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Standardisation of controlled vestibular stimulation for optimal stress relief in albino wistar rats

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Abstract
Stress is a universal phenomenon to the living being. Man is in quest to relieve the stress in present era for his optimal smoothness of life. Vestibular stimulation [VS] is one among the several types of stress relieving mechanisms. Vestibular stimulation has been proven effective for its stress relieving capacity both in experimental and in human subjects. There are several methods of vestibular stimulation. In this work swinging method of VS was experimented in stressed albino wistar rats. Till date standardization of VS by swinging has not been conducted. In this experiment VS standardization was conducted by using a specially designed device aiming at maximum stress relief to the albino wistar rats. From the observation of stress markers, serum glucose and cortisol, it is seen that optimum stress relief was obtained in 0.17 frequency of swinging VS in stressed albino wistar rats.

Keywords*:* Controlled vestibular stimulation, Standardization, Wistar rats, Stress relief

1. Introduction
Stress has become an integral part of human life. SELYE [1] proposed that "any situation in life that makes demands upon our adaptive mechanism creates stress". The total mankind is behind the quest for different methods to relieve their stress. Vestibular stimulation [VS], music, yoga, tranquilizing agents are few of them. Vestibular stimulation technique in a controlled way is most recent and popularly discussed one [2]. Controlled vestibular stimulation techniques are of several types such as vertical, horizontal [3], electrical [4] etc. VS has shown many benefits including decreased self-stimulation, decreased hypersensitivity, increased postural security, increased concentration and attentiveness, increased balance, increased body awareness, calming effects, reduction of abnormal muscle tone at slow speeds and increased alertness at high speeds [5] So speed is one of the important factor which has to be considered while applying VS as it can cause both positive and negative effect in organism. But nobody has done the standardization of the procedure in terms of speed for the horizontal vestibular stimulations for optimal stress relief. This work is first one of such in albino wistar rats. This study aims to know the effect of CVS in stressed albino wistar rats as well as to standardize the CVS frequency for maximum stress relief in albino wistar rats.

2. Materials and methods
The present research experiments were carried out in adult male wistar rats weighing in the range of 180 ± 30 g, which were 60 days of age. a total of 164 albino wistar rats when considered for the experiment. Out of 164 taken only 150 were included under various groups and remaining of them were discarded from the experiments due to physical disabilities or due to death.
These rats were divided in to 5 groups. Each group included 30 animals. First Group was kept as control, without any interventions, second group was subjected to FS. And the other three Groups included rats that were subjected to FS followed by CVS of any one of the three frequencies V1, V2 or V3.
The rats were bred and maintained at the central animal research facility (Rodent house Register number: 496/01/a/CPCSEA) of the Little Flower Medical Research Centre (LFMRC), Angamaly. Rats were housed in polypropylene cages (30 x 22 x 14 cm). Paddy husk was used as bedding material, which was changed on alternate days. The colony was maintained in a well aerated room with exhaust and ceiling fans. The rats were maintained at 12 hrs light-dark cycle. The room temperature was 28 ± 4 °C. Three to four rats were housed in each cage. The rat diet included rat chow obtained from the local market and multivitamin syrup was provided along with drinking water (1 ml syrup/100ml water). Each adult rat was provided

approximately 25 g of rat chow per day, the pregnant and lactating mothers were given excess amount as required. All animals received food and water ad libitum. Forced swimming stress was given to the animals as per the method of Ferry A, B.Weill-1991.

LFMRC has devised a new machine for providing vestibular stimulation by swinging, to provide a stress relief in albino wistar rats. This instrument was designed out of iron frame with a metallic swing with it. The swing consists of a metallic cage suspended on metallic string of length 19 cms. The cage is so devised that, only one animal can occupy in it and there will not be any space for free movement. However, the cage was sufficiently spacious not to create an entrapment stress to the animal. The device works on electricity and the frequency of oscillation can be adjusted by adjusting its frequency modulator. The movement provided by this device gives vestibular stimulation in off axis sinusoidal pitching motion. The vestibular stimulation were given to rats exposed to force swimming stress, as swimming stressed rats were found to be the most effected ones as compared to the other rats which were exposed to other stresses such as Immobilization Stress, Noise Stress and Overcrowding stress [6]. Soon after the rats were subjected to FS, they were taken out from water and dried using a towel, then they were given CVS of any one of the following frequencies such as 0.17(V1), 0.3(V2) and 0.5(V3). Serum glucose and serum cortisol were taken as the stress markers in this experiment. Serum glucose estimation was done by GOD-POD method [7]. The serum cortisol was estimated by the Rat cortisol ELISA kit purchased from bio Compare Company [8]. Insta graph pad (2013) and Microsoft excel (2007) were used for statistical analysis of the ANNOVA, Dunnet Multiple comparison test and paired 't' test respectively.

