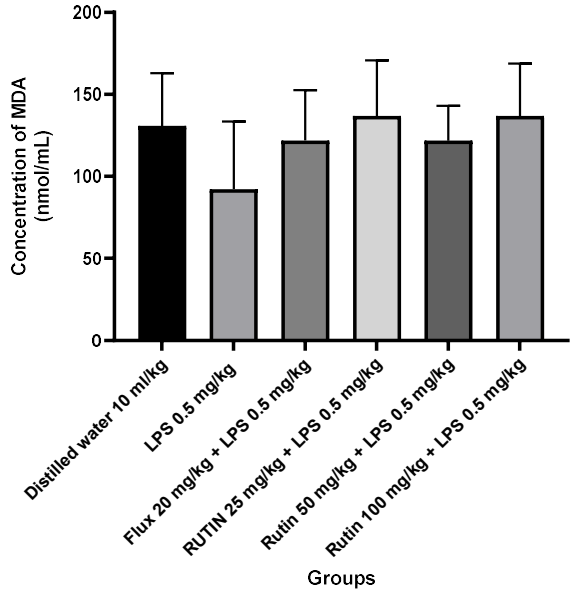


Figure 3: The effects of Rutin on lipid peroxidation in mice with LPS-induced depression. Results presented as mean ± SEM, $P \geq 0.05$ considered not significant (n = 6) using one-way ANOVA, DW: Distilled water, LPS: Lipopolysaccharide, Flux: Fluoxetine.

Our result for MDA concentration also showed an increased level of lipid peroxidation was slightly reversed by 50 mg/kg rutin and 20 mg/kg flux pretreatments (though not significant). However, compared to LPS alone, rutin pretreatment shows a slight increase in MDA≥ level i.e., lipid peroxidation. This coincides with the work of Kumar *et al.* (2014) who stated that a lower dose of rutin (20 mg/kg) showed no significant improvement in lipid peroxidation in the brain of rats. It is contrary to Enogieru *et al*. (2018) who stated that rutin acted as an antioxidant by reducing the production of MDA. In a study, the MDA level in the hippocampus of the rutin group was significantly lower than those in the control (Moghbelinejad *et al.,* 2014) LPS 0.5 mg/kg administration, though not significant, decreased the levels of MDA, and this decrease was reversed by 21 days of flux pretreatment (20 mg/kg) and rutin plus LPS (25, 50, and 100 mg/kg + 0.5 mg/kg). This disagrees with the study of Liu *et al.* (2018) which stated that LPS administration increased the levels of MDA and the increase was reversed by fluoxetine (20 mg/kg) treatment.

Effects of Pre-treatment with Rutin on Interleukin-6 in LPS-induced mice

The result of IL - 6 as a marker of inflammation using one-way ANOVA has shown a significant decrease in the concentration of IL-6 between the DW and treated groups [F (5, 30) = 2.977, P = 0.027]. Tukey posthoc test showed a significant difference is between Flux of 20 mg/kg + LPS 0.5 mg/kg (0.28 ± 0.24, P = 0.039) and model (LPS 0.5 mg/kg) (1.66 ± 0.75). Also, a significant decrease in the concentration of IL-6 was found when the model (LPS 0.5 mg/kg) was compared to rutin 100 mg/kg (0.31 ± 0.37, P = 0.046), but there is no significance when DW is compared to other treated groups (Figure 4).

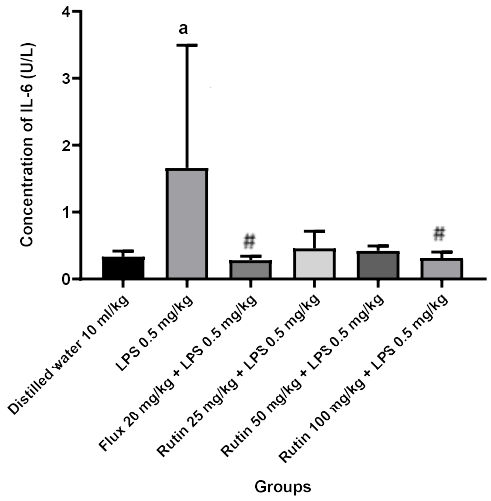


Figure 4**:** The effects of Rutin on inflammation (IL-6) in mice with LPS-induced depression. Results presented as mean ± SEM, $P \geq 0.05$ considered not significant (n = 6) using one-way ANOVA, DW: Distilled water, LPS: Lipopolysaccharide, Flux: Fluoxetine.
a: Significant compared to Flux, #: Significant compared to LPS

