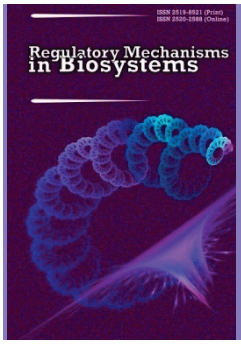

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Erucic acid treatment in lipopolysaccharide-induced anxiety and depression using ADMET properties / behaviour paradigms / interleukins pathways in rats

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The neuroimmune system plays a vital role in the etiology of anxiety and depression. In the current research, the aim was to investigate erucic acid's ability to counteract depression and anxiety induced by lipopolysaccharide (LPS) in rats. Erucic acid is known to have anti-inflammatory properties, and it was hypothesized that it would be able to reduce the inflammation and stress associated with LPS-induced anxiety and depression. The rats were distributed into five groups (n = 6). The impact of erucic acid with the dose administration of 10 and 20 mg/kg on anxiety depression-like behaviour was studied using the elevated plus maze test, staircase test, marble-burying behaviour, open field test, hyper emotionality, force swimming, and tail suspension test. Also, the biochemical parameters including cytokines i.e., interleukin-6 (IL-6) and IL-10 were performed. Swiss ADME, pkCSM, and ProTox-II served as as an integrated online platform for precise and complete predictions to determine ADME/T properties of erucic acid. LPS significantly increased anxiety and depression-like behaviour in rats. Erucic acid reduced the lengthening in time of immobility on LPS administration in the tail suspension test and forced swim test indicating the antidepressant type of action. Anti-anxiety effects of erucic acid were increased time spent in the open arms in the elevated plus maze test, decreased repetitive behaviors in the staircase test, marble-burying, and hyper emotionality tests. The high LD_{50} value (1.382) indicates that erucic acid is relatively non-toxic in acute oral exposures in rats. Erucic acid also prevented LPS-induced elevation of IL-6 and restored the IL-10 levels. It was demonstrated that erucic acid significantly attenuates LPS-induced depression and anxiety in rats. These findings suggest that erucic acid has potential antidepressant and anxiolytic effects, possibly through modulation of the neuroimmune system and inflammatory response. Erucic acid may be beneficial for neuropharmacological effects like anxiety and depression. Additionally, erucic acid may have potential benefits in treating other neurological disorders, such as Alzheimer's disease and Parkinson's disease. Further research is needed to better understand the potential therapeutic applications of erucic acid in neurological disorders.

Keywords: anxiety; depression; erucic acid; lipopolysaccharides; memory.

Introduction

Neuroinflammation has promptly been recognized as the main precursor in neuronic diseases such as anxiety and depression (Rossi et al., 2017; Beheshti et al., 2020). Depression is one of the most prominent diagnoses of mental distress signalized by observing dysphoria, psychic suffering, irregular sleep patterns, psychomotor slowness, a lack of desire, a drop in the standard of life and participation in communal interactions, and an increase in suicidal thoughts. Another neurological disease condition that frequently exists alongside depression is anxiety, which is usually managed as a comorbidity of depressed behaviours (Rubab et al., 2021). It is a negative emotional condition linked to feeling threatened with a sense of danger anxiety is indicated by a feeling of distress caused by anticipation of future dangers or adverse outcomes (Kraeuter et al., 2019). A type of anxiety termed an obsessive-compulsive disorder is characterized by time-consuming, upsetting, or detrimental obsessions or compulsive behaviours that are frequently accompanied by avoidance behaviours (Hirschtritt et al., 2017). In accordance with the medical examinations, elevated activities of transcription regulators are accountable for the formation of proinflammatory cytokines including interleukin-6 (IL-6) and IL-10 implicated in the commencement of depression (Shahidpour et al., 2021). The inflammatory cytokines in the brain and microglial activation are indications of depression and abnormal cerebral signalling demonstrating its dysfunctionality (Zhang et al., 2018). It has been determined that controlling several inflammatory cytokines, namely IL-6 and IL-10 is crucial as they are capable of actuating behavioural deficits (Lee et al., 2020). Lipopolysaccharides (LPS) when given to animals, cause their immune system to respond prominently. The most accepted animal test to determine the connection between depressive side effects and neuroinflammation is the acute infusion of the cytokine activator LPS (Jiang et al., 2017). The metabolic vitality both from the nervous system and several peripheral body parts moves to counteract the effects of invading microorganisms by reducing the processes that consume the energy, such as neurocognitive functions at the time of release of pro-inflammatory cytokines (Beheshti et al., 2020). The elevated inflammatory reaction is recommended to be considered within the etiology of anxiety and depression (Rubab et al., 2021). In the present study, natural treatment has been evaluated as a modern approach to the management of neuroinflammation and anxiety-like side effects (Lee et al., 2020).

It has recently been observed that the PPAR-δ ligand erucic acid enhances the function of cognition in animal models by reducing inflammation along with oxidative stress (Farag & Gad, 2022). Erucic acid is a naturally produced non-branched fatty acid with 22 carbons and a double bond on carbon-13. This mono-unsaturated fatty acid is a part of n-9 (“omega-9”) fatty acids (Vetter et al., 2020; Sayyed et al., 2023). Erucic acid is obtained from oilseed rape or rapeseed (Warner & Lewis, 2019).

The rationale for using different behavioral tests for studying the anxiety depression-like behavior using the elevated plus maze test, staircase test, marble-burying behaviour, open field test, hyper emotionality, force swimming, and tail suspension test in rats is to measure different aspects of the emotional and cognitive processes that are affected by the induction of inflammation and the treatment with erucic acid. Anxiety and depression are complex disorders that manifest in various ways, and single tests may not capture the full spectrum of behavioral changes associated with these conditions. In the present study, we employed a battery of tests that allows a more robust evaluation of the animals' behavioural phenotype and provides a comprehensive assessment of their emotional state. Each test has its advantages and limitations and can provide complementary information about the behavioral changes in the animals. The aim of our research was to investigate the potential of erucic acid, a monounsaturated fatty acid, in the treatment of lipopolysaccharide (LPS)-induced anxiety and depression in rats, while exploring its ADMET (absorption, distribution, metabolism, elimination, and toxicity) properties, behavioral paradigms, and involvement in interleukin pathways.

Materials and methods

Animals. Male Wistar rats with a weight of 180 ± 20 g were acquired for testing and kept in a typical lab setting at 24 ± 3 °C with a moisture control of 50–60%. Throughout the operation, the unrestricted allowance for water and pellet food was provided to all the rats. The animals were kept in 28 x 21 x 14 cm cages made of opaque polypropylene. The acclimatization period for animals was 10 days before the study was initiated. The animals were given naive treatment with care. They were randomly grouped into 5 groups, with 6 rats each. The regular circadian cycle and its effects on the results were retained by the experimental design. This research was undertaken with the approval of the Institutional Animal Care & Use Committee (IACUC/TRS/PT/021/010), obtained prior to the commencement and research conducted as per the ARRIVE guidelines. No previous procedures were performed on the animals used in the study.

