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Evaluation of Antistress, Anxiolytic and Hypnotic Activity of Vedic Calm, A Polyherbal Formulation

Bharathi KN*, Sivaramaiah N, Chowdary G Nagarjuna, Gupta AVSSS

Department of Pharmacology Visveswarapura Institute of Pharmaceutical Sciences, 22nd Main, 24th Cross, B.S.K II stage, Bangalore-560070, India.

**** Corresponding author:*** *knbharathijagan@yahoo.com*

ABSTRACT

The present study was undertaken to evaluate antistress, annxiolytic and hypnotic activity of Vedic calm, a polyherbal formulation comprising of *Bacopa monnieri, Centella asiatica, Evolvulus alsinides* and many other related plants extracts, all of which are classified in ayurveda as rasayanas which are reported to promote physical and mental health and also possess depressant activity. Antistress activity was evaluated by cold immobilization induced stress for 10 days in rats using *Withania somnifera* (100 mg/kg) as reference standard. Antianxiety activity was evaluated by elevated plus maze and light and dark box in acute study and elevated plus maze in chronic study of 10 days in rats, using diazepam (2 mg/kg) as reference standard. Hypnotic activity was evaluated by potentiation of thiopental induced sleeping time in mice. Stress was evidenced by occurrence of gastric ulcer, elevation of adrenals weight, liver weight, serum glucose, AST, ALT, cholesterol, WBC and reduction of spleen weight. Vedic calm pretreatment in rats with doses of 135 mg/kg, 270 mg/kg *p.o*. and standard drug *Withania somnifera* significantly reversed all the changes those were due to stress. The Vedic calm at 270 mg/kg significantly increased the time spent and number of entries into open arms in elevated plus maze and increased the time spent and number of entries into light box in light and dark box model. The Vedic calm at a dose of 390 mg/kg significantly potentiated duration of thiopental induced sleeping time in mice. The Vedic calm showed significant antistress, anxiolytic and hypnotic activity.

KEYWORDS: Antistress, Anxiolytic, Cold immobilization, Elevated plus maze, Hypnotic activity, Vedic calm.

INTRODUCTION

Stress is a response to physical, chemical, biological and emotional changes, consisting of a pattern of metabolic and behavioral reactions that helps in strengthening the organism (1). Stress happens whenever mind and body react to some real or imagined situation. Situations that cause stress reactions are called stressors. Homeostatic mechanisms are geared towards counteracting the everyday stresses of living. If they are successful, the internal environment maintains normal physiological limits of chemistry, temperature and pressure. If stress is extreme, unusual or long lasting, however, the normal mechanisms may not be sufficient. In this case the stress triggers a wide-ranging set of bodily changes, called the General Adaptation Syndrome (GAS). Some stress is beneficial and other types of stress, particularly if prolonged, can fatigue or damage the system to the point of malfunction or diseases. Stress has been defined as "the pattern of physiological reactions that prepares an organism for action" (2). Stress has been postulated to be involved in pathogenesis of variety of diseased states, for e.g., psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male impotency, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis. Allopathic drugs are available for counteracting the stress, but the side effects and cost associated with these allopathic

drugs necessitates the search for an alternative, which are with out side effects. In due regard Vedic Bio Labs, Bangalore has developed a polyherbal formulation, Vedic Calm, each 500mg capsule consisting of dried powder extracts of plants such as *Bacopa monnieri* (80 mg), *Centella asiatica* (50 mg), *Evolvulus alsinides* (50 mg), *Nardostacyhs jatamansi* (80 mg), *Rauwolfia serpentine* (50 mg), *Terminalia belerica* (20 mg), *Papaver somniferum* (20 mg), *Celastrus paniculatus* (50 mg), *Myristica fragrans* (10 mg), *Asphaltum* (10 mg), *Withania somnifera* (30 mg), *Sida cordifolia* (20 mg), *Acrous calamus* (10 mg), *Valeriana wallichi* (10 mg), *Mica* (10 mg), *Nelumbium speciosum* (Q.S), *Rosa centifolia* (Q.S). Vedic calm is already available in the market as anti stress capsules to reduce tension and induce sleep naturally in Ayurvedic system of medicine, but it lacks preclinical evidence. Hence, the present study was undertaken to evaluate antistress, anxiolytic and hypnotic activities of Vedic calm.

