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Probiotic Ameliorate Impaired Memory in Stressed Animal

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Abstract

Worldwide, around 50 million people have dementia every year; there are nearly 10 million new cases. The total number of people with dementia is projected to reach 82 million in 2030 and 152 in 2050. Probiotics are live microbial food supplements with certain benefits for consumers and are thought to maintain or improve the intestinal microbial balance. In this study animals were divided in to five groups. Stress was induced in rats by restraining rat for 6 hrs daily for 28 days. Stress in animal was determined by using open field and hole board method. Memory and learning were studied using Elevated Plus maze and water maze apparatus. Results shown increase in stress in negative control rats when compared with normal control but when treated with probiotic alone and along with quercetin stress were reduced compared to negative control using open field and hole board method. Results shown decrease in memory in negative control rats when compared with normal control but when treated with probiotic alone and along with quercetin stress were improved compared to negative control using Elevated Plus maze and water maze apparatus. From this it can be concluded that probiotic alone and along with quercetin may good option for the treatment of stress and Alzheimer disease.

Keywords- Stress, Alzheimer disease, probiotic, quercetin, dementia

Introduction

As per WHO (2019) Alzheimer disease (AD) is the most common form of dementia and may contribute to 60–70% of cases. Dementia is a syndrome usually of a chronic or progressive nature in which there is deterioration in cognitive function (i.e. Theability to process thought) beyond what might be expected from normal ageing. It affects memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Consciousness is not affected.

Worldwide, around 50 million people have dementia every year; there are nearly 10 million new cases. The total number of people with dementia is projected to reach 82 million in 2030 and 152 in 2050(Dementia, WHO,2019). The cognitive decline is associated with the AD pathogenesis which is due to decrease in acetylcholine, which also proposes that deficit of acetylcholine is life-threatening in the creation of the symptoms of AD. In addition, several researcher suggested that Reactive Oxygen species (ROS) is associated with etiopathogenesis of AD and it leads to a cumulative damage of cellular macromolecules and impairment of mitochondria function which further leads to a decrease in cellular energy production(Nagpal R et.al., 2019). Alterations in bidirectional brain-gut interactions are believed to be involved in the pathogenesis of well-known brain-gut

disorders such as irritable bowel syndrome (IBS) and related functional gastrointestinal (GI) disorders and have more recently been implicated as a possible mechanism in the pathophysiology of several brain disorders including autism spectrum disorders, parkinson's disease, disorders of mood and affect, and chronic pain (E A Mayer, et.al., 2015). Moreover, it has been shown that the absence and/or modification of the gut microflora in mice affects the hypothalamic–pituitary–adrenal (HPA) axis(Javier A. Bravo et.al. 2011).

Probiotics are live microbial food supplements with certain benefits for consumers and are thought to maintain or improve the intestinal microbial balance. Probiotics have been displayed to improve brain-gut-microbiota axis and regulate nervous system through neuroendocrine, neurometabolic and neuroimmunologic mechanisms. They can also reduce some oxidative stress biomarkers and inflammatory cytokines (Zahra Rezaei Asl et.al., 2019). Probiotics are beneficial to humans and animals when adequately administered. Probiotic bacteria make proficient interaction with the gut microbiota and provide health benefits. In recent years, attempts are devoted to find a link between the gutmicrobiome with neurological disease(Shima Mehrabadi, et.al.2020, Samaneh Bagheri,2019). Probiotics exhibit health promoting properties by improving the immune system, supplying antioxidants and improving mental health (Yodai Kobayashi, et.al.2017). Probiotics exhibit health promoting properties by improving the immune system, supplying antioxidants and improving mental health (B S Sivamaruthi et.al., 2019)

Materials and Methods

Animals

8 weeks old healthy female Sprague-dawley rats (weighing 150-250 gm) were used for this study. Animals were housed in polypropylene cages with wire mesh top and husk bedding and maintain under control condition of light (12h-light, 12h-dark), temperature(25±2ºC), and humidity (60±5%) and fed with a standard pellet diet and water ad libitum, were used for the entire animal study. The experiments were performed during day (8.00- 16hrs). The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocol for all the animal study was approved by Institutional Animal Ethics Committee (IAEC). For this study animals were divided in to following groups

1. Control Group: Animals were treated with vehicle alone

2. Negative control Group: Alzheimer's Disease in rats was produced by using Restraint stress for 28 days.

3. BL Group: Alzheimer's Disease in rats was produced by using Restraint stress and treated with Bifidobacterium longum probiotic (1x109 CFU) daily p.o. for28 days.

