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Modulatory Role of Rutin Supplement on Open Space Forced Swim Test Murine Model of Depression

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Summary: Flavonoids have been demonstrated to possess an anti-depressant effect and less adverse effects than tricyclic anti-depressants. For this reason, flavonoids in natural products have attracted growing attention. Rutin is a glycoside flavonoid which belongs to an important class of flavonoids, abundantly found in plants, such as buckwheat seeds, asparagus, red pepper, apples, citrus fruits and leaves of many herbs such as rosemary, dandelion or sage, and black and green tea. It is a vital nutritional component of food stuff. This study aimed at investigating the antidepressant potential of the rutin supplement on Swiss albino mice. For assessment of antidepressant activity, Open Space Forced Swim Test (OSFST), Tail Suspension Test (TST), Open-Field Test (OFT) and Novel Object Recognition Test (NORT) were used. Twenty-five Swiss albino mice were used for the study and divided into five groups. Group I received 10 mg/kg distilled water, group II received fluoxetine 20 mg/kg while group III, IV and V received rutin (30 mg/kg, 60 mg/kg and 120 mg/kg respectively) for sixteen days. The administration of the rutin supplement for sixteen days produced a reduction of immobility time in the TST (at 30 mg/kg, 60 mg/kg and 120 mg/kg), $p<0.05$. Likewise, a statistically significant difference was observed in line crossing in OFT, $p<0.05$. However, no significant effect was observed in percentage novel object preference in NORT. This study revealed that oral administration of rutin has an antidepressant potential in a dose dependent manner in OSFST mouse model of depression.

Keywords*:* Rutin, Flavonoids, Antidepressants, Immobility, Fluoxetine

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INTRODUCTION

Depression is a common mental disorder, characterized by persistent sadness and a loss of interest in activities that an individual normally enjoy, accompanied by an inability to carry out daily activities, for at least two weeks (WHO, 2017). Depression involves mood disturbances affecting brain regions such as hippocampus, temporal lobe, amygdala, caudate, anterior cingulate cortex, and frontal cortex that are involved in mood-regulating circuit (Jiao–Jie *et al.,* 2016). Depression may result in premature death, major social and economic consequences. Depressed mood, diminished interest/pleasure and fatigue are among the core symptoms of Major Depressive Disorder (MDD) (Marcia *et al.,* 2016). In adult subjects with MDD, long-term therapy with certain antidepressants may also be linked to cognitive side effects. For instance, treatment with antidepressants has been associated with increased cognitive deficits, such as apathy, inattentiveness, forgetfulness, word-finding difficulty, and mental slowing, in depressed individuals reaching partial or full remission (Beatrice *et al.,* 2016).

Interpersonal stress was demonstrated as an important factor in the emergence and exacerbation of depression and anxiety in clinical theories, while neuroendocrine research confirms the association of these syndromes with dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis (a major stress response system) (Sally *et al.,* 2016).

Rutin is a glycoside flavonoid which belongs to an important class of flavonoids abundantly found in plants, such as passion flower, buckwheat, tea, and apple. It is a vital nutritional component of food stuff. This flavonoid has been shown to exert several biological activities, such as: antimicrobial, anti-inflammatory, antioxidant, neuroprotective, antiviral, antiulcerogenic (Machado *et al.,* 2008). Rutin was suggested as the possible active component of *Hypericum perforatum* extract, a plant used in many countries for the treatment of mild to moderate forms of depression (Linde and Knuppel, 2005). In an acute study, reported by Machado *et al.* (2008) revealed that rutin isolated from ethanolic extract of *Schinus molle*, reduced immobility time in tail suspension test, but not in forced swimming test, with no effect in locomotor

activity in mice. In addition, Dimpfel (2009) in trying to predict efficacy and possible mechanisms of action of rutin and quercetin using rat electropharmacogram, suggested that both the flavonoids rutin and quercetin inhibit monoamine oxidase.

Therefore, the present study aimed to evaluate antidepressant potential of rutin supplement in open space forced swim test (OSFST) mouse model of depression. In addition, this study sought to examine effects of rutin supplement on working memory of mice subjected to OSFST using NORT.

