



EFFECTS OF RUTIN SUPPLEMENTATION ON ANXIETY-LIKE BEHAVIOURS ON CHRONIC UNPREDICTABLE MILD STRESS MOUSE MODEL

YUSHA'U, Y.^{1*}, MUHAMMAD, U.A.¹, KABIR, A.M.¹, ALIYU, F.U.¹, IMAM, M.K.² AND DAYYABU, M.M.¹

¹Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria-Nigeria, ²Aliko Dangote College of Nursing Sciences, Bauchi State, Nigeria

ABSTRACT

Exposure to chronic stress might lead to neurological disorders such as depression and anxiety. Limited efficacy due to pharmacological wear-off and increased side effects due to prolonged usage are associated with conventional antidepressants. Therefore, with this background, this study aimed to investigate the attenuative effects of rutin on anxiety and depressive-like behaviours induced in mice by chronic unpredictable mild stress (CUMS) using both open-field test (OFT) and elevated plus-maze (EPM) tests. Twenty-five (25) mice were grouped into 5 (n = 5) and subjected to CUMS followed by OFT and EPM tests. Group 1 were orally served distilled water (no treatment), while groups 2,3,4 and 5 were orally served rutin 30, 60, 120 mg/kg, and fluoxetine 20 mg/kg, respectively. The study was conducted in the span of 30 days. CUMS led to a decline in locomotion and muscle coordination abilities, anxiety, and depression associated with chronic stress. Administered graded doses of rutin produced a significant reduction in immobility time at a dose of 120 mg/kg ($p < 0.05$) compared to distilled water group. Furthermore, rutin significantly ameliorated anxiety-like phenotypes in both OFT and EPM tests. In conclusion, rutin showed the potential of positively reversing behavioural despair and anxiety-like behaviours in mice.

Keywords: Anxiety, anxiety-like behaviours, behavioral despair, depression, rutin

***Corresponding author:** yyushau@abu.edu.ng, yusufshau@gmail.com, +2348100872166

INTRODUCTION

Depression is a type of psychiatric or mood disorder characterized by significant and constant depressed mood and diminished levels of interest, with a high rate of morbidity, mortality, and disability [1, 2]. Clinical presenting symptoms of depression include pessimism, world-weariness, anxiety, agitation, hallucinations, and delusions; thus, the high cost of medical care imposes a huge financial burden on families and society with a more pressing concern that nearly 80% of depressed patients may go undiagnosed [1]. According to the latest statistical report of the World Health Organization, approximately 280 million people worldwide suffer from depression, with 700,000 people dying due to depression annually [3]. Currently, the most commonly used and commercially available antidepressants are the selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors designed to increase the concentration of these monoamines to relieve depressive symptoms. However, many drawbacks of these drugs limit their use as they are highly dependent and prone to relapse after discontinuation and they exhibit a high incidence of adverse reactions, delayed onset of action, and a substantial increase in patients' suicidal ideation. Furthermore, a one-half of depressed patients are

insensitive to the therapeutic targets of these drugs, so the treatment effect is poor [4, 5, 6]. Therefore, finding simple, safe, and effective new therapeutic targets is critical and has become a direction explored by many researchers [1, 2].

Stress causes several neuropsychiatric diseases with manifested clinical pathological alterations in discrete areas of the brain, thus, chronic stress is a risk factor for the development of depression, anxiety and deterioration of cognitive performance [7, 8], however, nowadays, the increased risk for many diseases and significant public health burdens results from comorbid anxiety and depression which are the major causes of psychiatric disorders [9]. Machawal and Kumar [7] reported that anxiety has been implicated in several psychiatric disorders such as depression, panic attacks, phobias, generalized disorder, obsessive compulsive disorder and post-traumatic disorders.

Traditional herbal treatments are called green medicine due to their harmlessness and effectiveness and are effective in the treatment of depression [10, 11, 12]. Researches abound that explore the potential neuroprotective effects of dietary supplements in stress management and its related neurobehavioral malfunctions, however, the exact molecular mechanism involved in the action of these supplements is not understood [7].

Rutin also known as quercetin-3-rutinoside or sophorin is a natural flavonoid or a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose widely found in vegetables, fruits, juices, tea and is consumed widely as a dietary supplement. Rutin is a well-known antioxidant and a neuroprotective agent well demonstrated against multiple disease states, including cancer, cardiovascular disease, and neurodegenerative disorders [7, 13] as well as its anxiolytic effect [14]. These therapeutic benefits of rutin have been proposed due to its antioxidant and free radical-scavenging properties, however, its exact mechanism is still not clear [7].

The CUMS model caused depressive-like and anxiety-like behavior in the animals when they were viewed in the Forced Swimming Test (FST) [15]. The EPM is usually performed to evaluate anxiety responses in mice and it pronounces significant effects of CUMS on the percentage of time in the open arms by prominently reducing the percentage of time in the open arms [16, 17]. Immobilization stress also produced anxiety-like behaviour in the EPM and OFT [7]. Meng *et al.* and Liu *et al.* [18, 19] also pronounced that behavioral testing using EPM and OFT suggested that chronic unpredictable mild stress does induce significant anxiety-like and depression-like symptoms in mice. In OFT the mice in the control group moved more in the central area and showed a strong desire to explore more than the mice in the CUMS that moved significantly less in the central area and only walked back and forth in the peripheral area, indicating that the mice were nervous [1, 19].

