GAVIN PUBLISHERS

Journal of Psychiatry and Cognitive Behavior

Sakthivel G, et al. J Psychiatry Cogn Behav: JPCB-122.

DOI: 10.29011/2574-7762. 000022

Research Article

Protective Effect of Myricetin Microemulsion against Psychological Stress in Rat Model

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Citation: Sakthivel G, Prajisha P, Karunya MD, Ravindran R (2017) Protective Effect of Myricetin Microemulsion against Psychological Stress in Rat Model. J Psychiatry Cogn Behav: JPCB-122. DOI: 10.29011/2574-7762. 000022

Received Date: 03 July, 2017; **Accepted Date:** 09 August, 2017; **Published Date:** 19 August, 2017

Abstract

Undoubtedly stress is an integral part of human life. Stressful experiences disturb the normal biological homeostasis, which has a deleterious effect on normal physiological and psychological function. In the past two decades, there was not much progression in the development of drugs, effective drug delivery or therapy for Central Nervous System (CNS) related problems. Myricetin is a flavonoid, which is present in many dietary products and it has biologically diverse actions such as anticancer, antihypertensive, antioxidant etc., nevertheless, it is unable to deliver to the brain due to its polar soluble nature. Hence, we developed microemulsion formulation of myricetin (MYR-ME) to enhance its bioavailability into the brain. An animal model of psychological stress was developed by using restrainer (6h/day for 21 days of restraint stress) and treatment with MYR-ME (10mg/kg BW) was administrated for 21 days orally. The experimental animals were randomly divided into four groups, Group I - Control, Group II - Vehicle, Group III - Restraint stress exposed animals and Group IV - Stress with MYR-ME treated animals. At the end stress procedure and stress with treatment, the next day animals were tested cognitive function and anxiety behaviors. The animals were exposed to chronic stress showed deleterious effects on cognitive function, learning and memory, as well as anxiety-related behavior. However, treatment with MYR-ME improved performance in the cognitive function and anxiety-related behavior, indicating that myricetin in the form of the microemulsion can enhance bioavailability of myricetin in the brain which could be demised stress-induced alterations and ultimately acts as a good nootropic and anxiolytic compound.

Keywords: Behavior; Chronic stress; Cognition; Learning and Memory; MYR-ME

Introduction

When homeostasis is threatened by stressful experience, the body undergoes myriad changes. The brain is the central target to stress, where hippocampus, amygdala and prefrontal cortex are the primary areas that undergo stress-induced structural remodeling which can ultimately alter the physiological and behavioral response. The stress can acts on the brain via multiple interacting pathways mediatedby glucocorticoids, excitatory amino acids, and ROS (reactive oxygen species) production. The result of this stressorcan lead to structural remodeling of neuronal architecture and thus, affectcognitive dysfunction, behavioralalteration, learning and memory impairment [1] and other neurological problems.

The stressful responseshas been regulated by two major hormonal system Hypothalamic-Pituitary-Adrenal (HPA) axis and Sympathetic Adrenal Medullary (SAM) axis. Further,elevated circulating glucocorticoidsactivates the release of excitatory amino acid glutamate, resulting in increased cellular Ca^{2+} concentration, the excess level of Ca^{2+} is toxic to the cell and causes structural and functional changes and also shrinkage of the hippocampal neuron [2] and leads to many pathological conditions such as cognitive decline, neurodegenerative diseases and neuronal death [3].

The earlier studies reported stress can induce behavioral alteration, depression, cognitive decline and may risk for many other neuropsychiatric and neurodegenerative diseases. Hence, it is important to develop ananimal model of human diseases for understanding pathophysiology and developing new therapeutic strategy against stress induced cognitive dysfunction and anxiety behavior. In the present study, spatial learning and memory was tested by eight arm radial maze [4], novel object recognition test [5].

Elevated plus maze activity considered as a valid animal behavioral study to detect anxiety by using natural stimuli like fear

of a novel open space and fear of balancing on a relatively narrow, raised platform [6]. Open field behavior is another method to test the exploratory, locomotor and anxiety or increased fear condition in rodents [7]. The light/dark test is based on the innate aversion of rodents to brightly illuminate areas and on the spontaneous exploratory behavior of rodents in responses to mild stressors that is novel environment and light [8].

There is a lack of effective drug delivery or therapeutic treatment for Central Nervous System (CNS) related diseases and disorders. There are many potentially active polar soluble drugs are unable to access to the brain. The biggest obstacle for achieving effective therapeutic treatment for the CNS related problem is the presence of Blood-Brain Barrier (BBB). There are many studies on neuroprotective and radical scavenging activity of polyphenols due to the antioxidant capacities. Accumulating studies shows that polyphenols markedly reduce the risk of dementia [9] and improves the cognitive performances [10].

Myricetin is the drug in the present study,among many other flavonoids it has more a number of replaceable numbers of a hydrogen atom in its chemical structure [11]. The hydrogen atom is responsible for the free radical scavenging activity of the compound and other health promising activity of myricetin. Myricetin is present in many plant-derived foodstuffs, notably in grapes, berries, onions, walnuts, herbs, wines, vegetables, fruits and medicinal plants and is classified as a flavonoid with strong antioxidant effects [12]. In recent years, it has been reported that myricetin exhibits a variety of beneficial effects on hypoglycemic [13], antioxidant [14], anticancer effect, neuroprotective activity, anti-inflammatory, and antiviral actions in humans [15], it received great attention because of its varied biological activities. Myricetin is a partial water-soluble compound, which limits its curative effect of the drug due to the presence of BBB [16]. Myricetin has the biologically diverse actions though, it is inaccessible to the braindue to polar soluble nature [17]. Hence, we need an alternative effective method for drug delivery techniques or drug formulation methods to reach polar soluble compounds into the brain. Hence, we have developed MYR-ME delivery system to enhance bioavailability polar soluble drugs into the brain, this was achieved by technique described in this article.

