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Comparative Study on the Effect of *Bacopa monniera* and Omega3 Fatty Acids on the Cortisol Hormone Level in Cold Stress Induced Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In recent years, researchers have identified several natural compounds that could potentially help to retard mental stress. Extracts of *Bacopa monniera* (BM), a traditional ayurvedic medicine and omega3 faffy acids (OFA) have been reported to enhance effects mental functions by combating the stress. However, there is still an lacunae studies on which of those two drugs has better results on cortisol hormonal changes due to stress. The current study aimed to compare the effects of BM and omega3 fatty acids on cold stress induced Wistar rats. We divided the animals into seven groups. Group I was control in which rats were kept under ideal laboratory conditions, Group II was given cold water swim stress for a period of one month after which the rats were sacrificed for hormonal assay, Group III in which cold water swim stress given for a month followed by oral administration of normal saline as vehicle for a month, Group IV and V in which cold water swim stress given for a month followed by oral administration of BM extracts 40mg/kg and 80mg/kg respectively as treatment for a month. Group VI and VII includes cold stress followed by oral gavages of OFA 60mg/Kg of Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) for 2 weeks (short-term model) and 4 weeks (long term model) respectively. After the treatment period blood was collected and cortisol assay was done. The data were compared with those of control rats. The results showed an increase in the cortisol level in the stressed group of rats when compared to the treatment group. These results indicate that oral administration of high dose of BM and long-term administration of OFA improves the cortisol level and helps in controlling the stress in rats.

Keywords: Bacopa; omega fatty acids; cold stress; cortisol; hippocampus.

1. INTRODUCTION

Stress is a worldwide spectacle that shows definite response in our body. Inescapable stress is related with deficits in learning and memory that causes major alterations in brain structures linking cortisol hormone that plays a direct role in it. Even though majority of the global population depends upon the herbal or natural source drugs for their health care, there has been an increase demand for the pharmaceutical products all over the world because of the side effects of the allopathic drugs.

"Bacopa monniera, (BM, Bacopa) has been used in an ayurvedic system of medicine for centuries. Traditionally, it was used as a brain tonic to enhance memory development and learning. The constituents responsible for Bacopa's cognitive effects are bacosides A and B. Their mechanism of action depends on the ability to enhance nerve impulse transmission" [1]. "Omega-3 fatty acids (OFA) are long chain, polyunsaturated fatty acids (PUFA) are the essential fatty acids (EFAs) cannot be synthesized in the human body and so they must be derived from dietary sources. Omega-3 fatty acids are an essential component of phospholipid-acyl chains and are critical to the dynamic structure of neuronal membranes" [2]. In order to appreciate the potential role of omega-3 fatty acids in mental health, some of the neurobiological alterations that exist in stress must first be examined. The role played by EFAs in the human body has been the subjected to research, particularly in recent years.

Having this in mind, the present context we experimented in two phases to compare natural and ayurvedic drug therapy on the cortisol hormonal assay to suggest an improved treatment to combat stress in our day-to-day life.

2. MATERIALS AND METHODS

2.1 Animal Model

For this experiment male Wistar albino rats weighing 150-180 gm were used. The study was conducted at BRULAC, Saveetha University, Tamil Nadu, India. The care and maintenance of the animal was as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2 Experimental Design

A total of forty-two rats were divided into seven groups (n=6). Group I was control in which rats were kept under ideal laboratory conditions, Group II was given cold water swim stress for a period of one month after which the rats were sacrificed for cortisol hormonal assay, Group III in which cold water swim stress given for a

month followed by oral administration of normal saline as vehicle for a month, Group IV and V in which cold water swim stress given for a month followed by oral administration of BM extracts 40mg/kg and 80mg/kg respectively as treatment for a month. Group VI and VII, cold stress followed by oral gavages of OFA 60mg/Kg of Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) for 2 weeks (short-term model) and 4 weeks (long term model) respectively. The total study was carried out for a period of sixty days.

2.3 Stress Protocol

The rats were forced to swim in the cold water for 10 minutes a day in the morning for a period of one month. Animals were forced to swim in a plastic bucket (Dimensions 45 cm height, 20cm in diameter) filled with 25cm depth of cold water (18 ±2 ºC) under observation. The stress period was selected based on a pilot study.

2.4 Extraction and Administration of BM

Through proper channel, Standardized plant extract of *BM* was obtained from herbal manufacturer, Natural Remedies Private Limited, India. This extract was manufactured from the dried aerial parts of *BM* from India. We used 180 ml of 95% ethanol for 3 days at room temperature and the resulting extract was processed and used. The plant extract was administered by using an oral feeding needle attached to a syringe. During this procedure if there is any resistance or the animal struggles, the gavage needle was immediately withdrawn, and another attempt was made. It was made sure those gavage volumes did not exceed 1% of the body weight of the rats.

