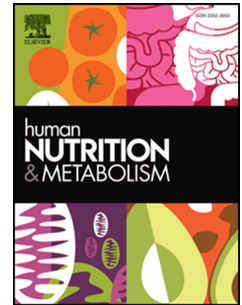


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Effect of *Ganoderma lucidum* supplementation on biochemical and psychophysiological biomarkers in female college students: a dose-response study

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Title:**Effect of *Ganoderma lucidum* supplementation on biochemical and psychophysiological biomarkers in female college students: a dose-response study**Sudeep Mitra¹, Mousumi Mitra², Dilip Kumar Nandi²,Amit Bandyopadhyay^{1*}¹Sports & Exercise Physiology Laboratory, Department of Physiology, University of Calcutta, University Colleges of Science & Technology, 92 APC Road, Kolkata:700009, India.²Laboratory of Human Performance, Department of Human Physiology & Department of BMLT, Raja Narendra Lal Khan Women's College (Autonomous), Midnapore:721102, India.

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Declaration of Competing Interest

Declared none.

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Title:**Effect of *Ganoderma lucidum* supplementation on biochemical and psychophysiological biomarkers in female college students: a dose-response study****Abstract**

Purpose: Academic stress negatively influences female college students' by modulating the autonomic nervous system (ANS) and antioxidant indices, causing poor cardiovascular health and well-being. The effectiveness of *Ganoderma lucidum* (GL) in ANS regulation for parasympathetic efficiency by antioxidant enrichment in female college students is not well investigated.

Method: A randomized, double-blinded, placebo (PL) controlled study was conducted to examine GL supplementation's effects at 500mg and 1000mg per day on selective anthropometric, biochemical and psychophysiological parameters for one month. Through the block-randomization method, 60 participants (mean age = 21.42 ± 2.07 years) were evaluated for the pre-experimental trial (0 day), mid-experimental trial (15th day), and post-experimental trial (30th day) phases.

Results: Physiological parameters, including systolic blood pressure, heart rate, and heart rate variability (high frequency, low frequency, ratio of low frequency to high frequency, root mean square of successive differences) showed significant improvements ($p < 0.01$) after the post-experimental GL_{1000mg} supplementation compared to the PL group. The Perceived stress scale (PSS) also exemplified similar significant changes ($p < 0.01$). For biochemical parameters, the post-experimental GL_{1000mg} trial reveals a significant improvement in superoxide dismutase ($p < 0.001$), glutathione reductase ($p < 0.05$) and interleukin-6 ($p < 0.01$) levels in comparison to PL. The GL_{1000mg} post-experimental supplemented group also showed significant improvements ($p < 0.05$) in glutathione reductase, cortisol and tumour necrosis factor α levels when compared to the baseline (pre) values.

Conclusion: Present findings indicate that a 30-day GL_{1000mg} intervention could be a viable preventive measure to enhance the psychophysiological health of female college students by reducing sympathetic efficacy, immune system dysfunction, and cardiometabolic risk factors for better psychophysiological well-being.

Keywords: Perceived stress, *Ganoderma lucidum*, Psychophysiology, Wellbeing.

1.Introduction

College students encounter stress due to unpleasant academic experiences, which affects their health and overall well-being. Academic stress is frequently related to scholastic neuro-depression, anxiety, failures, financial difficulties, marital issues, social isolation, and a lack of parental support, leading to extended dysphoric mood and behaviour[1]. Continuous academic stress can result in increased free radical production, which can induce cellular oxidative strain by depleting antioxidants through an overabundance of reactive oxygen species (ROS), which can have detrimental psychophysiological consequences [2,3]. Stress caused by anxiety promotes cortisol ejection from the adrenal cortex through the hypothalamic-pituitary-adrenal (HPA) axis, which affects the action of the autonomic nervous system (ANS) by increasing sympathetic activity and diminishes the parasympathetic response towards the heart, stimulating noradrenergic impulses for high blood pressure (BP) and heart rate (HR)[4].

About thirty-eight percent of Indian undergraduates experience distress, with a female preponderance, caused by high academic demands and failures that are strongly linked to suicidality and disastrously harmful interpersonal relationships. Indian female college students report a greater degree of perceived stress than other adolescents due to an impairment in inflammatory markers, which compromises the cortisol's feedback and reduces ANS and HPA quick responses, delaying the stress alleviation system. The HPA axis and the sympathetic nervous system (SNS) react with inflammatory cytokines and glucocorticoids to induce systemic chronic inflammation, which might contribute to the development of non-communicable illnesses[2].

Relying on the physiological function of baroreceptors and baroreflexes, heart rate variability (HRV) is the gold-standard technique for real-time quantitative measurement of neuro-cardiac stress dynamics. HRV comprises frequency-domain indices which include low frequency (LF), high frequency (HF), and sympathovagal index (LF/HF), as well as time-domain metrics such as the standard deviation (SD) of each normal RR peak points (SDNN) and the root mean square of successive differences (RMSSD) between normal rhythmic heartbeats. LF indicates the sympathetic response to stress, whereas parasympathetic activity is indicated by HF. SDNN and RMSSD are influenced by parasympathetic and sympathetic activities. Therefore, HRV may be used to monitor stress levels to improve health, fitness, and wellness conditions. [5,6].