Chemicals. LPS and erucic acid were used for the study and obtained from Sigma-Aldrich, USA. Cytokines interleukins-6 (IL-6, MSW-IL-6) and IL-10 (MSW-IL-10) were analyzed by rat enzyme-linked immunoassay (ELISA) kit (MSW Pharma, M.S., India). The multiple evaluations in the recent study were performed with high-grade chemicals.

Experimental design. To elicit depression and anxiety in laboratory animals, LPS was given intraperitoneally with a concentration of 1 mg/kg and freshly dissolved with 0.9% saline (pH 7.4) for 7 days (Zhang et al., 2018). The following treatments were administered to all of the five groups (n = 6) where:

Group I: Normal was treated with saline solution;

Group II: LPS control (LPS injected);

Group III: LPS + erucic acid treated at 10 mg/kg/p.o. (Kim et al., 2016, Liang et al., 2020);

Group IV: LPS + erucic acid treated at 20 mg/kg/p.o. (Liang et al., 2020, Sayyed et al., 2023);

Group V: erucic acid per se at 20 mg/kg/p.o.

Erucic acid was orally given at doses of 10 and 20mg/kg/day in the morning time post-LPS administration. Erucic acid was suspended in a 10% tween 80 solution (Kim et al., 2016). Behavioural parameters were manually detected by an observer from direct observations. After the treatment, behavioural tests were performed on the 6th and 7th days. After behvioural experiments, animals were sacrificed for biochemical estimations on 7th day. The experimental design (Fig. 1) provides a schematic overview of the research methodology employed for the conduct of the study. The initial and final body weight of the animals were evaluated.

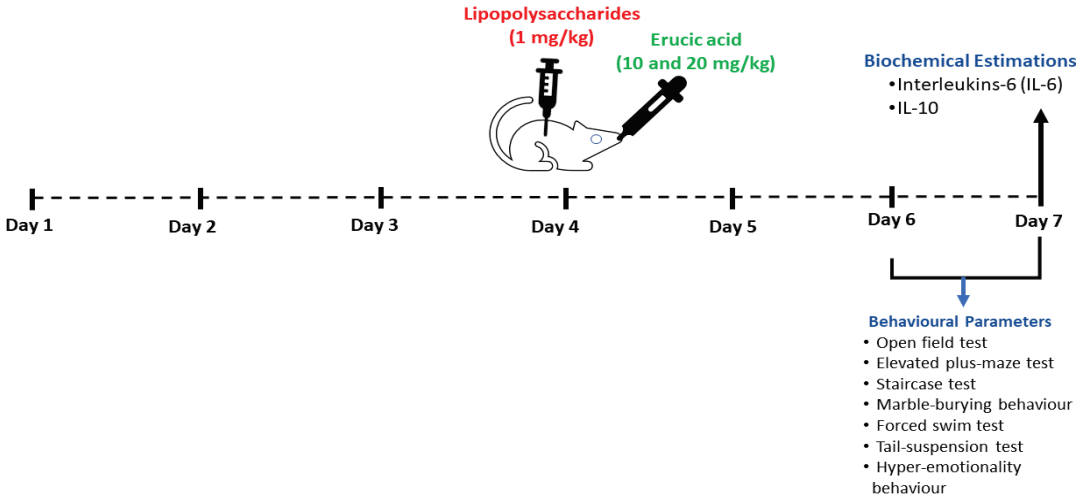

Fig. 1. Schematic overview of the research methodology

ADMET analysis. In terms of open-access databases, PubChem is one of the most comprehensive databases that contain essential information related to pharmaceutical development and chemical biology research. The chemical formula, SMILES and CAS number of erucic acid were found using the same term in the search box. Using pkCSM (http://structure.bioc.cam.ac.uk/pkcsm), ADMET analysis of erucic acid was performed to rapidly assess the pharmacokinetic and toxicity properties (Pires et al., 2015). To create an accurate predictive model, the brain or intestinal estimated permeation method (BOILED-Egg) is used to compute the lipophilicity and polarity of small molecules (Swiss ADME, www.swissadme.ch/index.php) (Daina et al., 2017). ProTox-II: a webserver for the prediction of toxicity of erucic acid was used (Banerjee et al., 2018).

Behavioural parameters. Open field test. The purpose of an open field test is to access the investigative drive, curiosity, and fear or anxiety along with motor movement. The equipment was composed of a platform along with the zone of 100 by 100 cm and a height of 40 cm. There were 16 similar squares divided up inside the box. Every box was further partitioned into a centre and periphery area. A rat was positioned within the centre of the field and allowed to explore for the duration of 5 min with capturing of its behavioural activity by digital camera. Animals who exhibit higher levels of anxiety in open field tests enter the central zone less frequently, move farther, and remain there for a shorter period of time. Additionally, they have a lower overall crossing number and total distance traveled (Shahidpour et al., 2021).

Elevated plus-maze test. The elevated plus maze comprises a centred platform and a system of four lifted arms extending out from it. Two of the opponesed arms were walled, while the other two arms must be open except from the platforms (other than entrance, exit points, and ceiling). In this test, rats are placed in the centre and given a set amount of time to explore the maze. The total time being spent in walled arms was matched against the total amount of time in open arms to measure fear or anxiety. The quantification of entries and the duration spent in the open arms and time spent in the centre arm was conducted over a 5-minute timeframe. The test relies on rodents' natural propensity to keep a safe distance from elevated or open areas in contrast to their innate eagerness to investigate unfamiliar areas. According to the theory, a rat with lower anxiety will be

more likely to visit the open unexposed arms of the labyrinth pretty often, whereas a rat with higher anxiety will take more time in closed arms (Kraeuter et al., 2019).

Staircase test. In the staircase test, each rat was put separately on the staircase. A step was known to be climbed when the rat put all of its four paws on one particular stair. Ascending stairs was a recognized locomotor and exploratory measure. The plunge steps were not counted. The rearing was noted whenever the rats stood up on its hind legs, either against a wall or on a step, to sniff the air. Rearing was regarded as an anxiety index and exploratory index. The staircase test was performed for a period of three minutes, after three minutes, the rat was removed, and the stairs were cleaned to remove lingering odors, if any (Farbstein et al., 2021).

Marble-burying behaviour. The rats were housed in cages of Plexiglas (inner measurements: length 33 cm × width 21 cm × height 19 cm) with approximately 3 cm of woodchips at the bottom and a total of 20 marbles were divided into grid type of position (4×5). The subjects were gently taken out to lessen unsettling influence on the bedding, post completion of 20 min test duration. Marbles were considered to be buried when at least 75% of them are covered with bedding (Wilkerson et al., 2018).