The microemulsion can be prepared by oil-in-water (o/w) or of water-in-oil (w/o), microemulsion producing a transparent product that has a particle size of 10 to 100 nm and does not have the tendency to coalesce. The particle size determines the rate of drug release as well absorption. It is composed of the oil phase, surfactant, co-surfactant and an aqueous phase at appropriate ratios [18]. The earlier studies have reported that myricetin in the oil are phase protected from the microemulsion and thus avoiding the gastrointestinal and liver metabolism, thereby, it could increase the drug bioavailability by the microemulsion delivery system, microemulsion increase the bioavailability of drug (16.05 fold) when compared to a myricetin suspension by the oral administration [16].

Based on the earlier literatures we developed a hypothesis that, myricetin in the form of microemulsion may enhance the drug delivery to the brain and thereby it can protect or prevent psychological stress induced cognitive dysfunction and anxiety behavior the Wistar albino rats.

Materials and Methods

Chemicals and Agents

Myricetin was purchased from Sigma-Aldrich and all other chemicals used are of an analytical grade, purchased from Sisco Research Laboratories (SRL), India.

Animals and Maintenance

Adult male Wistar Albino rats weighing 200-220g were randomly divided into four groups used for the study. All animal procedures were approved by the institutional animal ethical committee and CPCSEA (IAEC No: 01/26/2014). Animals were housed in a group of three rats per cage and animals were maintained at controlled room temperature 23 ± 2ºC with 12:12 hour light: dark cycle and allowed to free access to food and water. The animals were fed ad libitum with a standard rat pellet diet and drinking water.

Experimental Design

Animals were divided into four groups, each group consists of six animals: Group I- Non-stress control animals, Group II- Non stress control animal with vehicle administrated, Group III- Restraint stress exposed and Group IV- restraint stress with MYR-ME treated group.

Psychological Stress Model

According to the previously described method, psychological stress was developed by restraint the animals for 6 hours daily for twenty-one days [19]. The restrainers were designed by wire mesh, contains the steel base, and steel mesh, a padlock and latch will help to secure the rat in the restrainer. Wire mesh is designed tightly to fit and restrict the movement of the animal, without any discomfort, not restricting breathing, interfering with thermoregulation, or causing pain. Animals were subjected to chronic restraint stress 6h/day for 21days; the time period was randomly changed in ordered to avoid the habituation. Each group contains six animals.

Preparation and treatment of myricetin microemulsion (MYR-ME)

The microemulsion was introduced by Hoar and Schulman, MYR-ME(O/W) was prepared by the previously described method by Wang, et al. [16]. The microemulsion was prepared by dis-

solving Tween 80 and Tween 20 in 1:2 ratio (surfactant) in ethanol (co-surfactant), then mixed well with oleic acid (oil phase), sub sequently the appropriate amount of myricetin was added into the mixture. The formulations were formed spontaneously at room temperature when the appropriate amount of phosphate buffer (pH p6.5) was added by gently vibrating. MYR-ME (10mg/kg BW) dosage as described in the previous study [17] was administrated orally for 21 days, before an hour of stress exposure.

Behavior Analysis

Open Field Test

Open Field Test (OFT), is used to measure the exploratory and anxiety-related behavior in the novel place. The test was performed accordingly by apreviously described method with slight modification [20], the apparatus made up of (40 cm × 40 cm × 49 cm) black arena with wooden floor consists of equally divided (5 × 5) square. The apparatus is illuminated with white light in the center. Rats were placed in the center or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5-minute test, rats were returned to their home cages. The test provides a unique opportunity to assess three independent behavioral dimensions relating to the locomotor activity, exploration, and emotional activity, by placing the animal in the corner of brightly lighted a large rectangular box. The tested area was cleaned after every entry of an animal with 70% alcohol. Emotional status of the animal and locomotor activity of the rat was assessed by the parameters like peripheral ambulation, central ambulation, rearing, grooming, immobilization, and defecation.

Elevated Plus Maze (EPM)

The Elevated Plus Maze (EPM) test is used to assess anxiety-related behavior in a rodent model. The elevated plus maze was made of wooden Perspex, with two opposite open arms and two opposite closed arms of the same size, the entire apparatus was elevated 50 cm above the floor. The apparatus was situated in a darkened room, illuminated by a single 60 W white light bulb located approximately 100cm above from the center of the maze. Rats were placed in the central square of the maze, facing one of the open arms. Each animal tested to elevated plus maze test only once, rats were randomly removed from their home cages and tested for 5 min in an elevated plus maze to ensure anxiety levels. The number of entries into open, closed arms and time spent in each arm were scored for the first 5 min. The tested area was cleaned with 70% alcohol prior to the introduction of each animal. Animals falling off the maze were eliminated from the analysis. The parameters include the number of open, closed arm entries and the number of head dips (dipping the head below the open arm of the EPM, with all four paws on an open arm) (Walf & Cheryl, 2007).