According to the mechanism of action of rutin, it has been proposed that rutin produces a dose-dependent anxiolytic-like effect via GABA_A receptors, hence, rutin prevented anxiety behavior of [PTZ + rutin]-treated mice, in both OFT and EPM test [14]. An EPM study by Foudah *et al.* [12] also showed that depressed rats treated with rutin at 80 mg/kg produced anxiolytic effects by exhibiting less fear of open arms over time. In keeping with the above finding, an increase in the number of crossings and rearing in the rats treated signifying an improvement in locomotor activity of the rats was observed. More so, rutin was shown to exhibit a remarkable antidepressant activity in FST and Tail Suspension Test (TST) by reducing despair and immobility time [12, 20]. Concurrent oral treatment with rutin in CUS in adult Swiss albino mice for 21 days effectively revealed a significant CUS-rutin interaction by rescuing locomotion deficits and producing anxiolytic effect as animals showed improved locomotion and are observed to freely explore in the OFT and it further acted as a potent antidepressant in depressive-like behavior associated with chronic stress [13], hence, our research would explore the potency of rutin after CUMS exposure without treatment.

MATERIALS AND METHODS

Drugs and reagents used

Rutin hydrate (Sigma aldrich; cat no.: 10141790; LOT: #BCBL7548V, Bavaria, Germany). Fluoxetine (Flutex) (V. S. International Pvt. Ltd, Dabhel Daman-3096215, India).

Experimental animals

A total of 25 Swiss albino mice (weight: 18-24 g) were purchased from the laboratory Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, ABU, Zaria. All animals were restrained in separate plastic cages which contained saw dust as bedding and were fed with pellets made from vital feed finisher, maize offal as a binder of ratio 3:1 and water *ad libitum*. All animal experiments were conducted according to the guidelines for the management and use of experimental animals. This work was approved by the institution of Ahmadu Bello University Zaria Committee on Animal Use and Care (Approval No: ABUCAUC/2022/002).

Experimental design

Tail suspension test (labelled as TST1) was conducted which served as the basis to randomly grouped the animals into 5 groups (n=5).

Group 1: Served as control and received distilled water 10 mL/kg

Group 2: Received rutin 30 mg/kg

Group 3: Received rutin 60 mg/kg

Group 4: Received rutin 120 mg/kg [21]

Group 5: Received fluoxetine 20 mg/kg [22]

Thereafter, the animals were exposed to chronic unpredictable mild stressors for two weeks to induce depressive-like behaviours [11, 23, 24]. Next, Tail suspension test (labelled as TST2) was conducted to ascertain the success of induction of depression in the animals. Then, treatment with rutin was followed: in this the depressed mice were still exposed the chronic mild stressors while simultaneously treated with rutin daily *via* oral route for two weeks. Finally, the third Tail suspension test (labelled as TST3) was conducted to evaluate the effectiveness of the treatment.

Drug preparation

Rutin hydrate 0.12 g was dissolved in 10 mL of distilled water to obtain a stock concentration of 12 mg/mL and a dose of 120 mg/kg. Serial dilution was carried out to form doses of 60 and 30 mg/kg respectively. Fluoxetine 0.02 g was dissolved in 10 mL distilled water to form a stock concentration of 2 mg/mL and a dose of 20 mg/kg.

Neurobehavioral assays

Chronic mild stress

This animal model of stress consisted of chronic exposure to variable unpredictable stressors, none of which was sufficient alone to induce long lasting effects [25]. Chronic stress disrupts the overall homeostasis of the organism and contributes to the etiology of major depression in causing cognitive, behavioural, and physiological impairments [26, 27]. These stressors include: 45 tilted cage, overnight illumination, white noise, damp bedding, empty cage plus water, new clean cage, social stress, and meowing sound [11, 28, 29]. Two different stressors were applied daily (one in the morning and one at night) for a duration of one month. In the first two weeks of the experiment only the stressors were applied, while in the last two weeks, the stressors were applied concurrently with the treatments. Stressed animals display a worsening of the coat state compared to control, non-stressed animals. This change in coat state is reversed by chronic administration of antidepressants [30].

Tail suspension test

This procedure was conducted according to the protocol of Adem *et al.* and Can *et al.* [31, 32]. Each mouse was suspended by its tail with an adhesive tape (17 cm in length) wrapped around the animal's tail placed on a bar that is placed on a wood with dimension (55 height x60 width x12 cm depth). The mice are maintained in this position for 6 mins in a position from which they cannot escape [33]. The immobility time was recorded by an observer blind to the treatment [25]. This inactivity is interpreted as a measure of depressive-like behaviour [34, 35]. 70% ethanol was used to clean the apparatus between animals.

Open field test

Rodents tend to favor outer zones due to their aversion to open or unfamiliar environments [36]. The open field apparatus, measuring 72 x 72 cm with 36 cm walls, includes a clear Plexiglas ball for mouse visibility. It features blue lines creating sixteen (18 x 18 cm) squares with a central square (18 x 18 cm) in the middle [37]. Mice were consistently placed in the central area for 5 minutes, and parameters such as distance traveled, time in the center area, fecal pellet count, velocity, thigmotaxis, and crossings are standard indicators for assessing anxiety-like behavior [36, 38, 39]. To maintain cleanliness, 70% ethanol was used for apparatus cleaning before introducing each animal.