Forced swim test. The forced swim test is typically for depression-related behaviour in rats. Ordinarily, two barrels made of transparent Plexiglas measuring 45 cm in height by 20 cm in diameter and midway filled with water (24 ± 1 °C) were used to test the rats simultaneously. The two restricted swim tanks were divided by a hazy plastic screen to prevent the animals from seeing one another. Before the procedure of testing, the rats were moved to the testing area in their cages and given about an hour to familiarize themselves with the surroundings. The rats were carefully positioned in the centre of the tank at the start of each experiment and were free to swim around. A camera system (Intex Webcam, India) was used to monitor the animal's movements during the test. The camera system was mounted on a level plane and faced the sidewalls of the two tanks. The duration the rats remained immobile during a 5-min observation period was recorded. Several indicators were measured, including immobility, activity, and climbing time. The rats were rapidly taken out of the tank after the test, dried with a paper towel, and put back inside its cage (Fitzgerald et al., 2019).

Tail-suspension test. The rats were positioned 50 cm from the ground with their bodies swinging down in the air and their tails hanging in the test of tail suspension. The premise of the tail suspension test is that the animal will attempt to flee the stressful surroundings. Every rat was confined for a total of 6 minutes, and the latter 4 minutes of immobility were recorded. The animal ceases to strive and becomes static; a prolonged period of immobility phases is an indication of depressive behaviour (Belovicova et al., 2017; Zhang et al., 2018).

Hyper-emotionality behaviour. Based on the behaviour in response to fight and struggle stimuli, hyper-emotionality was assessed. The rat's reaction to being touched with a gloved hand was rated as a fight response, and its reaction to having its tail pinched by blunt forceps was rated as a struggle response. The hyperemotionality behaviour test was conducted for a duration of 5 minutes. On the scale of 0 to 4, the results were graded, where 0 represents no response at all and 4 represents a strong response. Hyper-emotionality scores were calculated as the sum of the assessed score (Afzal et al., 2022).

Serum biochemical parameters. The ELISA kit was tested on rats to assess compounds associated with biomarker activity, including IL-6 and IL-10 and measured in pg/mL.

Statistical analysis. Statistical analysis was presented in the form of standard error mean (SEM) using Graph pad prism software (Version 8.0.1). The behaviour and biochemicals test were examined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis. The P-value $P < 0.05$ was considered a significant value.

Results

ADMET properties. The ADMET properties of erucic acid are presented in Table 1. The predicted acute toxicity of erucic acid LD_{50} was 1.382 mol/kg. The distribution of molecular weight and dose value as well as BOILED-Egg Model for gastrointestinal absorption and blood-brain barrier (BBB) penetration was shown in Figure 2. Bioavailability radar showed that the colored zone is the most suitable physicochemical space for oral bioavailability based on the following properties: flexibility, lipophilicity, saturation, size, polarity, and solubility (Fig. 2).

Table 1
Predicted ADMET properties of erucic acid

Property	Model name	Predicted value	Unit
Absorption	Water solubility	-6.189	Numeric (log mol/L)
	Caco2 permeability	1.084	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	90.449	Numeric (% Absorbed)
	Skin Permeability	-2.734	Numeric (log Kp)
	P-glycoprotein substrate	No	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution	VDss (human)	-0.58	Numeric (log L/kg)
	Fraction unbound (human)	0.007	Numeric (Fu)
	BBB permeability	-0.337	Numeric (log BB)
	CNS permeability	-1.435	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	Categorical (Yes/No)
	CYP1A2 inhibitior	Yes	Categorical (Yes/No)
	CYP2C19 inhibitior	No	Categorical (Yes/No)
	CYP2C9 inhibitior	No	Categorical (Yes/No)
	CYP2D6 inhibitior	No	Categorical (Yes/No)
	CYP3A4 inhibitior	No	Categorical (Yes/No)
Excretion	Total Clearance	2.016	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
	Max. tolerated dose (human)	-0.833	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	No	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	1.382	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	3.567	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	Yes	Categorical (Yes/No)
	Skin Sensitisation	Yes	Categorical (Yes/No)

Note: BBB – blood-brain barrier, CNS – central nervous system; CYP – cytochrome; hERG – human ether-go-go gene; LD_{50} – Lethal dose 50%, OCT2 – organic cation transporter 2; VDss – volume of distribution.

Body Weight. Figure 3 shows the comparison between the initial and final body weight of each rat after the LPS injection. Initially, there was no difference in rats' body weight between the groups. Normal group rats' body weight increased over time. The body weight loss caused by LPS usually does not recover to normal levels or increases more slowly than in the normal group. In contrast, rats treated with LPS showed a significant reduction in body weight compared with normal rats ($P < 0.05$). However, the group receiving erucic acid showed body weight recovery ($F_{4, 25} = 7.114$; $P = 0.0006$).

Open field test. Figure 4 depicts the effect of erucic acid in rats subjected to open field test. It measures spontaneous locomotor activities. As per the research conducted, the number of box crossings decreased in the group treated with LPS. However, one-way ANOVA followed by Tukey's post-hoc test reveals that administration of 10 and 20 mg/kg doses of erucic acid significantly restored these activities ($F_{4, 25} = 14.01$; $P < 0.0001$). Similarly, the distance traveled by the rats ($F_{4, 25} = 69.65$; $P < 0.0001$) and time duration of nearness to the central time ($F_{4, 25} = 24.51$; $P < 0.0001$) decreased in the LPS treated group, which was normalized post-injection of 10 and 20 mg/kg of erucic acid. Erucic acid therapy significantly improved the mobility of LPS-treated rats. In the erucic acid per se group, notable changes were not observed.

Elevated plus maze test. The LPS-induced rats showed significantly decreased entries, time spent in the open arm and centre time as shown in Figure 5. The one-way ANOVA followed by Tukey's post-hoc test demonstrated that erucic acid at the dose administration of 10 and 20 mg/kg prevented this alteration and tend to restore it in open arm entries ($F_{4, 25} = 45.03$; $P < 0.0001$). On the other hand, time spent in the open arm decreased on inducing LPS that was substantially increased after both dose of erucic acid treatment ($F_{4, 25} = 14.67$; $P < 0.0001$). Moreover, LPS-treated rats increased centre time ($P < 0.01$), erucic acid treatment at both doses decreased the centre time ($F_{4, 25} = 49.45$, $P < 0.0001$). Per se group erucic acid did not show any significant changes.

Staircase test. The outcomes of the erucic acid effect in rats subjected to the staircase test are revealed in Figure 6. The total number of steps climbed by the rat shows the accepted locomotor and exploratory index. The one-way ANOVA followed by Tukey's post-hoc test showed that the LPS control group indicated a rise in the number of steps climbed, which was significantly drawn towards the normalization after the administration of erucic acid with a dose of 10 and 20 mg/kg ($F_{4, 25} = 25.57$; $P < 0.0001$). By contrast, the erucic acid per se group showed no significant changes.

Marble-burying behaviour. The treatment of rats with erucic acid at the amount of 10 and 20 mg/kg undergoing marble-burying behaviour is shown in Figure 6. The results of the analysis using one-way ANOVA followed by Tukey's post-hoc test indicated that the number of buried marbles was increased on the administration of LPS in rats that were significantly suppressed by erucic acid administered at the dose of 10 and 20 mg/kg ($F_{4, 25} = 37.56$; $P < 0.0001$). Administration of erucic acid in the per se group did not show any significant changes.