A well-known herbal supplement, *Ganoderma lucidum* (GL), is grown extensively across the Indian Himalayan regions and has good phytoactive richness, which includes a variety of proteins, peptides, amino acids, polysaccharides, trace minerals, sterols, steroids, triterpenoids and fatty acids. However, the fruiting bodies, mycelia, and spores of GL have been estimated to contain 400 different therapeutically active compounds [7]. GL's medicinal benefits, such as anti-oxidative, anti-inflammatory, and anti-hypercholesterolemia are mostly due to the presence of triterpenoids and polysaccharides [7,8]. GL fruiting bodies contain various triterpenoids, such as ganolucidic acid, ganodermic acid, ganoderenic acid K, and farnesyl hydroquinone, which possess an antagonistic effect against dyslipidaemia [9]. Additionally, the high levels of vital amino acids (lysine, histidine, phenylalanine, and leucine) in GL can activate the gene associated with antioxidant enzymes, preserving the balance between antioxidants and free radicals [10]. These phytochemicals also improve sleep length and quality for psychological well-being [11], which helps to relieve mental exhaustion, insomnia, and dizziness by physically activating the hippocampus neurons for defence against oxidative stress through the depletion of cortisol. Furthermore, research has shown that GL decreases sympathetic outflow and arterial blood pressure, thereby improving peripheral nervous system (PNS) functioning to cure neurocardiac diseases [12,13]. There is, however, a dearth of scientific information regarding the most suitable dosage and duration for GL.

The present research thus intended to determine if supplementing GL at dosages of 500 mg and 1000 mg for 15 and 30 days would improve the psychophysiological and biochemical well-being of college females. Furthermore, there is currently no published evidence on the efficacy of GL supplements for Indian students.

2. Materials and methods

2.1. Selection of participants

The minimal sample size for this study was established using PS Power and Sample Size Calculation software (version 3.1.2, 2014), which uses the mathematical algorithm and methodology proposed by Dupont and Plummer [14].

The expected difference in mean between the pre and post supplementation values (D) and the variation in standard deviation (SD) with response to matched pairs (σ) were calculated using a prior study [15] and from a pilot study conducted in this laboratory. The significance threshold was set at 0.05 and the power ($1-\beta$) at 0.8. Thus, the highest possible sample size was predicted to be 13 for each group. However, assuming a 20% dropout

probability, 16 subjects for each group are to be recruited in the present study. To be safe sided, 20 subjects for each group were recruited.

Eighty (n=80) sedentary female volunteers (aged 18–23 years), were initially selected for the present study by a simple randomization selection technique. To ensure an appropriate array of opinions regarding inclusion and exclusion criteria, participants were contacted personally, over the phone, or via email for participation. The inclusion and exclusion criteria are illustrated as follows-

Inclusion criteria:

1. Must be an Indian citizen,
2. Sedentary college or university student (aged 18–23 years)
3. Normal menstrual cycle,
4. Healthy and disease-free
5. Non-users of mushroom

Exclusion criteria:

- 1) History of any disease
- 2) Subjects with varying menstrual cycle
- 3) Allergic to mushrooms
- 4) Use of contraceptive pills or psychoactive substances or medicinal/herbal supplementation
- 5) Regular practice of any form of exercise or meditation

Sixteen (n=16) participants were, therefore consequently eliminated since they violated the inclusion criteria. Thereafter, two weeks prior to the experimental investigation, familiarisation with various experimental procedures was conducted. Four volunteers (n=4) were discomfited and failed to satisfy the experimental requirements and were further eliminated from participation. Therefore, for the present study, sixty (n=60) participants (mean age = 21.42 ± 2.07 years) were finally selected.

2.2. Ethical Consent

The Institutional Ethical Committee, University of Calcutta approved the experimental study (Ref no: CUIEC/02/03/2023-24, dated: 01.03.2024) as per the Helsinki Declaration (2013) and the Indian Council of Medical Research's (ICMR) recommendations. After explaining the study's procedure and protocol, each recruited participant gave signed informed consent before the start of the investigation. Figure 1 illustrates the present study's experimental design.

2.3. Dose and Duration

For general well-being treatment, the recommended *GL* supplementation dosage varies from 500mg to 1000mg per day[16]. Considering this, two separate *GL* dosages (500 mg and 1000 mg) were chosen for the present study. To prepare placebo capsules with identical size, colour, flavour, and odour non-calorific maltodextrin and magnesium stearate were used as excipients.

2.4. Experimental Protocol

A parallel experimental design with randomization, double blinding, and placebo (*PL*) control was conducted to evaluate *GL* supplementation's effects (500 mg and 1000 mg per day) on selective biochemical and psychophysiological parameters. Through the block-randomization method, sixty ($n=60$) participants (mean age = 21.42 ± 2.07 years) were divided evenly into three supplementing groups: *GL*_{500mg}, *GL*_{1000mg}, and *PL*. Therefore, twenty ($n=20$) participants in each group were allocated as per the sample size estimation. The pre-experimental trial (visit 1) was carried out before the initiation of *GL* supplementation. The mid-experimental trial (visit 2) and post-experimental trial (visit 3) were carried out after the 15th and 30th days of the supplementation period, respectively. Participants during each trial visited the laboratory at 7 AM following an overnight fasting. Laboratory temperature fluctuated between 18-20 °C and the relative humidity was estimated to be 65%-70% approximately.