Light and Dark box test (Place preference task)

The light/dark box also was used to assess the anxiety-like behavior of rodents. The box was divided into two compartments, 18 × 15 × 15 inches (long, wide and high) light compartment with open at the top and 12 × 15 × 15 inches (long, wide and high) the dark compartment that was fully enclosed. The divider between the two compartments and contained a 3 × 4 inch (wide, high) opening at floor level. This allows the animal entries between compartments. At the beginning of testing, each animal was placed in the center of the light compartment. Behavior subsequently was videotaped for 5 min. Behaviors were scored by an observer who was blind to the treatment conditions. The measures scored were (1) initial latency to enter the dark compartment, (2) time spent in the bright area and (3) time spent in a dark compartment [22].

Radial arm maze (RAM)

Spatial learning and memory were tested by using an eight-arm radial maze apparatus as described before with slight modification in the experimental procedure [23].The next day after the end of 21 days of restraint stress animals and restraint stress treated with MYR-ME animals were tested in the eight-arm radial maze for testing memory after stress and treatment. The eight-arm radial maze made of steel material, had an octagonal central platform, 33.5 cm wide, around which were arranged 60 cm long by 12 cm wide arms. The whole apparatus was elevated 40 cm from the floor in a sound proof chamber. Before the training session, the animals were 80% food deprived, during behavioral training the sucrose pellet is kept as its reward so that they could become habituated to the apparatus. Initially, animals were allowed to freely explore the maze for 2 days with all arms baited with sucrose pellets for 10 min. By the third day of training on the spatial task, only four arms (fixed for that animal) were always baited and food rewards placed at the end of the arms. Two training session were performed for eight days between 09:30-11:00am and 4:30-6:00 pm every day. Each individual rat had its own set of four rewarded arms. The room contained several visual reference cues on the wall. Each trial began with the placement of the animal on central platform facing arm number one and ended when the rat had visited the four baited. The following parameters were measured on ninth day based on the Olton's definition, i) Number of reference memory errors(i.e. each entry into a non-baited arms), ii) Number of working memory errors(i.e. re-entries into already visited baited arms were noted) and iii) Time is taken to visit all the baited arms.

Spontaneous alteration T-maze

Spontaneous alteration T-maze wasused to assess cognitive ability and memory retention ability of rodents. The test is based on the left and right arm discrimination, in this test, the rat has to discriminate either left or right arm of the T-maze to get the food reward. The T-maze consisted of a start box (12x12 cm), stem (35x12 cm), two arms (35x12 cm) and each arm had a goal area of (15x12 cm) and the side walls were off 40 cm height. The first day animals were habituated to apparatus and allow to explore

in theT-maze for 10 minutes in both the arms were baited with sucrose pellets. Followed by ten trials with inter-trial intervals of 2 minutes, rats were trained to reach either right or left arm and when they reached to the goal area pellets were provided. The rats were trained till reach 80% corrected response and noted a number of days taken for reaching 80%. Followed the 80% corrected response, the rats were tested for memory retention after two days as described before [24].

Novel object recognition test

The novel object recognition test is used to analyze recognition memory. Behavioral activity and neuronal activity were recorded first in the open field (40 cm × 40 cm × 49 cm), during the initial exposure and subsequent familiarization to two identical objects with same size and different shapes were used for the experiment. During habituation, animals were placed in the open field apparatus for 10 mins and the next day the identical object was placed and the rats were allowed to explore on objects for 5 mins after two hours of interval one old object is replaced with novel object and animals were allowed to explore for 5 mins. The objects and box were wiped by 70% ethanol after each trial to remove the odor of the previous rat. The positions of the objects were similar during training and test. Percentage of time spent was calculated as TNovel/(TNovel × TFamiliar) ×100 where TNovel is time spent with anovel object and TFamiliar is time spent with a familiar object.The experiment involved a three session, one habituation to open field apparatus and session two is for familiarization to identical objects. During the third session, the first test of novelty was performed as described before [25].

Statistical analysis

Data were analyzed by using (SPSS 20) version software and presented in the form of the bar diagram and expressed as mean ± SEM. One-way ANOVA, followed by the multiple comparisons by Tukey post hoc multiple comparisons was performed eight-arm radial maze, T-maze,novel object recognition, open fieldbehavior,elevated plus maze, and light and dark test. $p<0.05$ was considered as significant. The "a" represent control group, "b" represents a vehicle group,"c" represent the stress group and "d" represent stress with MYR-ME treated group.

Results

Open field behavior

Number of central square entries

The stress group animals demonstrated anxiety related behavior as tested by number of central squire entries which were significantly reduced (3.67 ± 0.42, 0.003), whereas those animals were treated with MYR-ME the entries were recorded at the same frequency as the non-stressed group (7.83 ± 0.60, p = 0.004). The parameters studied in the open field behavior are summarized inTable 1.