Elevated plus maze

The EPM theory is based on rodents' preference for enclosed dark spaces, aversion to open spaces/heights, and the innate desire to explore new environments

[33]. The maze can be constructed from various materials and typically comprises four arms, two open and two closed, with the mouse placed in the center for a 5-minute observation [24]. High open arm entries to total arm time ratio indicates low anxiety. Behavioral parameters recorded include spontaneous motor activity (total arm entries, distance traveled) and defensive behaviours (fecal boli, rears, stretching, freezing postures, and head dips) [40, 41]. Mice were removed from the maze, and it's cleaned with 70% ethyl alcohol (EtOH) to eliminate odor residue between tests. Percentages of open and closed arm entries and time spent in each are calculated. The index of open arm avoidance is calculated as $[100 - (\% \text{ time on open arms} + \% \text{ entries into open arms}) / 2]$ [42].

Statistical analysis

Statistical analysis involves mean \pm SEM presentation. One-way ANOVA with Tukey's *post hoc* test (or), and unpaired t-tests for TST comparisons were used. SPSS version 20 and GraphPad Prism version 8.0.2 were employed, with a significance threshold of $p < 0.05$.

RESULTS

Effects of rutin supplement on depression in mice subjected to chronic unpredictable mild stress model

Determination of behavioural despair in mice before exposure to chronic unpredictable mild stress using tail suspension test (TST1)

The mean immobility time in tail suspension test 1 (TST1) did not significantly differ in all the groups before subjecting them to chronic unpredictable mild stress, $p = 0.925, 0.982, 0.828$ and 0.999 for control vs rutin 30, 60, 120 mg/kg and fluoxetine 20 mg/kg respectively, demonstrating similar baseline in their behavioural despair (Table 1).

Assessment of behavioural despair in mice after a two-week exposure to chronic unpredictable mild stressors using tail suspension test (TST2)

Table 2 showed a paired t-test result which a statistically significant difference was observed between the mean immobility time of TST1 and TST2 in the control, rutin 120 mg/kg and fluoxetine 20 mg/kg groups at $p = 0.002, 0.01$ and 0.01 , respectively.

Evaluation of the effect of rutin supplement on behavioural despair in mice after a two-week exposure to chronic unpredictable mild stressors with concurrent treatment using tail suspension test (TST3)

As shown in Table 1, rutin 120 mg/kg significantly lowered immobility time when compared with the control group, $p = 0.048$. Although, no statistically significant difference was observed between the

control and rutin 30, 60 mg/kg and fluoxetine 20 mg/kg, $p = 0.972, 0.991$ and 0.265 , respectively.

Table 1: Effects of rutin supplement on depression in mice subjected to chronic unpredictable mild stress model

Groups	Immobility Time (seconds)		
	TST 1	TST 2	TST 3
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
Control (DW 10 ml/kg)	174.8 \pm 11.9	240.6 \pm 7.6	246.0 \pm 6.6
Rutin 30 mg/kg	194.0 \pm 4.8	220.4 \pm 18.8	225.0 \pm 15.9
Rutin 60 mg/kg	162.0 \pm 22.9	228.8 \pm 29.5	261.2 \pm 22.6
Rutin 120 mg/kg	149.0 \pm 19.5	206.4 \pm 27.9	142.4 \pm 37.9*
Fluo 20 mg/kg	180.6 \pm 18.4	210.2 \pm 19.2	174.4 \pm 26.6

*Statistically significant compared to control group, $p < 0.05$, ($n=5$), using one-way analysis of variance (ANOVA), Fluo: Fluoxetine, TST1: Tail Suspension Test before exposure to chronic unpredictable mild stressors, TST2: Tail Suspension Test after a 2-week exposure to chronic unpredictable mild stressors, TST3: Tail Suspension Test after a 2-week exposure to chronic unpredictable mild stressors and treatment with rutin and fluoxetine

Table 2: Comparison between the immobility time of TST1 and TST2 using paired t-test (two-tailed)

Groups	TST 1	TST 2	P-value	t-value
	Mean \pm SEM	Mean \pm SEM		
Control	174.8 \pm 11.9	240.6 \pm 7.6*	0.003	6.556
Rutin	194.0 \pm 4.8	220.4 \pm 18.8	0.255	1.327
30 mg/kg				
Rutin	162.0 \pm 22.9	228.8 \pm 29.5	0.088	2.247
60 mg/kg				
Rutin 120mg/kg	149.0 \pm 19.5	206.4 \pm 27.9*	0.017	3.964
Fluo 20 mg/kg	180.6 \pm 18.4	210.2 \pm 19.2*	0.017	3.971

*Statistically significant compared to TST1, $p < 0.05$, ($n=5$), using paired t-test (two-tailed), Fluo: Fluoxetine, TST1: Tail Suspension Test before exposure to chronic unpredictable mild stressors, TST2: Tail Suspension Test after a 2-week exposure to chronic unpredictable mild stressors.