3. Results

Table 1: Effect of FS in serum cortisol level

Mean Control	Mean Test	Mean difference	t	P
2.379 ± 0.607	4.139 ± 0.515	1.761 ± 0.211	8.35	<0.001

Table 2: Effect of FS in serum glucose level

Mean Control	Mean Test	Mean difference	t	P
86.626± 19.837	133.194 ± 23.608	46.568 ± 7.395	6.30	<0.001

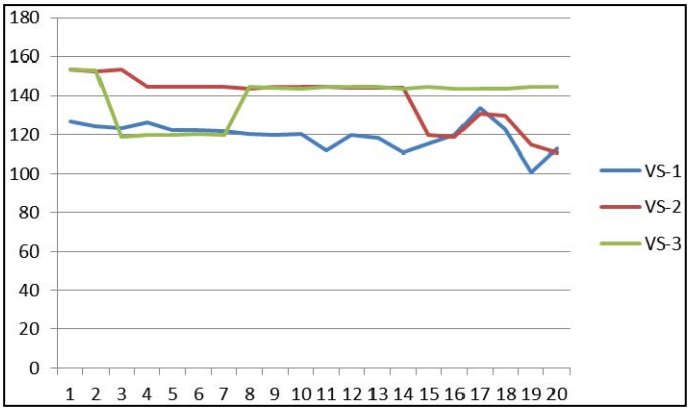

Fig 1: Results of Serum Glucose level on application of CVS

Table 3: Serum Glucose levels in stressed animals on application of different frequencies of CVS

Source of variation	df	SS	Mean square	f	P
Between the groups	3	2670.5	890.18	6.057	<0.001
Within the groups	76	11170	146.97		
Total	79	13840			

Table 4: Dunnett multiple comparison test of serum glucose values

Comparison	Mean difference	q	P value
Control Vs V-1	15.646	4.081	P<0.05
Control Vs V-2	3.908	1.019	p>0.05
Control Vs V-3	5.422	1.414	p>0.05

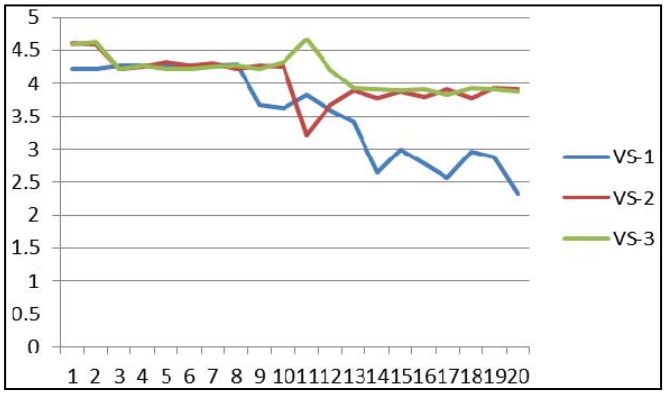

Fig 2: Results of serum Cortisol level on application of CVS

Table 5: Serum Cortisol levels in stressed animals on application of different frequencies of CVS.

Source of variation	df	SS	Mean square	f	P
Between the groups	3	4.663	1.554	6.670	<0.001
Within the groups	76	17.710	0.2330		
Total	79	22.373			

Table 6: Dunnett multiple comparison test for serum cortisol values

Comparison	Mean difference	Q	P value
Control Vs V-1	0.5695	3.731	P<0.05
Control Vs V-2	0.08600	0.5634	P>0.05
Control Vs V-3	-0.02550	0.1670	P>0.05

4. Discussion

The history of vestibular research dates back at least 400 years [9]. The speed has an important role in positive and negative effect of vestibular stimulation. Vestibular stimulation is double pronged, depending on the speed or frequency of vestibular stimulation applied; it can either excite [10] or inhibit [11] the hypothalamo pituitary adrenal cortical axis. When FS stress was applied in this experiment it showed an increase in cortisol and glucose level in a highly significant level ($p<0.001$), the elevated level of cortisol and glucose are considered as stress markers, which is due the stimulation of hypothalamo pituitary axis. When CVS was applied in different frequency the change in the values of glucose in between groups were highly significant ($p<0.001$) but in post doc analysis only the V1 group, on comparing with the control showed a significant change ($p<0.05$),which is proving the consistency of V1. Low frequency of 0.17 vestibular

stimulation showed the maximum relief which had a similarity to the previous studies conducted by electrically stimulated CVS stimulation, where the lower frequency showed the maximum relief [12]. The observation of cortisol, which is the chief stress marker [13] than the glucose, showed a similar observation as shown by glucose level. Interestingly V3 of cortisol level showed a negative value in the mean difference though it is insignificant statistically. This shows that with increased frequency, one of the important stress markers is increasing thus implicating the negative effect of higher frequency and certain negative effect of higher frequency were also reported in earlier studies [14]

Fig 3: LFMRC device for CVS

5. Conclusion

On application of FS a highly significant increase was seen in both glucose and cortisol levels of albino wistar rats, thus showing that the animals were stressed. When CVS was applied in three different frequencies, the change in the values of glucose in between groups were highly significant ($p<0.001$). However on detailed group wise comparative analysis it was seen that when a vestibular stimulation is applied in swinging motion in a frequency of 0.17 is optimum for stress relief shown by the values of serum glucose and cortisol whereas a frequency of 0.5 oscillations are implicative to cause a negative effect to produce stress to the animals.

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