Uttar Pradesh Journal of Zoology

Volume 45, Issue 6, Page 72-78, 2024; Article no.UPJOZ.3323
ISSN: 0256-971X (P)

Effect of *Bacopa monniera* on the Level of Cortisol Hormone in Forced Cold Water Swim Stress Induced Wistar Albino Rats

Saravana Kumar [a++*], Atmaram Thiruvengadam [b#], Punitha Poovan [c†], Priscilla Das [a‡], Thirupathirao Vishnumukkala [d], Jagadeesh Dhamodharan [e], Gayatri Devi [f^] and Jothi Priya [f^]

[a] Faculty of Medicine, SEGi University, Malaysia.
[b] Madha Medical College, Chennai, Tamil Nadu, India.
[c] Government Dharmapuri Medical College, Tamil Nadu, India.
[d] Human Biology Division, School of Medicine, International Medical University, Malaysia.
[e] Department of Anatomy, AIMST University, Kedah, Malaysia.
[f] Indira Medical College, Tiruvallur, Tamil Nadu, India.

Authors' contributions

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

Article Information

DOI: 10.56557/UPJOZ/2024/v45i63951

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3323

Original research Article

Received: 09/01/2024
Accepted: 13/03/2024
Published: 15/03/2024

[++] Associate Professor of Anatomy;
[#] Assistant Professor;
[†] Senior Assistant Professor;
[‡] Lecturer in Community medicine;
[^] Assistant Professor of Physiology;
**Corresponding author: Email: dr.sharan_anatomist@yahoo.com;*

ABSTRACT

The human brain remains a mystery even after years of research into the biological processes underlying its specific actions. In recent years, researchers have identified several natural compounds that could potentially help to retard mental deterioration. Extracts of *Bacopa monniera,* a traditional ayurvedic medicine, have been reported to have memory-enhancing effects in animals. However, there are no studies on bp in cold stress induced neuronal changes in hippocampus have been explored. The current study examined the effects of standardized extract of *Bacopa monniera,* on Wistar rats. We divided the animals into 4 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 histological studies to study the changes in the hippocampus caused by cold stress, 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 in which cold water swim stress given for a month followed by oral administration of *Bacopa monniera,* extracts 40mg/kg treatment for a month. Group V in which cold water swim stress given for a month followed by oral administration of *Bacopa monniera,* extracts 80mg/kg treatment for a month. 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 *Bacopa monniera,* extract improves the cortisol level and helps in controlling the stress in rats.

Keywords: Bacopa; brahmi; cold stress; cortisol; hippocampus.

1. INTRODUCTION

The human brain remains a mystery today, even after years of research into the biological processes underlying its specific actions. In addition to controlling and coordinating vital life processes, the brain regulates higher level operations such as memory and cognition, functions that provide us with the capacity to think, reason and act. These mental abilities, however, have been observed to diminish significantly with advancing age as early as in the fifth decade of life, and factors such as emotional stress could precipitate these effects even earlier in life. Gradual deterioration may progress to a point where the person, often in good physical health, is unable to perform even simple daily tasks.

"Brahmi has been used by Ayurvedic medical practitioners in India for almost 3000 years. The earliest chronicled mention is in the Ayurvedic treatise, the Charaka Samhita (100 A.D.), in which Brahmi is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition and lack of concentration. According to literatures Brahmi acts as an effective brain tonic that boosts one's capabilities to think and reason. In India, Brahmi is currently recognized as being effective in the treatment of mental illness and epilepsy. Pharmacologically, it is understood that Brahmi has an unusual combination of constituents that are beneficial in mental inefficiency and illnesses and useful in the management of convulsive disorders like epilepsy" [1].