MATERIALS AND METHODS

Experimental Design and Animal Treatment

Twenty-five apparently healthy mice weighing between 20–28 g were obtained from Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria and allowed access to feed and water *ad libitum*. The mice were grouped into five with five mice per group. Daily administration was carried out, based on daily body weight per mouse for sixteen days as described by Eric and Yan (2011). Group I: Received distilled water 10 ml/kg. Group II: Received fluoxetine 20 mg/kg orally. Group III, IV and V: Received rutin supplement 30 mg/kg, 60 mg/kg and 120 mg/kg orally respectively. All experiments on animals were in accordance with regulation on the case and use of laboratory animals (NIH, 1996) and Ahmadu Bello University Research Policy.

Drugs and Reagents

Rutin supplement was purchased from Sigma–Aldrich, Germany with a P Code: 101417910, LOT # BCBL 7548V. Other drugs and reagents used in the experiments were of analytical grades.

Open Space Forced Swim Test (OSFST)

This protocol describes a simplified method for inducing a chronic depression-like state in mice that is based on the repeated open-space forced swim method for rats (Eric and Yan, 2011). Tubs sized (24 × 43 × 23 cm) were filled with tap water at 32-34° C to a depth of 13 cm. mouse cages were transported to the lab and each mouse was placed gently in the water to swim for 15 min. After the swim, each animal was approached slowly, captured with the index and third fingers and placed in the home cage. The temperature of the water was maintained at 32-34° C in the tubs by replacing 2 litres with 2 litres of hot water (45-52°C) at 30 min intervals. Water in the tubs was changed completely after 8 mice have swum. Swim procedure was repeated for two additional days which need not to be consecutive. Treatment was instituted at 24 h after the fourth swim. The time of onset of drug action was determined by swimming the mice at days 2, 4, 7, 10, and 14 post pump implantation or at 1, 4, 7, 10 and 14 days after initiation of oral administration. Immobility and distance swum was recorded using videotape camera [JVC micro HDD by Victor Company of Japan Limited (35 x optical zoom, hybrid: LY36228–001A)].

Tail Suspension Test (TST)

The tail suspension test was performed as described by Gor *et al.* (2010). Mice were suspended from a metal rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. The duration of the test was 6 minutes and immobility was measured during the last 4 minutes to facilitate comparison with the forced swim test. Immobility was defined as the absence of any limb or body movements, except those caused by respiration.

Open Field Test (OFT)

The apparatus consists of floor space with dimension of 40 cm x 40 cm and 30 cm in height. The floor space was divided into 16 squares equally. Prior to the testing, the mouse was placed at the center of the floor space and allowed to acclimatize with the surrounding area for 2 minutes. Thereafter, each mouse was given 5 minutes to explore the open field arena (test session). Line crossing (an index of locomotor activity) as indicated by the total number of squares crossed was measured during the test session (Harish *et al.*, 2015).

Novel Object Recognition Test (NORT)

Novel object recognition apparatus is a rectangular arena that was made of opaque plastic and measured 42 cm × 52 cm. The walls are 40 cm high. A videotape camera [JVC micro HDD by Victor Company of Japan Limited (35 x optical zoom, hybrid: LY36228–001A)] was used to record the animals' behavior for subsequent analysis (Thur *et al.*, 2014). Mice were placed in the arena for 5-min where they encountered two identical sample objects (Sample Phase). At the end of the Sample Phase, mice were placed back in their home cages for a 5-min delay (±15 s). For the Testing Phase, animals were returned to the arena for 3-min where one of the familiar objects was replaced with a novel object. In addition to the arena, all objects were cleaned with 70% ethanol between each session. Successful novel object recognition was indexed by greater exploration of the novel compared to the familiar object. The discrimination ratio was calculated as the total time spent exploring the least recently seen object divided by the time exploring both objects sampled at test.

Statistical Analysis

Results were expressed as Mean ± SEM. Data for Open Field, Tail Suspension and Novel Object Recognition Tests were analysed using one way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparison. Data for Open Space Forced Swim Test were analysed using mixed (5 x 5 two–way) analysis of variance (ANOVA); with the between–subject factor as the treatment groups and within–subject factor as day. Where significant, the main effect of treatment groups was further analysed

using Tukey's post hoc, while the within–subject factor and the interactions (treatment with days) was analysed using Bonferroni post hoc test for repeated measurement, using SPSS version 22. Values with p< 0.05 were considered statistically significant.