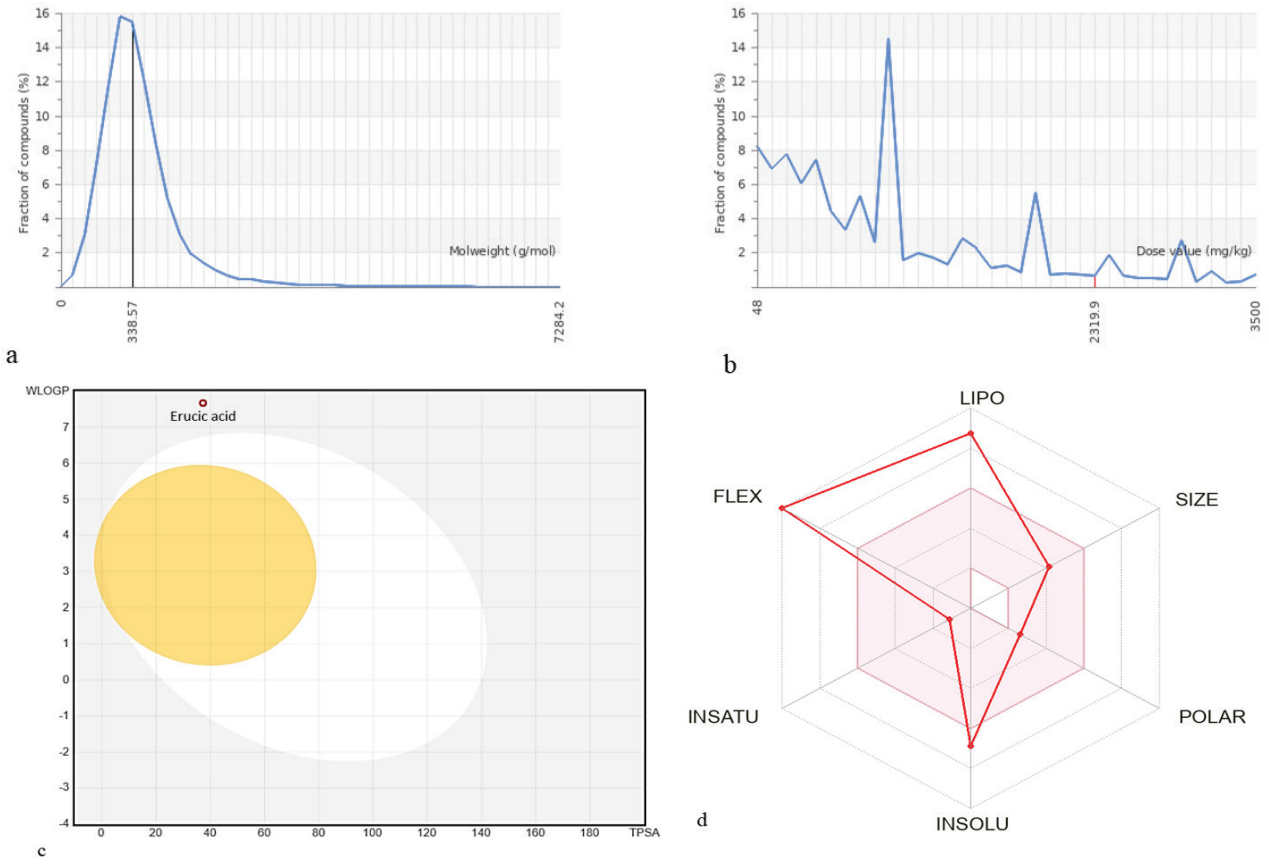

Fig. 2. Distribution of molecular weight (*a*), distribution of dose value (*b*), BOILED-egg model for gastrointestinal absorption and blood-brain barrier penetration (*c*) and bioavailability radar (*d*)

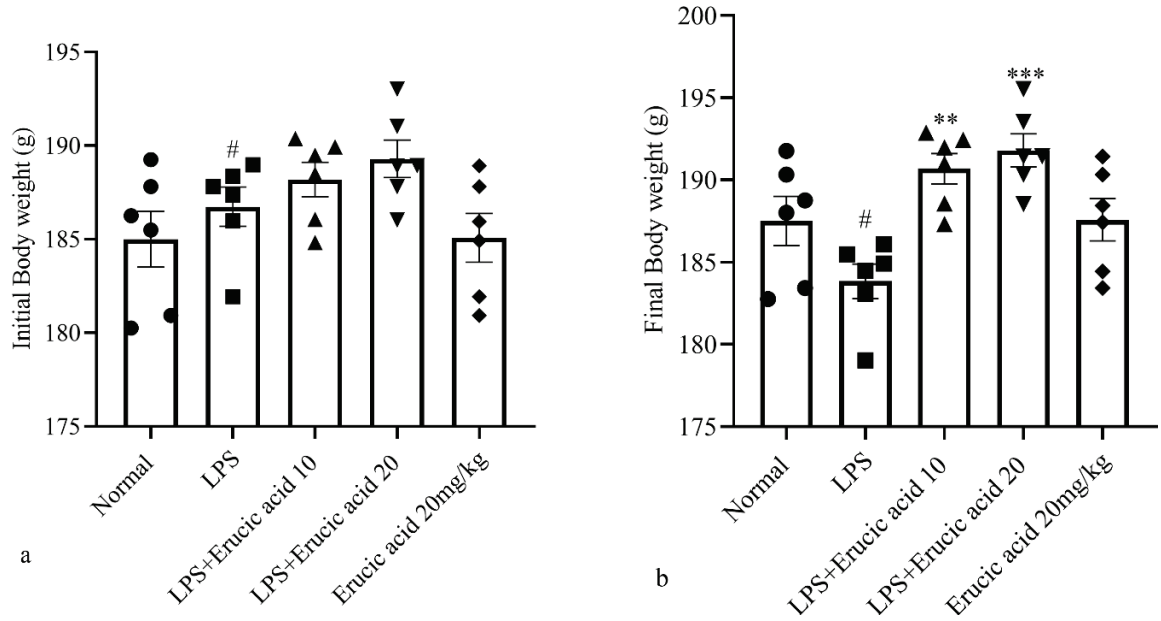

Fig. 3. Body weight of rats: initial (*a*) and final (*b*); values expressed as x ± SE (n = 6); one-way ANOVA followed by Tukey's post hoc test; $^{\#}P < 0.05$ vs. normal group; ** $P < 0.0001$, *** $P < 0.0001$ vs. the lipopolysaccharide (LPS) group