Parameters	Control	Vehicle	Stress	Stress + MYR-ME
Central squares entries (Count)	8.33 ± 0.61	8.3 ± 0.42	3.67 ± 0.42a,b,d	7.83 ± 0.60c
Peripheral squares entries (Count)	33.5 ± 3.27	33.83 ± 4.11	53.16 ± 4.35a,b,d	29.33 ± 3.14c
Number of grooming (Count)	9.33 ± 0.42	9.00 ± 0.73	14.66 ± 1.20a,b,d	7.83 ± 0.60c
Number of rearing (Count)	8.00 ± 0.73	7.83 ± 1.01	16.16 ± 1.57a,b,d	8.66 ± 0.96c
Number of faecal pellets (Count)	1.83 ± 0.30	2.16 ± 0.47	4.33 ± 0.42a,b,d	2.00 ± 0.36c

Table 1: Open field behavior-Describes parameters studied in the open field behavior. The data were expressed as mean ± SEM. *a compared to control, *b compared to vehicle, *c compared to stress group and *d has compared to stress with myricetin microemulsion treated and the value $p<0.05$ are considered as significant.

Number of peripheral square entries

The number of peripheral square entries were significantly-increased (53.16 ± 4.35, p = 0.004) in the restraint stress exposed group. Whereas, the number of peripheral square entries were significantly decreased (29.33 ± 3.14, p = 0.001) in stress with MYR-ME treated group, as similar to that of control group.

Number of grooming

There straint stress exposed group showed increased number of grooming (14.66 ± 1.20, p = 0.005), this was significantly reduced (7.83 ± 0.60, p = 0.001) in restraint stress with MYR-ME treated, almost similar pattern of grooming was observed as non-stressed group.

Number of rearing

There was markedly increased rearing (16.16 ± 1.57, p = 0.001) was observed in restraint stress exposed group, while this was reduced in MYR-ME treated group (8.66 ± 0.96, p = 0.003), this was restored almost as like that of control group.

Number of fecal pellets

The stress exposed group showed increased number of fecal pellets (4.33 ± 0.42, p = 0.001) whereas, MYR-ME treated group showed reduced number of fecal pellets at similar frequency as that of non-stressed group (2 ± 0.36, p = 0,003).

Elevated plus maze (EPM)

Number of entries in open arm

The one-way ANOVA followed by the Tukey posthoc test revealed stress exposed group animals were showed high anxiety levels as assessed by of open arms entries, which were decreased

in stress group (1.67 ± 0.33, P = 0.001), where as MYR-ME treated group showed significantly increased as similar that of non-stressed animals (5.33 ± 0.67, p = 0.006).The results of parameters studied were shown in Table 2.

Parameters	Control	Vehicle	Stress	Stress + MYR-ME
Open arm entries (Count)	7.00 ± 0.51	6.67 ± 0.49	1.67 ± 0.33^{a,b,d}	5.33 ± 0.67^{c}
Closed arm entries (Count)	4.33 ± 1.21	4.16 ± 0.75	8.33 ± 1.75^{a,b,d}	5.00 ± 0.89^{c}
Time spent in open arm (Count)	128.33 ± 4.64	119.0 ± 4.87	29.67 ± 2.18^{a,b,d}	95.83 ± 1.92^{c}
Time spent in closed arm (Count)	171.5 ± 4.50	177.16 ± 3.63	269.0 ± 3.74^{a,b,d}	197.83 ± 1.79^{c}
Head dipping (Count)	24.5 ± 1.78	24.5 ± 1.87	10.33 ± 0.67^{a,b,d}	24.5 ± 1.17^{c}

Table 2: Elevated plus maze-Describes parameters studied in the elevated plus maze. The data were expressed as mean ± SEM. *a compared to control, *b compared to vehicle, *c compared to stress group and *d has compared to stress with myricetin micro emulsion treated and the value p<0.05 are considered as significant.

Number of entries in closed arm

The increased number of closed arm entries (8.33 ± 1.75, p = 0.001) were observed in restraint stress exposed group, whereas it was significantly reduced in MYR-ME treated group, as close to that of non-stressed group (5 ± 0.89, p = 0.001).

Time spent in open arm

Stress group animals showed less time spent in the open arm (29.67 ± 2.18, p = 0.005), this was significantly increased in MYR-ME administrated group, as similar to that of non-stressed animals (95.8 ± 1.92, p 0. 006).

Time spends in closed arm

The time spent in the closed arm was higher in stress exposed group (269 ± 3.74, p = 0.005), whereas it was significantly decreased (197.83 ± 1.79, p = 0.004) in restraint stress with MYR-ME treated group.

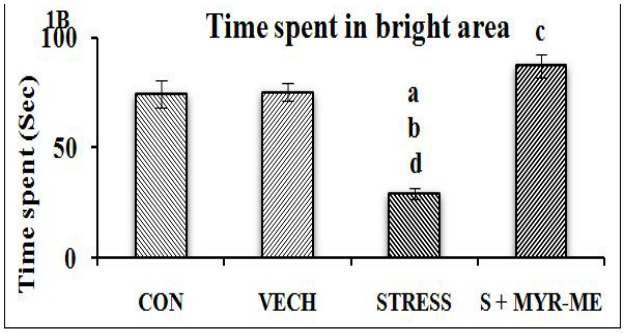
Number of head dips

The restraint stress exposed group showed significantly less (10.33 ± 0.67, p = 0.005) head dips whereas, it was almost restored in MYR-ME treated group, as like that of control animals(24.5 ± 1.17, p = 0.001).

