



## Original Research Article

## Effectiveness of vestibular stimulation on selected biochemical parameters in young adults

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## ABSTRACT

**Introduction:** Optimal stimulation of vestibular system has more soothing effects and is essential throughout the life. Though there are different methods like running, swinging, dancing, jumping to stimulate vestibular system, swinging on a swing is a simple method to stimulate vestibular system, which was an ancient practice incorporated in Indian tradition.

**Aim:** The aim of this research was to assess the effect of vestibular stimulation on selected biochemical parameters in young adults.

**Materials and Methods:** A total of 300 (130 males and 170 females) young adults were screened. 240 (120 males and 120 females) participants satisfying both inclusion and exclusion criteria were included in the study. This was a longitudinal follow-up study in which, participants were assessed three times. The first assessment was performed during regular classes (with no examination in preceding two weeks and forth coming two weeks), these are pre-intervention values. The second assessment was performed eight months after the intervention (during regular classes), and third assessment was performed sixteen months after the intervention in stressed state (A week before the University examinations).

**Results:** The mean salivary IgA in the experimental female group was 61 µg/mL. After 8 months, there was a slight increase in salivary IgA whereas after 16 months also there was a slight increase in salivary IgA. The mean salivary α amylase in experimental female group was 84 U/ mL. After 8 months, there was a slight decrease in salivary α amylase whereas after 16 months also, there was a slight decrease in salivary α-amylase.

**Conclusion:** The present study results support positive impact of stimulation of vestibular system using natural methods like swinging on a swing that exists as day to day activity in the tradition of India. The study recommends using the swing in routine life style for better wellbeing.

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## 1. Introduction

Autonomic nervous system, which regulates various body systems and contributes to homeostasis, can be impacted by stress. Autonomic and immune systems influence each other. Imbalance in the autonomic activity modulates immunity and stress through its connections with lymphoid organs.<sup>1,2</sup> Activation of sympathetic nervous system by heat stress increased the levels of interleukins, cortisol levels and growth related oncogenes in rats. This effect

is mediated by sympathetic projections to the lymphoid tissues, which regulates the maturation and development of the immune cells throughout the life.<sup>2</sup> Studies have shown that activation of central sympathetic nervous system during brain injury leads to suppression of immunity, altered inflammatory responses.<sup>3</sup> Inflammation was reported as one of the most common underlying cause for most of the cardiovascular diseases such as hypertension.<sup>4</sup> Activation of paraventricular nucleus increases interleukin levels and attenuates the blood pressure.<sup>3</sup> According to American Psychological Association, “Anxiety is an emotion characterized by feelings of tension, worried thoughts and

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physical changes like increased blood pressure” and other autonomic functions such as salivary alpha amylase levels. Positive correlation was reported between examination related anxiety and alteration in autonomic functions.<sup>5</sup>

Immunity refers to resistance of a host to pathogens and their toxic products.<sup>6</sup>The immune system has two lines of defense mechanisms. The first line defense mechanism against any invading organism is innate immunity, which is antigen-independent. The major function of innate immunity is to mobilize the immune cells to the infection site and inflammation through cytokines.<sup>7</sup>The immune cells involved in innate immunity are dendritic cells, mast cells, basophils, eosinophils, natural killer (NK) cells and lymphocytes.<sup>7</sup> In contrast, adaptive immunity is antigen dependent and is specific.<sup>8</sup>The major function of adaptive immunity is to identify specific antigen, activation of specific effector pathways and development of immunological memory. The cells involved are T cells and B cells.<sup>8</sup>Stress can often compromise the immune system function, which can lead to poor defense mechanisms against invading pathogens.