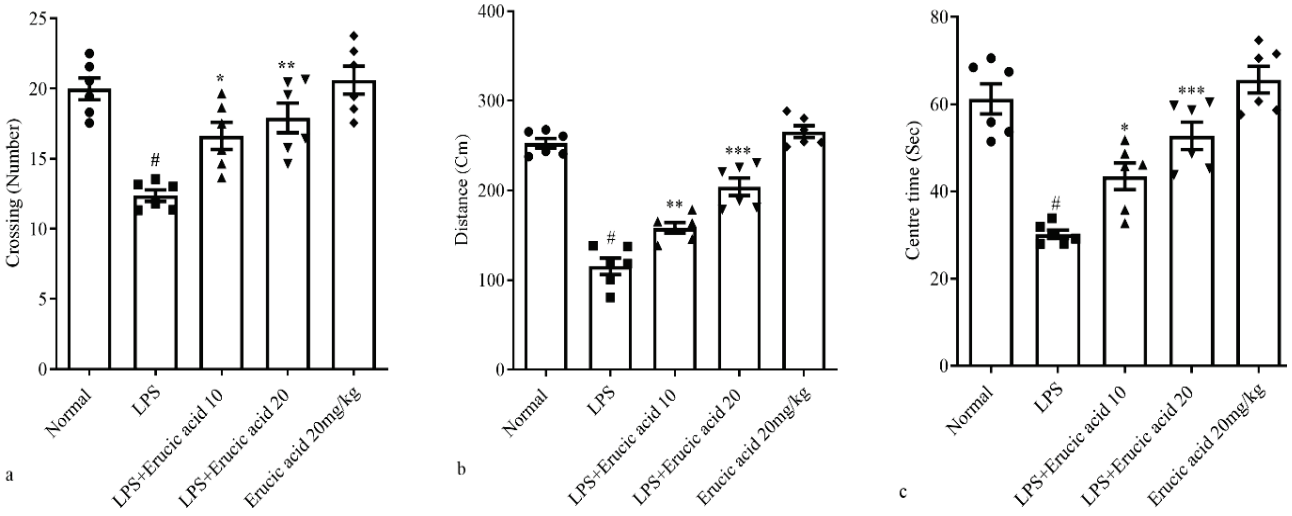

Fig. 4. The impact of erucic acid on open field test: *a* – total number of crossing, *b* – travelled distance, *c* – centre time; values are represented as x ± SE (n = 6); one-way ANOVA followed by Tukey's post hoc test; # P < 0.05 vs. normal group; * P < 0.05, ** P < 0.001, *** P < 0.0001 vs. the lipopolysaccharide (LPS) group

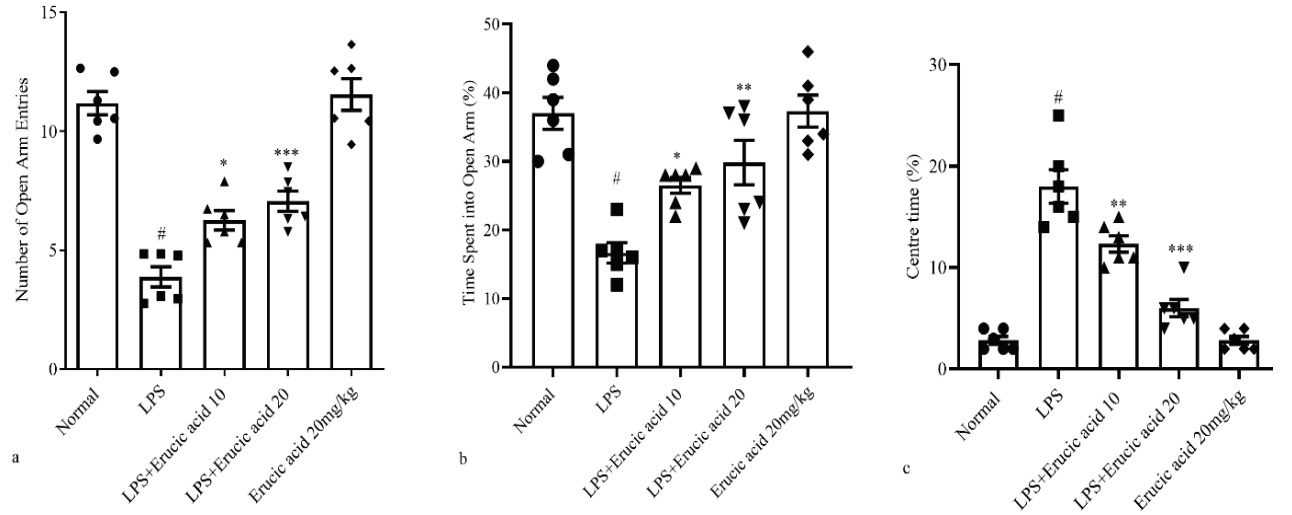

Fig. 5. Impact of erucic acid on elevated plus maze test: *a* – number of open arm entries, *b* – time spent in open arm entries, *c* – centre time; values are represented as x ± SE (n = 6); one-way ANOVA followed by Tukey's post hoc test; # P < 0.05 vs. normal group; * P < 0.05, ** P < 0.001, *** P < 0.0001 vs. the lipopolysaccharide (LPS) group

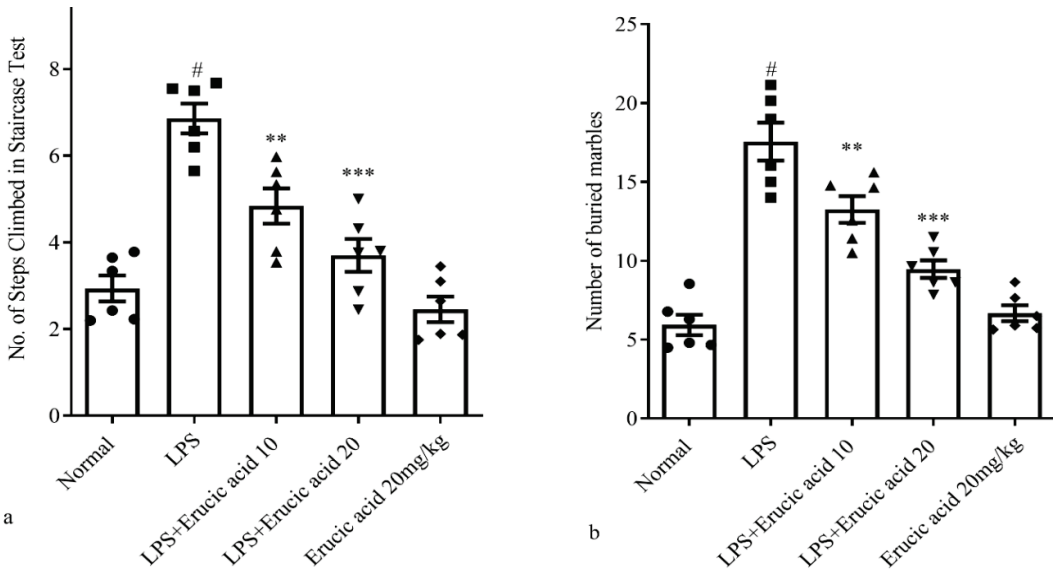

Fig. 6. Impact of erucic acid on Staircase test (*a*) and Marble-burying behaviour (*b*): values are expressed as x ± SE (n = 6); one-way ANOVA followed by Tukey's post hoc test; # P < 0.0001 vs. normal group; ** P < 0.001, *** P < 0.0001 vs. the lipopolysaccharide (LPS) group