A laboratory staff member was assigned to give the capsules with the prescribed amount to each participant regularly upon their arrival. He was responsible for assessing any negative effects of the supplements reported by pupils via a daily verbal communication or telephonic call. The laboratory assistant also kept a notebook to record the doses ingested and returned. Regular safety, toxicity, compliance, and adherence assessments were monitored similarly for a month. Although during the entire investigation period, no adverse side effects and false positives were documented. The lab assistant responsible for distributing capsules and dosages did not assist with data collection or statistical analysis. The researchers and participants were unaware of the capsules' identities (*GL* or *PL*) and correct dosages (500mg or 1000mg). Therefore, all the participants took part throughout the different phases of experimental trials, and thus no drop-out was recorded.

Prior to the trial visits, the selected participants were instructed to remain abstinent from any heavy exercise for 72 hours and avoid ingesting any fungal food supplements. Subjects further reported their dietary history for three days before the first trial and followed the same during each subsequent trial session. They were advised to maintain the same regimen. Records of physical activity and food intake throughout the previous 72 hours during each trial were acquired through self-administered, semi-structured questionnaire method.

2.5. Study Parameters

2.5.1. Anthropometric parameters

Participants stood barefoot, vertically upright on an adjustable height cum weighing measuring stadiometer (Delmer, India) while wearing minimal clothing, to estimate body mass (kg) and standing height (cm). Quetelet's index formula [17] was used to estimate the body mass index (BMI).

2.5.2. Psychophysiological estimations

Each subject's systolic (SBP) and diastolic blood pressure (DBP) were recorded in supine position by using an aneroid sphygmomanometer (Doctor-R, D1208, Japan) and stethoscope following the auscultatory method.

A short-term HRV was measured by recording an electrocardiogram (ECG) at 600 Hz in the supine position for 10 minutes by acquiring a steady-state HR. A two-channel BIOPAC MP-45 hardware recording device (Biopac, CA, USA) was telemetrically interfaced with computerised BSL pro-software (version 3.7.5) to record the time, duration and peaks between two recurrent R-R intervals (beat-to-beat recording). The software examined data from 5-minute (300 s) epochs and screened misclassified dropped beats that deviated from the mean normal R-R interval. Epochs that have non-normal R-R intervals of greater than 4% were not included in the analysis. Next, Fast Fourier transformation (FFT) was performed to quantify the time (SDNN, RMSSD) and frequency (LF, HF, LF/HF) domain properties of the R-R interval for 5 minutes after all artifacts and missing peaks were removed using Kubios HRV 2.0 version software (Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). Using normalised values for LF and HF is the most appropriate approach that has been used in the present investigation since the population's spectral power is not distributed normally. Additionally, the R-R intervals were used to compute the resting HR [18].

2.5.3. Perceived stress scale (PSS)

It is a 10-item questionnaire, that was used to gauge perceived stress among students. It is popular among adults as well as adolescents over the age of twelve years. A 5-point Likert scale, ranging from 0 (never) to 4 (very frequently), is taken into account for determining the total score (27–40 indicates a high level, 14–26 indicates a moderate level, and 0–13 indicates a low level of perceived stress) [19]. PSS was shown to have strong internal consistency, with a Cronbach α coefficient of 0.791 in the Indian Bengali student population [20].

2.5.4. Estimation of biochemical markers

A certified phlebotomist collected 5 ml of venous blood in ethylenediaminetetraacetic acid (EDTA) vials from the antecubital veins of participants who fasted overnight. Plasma was collected by centrifuging the blood samples for 10 minutes at -4°C at 2500g. To perform several biochemical tests, samples were kept at -20°C .

2.5.4.1. Lipid profile estimation

The high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG) values were estimated using standardized kit procedures [21, 22]. RMS analytica-705 semiautomated analyzer (Recorders & Medicare Systems Pvt Ltd, India) was used to read the absorbance.

2.5.4.2. Antioxidant estimation

Superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH) levels were measured using standardized sandwich and competitive enzyme-linked immunosorbent assay (ELISA) kit procedures [23–25]. BioRad iMark-1681130 (Bio-Rad Laboratories Pvt Ltd, India) microplate reader was used to interpret the absorbance.

2.5.4.3. Stress profile estimation

Cortisol and dopamine levels were measured using the standardized competitive inhibition ELISA kit procedure [26]. BioRad iMark-1681130 microplate reader (Bio-Rad Laboratories Pvt Ltd, India) was used to interpret the absorbance.

2.5.4.4. Inflammatory markers estimation

Tumour necrosis factor- α and interleukin 6 (TNF- α and IL-6) levels were measured using the standardized sandwich ELISA kit procedure [27]. BioRad iMark-1681130 microplate reader (Bio-Rad Laboratories Pvt Ltd, India) was used to interpret the absorbance.

2.5.4.5. Statistical analysis

The values for all variables have been presented as mean $\pm$ standard error of the mean (SE). GraphPad Prism statistical package, In-stat version 5.00 for Windows (GraphPad Software, San Diego, California, USA) was used for statistical evaluations. The significance level was set at 95% confidence level ($p < 0.05$). The Shapiro-Wilk test was used to evaluate the distribution of data's normality. The intragroup and intergroup variables were compared using analysis of variance (ANOVA) followed by the Bonferroni post hoc test to assess if there was a significant difference. Kruskal-Wallis and Dunn's comparisons test was used to evaluate PSS scores.

3. Results

3.1. Anthropometric Parameters

The anthropometric parameters of *GL* and *PL* subjects are presented in Table 1. Participants treated with *GL*_{500mg} and *GL*_{1000mg} at different time intervals did not exhibit any discernible changes in their anthropometric measurements.