Animal studies have shown that vestibular stimulation minimizes stress-induced changes in immunity.<sup>9</sup>Intact hippocampus was essential for normal functioning of immune system and a healthy vestibular system was essential for normal functioning of hippocampus.<sup>10,11</sup>Vestibular stimulation regulates sympathetic nerve activity through vestibulo-sympathetic reflex.<sup>12</sup>However, the link between vestibular and autonomic systems is complex and not been fully defined.<sup>13</sup>Vestibular stimulation was reported to decrease sympathetic activity and increase parasympathetic activity and reduce blood pressure.<sup>14</sup>Optimal stimulation of vestibular system has more soothing effects and is essential throughout the life. Though there are different methods like running, swinging, dancing, jumping to stimulate vestibular system, swinging on a swing is a simple method to stimulate vestibular system,<sup>15</sup> which was an ancient practice incorporated in Indian tradition. Vestibular stimulation influence mood states through its connections with cortical areas. Vestibular stimulation may relieve stress by decreasing the levels of cortisol through inhibition of hypothalamic-pituitary-adrenocortical (HPA) axis and sympathetic adrenomedullary (SAM) axis.<sup>16</sup> The aim of this research was to assess the effect of vestibular stimulation on selected biochemical parameters in young adults.

## 2. Materials and Methods

### 2.1. Study participants

A total of 300 (130 males and 170 females) young adults were screened. 240 (120 males and 120 females) participants satisfying both inclusion and exclusion criteria were included in the study. A detailed medical history

was obtained from all participants and standard physical examination was conducted. Written informed consent was obtained from all the participants included in the study. Selected participants were randomly assigned to four groups by simple random sampling.

Group Con-M (n = 60): Control male group (no vestibular stimulation was given)

Group Con-F (n = 60): Control female group (no vestibular stimulation was given)

Group Exp-M (n = 60): Experimental male group (vestibular stimulation was given)

Group Exp-F (n = 60): Experimental female group (vestibular stimulation was given).

### 2.2. Inclusion and exclusion criteria

Healthy young adults in the age group of 18-24 years who were willing to participate in the study were included in the study. Individuals suffering from any somatic or mental disorders, those with ear infections or any vestibular disturbances, visual disorders, cardio-respiratory disorders were excluded from this study.

### 2.3. Experimental design

The present study was conducted at Little Flower Institute of Medical Sciences and Research and Little Flower Medical Research Centre, Angamaly. The study was approved by Institutional Human Ethics committee. A written informed consent was obtained from all the participants and confidentiality of the data was maintained. This was a longitudinal follow-up study in which, participants were assessed three times. The first assessment was performed during regular classes (with no examination in preceding two weeks and forthcoming two weeks), these are pre-intervention values. The second assessment was performed eight months after the intervention (during regular classes), and third assessment was performed sixteen months after the intervention in stressed state (A week before the University examinations). Recording of all parameters were done between 1 to 2 pm to minimize diurnal variation. Filling up of the questionnaires and collection of salivary samples was done simultaneously.

### 2.4. Power analysis or sample size estimation

The sample size was estimated assuming the mean difference in the cortisol level to be 20% with 30% Standard deviation, for 3 groups (pre-test, 8 months and 16 months), 90% power and 0.05% significance. The estimated sample size was 58 and rounded off to 60 (control male-60; Experimental male-60; Control female-60; Experimental female-60). Sigma Plot 13.0 (Systat software USA) was used for calculating the sample size.

### 2.5. Vestibular stimulation

Vestibular stimulation was given by making the participants swing on a swing, according to their comfort (front and back direction) once a day, for 5 days in a week at their leisure timings (8:30-9:30 am, 11:00-12:00 am, 1:00-2:00 pm and 4:00-5:00 pm) in four groups.<sup>17</sup>

### 2.6. Duration, frequency and intensity of vestibular stimulation

Duration was recorded manually by using a timer. The mean and SD values for the duration of vestibular stimulation were calculated and recorded. One full two and fro movement of the swing was considered as a complete cycle. Number of cycles per minute was counted manually and recorded in similar pattern. The total width of the seat was 16 inches, when divided into equal halves, each half is of eight inches. The exact distance covered by the swing in to and fro directions was marked and the calculations were done accordingly. For example, when the swing moves in the front direction, say about sixty inches, subtract eight inches from sixty inches to get the exact distance covered from swing movement from the point of fixation to the forward movement and vice versa. Intensity was recorded manually with the help of observer's coordinators.