"Researchers believe that bacopa can stimulate gamma-aminobutyric acid (GABA), an amino acid that occurs in the central nervous system and that is associated with transmission of inhibitory nerve impulses. This enhanced ability to synthesize GABA leads to improved acquisition, memory, and adaptation to new conditions. Aqueous and alcoholic extracts of Bacopa administered to rats for 15 days significantly reduced mor an early study employed a sleep deprivation model to investigate the effect of Brahmi on the learning process in rats. When deprived of sleep, the levels of the stress hormone, serotonin, increased in rats. There was also an increase in glutamate levels, while GABA (gamma amino butyric acid), a chemical involved in the transmission of nerve impulses, showed marked reduction. Discrimination learning was significantly reduced following sleep deprivation stress. Brahmi significantly reduced the levels of stress hormones following sleep deprivation and improved discrimination learning in the animals. The authors concluded that Brahmi helps to regulate the altered levels of biogenic amines following stress, thereby improving learning" [2,3]. To date there is still a lacuna on the effect of BM on the cortisol level with low and high dose.

2. MATERIALS

2.1 Animal Model

For this experiment thirty male Wistar albino rats weighing 150-180 gms 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). Three rats were housed in a polypropylene cage under standard laboratory conditions with food and water provided ad libitum.

2.2 Experimental Design

"A total of thirty rats were divided into five 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 histological studies to study the changes in the hippocampus caused by cold stress, 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 in which cold water swim stress given for a month followed by oral administration of BM extracts 40mg/kg treatment for a month. Group V in which cold water swim stress given for a month followed by oral administration of BM extracts 80mg/kg treatment for a month. The total study was carried out for a period of sixty days" [4].

3. METHODOLOGY

3.1 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 to obtain the maximum percentage of cell degeneration in the CA1, CA3, and Dg region.

3.2 Extraction and Administration of *Bacopa monniera*

"Through proper channel, Standardized plant extract of BM was obtained from herbal manufacturer, Natural Remedies Private Limited, India. This ethanolic extract was manufactured from the dried aerial parts of BM from India. The plant extract obtained by the manufacturer was administered 40 mg/kg and 80 mg/kg for the respective groups orally by calculating the dosage with the body weight of the rats, using an oral feeding needle attached to a syringe. The distance from the oral cavity to the end of the xiphoid process was measured with the feeding needle, before performing the oral dosing procedure. The feeding needle glided down the esophagus with gravity alone and there was no resistance when passing the feeding needle" [4].

3.3 Blood Collection and Hormonal Assay

Under aseptic conditions, 5ml of animal's blood was collected from the internal jugular vein using a 21-gauge 5ml syringe. 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.

4. RESULTS: CORTISOL HORMONE ANALYSIS

The blood from each animal of all the groups were collected and analyzed for cortisol assay using ADVIA Centaur System. The ADVIA Centaur cortisol assay is a competitive immunoassay using direct chemiluminescent technology. 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 (cold stressed- negative control) was 1.74, in group III (cold stressed, treated with saline (placebo)- negative control) was 1.73, in group IV (cold stressed, treated with low dosage of 40 mg /kg of *Bacopa monniera* - positive control) was 1.27 and in group V (cold stressed, treated with low dosage of 80 mg /kg of *Bacopa monniera*- positive control) was 0.93. It is evident from the above data that there is an 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 dosage and high dosage of *Bacopa monniera* treatment positive control groups when compared with negative control groups. The mean cortisol value of group V that is treated

with high dosage of 80mg/kg *Bacopa monniera* was decreased when compared to that of the normal control group I which implies that treatment with high dose of *Bacopa monniera* certainly lowers the cortisol level there by reduces the stress. One way ANOVA test (Table 2 and Table 3) showed that there is statistically significant difference (F= 14.099, P<0.001). Tukey's HSD (Table 4 and Table 5) procedure was used for post-hoc pairwise comparisons between groups, which showed that treatment groups both low and high dosage of *Bacopa monniera* are statistically significant when compared to negative control stress groups.

The mean values of mean cortisol assay represented in µg/dl. Given are Group 1= control, Group II= Cold stress (CS), Group III Cold stress treated with saline, Group IV = cold stress treated with low dose *Bacopa monniera (BM)* 40mg/kg, Group V= cold stress treated with high dose *Bacopa monniera (BM)* 80mg/kg.

