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ORIGINAL ARTICLE

Comparative Study on Effect of *Bacopa monniera*, Omega Fatty Acids and Mesenchymal Stem Cells on Cold Stress Induced Neurodegeneration in Hippocampus of Wistar Rats

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Abstract:

*Background: N*atural and ayurvedic drugs are compared with stem cell therapy to suggest an improved treatment to enhance memory and combat stress in our day to day life. *Aim and Objectives*: The present study was aimed to compare the effect of traditional medicine *Bacopa monniera* (BM) with omega3 fatty acids and mesenchymal stem cells on cold stress induced neural changes in hippocampus of Wistar rats. *Materials and Methods*: Total 36 male rats divided into six groups. Group I was control in which rats were kept under ideal laboratory conditions, Group II was cold water swim stress in which rats were forced to swim in the cold water maintained at 18±2°C for ten minutes for a period of one month, Group III was given stress followed by oral administration of normal saline as a control, Group IV was given stress for a month followed by oral administration of 80 mg/kg of BM extract, Group V in which cold water swim stress given for a month followed by oral administration of 60 mg/kg omega3 fatty acid treatment for a month, Group VI in which cold water swim stress given for a month followed by intravenous injection of mesenchymal stem cells treatment. The animals were studied on their behavioral changes, cortisol assay and histological analysis. *Results*: The results showed P<0.001, that is significant difference between the groups in their behavioral study, cortisol assay and total number of cells. *Conclusion*: All three drugs have significant effect in improving the memory, but when comparison was made, it was suggested that intravenous infusion of bone marrow derived mesenchymal stem cells was superior followed by BM and omega3 fatty acids.

Keywords: Memory, Cold Stress, CA1, Neurons, Hippocampus

Introduction:

Stress may be described as the response to stimulus. Over the past decades, a diversity of animal models have been proposed for the study of stress and its effects. Compulsive swimming in cold water produced increased output of nor-adrenaline in parts of the brain structures and also roots alteration in blood cortisol level. These stresses are linked with damages in learning and memory that are established by their behaviors [1]. They also modify brain structures involved in memory, mainly on hippocampus. The recent researchers focused on the Indian ayurvedic medicine which has proven to have no side effects. Many researchers believe that omega3 fatty acids

and stem cells helps in neuroprotection and neuroregeneration [2]. Even when the modern medicine and recent drug therapies have reached its peak, people look for substitute methods of management. More than half of the global population still depends upon the herbal or natural source of drugs for their health care. Having this in mind, we still have lacunae which of those treatments could provide us healthier assistance to improve the memory. In the present context the natural and ayurvedic drugs are compared with stem cell therapy to suggest an improved treatment to enhance memory and combat stress in our day to day life.

Material and Methods:

Total of thirty six male Wistar rats divided into six groups were used for this study. In each group, six animals were considered. The study was conducted at Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha University, Chennai, Tamil Nadu, India. The care of the animal were upheld as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were housed in each polypropylene cage under normal laboratory conditions with food and water provided *ad libitum*. The experimental procedure was subjected to scrutiny by an Institutional Animal Ethics Committee (IAEC) for experimental clearance no - SU/BRULAC/RD/016/2014. Group I was control group in which rats were kept under ideal laboratory situations, Group II was cold water swim stress in which rats were forced to swim in the cold water kept at 18 ± 2°C for ten minutes for a period of one month, in Group III stress was given, followed by oral administration of normal saline as a control, Group IV was given stress for a month followed by oral administration of 80 mg/kg of *Bacopa monniera* (BM) extract, in the Group V cold water swim stress given for a month followed by oral administration of 60 mg/kg omega3 fatty acid treatment for a month, in Group VI cold water swim stress given for a month followed by intravenous injection of mesenchymal stem cells treatment.

Stress Protocol:

The rats were forced to swim in the cold water for ten minutes a day in the morning for a period of one month. Animals were compulsorily made to swim in a plastic bucket that measures with dimensions of forty five centimeter height and twenty centimeter in diameter filled with twenty five centimeter depth of cold water that was maintained at the temperature of 18 ± 2ºC which was measured using thermometer. The stress period was selected on the basis of previous studies that suggested maximum percentage of cell degeneration in the CA1 of the hippocampus.

Effect of BM on Cold Stress:

Homogenous plant extract of BM was obtained from herbal manufacturer, Natural Remedies Private Limited, India. The extract was administered 80 mg/kg orally by calculating the dosage with the body weight of the rats, using an oral feeding needle (gavage) attached to a syringe.

Effect of Omega3 Fatty Acids on Cold Stress:

Cold water swim stress was given for a month followed by oral administration of omega3 fatty acids 60 mg/kg per day treatment for four weeks as long term drug therapy. The total study carried out includes one month of stress period followed

by treatment period of four weeks as stated to be long term therapy.

Effect of Mesenchymal Stem Cells on Cold Stress:

In this study, a six-week-old Wistar rat weighing 80 g was used for the isolation of Bone Marrow Derived Mesenchymal Stem Cells (BMSCs). Incisions made, femur were removed and meschymal cells were isolated and cultured [3]. The sixth passage cells were then re-suspended and measured by a flow cytometer to analyze (FlowJo, India) for the expression of CD44, CD90 for positivity and CD45 for negativity. All these are specific markers for BMSCs.

Outcome Measures:

Behavioral test was done using Y-maze to assess the memory function. This is particularly used for measuring cognitive deficits including memory and learning by assessing hippocampal damage and evaluating the effects of drugs on memory. Cortisol assay was done using CLIA (Chem-iluminescence) method which is competitive immunoassay using direct ADVIA Centaur System. Routine stain of Hematoxylin and Eosin (H & E) was done to study the entire cells in CA1. The cell count was adjusted by using the Abercrombie's formula. Round, clear and medium or large neurons with distinct nucleus were counted. Darkly stained, shrunken cells and cells with fragmented nuclei were exempted from counting. Only sufficiently impregnated CA1 neurons were selected for study.