MATERIALS AND METHODS

Drugs and chemicals

Vedic Calm (procured on 02-08-2007 from Vedic Bio Labs Pvt Ltd, BTM, II stage, Bangalore), *Withania somnifera* (Ashwagandha capsules 250mg, Himalaya Herbal Healthcare, Bangalore), Diazepam (Nicholas Piramal India Ltd., Mumbai), Glucose, Cholesterol, AST and ALT kits (Recorders and Medicare systems (P) Ltd., Baddi), Sodium carboxy methyl cellulose (S.D fine chemicals, Mumbai).

Experimental animals

Albino Wistar rats weighing 200 ± 25 gm of both sex and Swiss albino mice weighing 20 ± 5 gm were procured from NIMHANS and Veterinary College, Bangalore. They were maintained under standard laboratory conditions (25°±2°C, relative humidity 50±15%, light and dark cycle of 12h) and fed with standard pellet diet and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethics Committee. Test doses of Vedic calm was selected for rat (135, 270mg/kg b.w.) and mice (195, 390mg/kg b.w.) based on the human dose (1500 mg/day) by using dose conversion factor based on body surface area.

Rat dose = Human dose X 0.018 … (For 200g rat)
Mouse dose = Human dose X 0.0026 … (For 20g mouse)

The dried powder extracts of polyherbal formulation was suspended in 0.5 %w/v sodium carboxy methyl cellulose and suspensions of standard drugs were prepared in the same manner.

Cold immobilization induced stress (1)

Albino Wistar rats were divided into 5 groups of 6 animals each. Group-I treated as vehicle control, Group-II treated as stress control, Group-III, Group-IV and Group-V animals were administered the Vedic calm 135 mg/kg b.w., 270 mg/kg b.w. and *Withania somnifera* 100 mg/kg b.w.,*p.o.* respectively, for 10 days, daily at 11am. Group II, III, IV, V animals were subjected to stress after 3 hr of administration of corresponding drugs, by making the animals immobilized on wooden planks by placing it on its back, with the help of a twine. The wooden planks with the animals were then placed in a cold plastic chamber maintained at 4° C for 2 hours daily. The animals were then released and placed back into their respective cages. The procedure was followed for 10 days continuously. The animals of all the five groups were fasted from the 9th day onwards. On the 10th day after 1 hour of induction of stress, blood samples were withdrawn by puncturing retro orbital plexus under light ether anesthesia for estimation of WBC count, serum glucose, AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase) and cholesterol. Glucose and cholesterol was estimated by end point method where as AST and ALT was estimated by Kinetic method using semi auto analyzer. Then the animals were sacrificed by anaesthetic ether, and the stomach was isolated immediately for measuring ulcer score. The stomach was opened by cutting through greater curvature and degree of ulceration was examined with a hand lens. The scoring system followed is zero ulcers-0, 1-2 ulcers-20, 3-5 ulcers-30, 6-8 ulcers-40 and more than 8 ulcers-50. Organs such as adrenals, spleen and liver were separated, the extraneous matter was removed and weighed immediately to avoid weight loss (3–5).

Elevated plus maze model

Albino Wistar rats were divided into 4 groups of 6 animals each. Group-I animals were administered the vehicle, Group-II, Group-III and Group-IV animals were administered the Vedic calm 135 mg/kg b.w., 270 mg/kg b.w. and diazepam 2 mg/kg b.w. *p.o.* respectively, single dose-only once in acute study and single dose for 10 days in case of chronic study. The experiment was conducted in a sound attenuated room. In acute study the animals of all groups were treated with respective drugs 30 min. prior to the experiment. In chronic study animals of all groups were treated with respective drugs for 10 days and on 10th day, the treatment was given 30 min. prior to the experiment. In both acute and chronic studies, during experiment each rat was placed in the center of the maze facing one of the closed arms and observed for ten minutes session. Number of entries into open arm,

number of entries into closed arm, time spent in the open arm, time spent in the closed arm and total number of entries in open and closed arm were recorded (6–10).