4. BL+Q Group: Alzheimer's Disease in rats was produced by using Restraint stress and treated with Bifidobacterium longum (1x109 CFU) daily p.o. and quercetin (50mg/kg) daily i.p. for 28 days.

5. STD Group: Alzheimer's Disease in rats was produced by using Restraint stress and treated with Donepezil (5 mg/kg) orally for 28 days.

Induction of Stress in animals

All groups were subjected for 28 days for restraint stress except normal control group which was placed in normal condition in animal house.

A saline bottle was used to cause memory impairment in female Sprague Dawley rats. Whenrats were firmly packed in a saline container for 6 hours every day for 28 days (Madhyastha S et al., 2008). Animalmodels of depression are subjected to constant stressors such as food deprivation, waterdeprivation,and being

tightly packed in a saline bottle. Chronic stress may inhibit the immune system and increase the synthesis of interleukin 1β under such circumstance. In rats, persistent psychological stress promoted neuroinflammation and neurodegeneration.

Dosing of Probiotics, querecetin and Donepezil

Daily dose of Probiotic Bifidobacterium longum (1x109 CFU) p.o. and querecetin (50mg/kg) i.p. were given to animals for the duration of 28 days.

Donepezil (5mg/kg) was used as standard drug. All solutions were prepared freshly on test days and administered according to their standard routes.

Determination of stress in rats

Open-field test

A large plywood box (75×75×29 cm) painted grey with a black grid (15×15 cm squares) on the floor was used for investigational testing. The rat was placed into a corner of the box and allowed to explore freely for 10 min. The box was thoroughly cleaned between subjects with a disinfectant solution. All test sessions were videotaped and the following measures were later recorded: number of rears (animal on hind limbs), number of grid boxes entered (front 2 paws over a line), time in center 9 squares, and latency to leave the corner box initially [Angela M. Gouirand and Leslie Matuszewich, 2005].

Hole-board test

The apparatus was composed of a gray wooden box (50 cm×50 cm× 50 cm) with four

equidistant holes 3 cm in diameter in the floor. The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground and divided into squares of 10 cm×10 cm with a water resistant marker. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total locomotor activity (numbers of squares crossed), and the number and duration of head-dippings were recorded. A head dip was scored if both eyes disappeared into the hole [Armario A, 1991].

Study of Learning and Memory Impairment State After 28 Days by Following Model

1] Modified Elevated plus maze apparatus:-

Modified Elevated Plus Maze Apparatus (MEPMA) was used for the assessment of learning and memory enhancement activity. The test was performed by placing the rat in one of the open arm of maze, typically facing opposite to closed arm. Upon release, the animal is free to explore the apparatus. One measure of memory is then recorded, the transfer latency i.e the time (in second) taken by the rat to move from the open arm into one of the closed arm with all its four legs was measured (Mani Vasudevan & Milind Parle, 2007 and Vijendar Kumar et.al, 2013).

2] Morris Water Maze Apparatus:-

The Morris water maze apparatus (MWMA) is a test of learning and memory for rodents to navigate from start locations around the perimeter of an open swimming area to locate a submerged escape platform. Learning and memory is assessed across repeated trials and reference memory is determined by preference for the platform area when the platform is absent. (Charles V., et.al, 2006)

Results

Table 1: Effect of probiotics alone and along with quercetin on on stressed rats using Open field test apparatus

Sr. No.	Groups	No. Of box entered		No. Of rears		Latency to inside portion of the field (sec.)		Duration of time in the inside portion of the field (sec.)	
		0 Days	28 days	0 Days	28 days	0 Days	28 days	0 Days	28 days
1.	Normal Control	188.19±2.35	190.19±5.85	40.22±2.45	40.1±3.49	113.90±4.71	113.90±4.71	44.45±2.70	44.20±2.56
2.	Negative Control	189.12±3.45	123.96±6.53**	39.24±1.75	58.31±5.99**	112.24±4.71	92.34±3.79**	43.80±2.56	15.80±2.61**
3.	**BL Group**	190.25±2.51	199.13±3.80@@	41.15±1.45	44.96±2.01@@	112.80±4.71	105.37±2.28@@	43.50±2.56	32.92±3.26@@
4.	**BL+Q Group**	187.19±3.45	187.15±5.28@@	40.35±1.25	41.45±1.50@@	112.30±4.71	113.55±4.07@@	44.60±2.78	36.65±2.33@@
5.	Donepezil	188.25±2.72	122.56±9.87	40.22±2.28	59.56±3.63	111.25±4.71	97.64±3.32	44.45±2.77	17.86±5.16