Forced swim test. The effects of erucic acid on rats undergoing the forced swimming test are shown in Figure 7. The depression-related behaviour post-induction of LPS was evaluated using the forced swim test. The findings from the examination using one-way ANOVA followed by Tukey's post-hoc test demonstrated that LPS induces a substantial rise in the duration of immobility of rats and its effect was partially reversed by treating them with 10 and 20 mg/kg of erucic acid ($F_{4, 25}$ = 164.3; P < 0.0001). However, a group of rats showed up with delayed activity time (swimming time) in the required area after the administration of LPS. The rats treated with erucic acid were normalized to their activity time ($F_{4,25}$ = 51.35; P < 0.0001). Furthermore, reduction in climbing time was observed in the LPS group as compared to the normal group (P < 0.01). The climbing time in those with both doses of erucic acid was significantly lower than the LPS-control group ($F_{4, 25}$ = 45.91; P < 0.0001). There were no notable changes observed in the erucic acid per se group.

Tail suspension test. The impact of the tail suspension test on LPS-induced impairment is demonstrated in Figure 7. The proposed test was meant to analyze the ability of rats to escape the stressful situation arising on administration LPS and the record the immobility time. All the animal groups underwent tail suspension test paradigms where the data was analyzed by using a one-way ANOVA followed by Tukey's post-hoc test, which revealed that the LPS-induced group demonstrated higher immobility duration compared with the erucic acid (10 and 20mg/kg) group ($F_{4,25}$ = 35.21; P < 0.0001). Treating the animals with erucic acid tends to normalize the condition. The erucic acid per se group did not exhibit any notable alterations.

Hyperemotional behaviour. The summary of hyper-emotional behaviour test results is shown in Fig. 8. The struggle and fight response indicated the hyper emotionality behaviour increased on LPS administration, which was sequentially reversed on the administration of erucic acid treatment. The outcomes of the statistical analysis, employing one-way ANOVA followed by Tukey's post-hoc test showed that the treatment of rats with LPS raised the hyper-emotionality score, which showed remarkable improvement in struggle ($F_{4, 25}$ = 50.62; P < 0.0001) and fight response ($F_{4,25}$ = 59.68; P < 0.0001) in treatment with erucic acid. The per se group provided with erucic acid showed no changes.

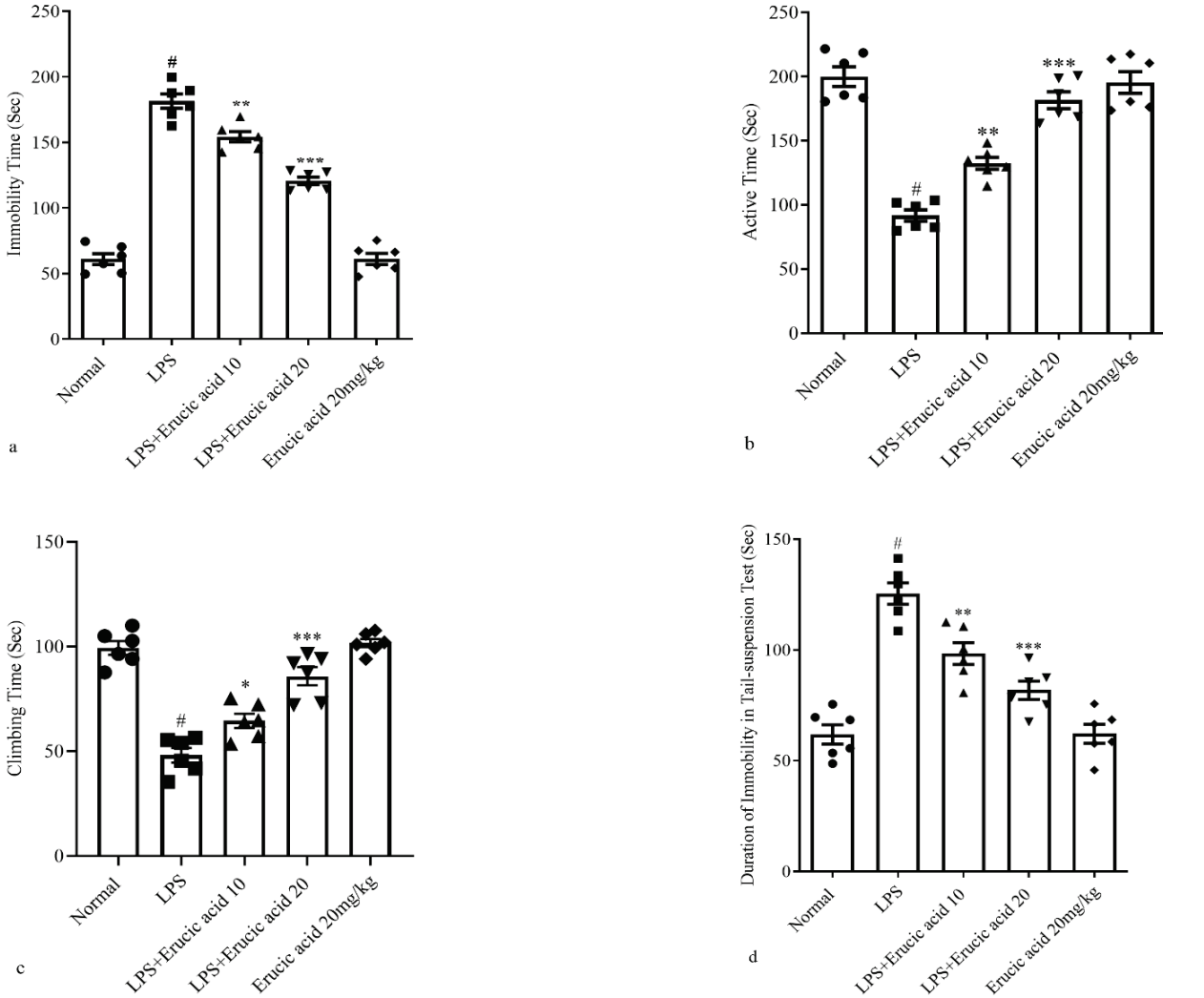

Fig. 7. Impact of erucic acid on forced swim test: *a* – immobility time, *b* – active time, *c* – climbing time, *d* – tail-suspension test; values expressed as x ± SE (n = 6); one-way ANOVA followed by Tukey's post hoc test; # P < 0.05 vs. normal group; * P < 0.05, ** P < 0.001, *** P < 0.0001 vs. the lipopolysaccharide (LPS) group

Estimation of cytokine markers. Figure 9 depicts possible functions for erucic acid in the regulation of cytokine markers i.e., IL-6 and IL-10 had substantially different measurements in the group treated with LPS when it was compared with the normal group (P < 0.05). The statistical analysis using one-way ANOVA followed by Tukey's post-hoc test reveals that both doses of erucic acid showed a substantial reduction in the pro-inflammatory biomarker levels of IL-6 ($F_{4, 25}$ = 82.49; P < 0.0001) while elevating the levels of IL-10 ($F_{4,25}$ = 58.94; P < 0.0001). The erucic acid per se group did not exhibit any remarkable alterations.