### 2.7. Saliva sample collection

The saliva samples were collected by using un stimulated passive drool. All the participants were advised to rinse the mouth thoroughly with plain water 10 minutes before the sample collection. This enabled to obtain the correct concentration and reduced the false values. Samples were not collected within 60 minutes of the meal as this may lower the pH of saliva and may lead to contamination like bacterial growth. Participants were made to bend the head forward, to facilitate the pooling of saliva on the floor of the mouth. The saliva thus collected was transferred to polypropylene vial using Saliva Bio-collection aid (SCA). Date and time of the specimen collection was recorded as there would be diurnal variation in cortisol levels with time. Samples were stored below -20°C within 30 minutes after collecting the sample till the batch analysis. Samples could be stored for 6 months at this temperature.

### 2.8. Salivary $\alpha$ -amylase

Salivary  $\alpha$ -amylase was estimated by using Salimetrics Salivary  $\alpha$ -amylase Assay, an enzymatic method. 2-chloro-p-nitrophenol is a chromogenic substrate that links with maltotriose that determines the  $\alpha$ -amylase activity yielding 2-chloro-p-nitrophenol. The amount of  $\alpha$ -amylase activity present in the sample is directly proportional to the increase in absorbance at 405 nm, measured spectrophotometrically.<sup>18</sup>

### 2.9. Salivary IgA

Salivary IgA was assessed by using Secretary IgA ELISA Kit (Saliva) - Salimetrics Assays, 1-1602. The Salimetrics Salivary IgA (SIgA, Secretary Immunoglobulin-A) is an indirect competitive immunoassay. A constant amount of goat anti-human SIgA conjugated to horseradish peroxidase is added to tubes containing specific dilutions of standards or saliva. The antibody goat anti-human SIgA enzyme horseradish peroxidase conjugate binds to the SIgA in the standard or saliva samples. The amount of free antibody enzyme conjugate remaining is inversely proportional to the amount of SIgA present in the sample. Bound SIgA Antibody Enzyme Conjugate produces a blue colour which is read at 450nm.<sup>19</sup>

### 2.10. Statistical analysis

Data was analyzed by using Sigma Plot 13.0 (Systat software, USA). One way repeated measures analysis of variance (One Way RM ANOVA) was used to observe significance of difference between the groups. Student-Newman-Keuls (SNK) method was used for multiple comparisons. Unpaired t test was used to observe significance of difference between the two groups.  $P < 0.05$  was considered as significant.

## 3. Results

### 3.1. Duration, frequency and intensity of vestibular stimulation

The mean and SD values for the duration of vestibular stimulation were  $4.86 \pm 0.99$  minutes in males and  $4.58 \pm 1.61$  minutes in females. The mean and SD values of frequency in males is  $20.60 \pm 2.45$  cycles/min and in  $21.0769 \pm 1.168$  cycles/min in females. The mean and SD values obtained for the intensity covered by the swing to and fro direction in males is  $1.88 \pm 0.28m$  and  $1.73 \pm 0.27m$  respectively and  $2.05 \pm 0.28m$  and  $1.9 \pm 0.25m$  in females respectively. All the above data are not statistically significant.