Effects of rutin supplement on anxiety-like behaviours in mice subjected to chronic unpredictable mild stress model using open field test

As shown in Table 3, a significantly ($p = 0.002$) increased frequency in line crossing between the control and rutin 120 mg/kg was revealed. However, no statistically significant difference was observed between the control and rutin 30, 60 mg/kg & fluoxetine 20 mg/kg, $p = 0.788, 0.662$ and 0.335 , respectively. In frequency of centre crossing, results showed no statistically significant difference between the control and groups treated with rutin 30, 60, 120 mg/kg and fluoxetine 20 mg/kg, $p = .999, 0.932, 0.689$ and 0.831 , respectively (Table 3). Although a

statistically significant increase in rearing was seen between the control and rutin 30, 60, 120 mg/kg and fluoxetine 20 mg/kg, $p = 0.001, 0.005, 0.000$ and 0.000 , respectively. Similarly, no statistically significant difference was observed in the frequency of defaecation between the control and groups administered with rutin 30, 60, 120 mg/kg and Fluoxetine 20 mg/kg, $p = 0.983, 1.000, 0.684$ and 0.999 respectively (Table 3). In the frequency of urination, *Tukey's post hoc* test revealed no significant difference between the control and rutin 30, 60, 120 mg/kg & fluoxetine 20 mg/kg administered groups, $p = 0.666, 1.000, 1.000$ and 1.000 , respectively (Table 3).

Table 3: Effects of rutin supplement on anxiety-like behaviours in mice subjected to chronic unpredictable mild stress model using open field test

Groups	Frequency of Line Crossing	Frequency of Centre Crossing	Frequency of Rearing	Frequency of Defaecation	Frequency of Urination
Control	55.4 \pm 9.2	1.4 \pm 0.9	1.6 \pm 0.4	1.6 \pm 0.4	0.2 \pm 0.2
Rutin 30 mg/kg	69.6 \pm 8.8	1.2 \pm 0.5	23.4 \pm 2.0*	2.0 \pm 0.6	0.6 \pm 0.2
Rutin 60 mg/kg	72.4 \pm 7.6	0.8 \pm 0.4	19.8 \pm 4.2*	1.6 \pm 0.8	0.2 \pm 0.2
Rutin 120mg/kg	111.8 \pm 7.3*	2.4 \pm 0.4	28.0 \pm 2.9*	0.6 \pm 0.2	0.2 \pm 0.2
Fluo 20 mg/kg	79.6 \pm 11.0	0.6 \pm 0.4	27.6 \pm 4.6*	1.4 \pm 0.4	0.2 \pm 0.2

*Statistically significant compared to the control (distilled water 10 ml/kg), $p < 0.05$, (n=5), using one-way ANOVA, *Tukey's post hoc* test, Fluo: Fluoxetine.

Assessment of the effect of rutin supplement on anxiety-like behaviours in mice subjected to chronic unpredictable mild stress model using elevated plus maze test

As shown in Table 4, *Tukey's post hoc* test revealed no statistically significant difference in % entry into opened arm between the control and groups administered with rutin 30, 60, 120 mg/kg & Fluoxetine 20 mg/kg at p values = 0.993, 0.929, 0.907 and 0.962. However, a statistically significant decrease was observed in % entry into enclosed arm between the control and rutin 120 mg/kg, $p=0.008$. Although, no significant difference was observed between the control and groups administered with rutin 30, 60 mg/kg and fluoxetine 20 mg/kg at p = 0.347, 0.276 and 0.560, respectively. Also, EPM results showed a significant difference between fluoxetine 20 mg/kg and rutin 30 mg/kg, $p = 0.008$, in

percentage time spent in opened arm. However, no statistically significant difference was seen when the control group was compared with rutin 30, 60 and 120 mg/kg and fluoxetine 20 mg/kg, $p = 0.096$, 0.649, 0.972 and 0.768, respectively (Table 4). The % time spent in enclosed arm was significantly lower in rutin 60 mg/kg when compared with the control, $p = 0.044$. However, no significant difference was observed when the control was compared with rutin 30, 120 mg/kg and fluoxetine 20 mg/kg, $p = 0.078$, 0.158 and 0.453, respectively (Table 4).

In an index of opened arm avoidance, *Tukey's post hoc* test showed a significant decrease in rutin 60 and 120 mg/kg groups in comparison with the control, $p = 0.016$ and 0.012 (Figure 1). However, no significant difference was observed between the control group and rutin 30 mg/kg and Fluoxetine 20 mg/kg, $p = 0.077$ and 0.751, respectively (Figure 1).

Table 4: Effect of rutin supplement on anxiety-like behaviours in mice subjected to chronic unpredictable mild stress model using elevated plus maze test

Groups	% Entry into Opened Arm	% Entry into Enclosed Arm	% Time Spent in Opened Arm	% Time Spent in Enclosed Arm
Control	42.4 ± 3.8	77.6 ± 9.4	16.2 ± 2.6	80.8 ± 5.4
Rutin 30 mg/kg	37.4 ± 1.4	62.6 ± 1.4	28.3 ± 4.2 [#]	58.5 ± 7.1
Rutin 60 mg/kg	52.0 ± 12.3	61.4 ± 3.0	22.5 ± 4.2	56.2 ± 3.3 [*]
Rutin 120mg/kg	52.8 ± 2.4	47.2 ± 2.4	19.0 ± 0.8	61.6 ± 5.7
Fluo 20 mg/kg	50.4 ± 13.8	65.6 ± 7.0	19.4 ± 1.8	67.1 ± 6.2

*Statistically significant compared to the control (distilled water 10 mL/kg), #Statistically significant compared to Fluo20 mg/kg $p < 0.05$, (n=5), using one-way ANOVA, *Tukey's post hoc* test SPSS Version 20, Fluo: Fluoxetine.