Light and dark test (Place-preference task)

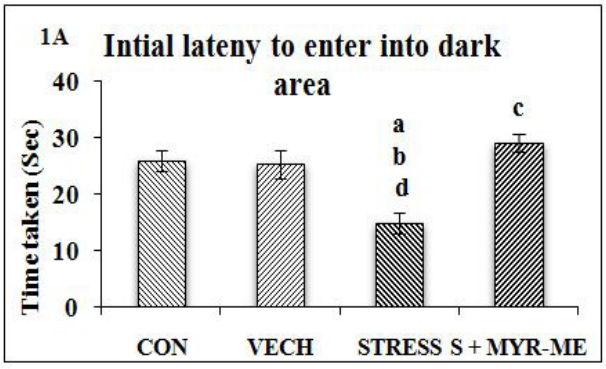
Initial latency to enter into dark area

The anxiety related behavior also tested by evaluating initial latency to enter into dark area, restraint stress exposed group has taken less time (15 ± 1.80, p = 0.003) to enter into dark area, whereas those who treated with MYR-ME animals showed significantly increased time was taken (29.16 ± 1.57, p = 0.001) to enter the dark area, as that of non-stressed animals, this is shown in Figure 1A.



Time spent in bright area

The decreased time spent in bright area was observed in restraint stress exposed group (29.16 ± 2.49, p= 0.001), while this was significantly increased in MYR-ME treated group, the time spent in bright area was as similar to that of non-stressed animals (87.16 ± 5.21, p = 0.02), the results are described in Figure 1B.



Time spent in dark area

The stress exposed group animals showed increased time spent (233.5 ± 2.83 sec, p = 0.01) in dark area,however this was

significantly decreased when treated with MYR-ME, as same that of non-stressed animals (176 ± 5.4sec, p = 0.001). Time spent in the dark area of all groups is shown in Figure 1C.

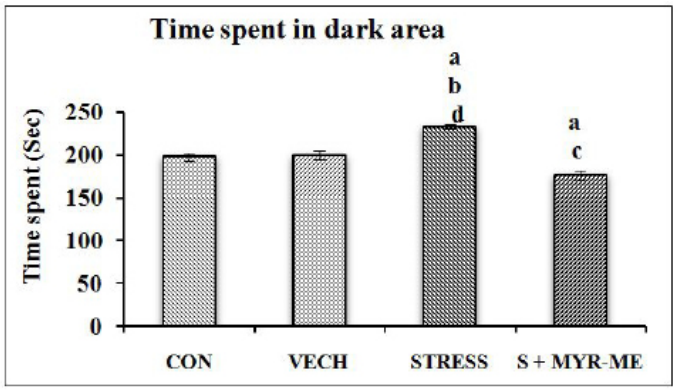
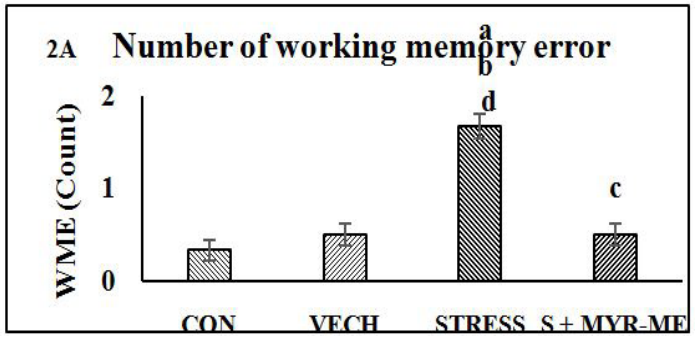


Figure 1: Light and dark box test, initial latency to enter into a dark area (1A), time spent in bright areas (1B) and time spent in a dark area (1C). The data were expressed as mean ± SEM. "a" represents compared to control, "b" represents compared to Vehicle, "c" compared to stress group and "d" represents compared to stress with myricetin microemulsion treated and the value p<0.05 are considered as significant.

Spatial memory assessment in eight-arm radial maze

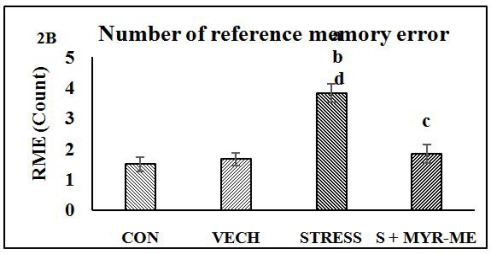
Working memory error

The stress exposed animals showed deficits in the spatial memory, this was tested by assessing working memory error,the resulats are summarized in Figure 2A. Tukey posthoc test revealed that the working memory error was significantly increased (1.67 ± 0.12, p = 0.02) in restraint stress induced group, whereas MYR-ME treated group showed significantly decreased (0.5 ± 0.12, p = 0.006), the similar trend was recorded as like non-stressed animals.



Reference memory error

The number of reference memory error was more in restraint stress exposed (3.83 ± .30, p = .003) group, whereas MYR-ME treated group showed a significantly less number of reference memory error (1.83 ± .30, p = .004), as same kind that of non-stressed animals, the results summarized in Figure 2B.