Significant antidepressant-like effects of 21 d of 100 mg/kg rutin pretreatment in a 0.5 mg/kg LPS-induced mouse model were exerted by decreasing the level of pro-inflammatory cytokine IL-6 in the hippocampus.

The IL-6 concentration showed a significant reduction in concentration, in a similar manner as fluoxetine (20 mg/kg). So, the administration of rutin counteracted the effect of LPS and produced a significant decrease in IL-6 compared to the group with neuroinflammation (model). Also, the model (LPS 0.5 mg/kg alone) has shown a significant increase in the level of IL-6 compared to the positive control (Flux 20 mg/kg + LPS) because LPS is known to cause inflammation. Studies have also demonstrated that administration either of the bacterial endotoxin LPS or of proinflammatory cytokines can cause depressive symptoms in rodents and humans (Sekio and Seki, 2014). Dowlati *et al.* (2010) have shown that elevation of interleukin IL-6 is a reliable biomarker of depression.

Effects of Pre-treatment of Rutin on Tumour necrosis factor-alpha in LPS-induced mice

The result of the effects of rutin on inflammatory cytokine TNF-α using one-way ANOVA showed no statistically significant difference between DW (0.67 ± 0.10) and LPS 0.5 mg/kg, Flux 20mg/kg, rutin 25 mg/kg, rutin 50 mg/kg, and rutin 100 mg/kg (0.67 ± 0.10, 0.76 ± 0.12, 0.60 ± 0.07, 0.52 ± 0.10, 0.61 ± 0.09) [F (5, 30) = 0.8523, P = 0.694] (Figure 5).

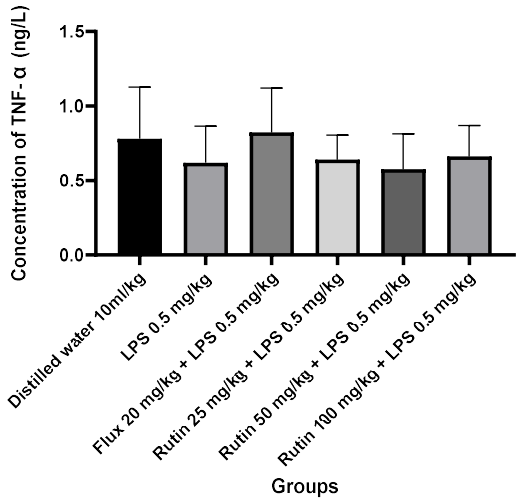


Figure 5: The effects of Rutin on inflammation (TNF-α) in mice with LPS-induced depression. Results presented as mean ± SEM, $P \geq 0.05$ considered not significant (n = 6) using one-way ANOVA, DW: Distilled water, LPS: Lipopolysaccharide, Flux: Fluoxetine.

However, TNF-α has shown a decrease which is not significant compared to normal control and LPS groups. Rutin affects inflammation by reducing and modulating the production of proinflammatory cytokines by reducing TNF-α generation (Enogieru *et al.,* 2018).