Statistical Analysis:

Normality tests Kolmogorov-Smirnov was calculated to show that the variables follow normal distribution. To analyze the data parametric tests were applied. One way ANOVA was useful to compare mean values. SPSS statistics version 22.0 was used to analyze the data. If P-value is less than 0.05 then was considered to be statistically significant.

Results:

Behavioural Study Analysis:

All the three treatment groups showed statistically significant but when compared to each other BMSCs treated group showed the high percentage when compared to the other two treatment groups in Table 1.

Cortisol Assay Analysis:

All the three treatment groups showed statistically significant difference but when compared between the three drugs there was no difference. So all the three treatment groups has the same effect on the cortisol assay in Table 2.

Total Number of Cells in CA1 Region:

All the three treatment groups showed statistically significant difference but when compared to each other BMSCs treated group shown the high percentage of cells proliferation when compare to the other two treatment groups. When the comparison was made between BMA and omega 3 fatty acids there is no evident increase in the number of cells in Table 3.

Table 1: Behavioural Study Analysis

Treatment group	No.	Mean	SD	F	*P*
Control	6	67.333	5.574	42.548	<0.001
Cold Stress	6	47.500	4.183		
Cold Stress + Saline	6	47.833	5.154		
Cold Stress + BM 80	6	68.667	3.670		
Cold Stress + LT Omega	6	62.167	1.329		
Cold Stress + BMSCs	6	77.167	2.639		

ANOVA

Sum of Squares		df	Mean Square	F	*P*
Between Groups	4529.583	7	647.083	42.548	<0.001
Within Groups	608.333	40	15.208		
Total	5137.917	47			

Table 2: Cortisol Assay Analysis

Treatment group	N	Mean	SD	F	*P*
Control	6	1.067	0.102	13.807	<0.001
Cold Stress	6	1.738	0.275		
Cold Stress + Saline	6	1.725	0.299		
Cold Stress + BM 80	6	0.933	0.216		
Cold Stress + LT Omega	6	0.933	0.216		
Cold Stress + BMSCs	6	0.955	0.078		

ANOVA

Sum of Squares		df	Mean Square	F	*P*
Between Groups	5.126	7	0.732	13.807	<0.001
Within Groups	2.122	40	0.053		
Total	7.248	47			

Table 3: Total Number of Cells in CA1 Region

Treatment group	N	Mean	SD	F	*P*
Control	6	63.500	12.438	9.087	<0.001
Cold Stress	6	50.667	3.933		
Cold Stress + Saline	6	50.833	3.869		
Cold Stress + BM 80	6	59.833	3.251		
Cold Stress + LT Omega	6	59.500	2.665		
Cold Stress + BMSCs	6	72.833	6.616		
Total	48	58.667	8.711		

ANOVA Table

Sum of Squares		df	Mean Square	F	*P*
Between Groups	2189.667	7	312.810	9.087	<0.001
Within Groups	1377.000	40	34.425		
Total	3566.667	47			

Discussion:

Neuroscientists have exposed that long-lasting *stress* and cortisol can damage the brain. Stress is capable to effect on memory tasks. The forced water swimming test has currently become widely accepted stressor model for studying physical stress in animals. BM has been studied broadly in animal models and *in-vitro,* as it is implicated in the treatment of anxiety, epilepsy and other neurodegenerative disorders, with the special attention on cognition, learning, and memory [1].

The clinical studies mostly focus on memory, omitting other facts of cognition comparable intelligence or creativity [2]. Homogenous adult mesenchymal cells were attained and colony of adult mesenchymal stem cells was seen. Similar study on cultured the bone marrow, reported the linear increase in the number of colonies with increase in number of explanted cells. The star shaped cells become spindle shaped cells and yielded growth [3].

In the present study, there was rise in the cortisol level in the forced cold water swim stressed rats. Comparable results have been reported using stress paradigms diets enriched with polyunsaturated fatty acids including omega-3 family that decreased both the adipose tissue mass and plasma

leptin levels in rats. On the other hand, omega-3 supplementation suggestively decreased cortisol levels in the positive control group rats. This recommends that omega-3 supplementation prevents hyper activation induced by stress, decreasing the effects of corticosteroids on dendritic morphology and neuronal action in the basolateral amygdale [4-6]. Our study explained Y-maze task was a powerful memory recognition test for chronically stressed rats, and because behavioral assessment can be started a day after the previous stress session and finished within hours after its commencement [7].

In the present study, our results established that BM treated rats with both low and high dosage showed improved performance when compared with the stressed negative control groups[8]. In the present study, positive control groups revealed an increase in the total number of neuronal cells in CA1 region. It can be noted from the results that total number of cells in CA1 region of treatment groups both low and high dose produce significant number of neurons when compared to negative control groups, which may be one of the reasons for the enhanced learning and memory in these animals reported earlier [9].

Neurogenesis shown by an increased number of proliferating neurons and neuritogenesis, evidenced by increased density of dendritic spines of CA1 pyramidal neurons in the hippocampus [10]. In the present study, we showed that intravenous injection of BMSCs reach the CA1 region of hippocampus and differentiate into glial cells and these cells could have perhaps given raise to new neurons [10].

Conclusion:

Intravenously injected BMSCs induces neuroprotective and neurogenesis action that possibly found to be superior when equated with other therapies and may be used as an adjuvant to improve memory and combat stress.

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