Values are expressed in Mean±SD, (n=6)

** P<0.01, compared to Group I; @@P<0.01, compared to Group II

Table 2: Effect of probiotics alone and along with quercetin on stressed rats using Hole board test

Sr. No.	Groups	Number of box crossing		Number of nose poking	
		0 Days	28 days	0 Days	28 days
1.	Normal Control	36.33 ± 1.86	36.33 ± 1.86	42 ± 1.58	43 ± 1.78
2.	Negative Control	36.33 ± 1.86	6.33 ± 1.36**	43 ± 1.57	5 ± 2.36**
3.	**BL Group**	36.33 ± 1.86	25.33 ± 1.36@@	43 ± 1.45	31.66 ± 2.25@@
4.	**BL+Q Group**	36.33 ± 1.86	32.33 ± 2.73@@	42 ± 1.22	38.66 ± 2.73@@
5.	Donepezil	36.33 ± 1.86	7.33 ± 1.36	43 ± 1.35	6 ± 0.89

Results are expressed as mean ± SD, (n=6)

@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with negative control group, *p<0.05 compared with negative control group

Table 3: Effect of probiotics alone and along with quercetin on transfer latency (TL) of rats in EPM apparatus

Sr. No.	Groups	Transfer latency in seconds on Day 0	Transfer latency in seconds on Day 28
1.	Normal Control	21.61 ± 2.22	21.35± 2.26
2.	Negative Control	23.36± 2.89^{ns}	36.1± 2.37@
3.	**BL Group**	23.1± 1.79^{ns}	20.67± 1.37**
4.	**BL+Q Group**	22.1± 1.79^{ns}	25.67± 1.37**
8.	Donepezil (5 mg/kg)	22.67 ± 1.87^{ns}	12.67± 1.87**

Results are expressed as mean ± SD, (n=6)

@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with negative control group, *p<0.05 compared with negative control group

Table 4: Effect of probiotics alone and along with quercetin on Escape latency of rats in EPM apparatus

Sr. No.	Groups	Escape latency in seconds on Day 0	Escape latency in seconds on Day 28
1.	Normal Control	31.31± 2.60	30.31± 3.62
2.	Negative Control	31.64± 0.52^{ns}	69.67± 2.39@
3.	**BL Group**	31.13± 2.70^{ns}	35.98± 1.79**
4.	**BL+Q Group**	31.23± 1.19^{ns}	39.34± 1.18**
8.	Donepezil (5 mg/kg)	31.21±2.29^{ns}	30.64± 2.26**

Results are expressed as mean ± SD, (n=6)

@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with negative control group, *p<0.05 compared with negative control group

Table 5: Effect of Probiotic alone and along with quercetin on Retention time (RT) of rats in MWM apparatus

Sr. No.	Groups	Retention time in seconds on Day 0	Retention time in seconds on Day 28
1.	Normal Control	39.31± 0.53	42.62± 2.43
2.	Negative Control	37.80± 1.79^{ns}	30.64± 0.98$^{@}$
3.	**BL Group**	40.18±1.23^{ns}	38.31± 1.31**
4.	**BL+Q Group**	37.21±1.73^{ns}	41.91± 1.65**
8.	Donepezil (5 mg/kg)	38.00± 0.90^{ns}	44.43± 1.87**

Results are expressed as mean ± SD, (n=6)
@p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with negative control group, *p<0.05 compared with negative control group

Table 1 shows the effect of probiotic alone and along with quercetin on stressed rats using Open field test. In negative control there was significant decrease (p<0.01) in the number of box entered or latency to inside portion and significant increase (p<0.01) in the number of rears as compared to control, but probiotic (1x109 CFU) daily p.o. alone and along with quercetin (50mg/kg) daily i.p. treated group shows significant increase (p<0.01) in the number of box entered or latency to inside portion and significant decrease (p<0.01) in the number of rears as compared to negative control.

Table 2 show the effect of probiotic alone and along

with quercetin on stressed rats using Hole board test. Negative control shows significant decrease (p<0.01) in the number of box crossing and nose poking behavior as compared to control, but probiotic (1x109 CFU) daily p.o. alone and along with quercetin (50mg/kg) treated group shows significant (p<0.01) increase in the number of box crossing and nose poking behavior as compared to negative control.