Discussion

The proposed study is the very first to depict that erucic acid, an omega-9 fatty acid, reduced the behavioural and biochemical changes that occurred on LPS induction and it is accepted as a potential animal model the evaluate depression and anxiety type of behaviour in Wistar rats (Sulakhiya et al., 2016). Acute LPS injection activates microglial cells, which in turn triggers an inflammatory cascade that results in neuroinflammation (Jangra et al., 2016). The pathophysiological mechanisms related to anxie-

ty and depression showed more complications and varied due to several detrimental molecular pathways. Numerous experimental research has indicated that inflammation and oxidative stress play a part in anxiety and depressive disorders (Jangra et al., 2016). The underlying mechanism of depression and the effectiveness of antidepressants have both been studied using the LPS-induced depression rodent model (Shao et al., 2020).

In this study we have predicted ADMET properties, using Swiss ADME AND Pkcsm, and Protox for erucic acid. The high LD_{50} value (1.382) indicates that erucic acid is relatively non-toxic in acute oral exposures in rats. As predicted by ADMET properties, erucic acid is well absorbed, but its distribution may be limited by high plasma protein binding and poor penetration across barriers like the blood-brain barrier. It is metabolized by CYP3A4 but inhibits CYP1A2, potentially affecting other drugs metabolized by these enzymes. Based on animal studies, erucic acid appears to have a low acute toxicity, but chronic exposure may have adverse effects. The BBB penetration of erucic acid was predicted to be < 1. The BOILED-egg model suggests that erucic acid may be able to cross the BBB due to the lack of efflux by the P-glycoprotein transporter. The findings are consistent with previous findings (Azzam, 2023).

The LPS administration resulted in a depressive kind of behaviour along with unsafe behaviour (Jiang et al., 2019). Rats exhibit sickening behaviour after two to six hours of receiving LPS injections, including decreased hunger, decreased hydration intake, decreased locomotion, weariness, disturbed sleep, and increased sensitivity to pain (Taniguti et al., 2018). Following repeated LPS exposure, sick behaviour, and depressive-like behaviour were linked to peripheral and CNS immunological pro-inflammatory activation (Moraes et al., 2017). Animals that ingested LPS displayed a remarkable lowering in rearing, crossings, and duration time (Jangra et al., 2016). Fatty acids, including omega-3 and omega-6 polyunsaturated fatty acids, have been studied for their potential effects on mental health, including anxiety and depression. They show the ability to inhibit the production of pro-inflammatory cytokines like IL-6 and reduce inflammation, which contributes to the improvement of anxiety and depression symptoms (Bhat & Ara, 2015). Omega-3 fatty acids have antioxidant properties that help in reducing oxidative stress that may positively impact anxiety and depression symptoms (Su et al., 2015; Sayyed et al., 2023). As a result of the administration of erucic acid at both doses, the duration of immobility was reduced in the tail suspension test and forced swim test, indicating an antidepressant effect. In addition to erucic acid's anti-anxiety effects, open arm activity improved in the elevated plus maze test, the number of stairs climbed in the staircase test was reduced, and marble-burying as well as hyper-emotional behaviour was suppressed. Also, erucic acid elevated the level of IL-10 and decreased IL-6 cytokine in LPS-induced rats.

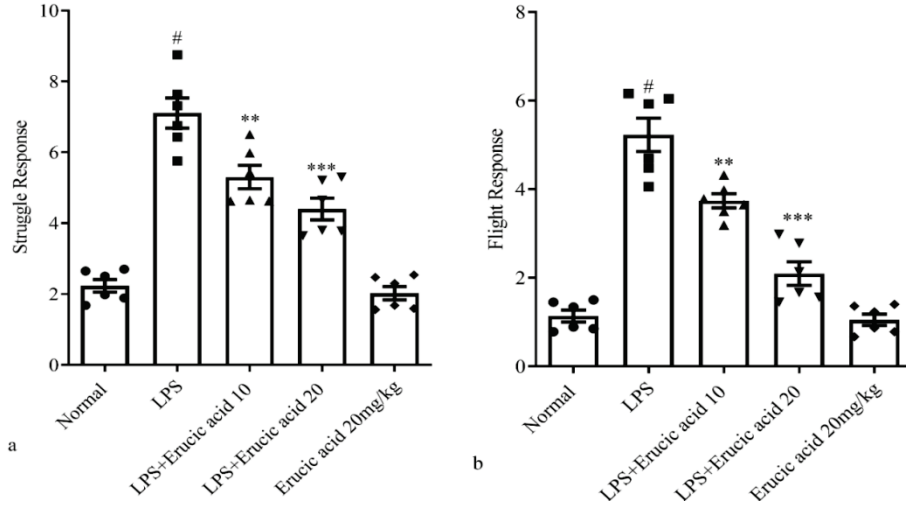

Fig. 8. Impact of erucic acid on hyper-emotionality behaviour: *a* – struggle response, *b* – fight response: one-way ANOVA followed by Tukey's post hoc test; # $P < 0.05$ vs. normal group; * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$ vs. the lipopolysaccharide (LPS) group

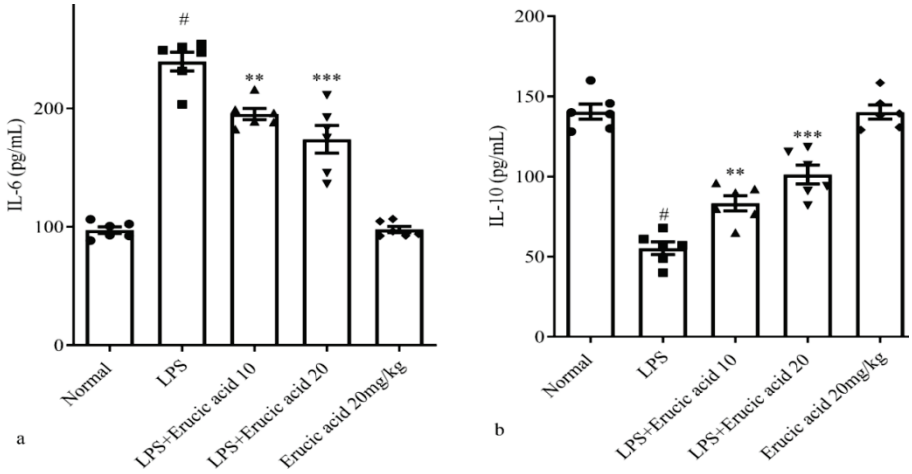

Fig. 9. Impact of erucic acid on biomarkers: *a* – interleukin-6 (IL-6), *b* – IL-10; one-way ANOVA followed by Tukey's post hoc test; # $P < 0.05$ vs. normal group; * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$ vs. the lipopolysaccharide (LPS) group