### 3.2. Salivary $\alpha$ -amylase

The mean salivary  $\alpha$ -amylase in control male group was 79 U/mL. After 8 months, there was a slight increase in salivary  $\alpha$ -amylase which was not statistically significant. After 16 months, there was a slight increase in salivary  $\alpha$ -amylase. The increase in salivary  $\alpha$ -amylase was statistically significant ( $p < 0.001$ ). The mean salivary  $\alpha$ -amylase in experimental male group was 77U/mL. After 8 months, there was a decrease in salivary  $\alpha$ -amylase whereas after 16 months, there was a decrease in salivary  $\alpha$ -amylase. The decrease in salivary  $\alpha$ -amylase was statistically significant ( $p < 0.001$ ). The mean salivary  $\alpha$ -amylase in control female group was 85U/mL. After 8

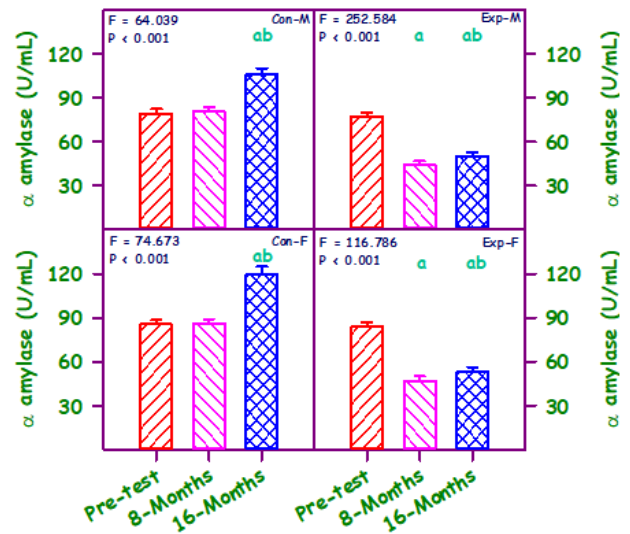
months, there was an increase in salivary  $\alpha$ -amylase which was not statistically significant. After 16 months, there was a slight increase in salivary  $\alpha$ -amylase. The increase in salivary  $\alpha$  amylase was statistically significant ( $p < 0.001$ ). The mean salivary  $\alpha$  amylase in experimental female group was 84 U/mL. After 8 months, there was a slight decrease in salivary  $\alpha$  amylase whereas after 16 months also, there was a slight decrease in salivary  $\alpha$ -amylase. The decrease in salivary  $\alpha$  amylase was statistically significant ( $p < 0.001$ ). After 8 months of vestibular stimulation, there was significant decrease in the salivary  $\alpha$ -amylase in the experimental male ( $t=9.0532$ ;  $p < 0.001$ ) and female ( $t=9.1335$ ;  $p < 0.001$ ) groups when compared to control male and female groups respectively (Figure 1).

### 3.3. Salivary IgA

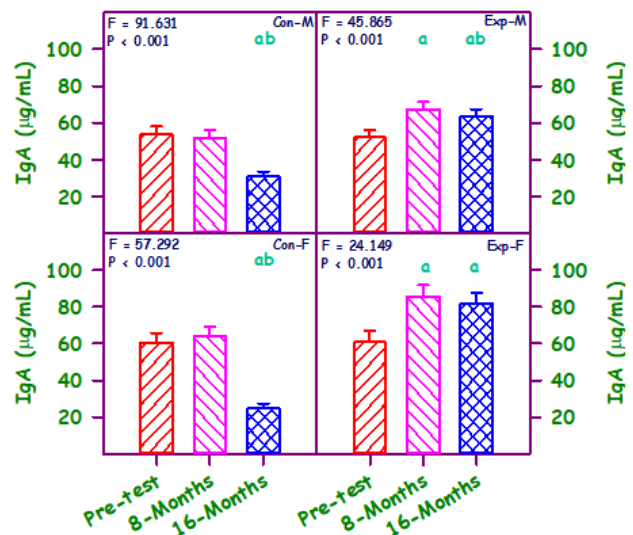
The mean salivary IgA in the control male group was  $54 \mu\text{g/mL}$ . After 8 months, there was a slight decrease in salivary IgA which was not statistically significant. After 16 months, there was decrease in salivary IgA. The decrease in salivary IgA was statistically significant ( $p < 0.001$ ). The mean salivary IgA in the experimental male group was  $52 \mu\text{g/mL}$ . After 8 months, there was a slight increase in salivary IgA whereas after 16 months also, there was a slight increase in salivary IgA. The increase in salivary IgA was statistically significant ( $p < 0.001$ ). The mean salivary IgA in the control female group was  $61 \mu\text{g/mL}$ . After 8 months, there was a slight increase in salivary IgA which was not statistically significant. After 16 months, there was a decrease in salivary IgA which was statistically significant ( $p < 0.001$ ). The mean salivary IgA in the experimental female group was  $61 \mu\text{g/mL}$ . After 8 months, there was a slight increase in salivary IgA whereas after 16 months also there was a slight increase in salivary IgA. The increase in salivary IgA was statistically significant ( $p < 0.001$ ). After 8 months of vestibular stimulation, there was significant increase in the salivary IgA in the experimental male ( $t=2.6575$ ;  $p < 0.001$ ) and female ( $t=2.6023$ ;  $p=0.0104$ ) groups when compared to control male and female groups respectively (Figure 2).