Time is taken to visit the entire baited arm

The restraint stress exposed animals were showed significantly increased (153. 16 ± 2.77, p = .003) time taken to visit all baited arms. However, MYR-ME treated group showed significantly less time taken (47.17 ± 3.26, p = .009) to visit all baited arms, alike non-stressed animals, the results are described in Fig-

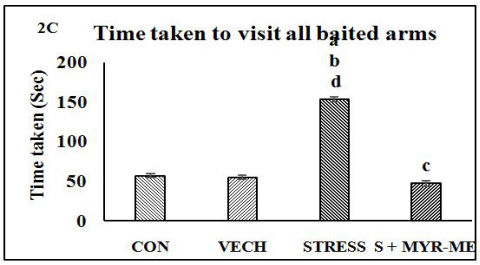
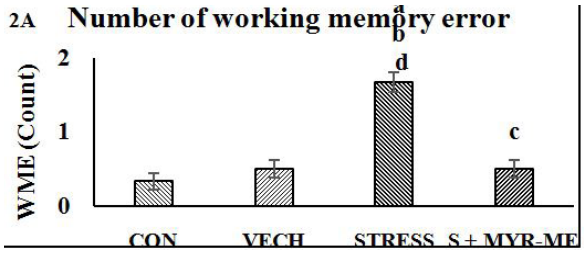


Figure 2: Eight-arm radial maze, working memory error (2A), reference memory (2B) and time was taken (2C). The data were expressed as mean ± SEM. "a" represents compared to control, "b" represents compared to Vehicle, "c" compared to stress group and "d" represents compared to stress with myricetin microemulsion treated and the value p<0.05 are considered as significant.

Novel object recognition test

The restraint stress exposed group showed significantly-less exploration to novel object (33.71 ± 3.43, p = 0.01), whereas MYR-ME treated group showed significantly increased novel exploration (74.58 ± 2.68, p = 0.003), this was restored almost as that of control animals. The results are shown in Figure 3.



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Spontaneous alteration T-maze

Spontaneous alteration T-maze was used to assess the cognitive ability of rodents. The parameters obtained are the number of days taken to attain 80% corrected response, spontaneous alteration scoring, theaverage time taken to each entry, memory retention scoring and the average time taken for memory retention scoring. The results described in Table 3.

Parameters	Control	Vehicle	Stress	Stress + MYR-ME
Days taken to attain 80% (Days)	1.5 ± 0.22	1.66 ± 0.21	3.1 ± 0.30a,b,d	1.33 ± 0.21c
Spontaneous alteration scoring (%)	85.00 ± 2.23	85.00 ± 2.36	81.67 ± 1.67	85.00 ± 2.16
Average time taken to each entry (sec)	15.93 ± 1.36	15.9 ± 1.23	24.51 ± 1.99a,b,d	9.73 ± 0.53a,c
Memory retention scoring (%)	81.67 ± 1.67	80.00 ± 2.58	61.67 ± 3.07a,b,d	81.67 ± 3.07c
Average time taken for memory retention scoring (sec)	15.70 ± 0.89	15.00 ± 1.32	44.11 ± 2.25a,b,d	13.26 ± 0.517

Table 3: Spontaneous alteration T-maze test-Describes parameters studied in the spontaneous alteration T-maze. The data were expressed as mean ± SEM. *a compared to control, *b compared to vehicle, *c compared to stress group and *d has compared to stress with myricetin microemulsion treated and the value $p<0.05$ are considered as significant.

Number of days taken for 80%correct choice

Restraint stress exposed group showed impaired cognitive function, this was tested by number of days taken to attain 80% correct choice (3.166 ± 0.30, p = .001), where as, those who treated with MYR-ME group showed similar days was observed as non-stressed animals (1.33 ± 0.21, p = 0.001).

Spontaneous alteration scoring

Spontaneous alteration scoring was less (81.67 ± 1.67, p = 0.683) in restraint stress exposed group animals, although, this was restored in MYR-ME treated group,as like non-stressed animals (85 ± 2.16).

Average time is taken to each entry

Restraint stress exposed animals demonstrated impaired decision-making ability was tested by average time to visit each arm entry (24.51 ± 1.99 sec, p = 0.01), however animals those were treated with MYR-ME the average time taken was noted at the same frequency as the non-stressed group (14.73 ± 1.53sec, p = 0.001).

Memory retention scoring

Impaired memory retention ability was observed in restraint stress exposed (61.67± 3.07, p = 0.004), while MYR-ME treated group showed significantly ($p<0.05$) increased memory retention scoring as that of non-stressed animals (81.66 ± 3.07, p = 0.002).

Average time is taken in memory retention scoring

Average time taken for each entry during memory retention scoring significantly was more in rrestraint stress exposed group (44.11 ± 2.25sec, p = 0.004), whereas MYR-ME administrated group animals were taken significantly less time, as similar result observed in non-stressed animals (13.26 ± 051sec, p = 0.006).

Discussion

Stress has become an integral part of human life, stressful experience disturbs the imbalance in the body's physiological function by elevated secretions of stress hormones, which damages the brain and it leads to impairment in cognitive function, learning and memory and also alters the behavior [26].

Restraint stress causes a wide range of anxiety behavior which leads to an alteration in learning and memory [27]. In the present study, twenty-one days of chronic restraint stress exposed animals showed increased anxiety level in the open field test, which is characterized by increased peripheral squares entries and increase grooming and rearing. However, MYR-ME treated group showed significantly reduced peripheral squares entries, grooming, rearing and increased central square entries. The similar result was found in chronic restraint stress exposed group [19]. Further, it was confirmed by elevated plus maze test, restraint stress exposed animals showed less open arm entries, more closed arm entries and also the time spent was more in the closed when compared to open arm, the anexiety level was significantly decreased in MYR-ME treated group. Our result is consistent with earlier findings, six days of restraint stress significantly increased anxiety behavior in the elevated plus maze behavior this was improved by treatement with *Ocium santum* and *Camellia sinensis*. Further, Mohan et al, [28] reported that myricetin treated group significantly decreased anxiety behavior by altering 5-HT levels.