Light and dark box model

Albino Wistar rats were divided into 4 groups of 6 animals each. Group-I animals were administered the vehicle, Group-II, Group-III and Group-IV animals were administered the Vedic calm 135 mg/kg b.w., 270 mg/kg b.w. and diazepam 2 mg/kg b.w. *p.o.* respectively. The experiment was conducted in a sound attenuated room. The animals of all groups were treated with respective drugs 30 min. prior to the experiment and during the experiment the rat was placed in the illuminated part of the box. The following parameters were recorded during the test session of 5 min, number of crossings between the light and dark area, total time spent in the light box and rearings in Light box (10).

Potentiation of thiopental induced sleeping time

The male Swiss albino mice were divided into 4 groups of 6 animals each. Group-I animals were administered the vehicle, Group-II animals were administered the thiopental sodium (25 mg/kg b.w. i.v) only, Group-III and Group-IV animals were administered the Vedic calm, 195 mg/kg b.w. and 390 mg/kg b.w. *p.o.* respectively 30 min prior to thiopental sodium (25 mg/kg b.w. i.v). After the loss of righting reflex the animals were placed on their back. The time period of loss of righting reflex and regaining of righting of reflex were recorded from the time of administration of drug. Duration of sleep was determined and compared between the groups (11).

Statistical Analysis

The values were expressed as mean ± standard error of mean (SEM). The results were subjected to statistical analysis by using one- way ANOVA followed by Tukey-Kramer test to calculate the significance difference if any among the groups. $P<0.05$ was considered as significant.

RESULTS

As shown in Table 1, in cold immobilization induced stress model, the biochemical parameters such as glucose, cholesterol, AST, ALT and WBC Count, weight of adrenal gland, liver, and gastric ulceration score were increased, with decrease in spleen weight, in stress control animals as compared to control animals. Pretreatment with Vedic calm and standard (*Withania somnifera*) drugs significantly reversed the all above said parameters.

In acute and chronic study of elevated plus maze model (Table 2 and 3) the number of entries into open and closed arm, time spent in open arm were increased and time spent in closed arm were decreased in Vedic calm treated animals when compared to control animals, which is comparable with that of reference standard, diazepam. Diazepam at a dose of 2 mg/kg b.w. does not inhibit motor activity. In light and dark box model (Table 4.) the number of entries between light and dark compartments and the time spent in light compartment, were increased significantly in Vedic calm and standard treated animals compared to control animals.

As seen from Table 5, there was significant increase in duration of thiopental induced sleep in Vedic calm pretreated animals when compared to only thiopental treated group animals.

DISCUSSION

In the present study, antistress activity was studied on both physical stress and psychological stress of chronic nature. There was a significant increase in blood glucose level in stress induced animals compared to normal control animals because, under stressful conditions adrenal cortex secretes cortisol in man and corticosterone in rats. Hyper secretion of cortisol helps in maintainance of internal homeostasis through the process of gluconeogenesis and lipogenesis (1). After treatment with the Vedic calm at dose of 135 mg/kg b.w. significantly ($P<0.01$) reduced the elevated glucose levels, Vedic calm at dose of 270 mg/kg b.w. and standard (*Withania somnifera*) also significantly ($P<0.001$) reduced the elevated glucose levels when compared with the stress control animals. This may be due to inhibition of the corticosterone secretion.

The significant raise in serum cholesterol in stress induced animals when compared to normal control animals, due to enhanced activity of hypothalamohypophyseal axis resulting in increased liberation of catecholamines and corticosteroids, which facilitates lipolytic effect of other agents such as growth hormone resulting in lipogenesis (1), (12). After treatment with the Vedic calm at dose of 135 mg/kg b.w., 270 mg/kg b.w. and standard (*Withania somnifera*) significantly ($P<0.001$) reduced the elevated cholesterol levels when compared with the stress control animals, may be due to inhibiting the activity of hypothalamohypophyseal axis.