### 4. Discussion

Stress can impact the functions of autonomic and immune system. Examinations are natural stressors, which can significantly impact general health<sup>20</sup> often by diminishing functions of autonomic and immune system.<sup>21</sup> Studies have shown a significant change in the immunoglobulin (IgE), cortisol levels, autonomic functions and blood cytology during stress.<sup>22</sup> Hence the present study analyzed the impact of examination stress on various immunological, cytological and autonomic parameters among study cohorts, both in male and female young adults, and tested if vestibular stimulation can improve these parameters.



**Fig. 1:** Effectiveness of vestibular stimulation in young adults on salivary alpha amylase. Con = Control; Exp = Experimental; M = Male; F = Female Values are Mean  $\pm$  SE (n-Control=60 each; n-Experimental=60 each) The 'F' and 'P' values are by oneway RM ANOVA with SNK multiple comparison test. <sup>a</sup>Significantly different from the pre-test group. <sup>b</sup>Significantly different from the 8 months group



**Fig. 2:** Effectiveness of vestibular stimulation in young adults on salivary IgA. Con = Control; Exp = Experimental; M = Male; F = Female Values are Mean  $\pm$  SE (n-Control=60 each; n-Experimental=60 each) The 'F' and 'P' values are by oneway RM ANOVA with SNK multiple comparison test. <sup>a</sup>Significantly different from the pre-test group. <sup>b</sup>Significantly different from the 8 months group

Autonomic nervous system, which is fundamental to systemic wellbeing, can directly and indirectly influence the immune function and stress response.<sup>23</sup> This study measured the parameters such as systolic pressure, diastolic pressure, pulse pressure, mean arterial blood pressure and pulse rate, which are well known to be directly regulated by autonomic nervous system. This study also evaluated salivary  $\alpha$ -amylase levels, which reflects functional-anatomy of autonomic nervous system.<sup>23</sup> All the indexes of autonomic nervous system tested in this study were significantly improved in both female and male cohorts following vestibular stimulation. The observations from the present study validate the previous reports of beneficial effects of vestibular stimulation for shorter duration on autonomic nervous system.<sup>9</sup> Such a benefit of vestibular stimulation on autonomic functions can possibly be mediated by involvement of vestibulo-sympathetic reflex in the brain stem region.<sup>24</sup> The beneficial effects of vestibular stimulation observed on both direct and indirect measures of autonomic nervous system, indicates the involvement of several complex networks within the cortical regions of the brain such as hypothalamic paraventricular nucleus, rostral ventrolateral medulla, posterior hypothalamus, supracolliculi and/or locus ceruleus.<sup>25</sup> Previous studies have shown that parabrachial and adjacent Kolliker-Fuse receives inputs from vestibular nuclei which mediates vital functions of autonomic nervous system such as systolic pressure, diastolic pressure, pulse pressure, mean arterial blood pressure and pulse rate.<sup>26</sup>