In addition, the restraint stress group animal spent more time in the dark area whereas, less time was spent in the bright area in the light and dark test, this was reduced in MYR-ME treated group,the anxiety-like behaviors as the time spent in the lighter area was increased and latency for entering the dark area was significantly reduced in MYR-ME treated group.

From the present study, it is observed that MYR-ME effectively protects from deteriorating effects of in the rat model. MYR-ME treated animals showed significantly increased spatial learning and memory. It reduces working memory error, reference memory error and time taken to visit the baited arms compare to restraint stress exposed animals. The present study supports the previous study that 21 days of restraint stress can impair spatial learning [29].

Chronic restraint stress exposed animals showed impaired object recognition memory by changing the dendritic morphology of limbic areas of the rat brain, such as hippocampus, amygdaloid complex, and prefrontal cortex [30], this alteration increases anxiety and impair both memory and spatial learning [31].

Restraint stress exposed group showed an average 3 days taken to attain 80% corrected response in the spontaneous alteration T-maze. In the 1st day, time taken for each entry to the baited arm was increased when compared to that of control, after two days of T-maze training stress group showed average memory retention scoring 60% and increased time has taken to reach the baited arm (i.e. average time taken for each entry) was more in the restraint group, this indicates the impaired decision-making ability in the restraint stress groups. Where as MYR-ME treated group showed increased memory retention ability and less time taken for visiting arms, this indicates that MYR-ME treatment improved or protect the deleterious effect caused by restraint stress, the reason would be increased bioavailability, controlled drug delivery and longer half-life (t1/2) of MYR-ME when compare to myricetin [32].Twenty-one days of restraint stress exposure leads to a deficit in both acquisition and retentions in the T-maze [5]. Chronic stress impairs the maintenance of novel short-term memory, i.e. working memory, which is the term applied to the aspect of memory responsible for the recall of information immediately after it has been presented. These results may support that chronic psychosocial stress exaggerates the acquisition of spatial information/object recognition [33]. This memory impairment was reduced by MYR-ME treatment.

Myricetin in the form of microemulsion showed (16.05) fold increased bioavailability, increased half-life and controlled delivery system thesewould be reason for the increased activity of myricetin as anxiolytic and nootropic compound [16]. Mohan et al. have reported that myricetin acted as an anxiolytic compound [28], this was further confirmed by the present study MYR-ME was acted as anxiolytic as a compound. Myricetin as such or in the form of microemulsion it acts as antioxidants and it provides the protection against oxidative stress [34-36], this would be a possible protective mechanism of action of myricetin against restraint stress.

The characteristic features of the study are, microemulsion formulation is one of the suitable alternative drug delivery methods for polar soluble or partially polar soluble compounds to enhance the drug delivery to the brain. The present study extensively validated the anxiety related behavior by appropriate and three different behavioral techniques and spontaneous alteration T - maze test to assess cognitive functions such as decision making, memory retention ability. However, the study also has limitations, the study doesn't includes biochemical, neurochemical and also molecular mechanism behind the ameliorative effect MYR-ME. Hence, it is necessary to carry out neurotransmitter estimation, genomic and proteomic expressions to identify the clear mechanism behind the ameliorative effect of MYR-ME, this will be carried out in future research.

Conclusion

Restraint stress have deleterious effect on cognitive function and spatial memory, and anxiety-related behavior. The compound of our interest, myricetin in the oil phase protected from the microemulsion and avoiding the gastrointestinal and liver metabolism thereby it could increase the drug bioavailability and controlled drug delivery by microemulsion delivery system. So, thereby myricetin enter the hepaticenteral circulation and extend drug release, results in longer residence time in rats. Restraint stress exposed animals were showed decreased memory retention ability, decision-making capacity,increased memory retention ability, spatial learning and increased anxiety related behaviour, this was effectively protected by oral administration of (10 mg/kg) MYR-ME.The microemulsion form of the myricetin may have protective effect against restraint stress-induced cognitive dysfunction, spatial memory impairment, and other behavioral alterations.

Declarations of interest

The authors declare no conflict of interest.

Acknowledgement

The financial assistance from University of Madras and ICMR -SRF fellowships are greatly acknowledged.

References

1. Kim JJ, Diamond DM (2002) The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 3: 453-462.
2. McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, et al. (2015) Mechanisms of stress in the brain. Nat Neurosci 18: 1353-1363.
3. Wulf Dröge, Hyman M Schipper (2007) Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell 6: 361-370.
4. Gomez JL, Lewis MJ, Luine VN (2013) Alcohol Intake : Effects on Spatial Memory in Male Rats. Alcohol 46: 499-504.
5. McLaughlin (2007) The function : An evaluation of chronic restraint paradigms. Brain Res 3: 56-64.
6. Dawson GR, Tricklebank MD (1995) Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol Sci 16: 33-36.

7. Pijlman FT a, Herremans AHJ, van de Kieft J, Kruse CG, van Ree JM (2003) Behavioural changes after different stress paradigms: prepulse inhibition increased after physical, but not emotional stress. Eur Neuropsychopharmacol 13: 369-380.

8. Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13: 167-170.

9. Beking K, Vieira A (2010) Flavonoid intake and disability-adjusted life years due to Alzheimer's and related dementias: a population-based study involving twenty-three developed countries. Public Health Nutr 13: 1403-1409.