3.2. Perceived stress scale

The self-assessed perceived stress scale of *GL* and *PL* subjects are presented in Table 2. After *GL*_{1000mg} post-experimental trial, perceived stress significantly declined ($p < 0.05$) in comparison to *PL*.

3.3. Psychophysiological parameters

The psychophysiological parameters of *GL* and *PL* subjects are presented in Table 3. SBP, HR, HF, LF, LF/HF and RMSSD significantly improved ($p < 0.01$) with *GL*_{1000mg} post-experimental trial compared to *PL*. Intra and inter-group comparisons showed non-significant changes in other physiological variables.

3.4. Lipid profile parameters

The lipid profile parameters of *GL* and *PL* subjects are presented in Table 4. The lipid profile showed no significant changes after *GL* treatment; however, a small but statistically insignificant improvement in HDL, LDL and TG levels was observed for intra- and inter-group comparisons.

3.5. Anti-oxidative stress markers

The anti-oxidative stress markers (SOD, CAT and GSH) of *GL* and *PL* subjects are presented in Table 5. Post-experimental *GL*_{1000mg} trial reveals a significant increase in SOD ($p < 0.001$) and GSH ($p < 0.05$) levels in comparison to *PL*. On comparison to baseline (pre), the *GL*_{1000mg} supplemented group also showed significant improvement ($p < 0.05$) in GSH level after 30 days (post) treatment.

3.6. Stress and neurotransmitter markers

The stress marker (Cortisol) and neurotransmitter (Dopamine) of *GL* and *PL* subjects are presented in Table 6. *GL*_{1000mg} post-experimental trial showed a significant decline ($p < 0.05$) in cortisol when compared to the baseline (pre) value. Dopamine didn't show any significant increase statistically for intra and inter-group comparison.

3.7. Pro-Inflammatory markers

The pro-inflammatory markers (TNF α , IL-6) of *GL* and *PL* subjects are presented in Table 7. TNF α level significantly improved ($p < 0.05$) in *GL*_{1000mg} post-experimental trial when compared to the baseline (pre) value. Additionally, the *GL*_{1000mg} post-experimental trial also illustrated a significant decline ($p < 0.01$) in the IL-6 level from the *PL*.

4. Discussion

The current study population consisted of healthy participants, as evidenced by normal ranges for body height, weight, and BMI. The individuals' physical measurements following *GL* supplementation for a month at varied dosages did not reveal any significant changes, illustrating that *GL* was devoid of any favourable and adverse effects. *GL* polysaccharides and triterpenoids possess anti-obesity and anti-adipogenic properties [28] that may result in a reduction of weight gain, as reported insignificantly in our study.

The assessment of HRV in this study is essential to understand the anti-stress effects of *GL* extract. Our findings revealed that *GL*_{1000mg} supplementation for a month modulated the influence on both the time domain (ANS activity) and frequency domain (ANS balance) parameters of HRV for determining better psychophysiological fitness. Significant improvements in HF, LF, LF/HF and RMSSD were detected in this present study. Additionally, the present study also showed a reduction in HR that significantly improves vagal activity. This may have a calming impact on the heart and further lower blood pressure through regulating neurocardiogenic sympathetic nerves. Therefore, *GL*'s potential to control stress resistance, stress index, and ANS balance was revealed by a general overall

improvement in psychophysiological measures; nevertheless, the PNS was the primary influencer that improved coping abilities in female college students as reported in the PSS questionnaire survey. Lower LF coupled with elevated HF may suggest repression of the hypothalamic sympathetic region, thereby improving the body's parasympathetic response to stress. HF signifies parasympathetic domination whereas LF represents sympathetic activity, and is related to blood pressure control. In contrast to sympathetic activity, a lower LF/HF ratio reflects parasympathetic superiority. The SDNN, which measures cardiac capacity to function normally, is affected by the variability in diastolic latency. Increased RMSSD suggests a parasympathetic supremacy, which assists in chronotropic acclimatisation and indicates a longer cardiac cycle [6,18]. As a result, the above possible indices reveal a reduction in stress following *GL* treatment, substantially supporting our findings for psychophysiological wellness. The distinctive effect of *GL* on ECG recordings in rats and rabbits showed that its hypotensive action was dose-dependently mediated via suppressing sympathetic outflow [13]. *GL* is therefore necessary for the effective functioning of the cardiovascular system, which has been demonstrated to affect general health and might be a measurable indicator for preserving the balance between sympathetic and parasympathetic activity. As a result, this is the first study to report how *GL* and HRV have a favourable association for improving psychophysiological stress in a human model.

Among college students, oxidative stress is primarily responsible for an upsurge in psychological discomfort [2,3]. However, in the present study, the anti-oxidative components of *GL* must have been easily absorbed and rapidly dissolved in the circulation, reducing oxidative stress in healthy female college students and aiding in the regulation of psychological well-being after supplementation of 1000 mg for 30 days. Our study revealed that encapsulated *GL* phytocompound (ganoderic acid) must have scavenging, chelating, and antioxidant properties which stimulated the antioxidant genes, increased the expression of antioxidant enzymes (SOD, CAT, and GPx), through the nuclear factor erythroid 2-related factor 2 (Nrf2) expression [29]. Furthermore, *GL* also includes trace amounts of many minerals (calcium, zinc, potassium, magnesium, and selenium), and essential amino acids (lysine, histidine, phenylalanine, and leucine) which aid antioxidant enzymes in quenching free radicals through a strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity [30,31]. Comparatively, studies on *GL* supplementation (225mg/day for 6 months) in healthy participants significantly enhanced the activity of antioxidant enzymes and decreased oxidative stress markers [29]. Moreover, in healthy persons, acute *GL*

consumption (1.1g for 10 days) raised plasma antioxidant capacity (FRAP value) by around 2-3% approximately [32]. Consistent with the past study results and mechanisms, the present findings report significant improvements in antioxidative enzyme activities (SOD, GSH) after a month *GL*_{1000mg} supplementation.