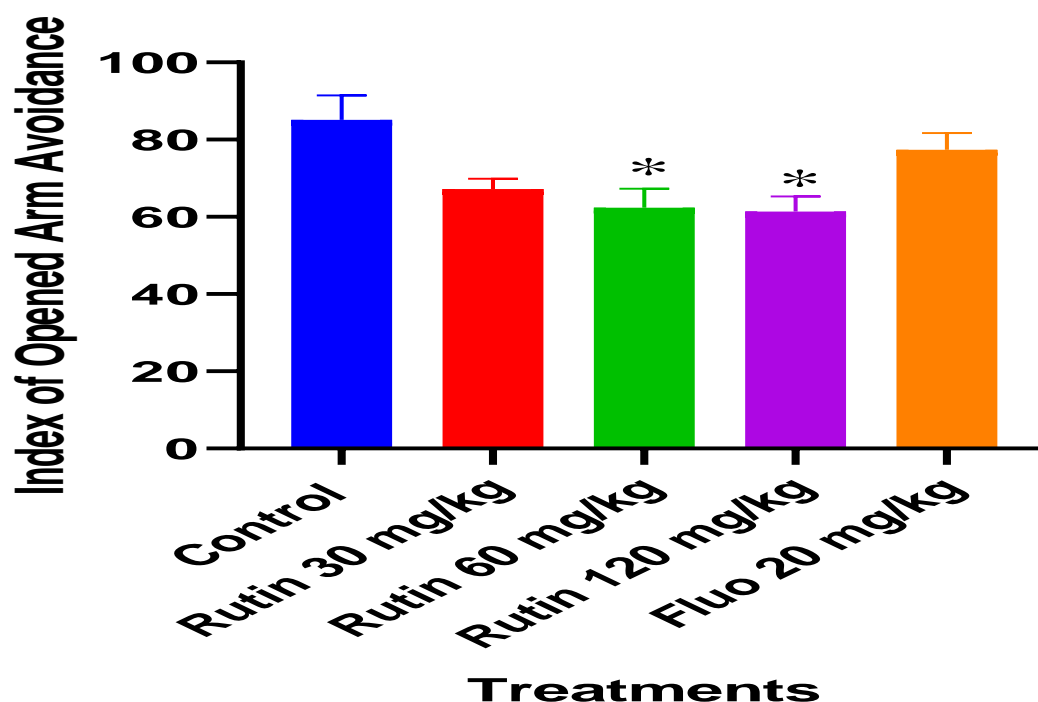


Figure 1: Effect of rutin supplement on index of opened arm avoidance in mice subjected to chronic unpredictable mild stress model using elevated plus maze test

*Statistically significant compared to the control (distilled water 10 ml/kg), $p < 0.05$, ($n=5$), using one-way ANOVA, Tukey's post hoc test, SPSS Version 20, drawn with Graph Pad Prism 8.0.2. Fluo: Fluoxetine.

DISCUSSIONS

The present study evaluated the effect of rutin on anxiety-like behaviours in mice exposed to CUMS model of depression. Determination of baseline behavioural despair in the experimental animals revealed no significant difference between the groups as demonstrated by the result of immobility time in TST1. Previous studies by Mustapha *et al.*, Yusha'u *et al.* and Yusha'u *et al.* [43, 23, 29] reported similar observations. Similarly, the behavioural despair of all the groups did not differ after a two-week exposure to chronic mild stressors as depicted in TST2. This is expected as all the groups including the control were exposed to the same stressors without any treatment. Studies by Mustapha *et al.* [43] and Yusha'u *et al.* [29] reported similar findings. However, comparing TST2 with TST1, a significant behavioural despair (increase was found, implying that the CUMS induced depressive-like behaviours in the animals. After treatment with rutin for a period of two-weeks, a reversal effect was observed in the group treated with 120 mg/kg of the rutin. This is an indication that rutin supplementation might have an antidepressant-like effect in the animals via decreasing immobility time (an index of behavioural despair). Yusha'u *et al.* [21] and Kabir *et al.* [11] reported similar findings in open-space forced swim test and chronic mild stress models of depression respectively. Furthermore, Ebokaiwe *et al.* [44] found that co-treatment with rutin at dose of

50 mg/kg for 35 days prevented depressive-like behaviours by reducing immobility time of TST in ethanol-induced depressive-like behaviours in rats. Similarly, administration of rutin at a dose of 80 mg/kg for 14 days showed antidepressant-like activity in forced swim test (FST) and TST in reserpine-induced depressed rats [12]. In addition, rutin reversed depressive-like symptoms in lipopolysaccharide-induced mouse model [43].