The marked increase in serum AST, ALT levels in stress induced animals due to the reason that stress induced hypothalamo-pitutary axis (HPA) and sympathetic system stimulation resulting in liberation of catecholamines and glucocorticosteroids, which inhibits the immune system at multiple sites like liver, kidney (12), since AST, ALT are

Table 1: Effect of the Vedic calm on chronic stress induced changes

	Biochemical Estimations				Organ Weights/100 gm Body weight				
Groups	Glucose (mg/dl)	AST IU/L	ALT IU/L	Cholesterol (mg/dl)	Adrenal gland weight (mg/100gm body weight)	Spleen weight (mg/100gm body weight)	Liver weight (gm/100gm body weight)	W.B.C Count (Cells/Cu.mm)	Gastric Ulceration Score
Control	86.21 ± 1.92	84.68 ± 0.97	76.73 ± 0.75	45.25 ± 1.01	38.83 ± 2.70	204.66 ± 1.30	3.18 ± 0.05	7033 ± 316.93	0.00 ± 0.00
Stress Control	127.10 ± 0.96+++	134.16 ± 1.84+++	133.26 ± 1.91+++	72.90 ± 0.36+++	64.5 ± 1.23+++	135.53 ± 1.45+++	3.77 ± 0.02+++	10350 ± 225.46+++	45.00 ± 2.23+++
Vedic calm (135 mg/kg)	118.33 ± 1.20**	124.40 ± 1.36***	115.75 ± 1.46***	66.70 ± 0.77***	56.5 ± 1.87*	157.33 ± 1.97***	3.69 ± 0.08	9183 ± 163.33**	38.33 ± 1.66*
Vedic calm (270 mg/kg)	103.85 ± 1.76***	104.53 ± 1.28***	98.46 ± 1.94***	55.33 ± 1.22***	53.16 ± 1.83**	178.50 ± 3.231***	3.50 ± 0.05*	7450 ± 158.64***	30.00 ± 0.00***
Standard *Withania somnifera* 100mg/kg	96.4 ± 1.74***	98.8 ± 1.66***	90.91 ± 1.00***	52.33 ± 1.07***	48.6 ± 1.56***	185.33 ± 1.94***	3.35 ± 0.06***	6983 ± 134.58***	25.00 ± 2.23***

Values are expressed as mean ± SEM, One way ANOVA followed by Tukey's multiple comparison test.

+++P< 0.001 Vs Control.

***P< 0.001 Vs Stress control.

*P<0.05 Vs Stress control.

**P< 0.01 Vs Stress control

Table 2: Effect of Vedic calm on number of entries and time spent in elevated plus-maze in acute study.

		Elevated Plus Maze				
		NUMBER OF ENTRIES (COUNTS/10 min)			TIME SPENT IN Sec./ 10 min.	
S.NO	TREATMENT	OPEN ARM	CLOSED ARM	TOTAL No. OF ENTRIES	OPEN ARM	CLOSED ARM
1	CONTROL (0.5% sodium CMC)	1.83 ± 0.30	2.66 ± 0.33	4.66 ± 0.61	13.83 ± 3.13	586.16 ± 3.13
2	VEDIC CALM (135 mg/kg, p.o)	4.16 ± 0.40**	4.50 ± 0.22**	9 ± 0.81**	61.83± 9.18**	538.16 ± 9.18**
3	VEDIC CALM (270 mg/kg, p.o)	5.33 ± 0.33***	5.33 ± 0.33***	10.66 ± 0.61***	87.83 ± 11.52 ***	511.83 ± 11.51***
4	DIAZEPAM (2 mg/ kg)	5.5 ± 0.42***	5.83 ± 0.30***	11.33 ± 0.71***	101 ± 9.30***	499 ± 9.30***

All values are given in mean ± SEM, n = 6. One way ANOVA followed by Tukey's multiple comparison test.

***P< 0.001,

**P< 0.01, Vs control.

Table 3: Effect of Vedic calm on number of entries and time spent in elevated plus-maze model in chronic study.