In the present experimental study, *GL* supplementation appeared not to improve plasma lipid concentrations significantly; nevertheless, a slight non-significant improvement in TG, HDL-C, and LDL-C levels was detected. However, the existence of several other chemical constituents (ganolucidic acid, ganodermic acid, and ganoderenic acid K) along with triterpenoids in encapsulated *GL* is responsible for its cholesterol-lowering action. These substances are the natural source of statins that efficiently reduce cholesterol overproduction by preventing the metabolism of 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) activity [9,33,34]. Tong et al (2008) observed that *GL* significantly elevated the plasma concentration of HDL-C by significantly lowering the levels of TG, TC, and LDL-C in hyperlipidemic rats [35]. Contradictorily, a non-significant TG decline and HDL-C rise in 26 individuals with borderline elevated blood pressure and/or cholesterol after a 12-week *GL* (1.44 g extract/d) supplementation was reported [33]. Consequently, there could be several reasons for the differences between the current study's nonsignificant findings and the previously mentioned significant ones. Initially, our research included healthy female college students, while other studies included rats with diet-induced hyperlipidaemia or high-pressure individuals. Additionally, *GL* extracts from a submerged mycelium culture or the fruiting body were used in other studies, whereas *GL* encapsulation consisted of a specific composition in the present study. Thus, it is apparent from the current study that individuals with normal cholesterol levels wouldn't likely benefit from *GL* supplementation; rather, unhealthy individuals are likely to observe improvements in their lipid biomarkers for longer *GL* supplementation periods.

Studies reveal that stress causes the synthesis of inflammatory mediators (TNF- α , IL-6), which results in psychophysiological discomfort [36]. This may have an adverse effect on serotonergic neuronal transmission, exacerbating depression symptoms. The current findings on healthy participants suggest that encapsulated *GL* components have anti-inflammatory qualities, as seen by the significant reduction in TNF- α and IL-6 indicators under academic stress following a 30-day 1000 mg *GL* treatment. The reason for this might be the action of triterpenoids generated from *GL*, which inhibit the production of TNF- α and IL-6, as well as the inflammatory mediators prostaglandin E(2) (PGE2) and nitric oxide (NO), by

delaying the inflammatory signalling process of nuclear factor- κ B (NF- κ B) and activator protein 1 (AP-1) [37]. In a clinical trial involving patients with ST-elevation myocardial infarction (STEMI), *GL* polysaccharides (750 mg/day in three separate dosages) decreased TNF- α , IL-1, and MDA values [38]. In another randomized, closed-label clinical study, polysaccharides of *GL* were taken 3 times a day (250mg/day) for 90 days. Pro-inflammatory markers (IL-6, and TNF- α) were significantly reduced in paroxysmal atrial fibrillation (AF) patients in comparison to *PL* after *GL* supplementation [39]. Therefore, it is clear from the information above that the results of the current study are in line with *GL*'s anti-inflammatory properties, which reduce pro-inflammatory effects and improve perceived stress levels, that could offer neuroprotective and cognitive benefits by limiting prolonged exposure to elevated cortisol levels.

Substantial improvements in HR, BP, and HRV are related to psychophysiological stress improvements and relaxation, as reported previously, eventually causing a decline in cortisol levels following a month of *GL*_{1000mg} consumption. Dopamine, which is directly associated with mental tranquillity, may inhibit the activity of adrenocorticotropin-releasing hormone to produce cortisol, and therefore minimise irregular vascular variability through neuro-cardiac interactions [6]. An increase in dopamine levels after *GL* supplementation is therefore also indicative of improved hippocampal capacities, causing inhibition of stress-related neuropsychophysiological imbalances. *GL*'s antagonistic binding characteristic with 5-hydroxytryptamine 2A (5-HT_{2A}) receptors [40] enables it to have an effect equivalent to that of an antidepressant, which lowers anxiousness through minimizing cortisol levels. According to reports, *GL* proteins, triterpenes, and ganoderic acid possess inhibitory effects on angiotensin-converting enzyme (ACE), which suppress sympathetic output in the CNS and alternatively enhance the properties of endothelial nitric oxide synthase enzyme [11,41]. This might cause vasodilation, and promote the maintenance of stress by lowering cortisol and perceived stress scores as reported in the present findings. Therefore, these effects were observed at the lowest possible dose and for the shortest duration (1000mg/day/ 30 days) among healthy female college students compared to the other clinical trials and thus the present study proved to improve overall well-being, quality of life by regulating the body's stress parameters.

5. Conclusion

The current study confirmed that *GL* had a strong impact on neuro-cardiovascular

wellness, through reducing sympathetic response in the CNS due to its antioxidant richness and anti-inflammatory properties. This elucidates the perceived stress level by balancing the detrimental ROS generations. Therefore, during challenging academic stress exposure, a month of *GL*_{1000mg/day} supplementation had a better soothing effect in female college students due to overall biochemical and psychophysiological improvements. This intervention protocol thus serves as a viable and affordable preventive technique for improving well-being.