The elevated plus maze test and open field test are the two validated behavioural paradigms for evaluating anxiety-related disorders in animals (Yang et al., 2017; Bouguiyoud et al., 2022). The use of LPS in the current study, resulted in a reduction of crossing numbers, distance traveled, and the total duration of time the rats remained in the open field test. These results are in accordance with previously reported findings, that the rats injected with LPS exhibited anxiety-like behaviour (Arab et al., 2020). The frequency of the rats entering the central area at the time of testing may be considered as an indicator of reduced anxiety. Furthermore, there was a reduction in total crossing numbers, time, and distance traveled in the LPS group. Locomotor activity is determined by the crossings and traveled distance. However, a reduction in motor activity led to anxiety-

like behaviours. Post administration of erucic acid, there was an increase in crossing numbers, time, and distance covered in the rats undergoing the open field test. The open-field test indicated increased exploratory behaviour and reduced immobility, suggesting an anxiolytic-like effect.

The rats administered LPS had a minimum number of entries and time spent in the open arms of the elevated plus maze when it was compared to that of the normal group. This outcome shows the alignment with the previous studies that indicated anxiety-like behaviours in LPS administration, where there is a lowering of entries and time spent in the open arms in comparison with normal group (Yang et al., 2017; Patil et al., 2020; Shahidpour et al., 2021). These results would suggest that the anxiety and depression type of behaviours induced by LPS administration were evident in motor activity deficits. The elevated plus maze test showed increased time spent in open arms, indicating that reduced anxiety may be due to erucic acid in both doses of treatment.

Similarly, the rats administered with LPS had anxiety-like behaviour that was evident in the marble-burying test and staircase case. There was an increase in the number of marbles buried post-administration of LPS. However, an increase in total marbles buried indicated depression as per the previous study (de Brouwer et al., 2019). The rise in the number of stairs climbed by rats is a mark of depressive behaviour that was also shown upon the LPS administration (Afzal et al., 2021). The post hoc test revealed that erucic acid decreased repetitive behaviours associated with anxiety and compulsivity in the marble-burying behaviour and staircase test.

In the present study, tail-suspension test and forced swim test revealed that rats exposed to LPS were less active and more immobile. Both techniques were used to demonstrate depression-like behaviour in rats. Progressive immobility is the key result of the forced swim test and tail-suspension test representing the amount of dejection and depression. Additionally, antidepressant medications shorten the duration of immobility in the tests and that makes the animal escape drowning (Shahidpour et al., 2021). As per the previous studies, rats' immobility in the forced swim test and tail-suspension test indicates depression-like behaviour (Abareshi et al., 2019, Arab et al., 2020). In consideration of these findings, the results indicated that LPS-induced depression type of behaviour was mitigated by treatment with erucic acid. Furthermore, the results of inducing LPS in rats to analyze the hyperemotionality behaviour showed the elevation in struggle and fight response in rats. The increase in the response while handling it with gloved hands and pinching of the tail with not-so-sharp forceps represented the depression behaviour. Erucic acid reduced the hyperemotionality behaviour a comparable with the LPS control group. Erucic acid showed reduced signs of anxiety and depression-like behaviour in the hyper emotionality test, force swimming test, and tail suspension test.

Our findings are consistent with the notion that erucic acid possesses anxiolytic and antidepressant-like effects. Several other studies have reported similar behavioral improvements following erucic acid administration in various animal models of anxiety and depression (Gnanasekar et al., 2014; Sulakhiya et al., 2016; Arab et al., 2020; Bouguiyoud et al., 2022).

Additionally, several studies have shown that the brain regions linked to anxiety and depression are affected by LPS therapy, which causes a rise in the expression and release of neuroinflammatory markers i.e. IL-6, and decrease in IL-10 (Zhu et al., 2015; Shahidpour et al., 2021). Conversely, this research has focused more on the inflammatory response to persistent LPS exposure, which results in memory impairment. The treatment with LPS lowered the levels of IL-10 and increased the IL-6 levels (Zhao et al., 2019; Da Ré et al., 2020). The neurological disorder was significantly influenced by inflammation. Previous research has shown how several phytochemicals can reduce LPS-induced behavioural abnormalities by preventing neuroinflammation (Jangra et al., 2016). The results of this study revealed that neurological disorders had alterations in IL-6 and IL-10 levels, which were reversed by both doses of erucic acid (Tiftik et al., 2021). Additionally, the modulation of cytokines (IL-6 and IL-10) observed in our study aligns with the anti-inflammatory effects of erucic acid reported in previous research (Sayyed et al., 2023).

Based on the available evidence, this is the first study to show that erucic acid can protect against LPS-induced anxiety and depression in rats. In LPS-induced anxiety or depression-like behaviour an inflammatory cytokine maintained by erucic acid might have acted as an antioxidant and accumulate longer chain fatty acids in the brain. The probable mechanism by which erucic acid exerts its anti-anxiety and anti-depressant effects may involve the modulation of cytokines, which are inflammatory mediators that can affect brain function and behaviour. Erucic acid may reduce the levels of pro-inflammatory cytokines, such as IL-6, which have been associated with increased anxiety and depression in animal studies. Erucic acid may also increase the levels of anti-inflammatory cytokines, such as IL-10, which have been shown to have neuroprotective and antidepressant effects in animal models. By modulating the cytokine balance, erucic acid may restore the homeostasis of the neuroimmune system and improve behavioural outcomes via the activation of PPAR-δ/β (Altinoz & Ozpinar, 2019). This study has some limitations, such as its short duration and the small number of animals it included for the behavioural and cytokine markers test. With only n = 6 per group, the number of animals is very small. This can affect the validity of the analysis, as well as the generalizability of the results. A larger sample size may increase the chance of finding outliers, as well as reduce the error and improve the precision of the estimates. The exact molecular mechanisms underlying erucic acid's effects were not extensively explored, and further research is warranted to elucidate the intricate pathways involved in immunohistochemistry and molecular studies including western blotting, RT-PCR, tissue immunohistochemistry also with another model, gene protein expression studies, and other genetic models. Additionally, the study's duration, dosing regimen, and potential long-term effects of erucic acid were not addressed, necessitating future investigations. Lastly, other behavioral and neurochemical parameters associated with anxiety and depression should be considered in future studies to provide a more comprehensive understanding.

Conclusion

The present study demonstrates that erucic acid administration exerts anxiolytic and antidepressant-like effects in animal models, as indicated by improvements in various behavioural tests. The modulation of IL-6 and IL-10 suggests a potential role of erucic acid in regulating the inflammatory response associated with anxiety and depression. However, further studies are required to elucidate the precise mechanisms of action, evaluate the translational potential of human subjects, and address the limitations identified in this study.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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