This investigation demonstrates that rutin supplementation potentially exhibits anxiolytic effects, indicated by enhanced line crossing and rearing (markers of anxiolytic behaviours) in the open field test (OFT). Notably, similar outcomes were observed in the study by Foudah *et al.* [12], where rutin at 80 mg/kg considerably increases crossings and rearing in the OFT using a reserpine-induced rat model of depression. Additionally, our research revealed that doses of rutin at 60 and 120 mg/kg effectively mitigated anxiety-like behaviours in depressed animals within the Elevated Plus Maze (EPM), as evidenced by decreased time spent in closed arms and reduced indices of avoidance of open arms, aligning with the characteristic behavior of anxious rodents seeking enclosed spaces and displaying natural fear of heights, which are evident in the EPM paradigm. Foudah *et al.* [12] findings further supported this, illustrating that rutin doses of 40 and 80 mg/kg decreased anxiety-like behaviours by

augmenting both entry frequency and time spent in the open arms of the EPM.

CONCLUSION

The study underscores rutin's promise as both an antidepressant and anxiolytic, as evidenced by its reduction of depressive and anxiety-like behaviours across TST, OFT and EPM models.

REFERENCES

1. CHEN, Z., GU, J., LIN, S., XU, Z., XU, H., ZHAO, J., FENG, P., TAO, Y., CHEN, S. & WANG, P. (2023). Saffron essential oil ameliorates CUMS-induced depression-like behavior in mice via the MAPK-CREB1-BDNF signaling pathway. *Journal of Ethnopharmacology*, **300**: 1-16. doi:https://doi.org/10.1016/j.jep.2022.115719
2. ZHOU, Z., WANG, Y., SUN, S., ZHANG, K., WANG, L., ZHAO, H. & ZHANG, Y. (2023). Paeonia lactiflora Pall. Polysaccharide alleviates depression in CUMS mice by inhibiting the NLRP3/ASC/Caspase-1 signaling pathway and affecting the composition of their intestinal flora. *Journal of Ethnopharmacology*, **316**: 1-14. doi:https://doi.org/10.1016/j.jep.2023.116716
3. WHO (2023). *Depression*. Retrieved 05 17, 2023., from World Health Organization: <https://www.who.int/news-room/fact-sheets/detail/depression>
4. RHEKER, J., WINKLER, A., DOERING, B.K. & RIEF, W. (2017). Learning to experience side effects after antidepressant intake - results from a randomized, controlled, double-blind study. *Psychopharmacology*, **234**(3): 329-338. doi:https://doi.org/10.1007/s00213-016-4
5. MONCRIEFF, J. (2020). Persistent adverse effects of antidepressants. *Epidemiology and Psychiatric Sciences*, **29**: e56. doi:https://doi.org/10.1017/s2045796019000520
6. QUILICHINI, J.B., REVET, A., GARCIA, P., BOUQUI'E, R., HAMARD, J., YRONDI, A. & MONTASTRUC, F. (2022). Comparative effects of 15 antidepressants on the risk of withdrawal syndrome: a real-world study using the WHO pharmacovigilance database. *Journal of Affective Disorders*, **297**: 189-193. doi:https://doi.org/10.1016/j.jad.2021.10.041
7. MACHAWAL, L. & KUMAR, A. (2014). Possible involvement of nitric oxide mechanism in the neuroprotective effect of rutin against immobilization stress induced anxiety like behaviour, oxidative damage in mice. *Pharmacological Reports*, **66**: 15-21. doi:https://doi.org/10.1016/j.pharep.2013.08.001
8. GAWALI, N.B., BULANI, V.D., GURSAHANI, M. S., DESHPANDE, P.S., KOTHAVADE, P.S. & JUVEKAR, A.R. (2017). Agmatine attenuates chronic unpredictable mild stress-induced anxiety, depression-like behaviours and cognitive impairment by modulating nitrergic signalling pathway. *Brain Research*, **1663**: 66-77. doi:https://doi.org/10.1016/j.brainres.2017.03.004
9. XIONG, T-W., LIU, B., WU, Q., XU, Y-Y., LIU, J. & SHI, J-S. (2023). The protective effects of *Dendrobium nobile* Lindl. alkaloids (DNLA) against CUMS-induced anxiety/depression and hippocampal transcriptome changes in rats. *Pharmacological Research - Modern Chinese Medicine*, **7**: 1-10. doi:https://doi.org/10.1016/j.prmcm.2023.100250
10. GANESHPURKAR, A. & SALUJA, A.K. (2017). The Pharmacological Potential of Rutin. *Saudi Pharmaceutical Journal*, **25**: 149-164. doi:10.1016/j.jsps.2016.04.025
11. KABIR, A.M., YUSHA'U, Y., ADAM, U.M., IBRAHIM, S. & MUHAMMAD, M.D. (2022). Ameliorative effect of rutin supplement on chronic unpredictable mild stress-induced depressive phenotypes in mice. *Nigerian Journal of Neuroscience*, **13**(4): 139-146. doi:https://doi.org/10.47081/njn2022.13.4/004
12. FOUDAH, A.I., ALQARNI, M.H., ALAM, A., DEVI, S., SALKINI, M.A. & ALAM, P. (2022). Rutin Improves Anxiety and Reserpine-Induced Depression in Rats. *Molecules*, **27**: 1-12. doi:https://doi.org/10.3390/molecules27217313
13. PARASHAR, A., MEHTA, V. & UDAYABANU, M. (2017). Rutin alleviates chronic unpredictable stress-induced behavioral alterations and hippocampal damage in mice. *Neuroscience Letters*, **1**-14. doi:10.1016/j.neulet.2017.04.058
14. ANESTI, M., STAVROPOULOU, N., ATSOPARDI, K., LAMARI, F. N., PANAGOPOULOS, N.T. & MARGARITY, M. (2020). Effect of rutin on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice. *Epilepsy & Behavior*, **102**: 1-7.