6. Limitations of the study

The study's primary analysis provides the trial's more significant, statistically rigorous, and clinically useful outcomes. However, multiple analyses at different periods may produce type 1 errors. The present study tried to mitigate the above error by merging the intervention groups and concentrating predominantly on 30-day analyses, as other intervention periods showed non-significant changes. The study was additionally underpowered due to various inferential hypotheses regarding *GL*'s accurate dose and duration. The dose and duration of *GL* supplementation used in the present evaluation can be considered a strength, proving that *GL* 1000mg/30 days is the lowest effective dose which enhances psychophysiological and biochemical indicators in stressed college females. A limited selection of healthy female college students is another drawback of the present study, although it was appropriate to investigate the responses in healthy participants before attempting to evaluate the effects in diseased individuals. The results of the study will promote the use of *GL* as a potent nutraceutical to enhance human health in an array of difficult environmental, situational, and social circumstances for individuals of all ages and genders. One significant drawback is the dearth of neuropsychophysiological mechanisms (molecular and cell signalling), which might have proved the most accurate pathway about the effectiveness of *GL*_{1000mg} supplementation. Nevertheless, the current study incorporated selective immunological, neurotransmitter, and stress biomarkers along with HRV assessments (psychophysiological marker), which provided the best feasible conclusion regarding the effectiveness of *GL*_{1000mg} capsules through multifaceted computations. The safety of *GL* has mostly been determined by empirical observations and numerous studies in animals and in-vitro, which corroborate the favourable benefits; nevertheless, there are limited human trials in stress management. The current study may thus serve as a prototype for a future investigation that employs a bigger sample size, a longer intervention period (>1 month) and by recruiting both males and females to fully uncover the potential therapeutic

effects of *GL* supplementation on various stress-related ailments.

Ethical approval statement

The Human Ethical Committee for Biomedical and Health Research involving Human Participants, University of Calcutta approved the experimental design (Ref no: CUIEC/02/03/2023-24), dated 01.03.2024 as per the guidelines of ICMR, Government of India.

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Data Statement

All data is included within this article.

Declaration of Competing Interest

Declared none.

Submission statement

The present study has not been published previously or is not under consideration for publication elsewhere. All authors have reviewed and approved the manuscript for submission.

References

- [1] Dyson R, Renk K. Freshmen adaptation to university life: Depressive symptoms, stress, and coping. *Journal of clinical psychology*. 2006 Oct;62(10):1231-44.
- [2] Batabyal A, Bhattacharya A, Thaker M, Mukherjee S (2021) A longitudinal study of perceived stress and cortisol responses in an undergraduate student population from India. *PLoS ONE*16(6): e0252579. [https://doi.org/ 10.1371/journal.pone.0252579](https://doi.org/10.1371/journal.pone.0252579).
- [3] Whirledge S, Cidlowski JA. Glucocorticoids, stress, and fertility. *Minerva endocrinologica*. 2010 Jun;35(2):109.
- [4] Assadi SN. What are the effects of psychological stress and physical work on blood lipid profiles?. *Medicine*. 2017 May 1;96(18):e6816.
- [5] Zhao S, Chi A, Yan J, Yao C. Feature of heart rate variability and metabolic mechanism in female college students with depression. *BioMed research international*. 2020;2020(1):5246350.
- [6] Mitra S, Mitra M, Nandi P, Pandey M, Chakrabarty M, Saha M, Nandi DK. Efficacy of Yoga for COVID-19 Stress Prophylaxis. *Journal of Physical Activity and Health*. 2023 Aug 25;20(11):1034-42.
- [7] Wachtel-Galor S, Tomlinson B, Benzie IF. *Ganoderma lucidum* ('Lingzhi'), a Chinese medicinal mushroom: biomarker responses in a controlled human supplementation study. *British Journal of Nutrition*. 2004b Feb;91(2):263-9.