		Elevated Plus Maze				
		NUMBER OF ENTRIES (COUNTS/10 min)			TIME SPENT IN Sec./ 10 min	
S.NO	TREATMENT	OPEN ARM	CLOSED ARM	TOTAL No. OF ENTRIES	OPEN ARM	CLOSED ARM
1	CONTROL (0.5% sodium CMC)	2 ± 0.25	3 ± 0.25	5.1 ± 0.51	17.66 ± 3.91	582.33 ± 3.91
2	VEDIC CALM (135 mg/kg, p.o)	3.66 ± 0.21***	4 ± 0.25	7.66 ± 0.33**	66.66 ± 6.63**	533.33 ± 6.63**
3	VEDIC CALM (270 mg/kg, p.o)	4.66 ± 0.21***	5.33 ± 0.33***	10 ± 0.51***	96 ± 14.16 ***	504 ± 14.16***
4	DIAZEPAM (2 mg/kg)	5.66 ± 0.33***	6.16 ± 0.30***	11.83 ± 0.60***	105.5 ± 7.90***	494.5 ± 0.60***

All values are given in mean ± SEM, n = 6. One way ANOVA followed by Tukey's multiple comparison tests.
***P< 0.001,
**P< 0.01 Vs control.

Table 4: Effect of Vedic calm on number of entries, time spent in light box and rearings in light box.

Sl. NO	TREATMENT	TOTAL NO.OF ENTRIES. Counts/ 5 min	TIME SPENT IN LIGHT BOX (Sec) / 5 min	NO. OF REARINGS IN LIGHT BOX. Counts/5 min
1.	Control	5.66±0.42	54.66±1.43	1.83±0.30
2.	Vedic calm (135 mg/kg)	7.33±0.21**	91.66± 1.56***	3.5±0.22**
3.	Vedic calm (270 mg/kg)	9.16±0.16***	112.16±2.16***	4.83±o.30***
4.	Diazepam (2mg/kg)	10±0.25***	122.3±1.47***	6±0.25***

All values are given in mean ± SEM, n = 6. One way ANOVA followed by Tukey's multiple comparison test.
***P< 0.001,
**P< 0.01 Vs control.

Table 5: Effect of thiopental and Vedic calm on thiopental induced duration of sleep in Mice.

S.No	Group	Duration of sleep in min	%of potentiation
1.	Normal Control	0 ± 0	——
2.	Thiopental (25mg/kg) i.v	22.56±0.98	——
3.	Vedic calm (195 mg/kg) + Thiopental (25 mg/ kg)	24.87±0.71	10.24%
4.	Vedic calm (390 mg/kg) + Thiopental (25 mg/ kg)	28.62±0.74**	26.86%

All values are given in mean ± SEM, n = 6. One way ANOVA followed by Dunnet's multiple comparison test.
**P< 0.01 Vs thiopental.

the indicators of liver function, as a result AST and ALT levels were increased (3), (13). After treatment with the Vedic calm at dose of 135 mg/kg b.w., 270 mg/kg b.w. and standard (*Withania somnifera*) significantly (P<0.001) reduced the elevated serum AST and ALT levels when compared with the stress control animals, may be due to inhibition of stimulation of sympathetic nervous system. Stress causes alteration in hematological parameters like increase in WBC counts (1). Treatment with Vedic calm at dose 135 mg/kg b.w. significantly (P<0.01) reduced the elevated WBC and Vedic calm at a dose of 270 mg/kg b.w. and *Withania somnifera* significantly (P<0.001) reduced the elevated WBC.

When the animals were subjected to stress, induction of gastric ulcers were observed when compared with the normal control animals. This may be due to involvement and hyper activation of Para ventricular Nucleus (PVN) in turn stimulates the paracrine system, which causes the release of histamine, there by leading to increased secretion of acids (3), (14). Vedic calm and *Withania somnifera* significantly reduced the stress induced gastric ulcers. The level of significance was high in Vedic calm higher dose and *Withania somnifera* (P<0.001) compared to Vedic calm lower dose (P<0.05). This may be due to inhibiting of hyper activation of Para ventricular Nucleus (PVN).