- doi:<https://doi.org/10.1016/j.yebeh.2019.106632>
15. GABRIELE DA SILVA, J.M., ANTUNES, F.T.T., FILLOT DA SILVA, L.B., PEDRON, C., BERBIGIER DE OLIVEIRA, I., FERRAZ, A.F., MARTINS, M.I.M., DALLEGRAVE, E. & HUBNER DE SOUZA, A. (2021). Adaptogenic effects of curcumin on depression induced by moderate and unpredictable chronic stress in mice. *Behavioural Brain Research*, **399**: 113002. doi:[10.1016/j.bbr.2020.113002](https://doi.org/10.1016/j.bbr.2020.113002)
16. WANG, D., WU, J., ZHU, P., XIE, H., LU, L., BAI, W., PAN, W., SHI, R., YE, J., XIA, B., ZHAO, Z., WANG, Y., LIU, X. & ZHAO, B. (2022). Tryptophan-rich diet ameliorates chronic unpredictable mild stress induced depression- and anxiety-like behavior in mice: The potential involvement of gut-brain axis. *Food Research International*, **157**: 1-16. doi:<https://doi.org/10.1016/j.foodres.2022.111289>
17. ZHANG, W., YU, M., ZHANG, Q., YANG, Z. & ZHANG, T. (2022). DFO treatment protects against depression-like Behaviours and cognitive impairment in CUMS mice. *Brain Research Bulletin*, **187**: 75-84. doi:<https://doi.org/10.1016/j.brainresbull.2022.06.016>
18. MENG, C., FENG, S., HAO, Z., DONG, C. & LIU, H. (2022). Antibiotics exposure attenuates chronic unpredictable mild stress-induced anxiety-like and depression-like behavior. *Psychoneuroendocrinology*, **136**: 1-13. doi:<https://doi.org/10.1016/j.psyneuen.2021.105620>
19. LIU, S-Z., YANG, J., CHEN, L-L., WANG, P., & LIN, L. (2023). Tanshinone IIA ameliorates chronic unpredictable mild stress-induced depression-like behavior and cognitive impairment in rats through the BDNF/TrkB/GAT1 signaling pathway. *European Journal of Pharmacology*, **938**: 1-10. doi:<https://doi.org/10.1016/j.ejphar.2022.175385>
20. ANJOMSHOA, M., BOROUJENI, S. N., GHASEMI, S., LORIGOOINI, Z., AMIRI, A., BALALI-DEHKORDI, S. & AMINI-KHOEI, H. (2020). Rutin via Increase in the CA3 Diameter of the Hippocampus Exerted Antidepressant-Like Effect in Mouse Model of Maternal Separation Stress: Possible Involvement of NMDA Receptors. *Behavioural Neurology*, **2020**: 1-9. doi:<https://doi.org/10.1155/2020/4813616>
21. YUSHA'U, Y., MUHAMMAD, U.A., NZE, M., EGWUMA, J.M., IGOMU, O.J. & ABDULKADIR, M. (2017). Modulatory Role of Rutin Supplement on Open Space Forced Swim Test Murine Model of Depression. *Nigerian Journal of Physiological Sciences*, **32**: 201-205.
22. KULKARNI, S.K. & DHIR, A. (2007). Effect of various classes of antidepressants in behavioral paradigms of despair. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, **31**(6): 1248-54. doi:[10.1016/j.pnpbp.2007.05.002](https://doi.org/10.1016/j.pnpbp.2007.05.002)
23. YUSHA'U, Y., ADAM, U.M., ANAVE, A.A., ZAYYAD, U., WAHAB, A.A., SALEH, M. I.A. & YA'U, J. (2019). Chronic Administration of Alpha Lipoic Acid Shows Antidepressant-Like Effect in Mice Subjected to Chronic Mild Stress. *Nigerian Journal of Neuroscience*, **10**(2): 41-46.
24. ALQURASHI, E., ALDOBYANY, A., TOUMAN, A., ALQAHTANI, A., ALSAGGAF, R., ALNASHIWAATY, O., GHALEB, N., MABAR, H. & ALBANNA, A. S. (2022). Prevalence of depression and its associated factors among patients with confirmed COVID-19 in Makkah, Saudi Arabia. *Frontiers in Psychiatry*, **13**: 863215. doi:[10.3389/fpsy.2022.863215](https://doi.org/10.3389/fpsy.2022.863215)
25. CHEN, C.Y.O., MILBURY, P.E. & BLUMBERG, J.B. (2019). Polyphenols in almond skins after blanching modulate plasma biomarkers of oxidative stress in healthy humans. *Antioxidants*, **8**(4): 95-103.
26. SURGET, A. & BELZUNG, C. (2009). Unpredictable chronic mild stress in mice. In A. V. LaPorte (Ed.), *Experimental animal models in neurobehavioral research* (pp. 79-112). Nova Science Publishers.
27. WILLNER, P. (2017). Reliability of the chronic mild stress model of depression: A user survey. *Neurobiology of Stress*, **6**: 68-77. doi:[10.1016/j.ynstr.2016.08.001](https://doi.org/10.1016/j.ynstr.2016.08.001)
28. ZHANG, J.C., YAO, W., DONG, C., YANG, C., REN, Q., MA, M., HAN, M. & HASHIMOTO, K. (2015). Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. *Psychopharmacology*, **232**: 4325-4335. doi:<https://doi.org/10.1007/s00213-015-4062-3>
29. YUSHA'U, Y. MUHAMMAD, U.A., MUSTAPHA, S., UMAR, A. H., IMAM, M. I., UMAR, B., ALHASSAN, A.W., SALEH, M.I.A. & YA'U, J. (2021). Alpha-lipoic acid attenuates depressive symptoms in mice exposed to chronic unpredictable mild stress. *Journal of African Association of Physiological Sciences*, **9**(1): 58-68.
30. NOLLET, M. (2021). Models of depression: Unpredictable chronic mild stress in mice.