- [8] Chiu HF, Fu HY, Lu YY, Han YC, Shen YC, Venkatakrishnan K, Golovinskaia O, Wang CK. Triterpenoids and polysaccharide peptides-enriched *Ganoderma lucidum*: a randomized, double-blind placebo-controlled crossover study of its antioxidation and hepatoprotective efficacy in healthy volunteers. *Pharmaceutical biology*. 2017 Jan 1;55(1):1041-6.
- [9] Chen B, Tian J, Zhang J, Wang K, Liu L, Yang B, Bao L, Liu H. Triterpenes and meroterpenes from *Ganoderma lucidum* with inhibitory activity against HMGs reductase, aldose reductase and α -glucosidase. *Fitoterapia*. 2017 Jul 1;120:6-16.
- [10] Rubel R, Dalla Santa HS, Fernandes LC, Bonatto SJ, Bello S, Figueiredo BC, Lima Filho JH, Santos CA, Soccol CR. Hypolipidemic and antioxidant properties of *Ganoderma lucidum* (Leyss: Fr) Karst used as a dietary supplement. *World Journal of Microbiology and Biotechnology*. 2011 May;27:1083-9.
- [11] Mitra S, Mitra M, Nandi DK, Saha M, Bandyopadhyay A. Efficacy of Lingzhi or Reishi Medicinal Mushroom *Ganoderma lucidum* (Agaricomycetes) Supplementation on Psychological Stress and Selective Fitness Profile Parameters in Female College Students in West Bengal, India. *International Journal of Medicinal Mushrooms*
- [12] Abdullah N, Ismail SM, Aminudin N, Shuib AS, Lau BF. Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. *Evidence-Based Complementary and Alternative Medicine*. 2012;2012(1):464238.
- [13] Lee SY, RHEE HM. Cardiovascular effects of mycelium extract of *Ganoderma lucidum*: inhibition of sympathetic outflow as a mechanism of its hypotensive action. *Chemical and Pharmaceutical Bulletin*. 1990 May 25;38(5):1359-64.
- [14] Dupont WD, Plummer WD. Power and sample size calculation involving linear regression. *Control Clin Trials*. 1998;19(6):589–601. doi:10.1016/S0197-2456(98)00037-3
- [15] Aziz AR, Goh PO, Teh KC. Effect of Thirty Days Supplementation of Standardized Lingzhi Extract on Aerobic, Anaerobic and Strength Parameters in Trained Athletes. *International Journal of Applied Sports Sciences*. 2006 Jun 1;18(1).
- [16] Chang R. Effective dose of *Ganoderma* in humans. In *Proceedings of the Contributed Symposium A 1994* (Vol. 59, pp. 101-113).
- [17] Jonson BL, Nelson JK. *Practical Measurements for Evaluation in Physical Education*. Macmillan Publishing Co.; 1996.
- [18] Mitra S, Mitra M, Saha M, Nandi DK. Yoga for Psychophysiological Wellbeing during Menstrual Phases in Eumenorrheic Females. *Applied psychophysiology and biofeedback*. 2025 Mar;50(1):135-48.
- [19] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *Journal of health and social behavior*. 1983 Dec 1;385-96.
- [20] Chakraborti A, Ray P, Sanyal D, Thakurta RG, Bhattacharayya AK, Mallick AK, Das R, Ali SN. Assessing perceived stress in medical personnel: in search of an appropriate scale for the Bengali population. *Indian journal of psychological medicine*. 2013 Jan;35(1):29-33.
- [21] Naito HK. High-density lipoprotein (HDL) cholesterol. Kaplan A, et al. *Clinical Chemistry*. The C.V. Mosby Co. Princeton; 1984: 1207–1213
- [22] Schettler G, Nüssel E. Cholesterol CHOP-PAP. *Arb Med Loz Med Prä v Med*. 1975;10:25.
- [23] Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol chem*. 1952 Mar 1;195(1):133-40.
- [24] Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione.

- [25] Marklund S, Marklund G. Assay of SOD activity in tissue. *J Biochem.* 1988;13(13):305-15.
- [26] Bacarese-Hamilton T, Cattini R, Shandley C, Howard C, Palmer R, McFarthing K. A fully automated enzyme immunoassay for the measurement of cortisol in biological fluids.
- [27] Joshi L, Ponnana M, Sivangala R, Chelluri LK, Nallari P, Penmetsa S, Valluri V, Gaddam S. Evaluation of TNF- α , IL-10 and IL-6 cytokine production and their correlation with genotype variants amongst tuberculosis patients and their household contacts. *PloS one.* 2015 Sep 11;10(9):e0137727.
- [28] Delzenne NM, Bindels LB. *Ganoderma lucidum*, a new prebiotic agent to treat obesity?. *Nature reviews Gastroenterology & hepatology.* 2015 Oct;12(10):553-4. <https://doi.org/10.1038/nrgastro.2015.13>
- [29] Chiu HF, Fu HY, Lu YY, Han YC, Shen YC, Venkatakrishnan K, Golovinskaia O, Wang CK. Triterpenoids and polysaccharide peptides-enriched *Ganoderma lucidum*: a randomized, double-blind placebo-controlled crossover study of its antioxidation and hepatoprotective efficacy in healthy volunteers. *Pharmaceutical biology.* 2017 Jan 1;55(1):1041-6.
- [30] Iranzo O. Manganese complexes displaying superoxide dismutase activity: a balance between different factors. *Bioorganic chemistry.* 2011 Apr 1;39(2):73-87.
- [31] Rubel R, Dalla Santa HS, Fernandes LC, Bonatto SJ, Bello S, Figueiredo BC, Lima Filho JH, Santos CA, Soccol CR. Hypolipidemic and antioxidant properties of *Ganoderma lucidum* (Leyss: Fr) Karst used as a dietary supplement. *World Journal of Microbiology and Biotechnology.* 2011 May;27:1083-9.
- [32] Wachtel-Galor S, Szeto YT, Tomlinson B, Benzie IF. *Ganoderma lucidum* ('Lingzhi'); acute and short-term biomarker response to supplementation. *International journal of food sciences and nutrition.* 2004a Feb 1;55(1):75-83.
- [33] Chu TT, Benzie IF, Lam CW, Fok BS, Lee KK, Tomlinson B. Study of potential cardioprotective effects of *Ganoderma lucidum* (Lingzhi): results of a controlled human intervention trial. *British Journal of Nutrition.* 2012 Apr;107(7):1017-27.
- [34] Shiao MS. Natural products of the medicinal fungus *Ganoderma lucidum*: occurrence, biological activities, and pharmacological functions. *The Chemical Record.* 2003 Jul;3(3):172-80.
- [35] C. C. Tong, Y. K. Choong, S. Mohamed, N. M. Mustapha and N. A. Umar, Efficacy of *Ganoderma lucidum* on plasma lipids and lipoproteins in rats fed with high cholesterol diet, *Nutr. Food Sci.*, 2008, 38, 229–238.
- [36] Maes M, Song C, Lin A, et al. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and Th1-like response in stress-induced anxiety. *Cytokine.* 1998;10(4): 313–318. doi:10.1006/cyto.1997.0290
- [37] Maes M, Song C, Lin A, et al. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and Th1-like response in stress-induced anxiety. *Cytokine.* 1998;10(4): 313–318. doi:10.1006/cyto.1997.0290
- [38] Sargowo D, Aissy CR, Kalsum U, Nugroho FW, Kamila PA, Irawan D, Sitio M, Adrian LH, Pratama AR, Arifin Y, Fadlan R. The role of polysaccharide peptide of *Ganoderma lucidum* as a potent protective vascular endothelial cell, anti inflammation, and antioxidant in STEMI and NSTEMI patients. In *AIP Conference Proceedings 2019 Jun 4 (Vol. 2108, No. 1).* AIP Publishing.
- [39] Rizal A, Sandra F, Fadlan MR, Sargowo D. *Ganoderma lucidum* polysaccharide peptide reduce inflammation and oxidative stress in patient with atrial fibrillation. *The Indonesian Biomedical Journal.* 2020 Dec 2;12(4):384-9.