The beneficial effects of vestibular stimulation on systolic pressure, diastolic pressure and pulse rate could also be a consequence to modulation of autonomic nervous system in medullary baroreflex network.<sup>27</sup> While the beneficial effects of vestibular stimulation on salivary  $\alpha$ -amylase levels could be a consequence to activation of autonomic nervous system in entire cerebellar vermis, the flocculus, the fastigial nucleus and the anterior and posterior interpositus nuclei.<sup>28</sup> Further the improvement in autonomic parameters following vestibular stimulation may also involve medial cortical regions of lobules, cerebellum networks, uvula, posterior vermis, nucleus tractus solitaries (NTS) and dorsal motor nucleus.<sup>29</sup> The support for the association of autonomic system with vestibular system is evident from the convergence of vestibular and baroreceptor signals on NTS neurons,<sup>30</sup> which is reported to positively modulate blood pressure and heart rate.<sup>31</sup>

The results obtained from the present study are consistent with other studies reporting, beneficial effects of caloric vestibular stimulation on sympathetic nerve activity, which can lead to decrease in blood pressure.<sup>32</sup> Transient changes in systolic blood pressure, were also observed following linear vestibular stimulation in human subjects.<sup>33</sup> In similar lines, rotatory vestibular stimulation was reported to cause significant decrease in heart rate in children with Down's

syndrome.<sup>34</sup> Such benefit on heart rate and blood pressure is also reported in human subjects following lateral rocking movement<sup>23</sup> or conventional swing.<sup>35</sup> Contrary to this study results, 30 minutes of rocking was reported to increase both systolic and diastolic blood pressure in patients with Alzheimer's disease.<sup>36</sup> Further, a few studies have demonstrated that rotational vestibular stimulation alters respiration but not the cardiovascular parameters.<sup>37</sup> Such contrasting observations in other studies may be due to variations in the study subjects, compromised neural anatomy and physiology as a consequence to disease, and/or variations in the duration and nature of vestibular stimulation employed.

The beneficial effects of vestibular stimulation are attributed to improvement in autonomic functions regulated by the hypothalamic-pituitary-adrenocortical (HPA) axis<sup>16</sup> and sympathetic adreno-medullary (SAM) axis. The role of HPA axis is further evident from the reduction in salivary cortisol levels observed in this study following vestibular stimulation. Vestibular stimulation is reported to inhibit paraventricular nucleus and decreases the release of corticotropin releasing hormone (CRH), which is the main integration site that regulates HPA axis.<sup>38</sup> In addition to these direct effects indirect pathways of vestibular stimulation such as secretion of GABA, activating limbic system, substantia nigra, preoptic area, dorsal raphe nucleus and fastigial nucleus are also reported to inhibit HPA axis.<sup>39</sup> The reduced salivary cortisol levels following vestibular stimulation could be mediated by inhibition of hippocampus and prefrontal cortex, as both these areas are known to influence release of the glucocorticoids and behavioral responses to stress.<sup>40</sup> Further the Presence of glucocorticoid and mineralocorticoid receptors in the hippocampus allows the hippocampus to detect the concentration of circulating glucocorticoids and to modulate the negative feedback inhibition of HPA axis.<sup>41</sup> The indirect effects of vestibular stimulation on HPA axis in regulating corticosteroid levels involves modulation of pre frontal cortex,<sup>42</sup> dorsal raphe nucleus<sup>43</sup> and fastigial nucleus of cerebellum.<sup>44</sup> The results of the present study on reduced salivary cortisol levels following vestibular stimulation is consistent with several previous reports in animals and humans.<sup>35</sup> Such beneficial effects are also observed in patients with conversion disorder.<sup>44</sup> Hence the significantly decrease in the salivary cortisol levels as observed in this study during regular classes and during pre-examination period warrants wider application to harvest the benefits of vestibular stimulation in wider population.