2.5 Blood Collection and Hormonal Assay

Under aseptic conditions, animal's blood was collected from the internal jugular vein using a 21-gauge 5ml syringe, in the morning time between 8.00 AM and 9.00 AM. The blood was then transferred to a sterile plain collection tube. The blood was allowed to stand for 30 minutes to enable clot formation. Then the blood was centrifuged at 3000rpm and 1ml of serum was extracted. The serum was analyzed for corticosterone hormone. Cortisol assay was done using CLIA (Chemiluminescence) method which is competitive immunoassay using direct technology by ADVIA Centaur System.

3. RESULTS

Cortisol levels of animals in each group were tabulated in units of µg / dl. The Mean cortisol assay values are interpreted in the bar graph [Fig. 1] shows as follows group I (normal control) was 1.07, in group II (with cold stress) was 1.74, in group III (stressed then treated with saline) was 1.73, , in group IV (cold stressed, treated with low dosage of 40 mg /kg of *BM*) was 1.27 and in group V (cold stressed, treated with high dosage of 80 mg /kg of *BM*) was 0.93. in group VI (stressed then treated with short term supplement, two-weeks of omega3 fatty) was 1.55 and in group VII (stressed then treated with long term supplement, four-weeks of OFA) was 0.93. It is evident from the above data that there is increase in the mean cortisol value in group II and Group III when compared with group I which indicates that blood cortisol level in the stressed groups is elevated.–Furthermore, the value was decreased in both low and high dosage treated with *BM*. Likewise, the value was also decreased in both short term and long-term therapy of OFA treatment too [Table 1]. When comparing the mean cortisol value between the groups and within the groups by using one-way anova test showed that there is statistically significant difference ($P<0.001$). [Table 2].

4. DISCUSSION

Neuroscientists have discovered chronic *stress* and cortisol *can damage* the *brain*. Earlier studies established that continuous cold exposure for only one week does not result in sensitization and so in this study protocol the stress period for one month was used, which is chronic and so have the effect on the neurons. One of the more striking findings in the earlier studied was prolonged exposure to stress over the course of twenty-one days produces release of glucocorticoids, which could cause atrophy of dendritic branches in pyramidal neurons of rat hippocampus [3]. In the present study, rats were exposed to stressor in which they repeatedly forced to swim in cold water once daily in the morning till it began to sink. Initially they began to sink within few minutes, but later it took approximately twenty minutes to sink. "The possible reason could be, they might begin to adapt to the situation. In this study the temperature of the water was maintained at 18±2^{o} C as earlier studies demonstrated that under two stress conditions, high stress (cold water, 19 °C); and low stress (warm water, 25 °C) and chronic exposure to cold enhances the

release of noradrenalin from nerve terminals in the hippocampus" [4].

"Bacopa has been studied extensively in animal models and *in vitro* as it is implicated in the treatment of neurodegenerative disorders. In the present study we focused on both low dose and high dosage impact on cortisol hormone in stress. In the earlier studies by researches they used twenty-four rats were divided into two, four and six week treatment groups and then further divided into three dosage groups of 20, 40, and 80 mg/kg with age matched controls. The results indicated an improvement in spatial learning and memory retention by various behavioural tests and a significant enhancement in dendritic length and branching within the amygdala at the doses of 40 and 80 mg/kg at the four and six week intervals. The lower dosage of 20 mg/kg had significant effects only at the 6-week mark, whereas the two higher dosages began to show significance as early as 2 weeks. For this reason, the present study we used low and high dose as 40 and 80 mg/kg respectively" [5,6].

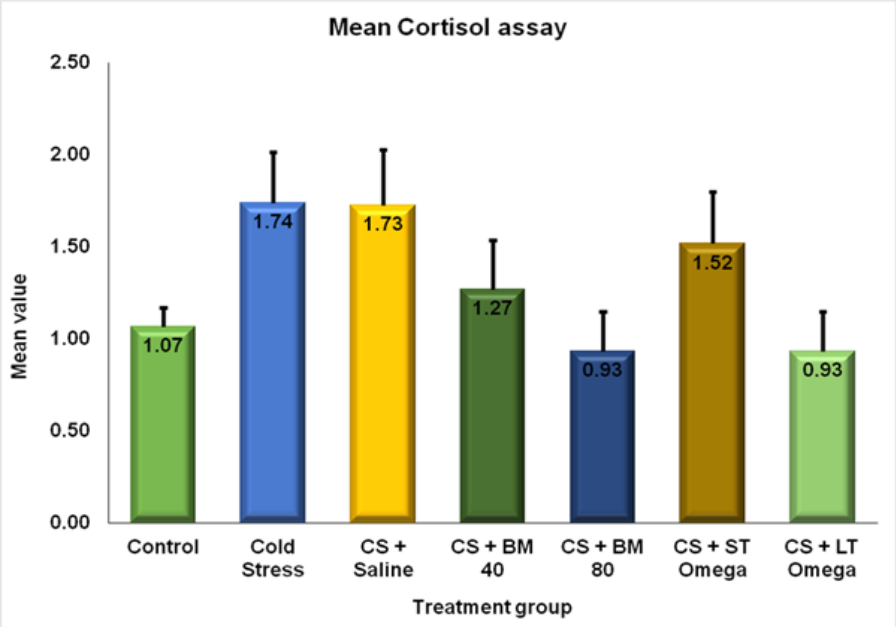

Fig. 1. Group 1= control;Group II= Cold stress (CS);Group III CS treated with saline; *Group IV = CS treated with Bacopa monneria (BM) 40mg/kg; Group V = CS treated with BM 80mg/kg; Group VI CS treated with short term (ST) [2 weeks] administraction of OFA; Group VII = cold stress treated with long term (LT) [4 weeks] administraction of OFA.*