Table 1. Cortisol Assay – Advia centaur system

S.No	Animals	Group - I	Group - II	Group - III	Group - IV
1.	No.1	0.94	2.22	1.14	1.26
2.	No.2	1.21	1.74	0.84	1.60
3.	No.3	1.13	1.54	1.23	0.91
4.	No.4	1.01	1.86	0.64	1.32
5.	No.5	0.99	1.47	0.85	1.50
6.	No.6	1.12	1.60	0.90	1.02

The above result gives the values of cortisol levels of animals of each group, tabulated in units of µg / dl.

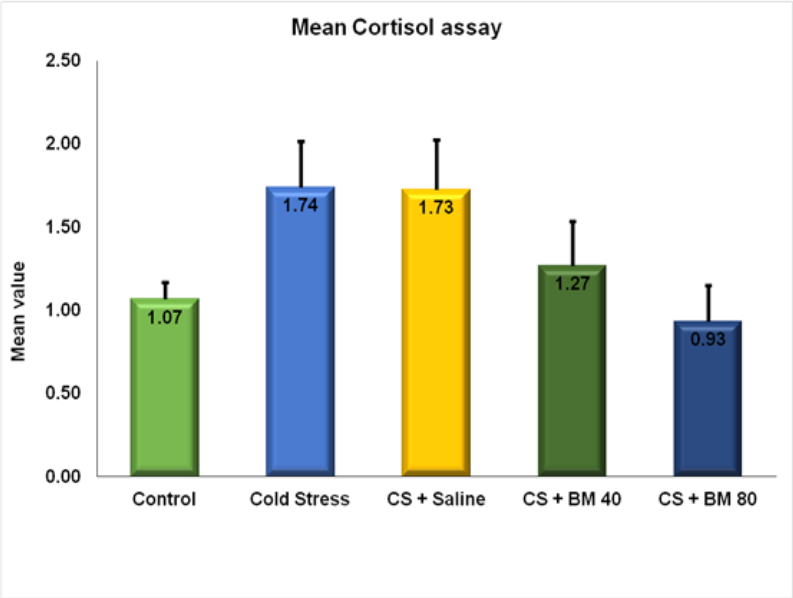

Fig. 1. Mean cortisol assay

Table 2. One way ANOVA to compare mean cortisol assay between treatments. Group = Effect of BM

Treatment group	N	Mean	Std. Dev	F-Value	P-Value
Control	6	1.0667	.10250	14.099	<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		
Total	30	1.3463	.40597		

Table 3. ANOVA table

Sum of Squares		df	Mean Square	F-Value	P-Value
Between Groups	3.312	4	.828	14.099	<.001
Within Groups	1.468	25	.059		
Total	4.779	29			

Table 4. Tukey HSD post Hoc tests for multiple comparisons

Treatment group		Mean Difference	P-Value
Control	Cold Stress	-.67167	0.001
	Cold Stress + Saline	-.65833	0.001
	Cold Stress + BM 40	-.20167	0.608
	Cold Stress + BM 80	.13333	0.873
Cold Stress	Cold Stress + Saline	.01333	0.999
	Cold Stress + BM 40	.47000	0.019
	Cold Stress + BM 80	.80500	<0.001
Cold Stress + Saline	Cold Stress + BM 40	.45667	0.024
	Cold Stress + BM 80	.79167	<0.001
Cold Stress + BM 40	Cold Stress + BM 80	.33500	0.150

Table 5. Homogeneous subsets

Treatment group	N	Subset	
		1	2
Cold Stress + BM 80	6	.9333	
Control	6	1.0667	
Cold Stress + BM 40	6	1.2683	
Cold Stress + Saline	6		1.7250
Cold Stress	6		1.7383