10. Christian a, Grethe S, Harald A (2009) Intake of Flavonoid-Rich Wine , Tea , and Chocolate by Elderly Men and women is associated with better cognitive test performance. J Nutr 139: 120-127.

11. Arora A, Nair MG, Strasburg GM (1998) Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic Biol Med 24: 1355-1363.

12. Ong KC, Khoo HE (1997) Biological effects of myricetin. Gen Pharmacol 29: 121-126.

13. Yoshikawa M, Shimada H, Nishida N, Li Y, Toguchida I, et al. (1998) Antidiabetic principles of natural medicines. II. Aldose reductase and alpha-glucosidase inhibitors from Brazilian natural medicine, the leaves of Myrcia multiflora DC. (Myrtaceae): structures of myrciacitrins I and II and myrciaphenones A and B. Chem Pharm Bull (Tokyo) 46: 113-119.

14. Chen W, Li Y, Li J, Han Q, Ye L, et al. (2011) Myricetin affords protection against peroxynitrite-mediated dna damage and hydroxyl radical formation. Food Chem Toxicol 49: 2439-2444.

15. Semwal DK, Semwal RB, Combrinck S, Viljoen A (2016) Myricetin: A dietary molecule with diverse biological activities. Nutrients 8: 90.

16. Wang S, Ye T, Zhang X, Yang R, Yi X (2013) Myricetin microemulsion for oral drug delivery : formulation optimization, in situ intestinal absorption and in-vivo evaluation. Asian J Pharm Sci 8: 18-25.

17. Barzegar A (2016) Antioxidant activity of polyphenolic myricetin in vitro cell- free and cell-based systems. Mol Biol Res Commun 5: 87-95.

18. Tenjarla S (1999) Microemulsions: an overview and pharmaceutical applications. Crit Rev Ther Drug Carrier Syst 16: 461-521.

19. Chen WQ, Zhao XL, Wang DL, Li ST, Hou Y, et al. Effects of epigallocatechin-3-gallate on behavioral impairments induced by psychological stress in rats. Exp Biol Med (Maywood) 235: 577-583.

20. O'Mahony CM, Clarke G, Gibney S, Dinan TG, Cryan JF (2011) Strain differences in the neurochemical response to chronic restraint stress in the rat: Relevance to depression. Pharmacol Biochem Behav 97: 690-699.

21. Walf A Alicia, Frye A Cheryl (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2: 322-328.

22. McGivern RF, Rittenhouse P, Aird F, Van de Kar LD, Redei E (1997) Inhibition of stress-induced neuroendocrine and behavioral responses in the rat by prepro-thyrotropin-releasing hormone 178-199. J Neurosci 17: 4886-4894.

23. Shif O, Gillette K, Damkaoutis CM, Carrano C, Robbins SJ, et al. (2006) Effects of Ginkgo biloba administered after spatial learning on water maze and radial arm maze performance in young adult rats. Pharmacol Biochem Behav 84: 17-25.

24. Deacon RMJ, Rawlins JNP (2006) T-maze alternation in the rodent. Nat Protoc 1: 7-12.

25. Singh P, Thakur MK (2014) Reduced recognition memory is correlated with decrease in DNA methyltransferase1 and increase in histone deacetylase2 protein expression in old male mice. Biogerontology 15: 339-346.

26. Moreira PS, Almeida PR, Leite-Almeida H, Sousa N, Costa P (2016) Impact of chronic stress protocols in learning and memory in rodents: Systematic review and meta-analysis. PLoS One 11: 1-17.

27. Sandi C, Pinelo-Nava MT (2007) Stress and memory: Behavioral effects and neurobiological mechanisms. Neural Plast 2007: 78970.

28. Mohan M, Jadhav SS, Kasture VS, Kasture SB (2009) Effect of myricetin on behavioral paradigms of anxiety. Pharm Biol 47: 927-931.

29. Kumar RS, Narayanan SN, Nayak S (2009) Ascorbic acid protects against restraint stress-induced memory deficits in wistar rats. Clinics 64: 1211-1217.

30. Magariños AM, McEwen BS, Flügge G, Fuchs E (1996) Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci 16: 3534-3540.

31. Magariños AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Comparison of stressors. Neuroscience 69: 83-88.

32. Guo RX, Fu X, Chen J, Zhou L, Chen G (2016) Preparation and Characterization of Microemulsions of Myricetin for Improving Its Antiproliferative and Antioxidative Activities and Oral Bioavailability. Journal of Agricultural and Food Chemistry 64: 6286-6294.

33. Krugers HJ, Douma BRK, Andringa G, Bohus B, Korf J, et al. (1997) Exposure to chronic psychosocial stress and corticosterone in the rat: Effects on spatial discrimination learning and hippocampal protein kinase Cg immunoreactivity. Hippocampus 7: 427-436.

34. Justino GC, Vieira AJ (2010) Antioxidant mechanisms of quercetin and myricetin in the gas phase and in solution- A comparison and validation of semi-empirical methods. J Mol Model 16: 863-876.

35. Roedig-Penman A, Gordon MH (1998) Antioxidant properties of myricetin and quercetin in oil and emulsions. J Am Oil Chem Soc 75: 169-180.

36. Pandey KB, Mishra N, Rizvi SI (2009) Myricetin may provide protection against oxidative stress in type 2 diabetic erythrocytes. Z Naturforsch C 64: 626-630.