RESULTS

Effect of Days of Swimming and Rutin Supplementation on Immobility in Open Space Forced Swim Test in Mice

A mixed 5 x 5 two-way repeated measure ANOVA was performed to determine the effects of days of swimming and treatment group on immobility time in open space forced swim test (OSFST). The mean and standard error of mean are presented in Table 1 and Figure 1. The interaction effect between the days of swimming and treatment groups was not statistically significant [Wilks lambda = 0.298, F (4, 20) = 1.60, p= 0.103]. There was statistically significant difference in main effect for days of swimming on immobility time [Wilks lambda=0.213, F (4, 20) =15.73, p=0.00, multivariate partial eta squared=0.75]. Similarly, the main effect of treatment groups on immobility time was statistically significant [F (4, 20) = 4.37, p=0.011].

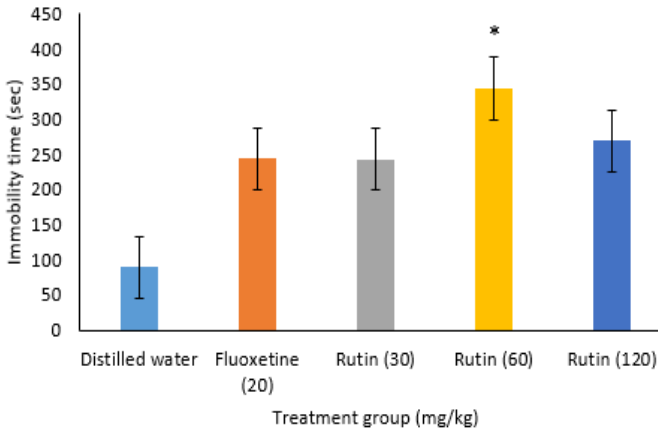

Figure 1: Effect of Rutin Supplement on Immobility Time in Open Space Forced Swim test in Mice. *The mean difference is significant when compared with distilled water group, p<0.05

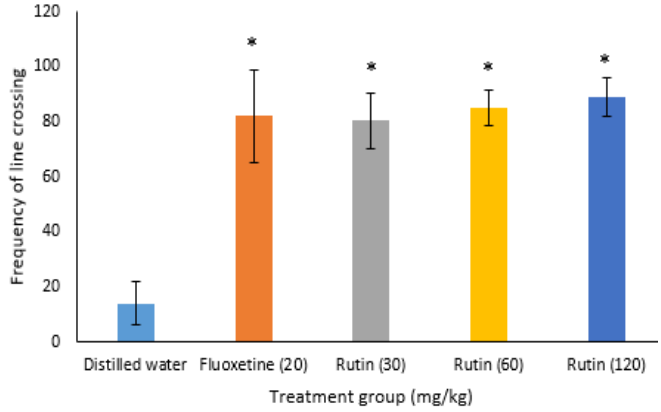

Figure 2: Effect of Rutin Supplement on Locomotion in Open Field Test. *The mean difference is statistically significant when compared with distilled water group, p<0.05, n=5.

Table 1: Effect of Days of Swimming on Immobility time in Open Space Forced Swim Test in Mice

Day	Immobility time
One	450.6 ± 33.6
Four	224.7 ± 23.4[a]
Seven	259.2 ± 27.5[a]
Ten	130.8 ± 30.1[a,c]
Fourteen	122.2 ± 28.9[a,b,c]

[a]The Mean difference is significantly differently, when compared with day one, p<0.05.
[b]The Mean difference is significantly differently, when compared with day Four, p<0.05.
[c]The Mean difference is significantly differently, when compared with day Seven, p<0.05.

Table 2: Effect of Rutin Supplement on Behavioural Despair in Tail Suspension Test

Treatment Groups (mg/kg)	Immobility time
Distilled water	221.2 ± 23.2
Fluoxetine (20)	66.2 ± 17.4*
Rutin (30)	115.6 ± 27.7*
Rutin (60)	71.8 ± 9.4*
Rutin (120)	49.6 ± 9.5*

*The mean difference is statistically significant when compared with distilled water group, p<0.05, n=5.

Effect of Rutin Supplementation on Immobility Time in Tail Suspension Test in Mice

There was a statistically significant effect of the treatment on the groups [F (4, 20) = 13.49, p= 0.000]. Post hoc comparisons using Tukey's HSD indicate that the means difference of immobility time for distilled water (221.2 ± 23.2) is significantly different from group two (66.2 ± 17.4), three (115.5 ± 27.7), four (71.8 ± 9.4) and five (49.6 ± 9.5). Group two, three, four and five did not differ significantly from either of the other groups (Table 2).