Table 1. Group = Effect of BM and OFA

Treatment group	N	Mean	Std. Deviation	F-Value	P-Value
Control	6	1.0667	.10250	12.435	<0.001
Cold Stress	6	1.7383	.27469		
Cold Stress + Saline	6	1.7250	.29938		
Cold Stress + BM 40	6	1.2683	.26701		
Cold Stress + BM 80	6	.9333	.21612		
Cold Stress + ST Omega	6	1.5167	.27926		
Cold Stress + LT Omega	6	.9333	.21612		
Total	42	1.3117	.39969		

Table 2. ANOVA

Sum of Squares		df	Mean Square	F-Value	P-Value
Between Groups	4.459	6	.743	12.435	<0.001
Within Groups	2.091	35	.060		
Total	6.550	41			

“In the present study cortisol level was estimated during stress and after the treatment with Bacopa. Concisely, stress reactions commence with establishment of the hypothalamic-pituitary-adrenal (HPA) axis. In the present study cold stress groups have elevated cortisol level, possibly the activity of the hypothalamic-pituitary-adrenal axis would have increased resulting in higher glucocorticoid levels, as seen in depression” [5,7]. Providentially, our current study treatments with low and high dosage of bacopa have resulted in decreased level of cortisol when compared with stressed groups with no treatment or saline treated. The cortisol levels were almost closer when compared with that of the normal control. Thus, clearly indicating that compounds and their derivatives formed when bacopa is consumed, crossing the blood brain barrier. Similar to the present study, there are vast number of studies using Bacopa evidently indicated the changes in the brain [8,6].

“Earlier cod-liver oil was ingested mainly by children with the usual dose being a teaspoon. In the present study we used OFA dosage for therapy was 60 mg/Kg [Docosahexaenoic acid 24 mg/Kg and Eicosapentaenoic acid 36 mg/Kg] administered orally, two weeks for short-term model and four weeks for long term model respectively. Similar to our study researchers proved that amnesia induced by pre-training scopolamine administration was significantly decreased in rats which had previously received 60 mg/Kg of omega-3 once daily for 2 weeks or 8 weeks” [9,8]. “In another experiment long-term treatment with low doses of omega-3 fatty acids suggested a suitable treatment for amnesia. Furthermore, study on animals fed a low omega-3 fatty acid diet has clearly shown improvement in behavioural disorders with deficient animals. On the other hand, omega-3 supplementation significantly decreased cortisol levels in the stressed rats. This suggests that omega-3 supplementation prevents hyperactivation induced by stress, thus decreasing the effects of corticosteroids on dendritic morphology” [7,10].

“Preclinical and clinical studies support the use of omega-3 supplementation in stress-related disorders such as depressive and anxiety disorders the reason being less movement of solutes across the blood-brain barrier, because OFA could reduce activities of the enzymes together with increased production of free radicals due to stress. In humans, a high dose of about 10 gm OFA lowered the low-density lipoprotein whereas lower doses do not” [11,12].

“Researchers conducted to compare high doses of fish oil (9.6 g/day) to placebo and standard antidepressant therapy in patients with major depressive disorder results showed that omega-3 fish oil capsule contained 440 mg EPA and 220 mg DHA had a significantly decreased score compared to those on placebo. Thus, relatively high dose of fish oil was well tolerated and has no adverse effects” [13].

“Comparable results have been reported earlier using stress paradigms, diets enriched with poly unsaturated fatty acids, particularly the omega-3 family, decreased both adipose tissue mass and plasma leptin levels in rats. On the other hand, omega-3 supplementation significantly decreased cortisol levels in the positive control group rats. This suggests that omega-3 supplementation prevents hyper activation induced by stress, decreasing the effects of corticosteroids on dendritic morphology and neuronal activity of the basolateral amygdala as described in earlier studies” [14,15,16]. Thus, the present findings of demonstrate that OFA supplementation in a long-term therapy with low dose possibly produces anti-stress effects, by improving memory tasks, decreasing plasma cortisol levels.

5. CONCLUSION

Results for phase I experiments with BM showed treatment with BM cause low blood cortisol level. High dosage of BM 80 mg/kg was better when compared with the low dose of BM 40 mg/kg. Phase II experiment showed long term omega-3 fatty acids is significant when compare with short term therapy. Taken together, comparing *BM and* OFA, high dosage of BM and long-term therapy of OFA was found to show better response in reducing the blood cortisol level induced by stress.

ETHICAL APPROVAL

Before the experiment, the experimental protocol was subjected to scrutiny by an institutional Animal Ethical Committee for experimental clearance (IAEC-SU/BRULAC/RD/002/2014).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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