- Current Protocols*, **1**: e208. doi:10.1002/cpz1.208
31. ADEM, C., DAVID, T.D., CHANTELLE, E.T., SEAN, C.P., SHAMBHU, B. & TODD, D.G. (2012). The tail suspension test. *Journal of Visualized Experiments*, **59**: 3769.
32. CAN, A., DAO, D.T., TERRILLION, C.E., PIANTADOSI, S.C., BHAT, S. & GOULD, T.D. (2012). The Tail Suspension Test. *Journal of Visualized Experiments* (59), e3769-75. doi:10.3791/3769
33. CHEN, F., ZHENG, D., LIU, J., GONG, Y., GUAN, Z. & LOU, D. (2020). Depression and anxiety /among adolescents during COVID-19: A cross-sectional study. *Brain, behavior, and immunity*, **88**: 36–38. doi:https://doi.org/10.1016/j.bbi.2020.05.061
34. YAN, H-C., CAO, X., DAS, M., ZHU, X-H. & GAO, T-M. (2010). Behavioral animal models of depression. *Neuroscience Bulletin*, **26**(4): 327-337. doi:10.1007/s12264-010-0323-7
35. ABELAIRA, H.M., RÉUS, G.Z. & QUEVEDO, J. (2013). Animal models as tools to study the pathophysiology of depression. *Brazilian Journal of Psychiatry*, **35**(2): S112-20. doi:10.1590/1516-4446-2013-1098
36. SEIBENHENER, M.L. & WOOTEN, M.C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visual Experiment*, **96**: e52434. doi:10.3791/52434
37. BROWN, J., COHEN, P., JOHNSON, J.G. & SMAILES, E.M. (1999). Childhood abuse and neglect: specificity of effects on adolescent and young adult depression and suicidality. *Journal of the American Academy of Child & Adolescent Psychiatry*, **38**(12): 1490-149.
38. SCHUCH, F.B., VANCAMPFORT, D., RICHARDS, J., ROSENBAUM, S., WARD, P.B. & STUBBS, B. (2016). Exercise as a treatment for depression: A meta-analysis adjusting for publication bias. *Journal of Psychiatric Research*, **77**: 42-51. doi:10.1016/j.jpsychires.2016.02.023.
39. KRAEUTER, A.K., GUEST, P.C., & SARNYAI, Z. (2019). The Elevated Plus Maze Test for Measuring Anxiety-Like Behavior in Rodents. *Methods in Molecular Biology*, **1916**: 69-74. doi:10.1007/978-1-4939-8994-2_4
40. SORREGOTTI, T., CIPRIANO, A.C., CRUZ, F.C., MASCARENHAS, D.C., RODGERS, R.J. & NUNES-DE-SOUZA, R.L. (2018). Amygdaloid involvement in the defensive behavior of mice exposed to the open elevated plus-maze. *Behavioural Brain Research*, **338**: 159-165. doi:https://doi.org/10.1016/j.bbr.2017.10.022.
41. WALF, A.A. & FRYE, C.A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *National Protocol*, **2**(2): 322-8. doi:10.1038/nprot.2007.44.
42. TRULLAS, R. & SKOLNICK, P. (1993). Differences in fear motivated Behaviours among inbred mouse strains. *Psychopharmacology*, **111**(3): 323-31. doi:10.1007/BF02244948.
43. MUSTAPHA, S., MAGAJI, R.A., MAGAJI, M., GAYA, I.B., YUSHA'U, Y., & CHIROMA, S. M. (2022). Rutin ameliorates lipopolysaccharide-induced depressive-like behaviours in mice model via inhibition of neuroinflammation. *Journal of African Association of Physiological Sciences*, **10**(1): 67-77.
44. EBOKAIWE, A.P., OBASI, D.O., OBETEN, U. & UNYEMUCHE, T. (2023). Rutin co-treatment prevented cognitive impairment/depression-like behavior and decreased IDO activation following 35 days of ethanol administration in male Wistar rats. *Alcohol*, **106**: 22-29. doi:https://doi.org/10.1016/j.alcohol.2022.10.002