Effect of Rutin Supplementation on Locomotor Activity in Open Field Test in Mice

One-way ANOVA demonstrated that there was statistically significant difference in the locomotor activity between the treatment groups [F (4, 20) = 9.16, p= 0.00]. Pairwise comparisons showed that the mean locomotor activity for distilled water (14.0 ± 7.8) was significantly different from group two (82.0 ± 17.0), three (80.4 ± 10.2) four (85.0 ± 6.3) and five (89.0 ± 7.1). The mean difference did not differ significantly among the other groups (Figure 2).

Effect of Rutin Supplementation on Working Memory in Novel Object Recognition Test in Mice

There was no statistically significant difference in percentage novel object preference between the treatment groups [F (4, 20) = 1.54, p= 0.23], (Figure 3).

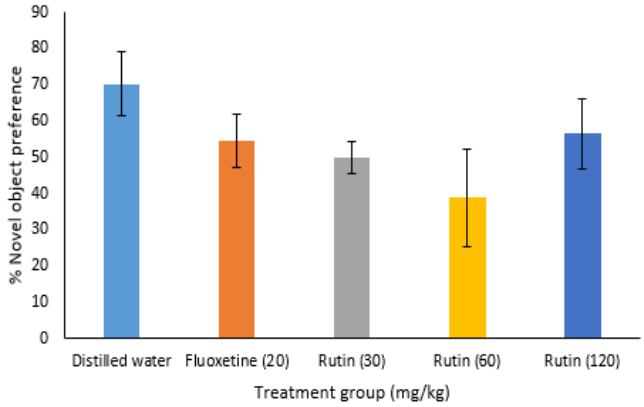

Figure 3: Effect of Rutin Supplement on Cognition in Novel Object Recognition Test. The mean difference is not statistically significant when compared with distilled water group, $p>0.05$, $n=5$.

DISCUSSION

In this study, rutin was found to cause a decrease in the immobility time in the open-spaced forced swim test at doses 30 mg/kg, 60 mg/kg and 120 mg/kg and this depicts decrease in depressive–like symptoms in a dose dependent manner. This is in agreement with the findings of Su *et al*., (2014) who reported that mice treated with rutin extracted from the ethyl acetate extract of *Saussurea involucrata* at a dose of 60 mg/kg, had longer swimming time than the control mice in force swimming test. Similarly, there was a significant decrease in the immobility time in TST in mice treated with 30 mg/kg, 60 mg/kg and 120 mg/kg of rutin supplement compared to control. This decrease in immobility time is an indication of antidepressant–like effect exerted by rutin. This is in concordance with the findings of Machado *et al.* (2008) that acute treatment with rutin isolated from ethanolic extract of *Schinus molle* exerts an antidepressant-like effect in TST. The decrease in immobility time observed in this study might be due to the ability of rutin to inhibit monoamino oxidase activity, as studies by Dimpfel. (2009) using electropharmacogram reported that rutin exerts its effects by inhibition of monoamine oxidase activity. This might lead to increase in brain monoamines (serotonin, adrenaline, noradrenaline) thereby alleviating depressive symptoms.

This study revealed that chronic administration of rutin (at doses of 30 mg/kg, 60 mg/kg and 120 mg/kg) significantly increased locomotor activity in the open field test. However, studies by Machado *et al*. (2008) reported that acute treatment with rutin isolated from ethanolic extract of *Schinus molle* has no effect on locomotor activity in mice. Probably, the increase in locomotor activity might be due to increase in brain monoamines (serotonin, noradrenaline) levels, as reported by Prinssen *et al*. (2006) that selective serotonin reuptake inhibitors increased locomotor activity in gerbils.

In the novel object recognition test, this study revealed that rutin did not significantly alter percentage novel object preference depicting no effect on short term memory. However, Grandhi *et al*. (2016) found that rutin reversed scopolamine–induced short term episodic memory, but in combination with another flavonoid naringin.

The present study provides evidence indicating that chronic administration of rutin supplement produced an antidepressant–like effect in tail suspension test, increased locomotor activity in open-field test, but has no effect in working memory in novel object recognition test on open space forced swim test mouse model of depression.

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