Table 3 shows the effect of probiotic alone and along with quercetin on transfer latency of rats on EPM apparatus in stressed rats. There was significant increase (p<0.01) in transfer latency in negative control group compared to normal control group on 28th day. Probiotic alone and combination with quercetin treated shows significant (p<0.05) decrease in the transfer latency at (1x109 CFU) daily p.o. and quercetin (50mg/kg) daily i.p. respectively compared to negative control group on 28th day.

Table 4 shows the effect of probiotic alone and along with quercetin on Escape latency of rats on Morris Water maze apparatus in stressed rats. There was significant increase (p<0.01) in Escape latency in negative control group compared to normal control group on 28th day. Probiotic alone and combination with quercetin treated shows significant (p<0.05) decrease in the Escape latency at (1x109 CFU) daily p.o. and quercetin (50mg/kg) daily i.p. respectively compared to negative control group on 28th day. Table 5 shows the effect of probiotic alone and along with quercetin on Retention time of rats on Morris Water maze apparatus in stressed rats. There was significant decrease (p<0.01) in Retention time in negative control group compared to normal control group on 28th day. Probiotic alone and combination with quercetin treated shows significant (p<0.05) increase in the Escape latency

at(1x109 CFU) daily p.o. and quercetin (50mg/kg) daily i.p. respectively compared to negative control group on 28th day.

Discussion

Numerous studies have demonstrated a connection between immunological network changes, stress exposure, and the advancement of disease, especially in neurodegenerative conditions like Alzheimer's disease (AD). However, nothing is known about how this interaction works. B-amyloid buildup, which results in plaques strewn throughout the brain, is the primary characteristic of AD neuropathology. It begins in the neocortical regions of the brain, moves progressively to the midbrain as the disease worsens, and eventually spreads to the cerebellum and brain stem. Like major depressive disorder (MDD), chronic stress frequently leads to cognitive impairment, which is similar to the pathology seen in AD.

Glutamate, a stress marker, has been discovered to be elevated in AD patients and those experiencing chronic stress, which may indicate that stress speeds up the onset of neurodegenerative diseases. (Feng Yilin and others, 2023) The primary cause of dementia, which is typified by a loss of thinking and independence in one's own everyday activities, is Alzheimer's disease (AD), a condition that results in the degradation of brain cells. The cholinergic and amyloid hypotheses are the two main theories put up as the causes of AD, which is thought to be a complex disease.

Scopolamine, streptozotocin, alcohol, and the dysregulation of heavy metals like aluminum (Al), copper (Cu), zinc (Zn), lead (Pb), and reducing sugar (D-galactose) are some of the typical substances used to imitate AD. (Mahdi Onesimus et al., 2019) Restraint stress, a modified version of immobilization stress, is one of the often used models.

Probiotics are living microorganisms that provide health benefits to the host when administered in adequate amounts. The health benefits of probiotics are living microorganisms that have a positive effect on human health when taken in sufficient amounts. Lactic acid bacteria, bifidobacteria, and yeast are commonly used as probiotics. Probiotic can easily get accommodated in human gut. So, in this study Bifidobacterium longum probiotics alone and with quercetin are studied and used to cure the disease. (Nicoleta Maricia Maftei et al., 2024) In elevated plus maze apparatus, there was significant increase in the transfer latency in negative control group as compared to the normal control group. Whereas probiotic (27 × 1010 CFU/gm) alone and in combination with quercetin (50 mg/kg) and Donepezil (5 mg/kg) treated group showed significant decrease in transfer latency as compared to negative control group after 28 days. In morris water maze apparatus, there was significant increase in the escape latency in negative control group as compared to the normal control group. Whereas probiotic (27 × 1010 CFU/gm) alone and in combination with quercetin and Donepezil (5 mg/kg) treated group showed significant decrease in escape latency as compared to negative control group after 28 days.

In morris water maze apparatus, there was significant decrease in the retention time in negative control group as compared to the normal control group. Whereas probiotic (27 × 1010 CFU/gm) alone and in combination with quercetin and Donepezil (5 mg/kg) group showed significant increase in retention time as compared to negative control group after 28 days. The brain AchE of rats plays an important role in AD.

Conclusion

The present finding indicates that the probiotics (27 × 1010 CFU/gm) alone and in combination with quercetin showed significant improvement in learning and

memory enhancing activity in rats hence probiotic bacteria may be useful in the treatment of Alzheimer's Disease.

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