Effects of Pre-treatment with Rutin on Brain-derived neurotrophic factor in LPS-induced mice

The result of BDNF on LPS-induced depression using one-way ANOVA indicated a significant difference between the groups [F (5, 30) = 2.543, P = 0.001)]. However, Tukey posthoc test showed a significant decrease in the concentration of BDNF between model (LPS 0.5 mg/kg) (1.56 ± 0.12) and flux (0.78 ± 0.12) (P = 0.006), model (LPS 0.5 mg/kg) and rutin 50 mg/kg, rutin 100 mg/kg (0.74 ± 0.12, 0.83 ± 0.15) (P = 0.004, 0.012). Moreover, a significant increase in the concentration of BDNF was found between DW (0.62 ± 0.16) and model (1.56 ± 0.12) (Figure 6)

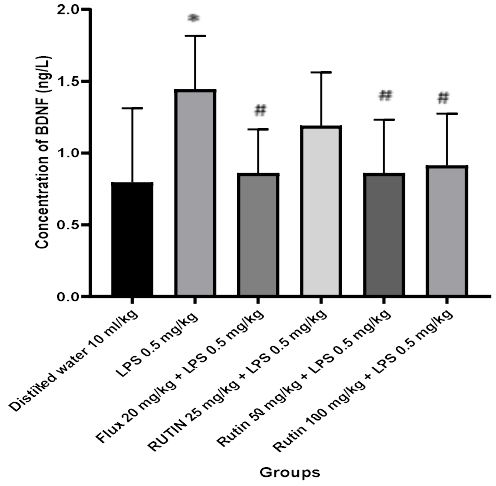


Figure 6: The effects of Rutin on depression biomarker (BDNF) in mice with LPS-induced depression. Results presented as mean ± SEM, $P \geq 0.05$ considered not significant (n = 6) using one-way ANOVA, DW: Distilled water, LPS: Lipopolysaccharide, Flux: Fluoxetine. *: Significant compared to DW, #: Significant compared to LPS

Our findings on the effect of 21 d of pretreatment with rutin on BDNF levels in the hippocampus indicated a significant decrease in BDNF at 50 and 100 mg/kg rutin which is in a similar pattern to fluoxetine, while 0.5 mg/kg LPS alone showed a significant increase compared to normal mice. This result is in contrast with the work of Moghbelinejad *et al.* (2014) who found that rutin (100 mg/kg i.p) for 21 d significantly increased extracellular BDNF gene expression in the hippocampus of rats. Also, Liu *et al.* (2018) stated that rutin (100 mg/kg) treatment also increased the levels of BDNF and tropomyosin receptor kinase (TrkB), its transcription

factor in rat hippocampus. However, there have been inconsistent findings on the relationship between BDNF levels and the severity of depression, four to eight weeks of antidepressant treatment are recommended to evaluate a change in BDNF or a treatment effect (Lee and Kim, 2010). Also, BDNF delivery into the hippocampus is very difficult because of its inability to cross the BBB and its short half-life time. Other factors that influence this antidepressant effect on BDNF include the length or duration of administration, route of administration, class of antidepressants, and doses of the drugs (Numakawa *et al.,* 2018). In general, increases in BDNF expression appear only after long-term treatment with antidepressants. Rutin may be acting like monoamine oxidase inhibitors. Typical monoaminergic (serotonin, and norepinephrine) antidepressants cause a delayed increase in BDNF expression (Ye *et al.,* 2021). Monoaminergic agents have a slow response rate of weeks to months and are only effective in approximately two-thirds of patients (Duman *et al.,* 2021)

Previous studies have shown that flavonoids attenuated depressive-like behaviors. In line with this, a number of pharmacological effects have been reported for rutin including antioxidative stress, anti-neuroinflammatory, and nephroprotective activities (Budzynska *et al.,* 2019; Caglayan *et al.,* 2019). Further studies should assess the effects of rutin doses alone to compare the difference with rutin pretreatment and increase the duration of administration.

Conclusion

Based on the results of this study, 21 d of rutin pretreatment, through proinflammatory cytokine IL-6, may possess an antidepressant-like effect in LPS-induced mice. We showed that rutin attenuated the negative effects of LPS on the hippocampus by decreasing IL-6 at the highest dose of 100 mg/kg. This was confirmed by the decreased immobility time in TST at 25 and 100 mg/kg, which are comparable to fluoxetine. Future researches should explore other mechanisms such as more oxidative and inflammatory markers, and histological studies.

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Conflicts of Interest

The authors declared none.

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