The increase in weight of adrenals and liver were observed in stress induced animals when compared to normal control animals may be due to the stress induced adrenomedullary response leading to increased production of corticotropic hormone that leads to increase in weight of adrenals and liver (1). After treatment with the Vedic calm at dose of 135 mg/kg, significantly reduced the elevated adrenal gland weight (P<0.05) but not liver weight. Vedic calm at dose of 270 mg/kg b.w., significantly reduced the elevated adrenal gland weight (P<0.01) and liver weight (P<0.05) and standard (*Withania somnifera*) also significantly (P<0.001) reduced the adrenal gland weight and liver weight. This may due to reversing the stress induced adrenomedullary response leading to decreased production of corticotropic hormone.

The decrease in spleen weight was observed in stress induced animals when compared to normal control animals

may be due to recruitment of lymphocytes to blood from spleen which results in squeezing of the spleen (3). After treatment with the Vedic calm at doses of 135 mg/kg b.w., 270 mg/kg b.w, and standard (*Withania somnifera*) significantly (P<0.001) increased the spleen weight, may be due to inhibition of recruitment of lymphocytes to blood from spleen.

The antistress activity of Vedic calm may be due to the presence of *Bacopa monnieri, Centella asiatica, Evolulus alsinoides* and *Withania somnifera* which individually possesses significant antistress activity.

In the evaluation of anti anxiety activity the two experimental models used in our study are elevated plus maze and light and dark box. These are based on the assumption that unfamiliar, non-protective and brightly lit environmental stress provokes inhibition of normal behaviour. This normal behavioural inhibition is further augmented in the presence of fear or anxiety like state (10).

In acute study after treatment with the Vedic calm at dose of 135 mg/kg b.w. (P<0.01), 270 mg/kg b.w. (P<0.001) and diazepam (P<0.001) significantly increased the time spent in open arm, decreased the time spent in closed arm and increased the number of entries between the arms (Table 2). This may be due to decreased fear, an increased exploratory behaviour and the behavioural disinhibitory effect of the Diazepam and Vedic calm (6). Similar findings were observed in chronic study also (Table 3). This may be due to the same mechanism as said above in acute study.

In light and dark box model, after treatment with the Vedic calm at dose of 135 mg/kg b.w., 270 mg/kg b.w. and standard (Diazepam) significantly (P<0.001) increased the time spent in light area and the number of entries between the compartments (P<0.01) (Table 4), may be due to the decreased fear, decreased aversion to bright light and increased exploratory behaviour of the animal (10).

In evaluation of potentiation of thiopental sodium induced sleeping time model, Vedic calm alone did not induce sleep at lower and higher doses but potentiated the thiopental induced sleeping time, which may be due to that the thiopental potentiates the GABA- induced chloride conductance and simultaneously depress voltage activated Ca^{2+} channels. The inhibition of these voltage Ca^{2+} channels could be the blockade of Ca^{2+} entry into presynaptic nerve terminals leading to inhibition of the release of excitatory neurotransmitters such as glutamate. This results in net reduction of excitatory synaptic transmission leads to depression which ultimately produces sleep (15). This mechanism may be further enhanced in presence of Vedic calm.

In this study results showed that the Vedic calm at lower dose (P>0.05) did not affect the thiopental induced sleeping time significantly, but higher dose (P<0.01) has significantly potentiated the thiopental induced hypnotic activity (Table 5).

The present experimental findings of both the pharmacological and biochemical parameters suggest that the herbal formulation is having antistress, anti anxiety activity and hypnotic activity, which was evidenced by potentiation of the thiopental induced sleep. Hence, the study supports the clinical use of Vedic calm. However the exact mechanism of Vedic calm as antistress, anti anxiety and hypnotic agent is not known, which requires further studies. The exact mechanism of action of Vedic calm can be established by estimating the norepinephrine, 5 Hydroxytryptamine, mono amino oxidase A and B and corticosterone levels.

CONCLUSION

Vedic calm possess significant antistress, anxiolytic and hypnotic activity, which substantiate its clinical use.

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