5. DISCUSSION

Neuroscientists have discovered chronic stress and cortisol can damage the brain. Stress is competent to effects on memory tasks whereas chronic stress can impose structural damage to hippocampal subfields [5]. In the present study the stressor that was used has complex tasks, consisting of forced water swim test, chronic cold exposure, relocation from room to another experimental room etc. We have concentrated on cold water as a chronic stressor because the alterations in noradrenergic functions that occur in rats after exposure resemble those observed in humans afflicted with brain disorders. The prelude exposure to the cold temperature itself is the cause for the alterations in the brain function, like the study that explains chronic or repeated stress can cause a wide range of physiological and neuroendocrine changes [5,7,8]. The study data indicated that swim durations for all animals were greatest when water temperature was at or slightly below normal core body temperature. When the temperature drops below 18^0C, the body may not be warm by itself, and so serious illness and tissue damage can damage can occur. In this study the temperature of the water was maintained at 18±2° C, that was considered to cause high stress, in which spatial learning was studied under two stress conditions, high stress (cold water, 19 °C); and low stress (warm water, 25 °C). Furthermore, chronic exposure to cold also enhances the release of noradrenalin from nerve terminals in the hippocampus [9,10,11].

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 [12]. “Additionally, chronic cold stress diminished the regulatory functions. The forced water swimming test introduced by has now become a widely accepted stressor model for studying physical stress in animals. It was designed as a primary screening test for antidepressants and became very popular because of low-cost, fast, and reliable model to test potential memory enhancing treatments with

a strong predictive validity" [13,14]. "In a study, the effect of Bacopa monniera on the dendritic morphology of neurons in the amygdala, a region implicated in learning and memory using three dosage groups of 20, 40, and 80 mg/kg. The results indicated an improvement in spatial learning and memory retention in the amygdala region at the dosage of 40 and 80 mg/kg. The lower dosage of 20 mg/kg does not had significant effects, whereas the two dosages began to show significance" [15]. Based on this earlier study, we have decided to use 40 and 80 mg/kg of Bacopa monniera.

The present study cortisol level was estimated during stress and after the treatment with Bacopa. The mechanism that exists between stress and cortisol, concisely the stress reactions commence with establishment of the hypothalamic-pituitary-adrenal (HPA) axis. "Hypothalamus release corticotropin-releasing hormone (CRH), which passes to the anterior pituitary gland, in turn stimulating the release of adrenocorticotropic hormone (ACTH) directly into the blood stream. This hormone then travels through the blood to cortex of adrenal gland to release the cortisol. This hormone release leads to favorable short-term responses. While long-term exposure to cortisol can cause harmful effects to the hippocampus" [14-16].

"In the present study cold stress groups have elevated cortisol levels, possibly the activity of the hypothalamic-pituitary-adrenal axis would have increased resulting in higher glucocorticoid levels, as seen in depression. This was like various studies that explain brain regions involved in the stress response, including the amygdala and hippocampus, modify activity of the hypothalamic-pituitary-adrenal axis" [4]. "While cortisol is vital for the body's response to stress, it's important that relaxation reaction to be activated so the body's functions can return to normal following a stressful event. Providentially, our current study treatments with low and high dosage of bacopa have resulted in decreased level of cortisol when compared with negative control" [15]. 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. Like the present study, there are vast number of studies using Bacopa evidently indicated the changes in the brain. The effect on upregulating antioxidant effect in the brain hippocampus, enhancing cerebral blood flow, protecting against neurotoxicity clearly show that bacosides, the active ingredient of bacopa have activity and cross the blood brain barrier. Finally, cortisol participates in inhibitory feedback by blocking the secretion of corticotrophin-releasing hormone, consequently preventing the HPA axis from occurring. Many neurobiologists hypothesize that chronic levels of high stress disrupt the feedback loop, resulting in the breakdown of feedback inhibition to control and the sustained release of cortisol. Higher and more prolonged levels of cortisol in the bloodstream have been shown to have negative effects including impaired cognition [16].