The role of sympathetic adreno-medullary axis in mediating the beneficial effects of vestibular stimulation is evident from studies reporting inhibition of paraventricular nucleus through GABA.<sup>45</sup> Further, injection of GABA receptor antagonist into the posterior hypothalamus is reported to relieve stress-induced tachy cardia and

adrenergic response.<sup>46</sup> Also several vestibulo-autonomic pathways are reported to be located from medial vestibular nucleus to the dorsal motor nucleus of the vagus nerve and the nucleus of the solitary tract.<sup>47</sup> Consequently, excitation of vagal efferent fibers is observed following vestibular stimulation.<sup>48</sup> Thus it is convincing that vestibular stimulation balances the autonomic activity through stimulation of parasympathetic nervous system and inhibition of sympathetic nervous system. Stimulation of parasympathetic system and inhibition of sympathetic system decreases heart rate and respiratory rate. Decrease in the heart rate and respiratory rate sends signals to medulla and inhibits locus coeruleus and decreases norepinephrine availability to hypothalamus which in turn secretes less corticotrophin releasing hormone.<sup>49</sup> This mechanism ultimately reduces the cortisol levels as observed in this study.

The effect of vestibular stimulation on the immune system was evident from significant increase in salivary IgA levels in the experimental group when compared with control group. This effect although was more prominent among female participants, but was observed in both gender. The results from this study hence support the concept that stress associated with pre-examination period can reduce humoral immunity and secretory immune response (salivary IgA levels) and this can be effectively restored by vestibular stimulation. The effect of vestibular stimulation on salivary IgA levels is likely to be mediated by sympathetic division of autonomic nervous system modulating the balance of type-1/type-2 cytokines.<sup>22</sup> It is also likely that the beneficial effects of vestibular stimulation observed on humoral immunity may be indirectly mediated by reduction of stress,<sup>50</sup> with potential involvement of the autonomic nervous system in this process. The improvement in innate and humoral immunity as observed in the present study following vestibular stimulation could also be mediated by the limbic system.<sup>51</sup> For instance, hippocampus, which is an essential part of the limbic system, is reported to decrease circulating corticosteroid levels,<sup>51</sup> regulate vegetative, visceral and endocrine functions and further influence primary and secondary immune response.<sup>51</sup> The specific benefits of vestibular stimulation on innate immunity observed among this study cohort could, additionally be influenced by the effects of autonomic system on endocrine, lymphoid and myeloid organs.<sup>51,52</sup> This complex network may involve hypothalamus, spleen, thymus and hypothalamic–pituitary–adrenal axis in addition to the involvement of hippocampus.<sup>53</sup> Other factors, which may have contributed to the beneficial effects of vestibular stimulation on innate and humoral immunity include neuronal tracts linking vestibule-cerebellum,<sup>54</sup> glutamatergic and gamma aminobutyric acid (GABA)-ergic projections from cerebellum to hypothalamus<sup>55</sup> and/or paracrine regulation from myeloid organs.<sup>56,57</sup> The results of the present study support the beneficial effects of vestibular stimulation on not only

humoral immunity and secretory immune response, but benefits are also observed in improving innate immune response. Such multi-dimensional benefits on immune system by vestibular stimulation are essential not only to combat stress associated with day to day life but more so to effectively address pre-examination stress among students. The hazardous effects of stress on cardiovascular system are widely reported in the literature to be predominantly mediated through the activation of sympathetic nervous system and vagal withdrawal. In the present study, it was observed that the pulse rate, systolic, diastolic, salivary IgA, cortisol and salivary  $\alpha$ -amylase levels were significantly decreased but remained within normal limits following vestibular stimulation. Further, positive impact of vestibular stimulation was observed on immunity and autonomic functions in relation to the stress. Hence, this study provides convincing evidence for the beneficial effects of vestibular stimulation on biochemical and physiological parameters in relation to pre-examination stress among young adults of both genders.

## 5. Conclusion

The present study results support positive impact of stimulation of vestibular system using natural methods like swinging on a swing that exists as day to day activity in the tradition of India. The study recommends using the swing in routine life style for better wellbeing.

## 6. Acknowledgement

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## 7. Conflicts of Interest

None declared.

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