6. CONCLUSION

To conclude, the present findings clearly demonstrate that *Bacopa monniera* extracts administered orally could possibly cause anti-stress effects, thereby decreasing plasma cortisol level and anxiety in forced cold water swim stressed rats. However, the limitations of this study could be its effect with lower dosage and its effects on various other stressors.

ETHICAL APPROVAL

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.

REFERENCES

1. Chaudhari KS, Tiwari NR, Tiwari RR, Sharma RS. Neurocognitive effect of nootropic drug Brahmi (Bacopa monnieri) in Alzheimer's disease. Ann Neurosci. 2017; 24:111–22.
2. Jain PK, Das D, Jain P, Jain P, Jain PK. Pharmacognostic and pharmacological aspect of Bacopa monnieri - a review. Innovare J Ayurvedic Sci. 2016;4: 796-11.
3. Kumar SK, Perumal S, Rajagopalan V. Therapeutic effect of bone marrow mesenchymal stem cells on cold stress induced changes in the hippocampus of rats. NeuralRegen Res. 2014; 9:1740-1744.

4. Kumar SS, Saraswathi P, Vijayaraghavan R. Effect of bacopa monniera on cold stress induced neurodegeneration in hippocampus of wistar rats: a histomorphometric study. Journal of clinical and diagnostic research: JCDR. 2015 ;9(1):AF05.
5. Kumar SS, Devi G, Renuga, Balima NS. Effect of Short- and Long-Term Omega-3 Fatty Acids on Cold Stress Induced Neuronal Changes in Hippocampus of Wistar Rats. J Neurol Sci 2014;31:672-679.
6. Kumar SS, Saraswathi P, Vijayaraghavan R. Effect of Bacopa monniera on cold stress induced neurodegeneration in hippocampus of Wistar rats: a histomorphometric study. J Clin Diagn Res. 2015;9(1):AF05–AF07.
7. Murthykumar K, Kumar SKS. Comparative Study on Effect of Omega-3 Fatty Acid and Bone Marrow Mesenchymal Stem Cells on Cold Stress Induced Neuronal Changes inthe Hippocampus of Rats. Int J Pharm Sci Res. 2016;7(10): 4152-56.
8. Praagh Van, Schinder Alejandro F, Christie Brian R, Toni Nicolas, Palmer Theo, Gage Fred. Functional Neurogenesis in the Adult Hippocampus. Nature. 2002;415:1030–34.
9. RosheneR, Saravanakumar S, Stem Cells on Hippocampus – A Short Review. J Pharm Sci Res 2015; 7(6): 377-380.
10. Russo A, Borrelli F. Bacopa Monniera, a Reputed Nootropic Plant: An Overview. Phytomedicine. 2005;12:305–17.
11. Saraswathi, K. Balaji, Julie Christy, S. Saravanakumar, R. Vijayaraghavan, Musculoskeletal Trauma Treated with Bone Marrow Mesenchymal Stem Cells in Wistar Albino Rat. Sch J App Med Sci. 2015;3(2D):768-772.
12. Saravana Kumar, Guru B. Bhuvanalakshmi, Mangala Gowri, Perumal Saraswathi., Morphological Comparison of Bone Marrow Derived Mesenchymal Stem Cell from Albino Rats and Indian Chicken. Eur J Anat. 2013; 17(1):17-22.
13. Savitha P, Saravana Kumar S. Effect of Omega-3 Fatty acids on Memory – Review. Res J PharmTech. 2014; 7(6):715-718.
14. Swarna Meenakshi, Saravana Kumar. Neurocognitive Enhancement. Int J Pharm Tech Res. 2014;5:1546- 1548.
15. Vollala VR, Upadhya S, Nayak S. Enhancement of basolateral amygdaloid neuronal dendritic arborization following Bacopa monniera extract treatment in adult rats. Clinics (Sao Paulo). 2011; 66(4):663-71.
16. Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. Front Ag Neurosci. 2010;2:12.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3323