- [40] Matsuzaki H, Shimizu Y, Iwata N, Kamiuchi S, Suzuki F, Iizuka H, Hibino Y, Okazaki M. Antidepressant-like effects of a water-soluble extract from the culture medium of *Ganoderma lucidum* mycelia in rats. BMC complementary and alternative medicine. 2013 Dec;13:1-8. <https://doi.org/10.1186/1472-6882-13-370>
- [41] Zhang GP, Jin HM, Long JJ, Qian R, Cao X, Zhang M, Luo B, Wang Z, Sen C. Effect of *ganoderma lucidum* (ling zhi) combined with hypotensor on blood sugar, plasma NO, microcirculation and hemorrheology in treatment of refractory hypertension. J Chin Micro. 1999;3:75-8.

Table 1: Anthropometric Parameters

Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
Body height (cm)	<i>PL</i>	159.26±6.63	-	-
	<i>GL 500 mg</i>	160.75±4.52	-	-
	<i>GL 1000 mg</i>	159.09±4.41	-	-
Body weight (kg)	<i>PL</i>	58.31±2.37	58.52±2.66	58.60±1.89
	<i>GL 500 mg</i>	60.19±3.01	60.15±2.52	60.09±2.55
	<i>GL 1000 mg</i>	60.96±2.55	60.79±2.92	60.56±2.10
Body mass index (kg/m ²)	<i>PL</i>	23±0.21	23.1±0.29	23.1±0.32
	<i>GL 500 mg</i>	23.3± 0.56	23.3 ±0.47	23.3 ±0.54
	<i>GL 1000 mg</i>	24.1±0.29	24.0±0.37	23.9±0.45

Abbreviations: *PL*= Placebo, *GL*= *Ganoderma lucidum*

Values are expressed as Mean ± SE

Table 2: Perceived stressed scale (PSS)

Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
PSS	<i>PL</i>	29.86	31.02	31.97
	<i>GL 500 mg</i>	30.03	29.54	28.95
	<i>GL 1000 mg</i>	29.57	28.81	27.13*

Abbreviations: *PL* = Placebo, *GL* = *Ganoderma lucidum*, *PSS* = Perceived stress scale

Values are expressed as Mean $\pm$ SE

*p < 0.05 when compared between *PL* and *GL*_{1000 mg} trials.

Table 3: Psychophysiological parameters

Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
Systolic blood pressure (mm of Hg)	<i>PL</i>	120.81±3.41	121.14±3.25	122.02±3.10
	<i>GL 500 mg</i>	121.32±2.68	121.08±2.44	120.70±1.91
	<i>GL 1000 mg</i>	118.84±1.86	118.08±1.66	117.69±1.02**
Diastolic blood pressure (mm of Hg)	<i>PL</i>	81.96±1.10	81.50±0.99	81.02±0.91
	<i>GL 500 mg</i>	80.65±1.00	80.11±1.07	79.67±1.01
	<i>GL 1000 mg</i>	81.48±1.44	80.89±0.81	79.40±0.83
Heart rate (beats/min)	<i>PL</i>	80.80±2.91	81.27±1.15	81.40±1.09
	<i>GL 500 mg</i>	79.85±3.08	78.98±2.32	78.01±2.13
	<i>GL 1000 mg</i>	78.11±2.36	77.73±2.10	75.79±1.61**
HF (nu)	<i>PL</i>	70.34±3.03	69.23±2.87	69.80±2.42
	<i>GL 500 mg</i>	70.20±2.43	71.62±2.45	72.67±2.27
	<i>GL 1000 mg</i>	69.73±2.61	71.60±2.80	74.10±2.70**
LF (nu)	<i>PL</i>	55.23±3.09	58.63±3.80	59.72±3.65
	<i>GL 500 mg</i>	55.60±3.65	53.80±3.29	53.10±3.06
	<i>GL 1000 mg</i>	55.59±3.62	54.04±3.32	51.28±2.70**
LF/HF	<i>PL</i>	0.78±0.07	0.84±0.09	0.85±0.11
	<i>GL 500 mg</i>	0.79±0.14	0.75±0.12	0.73±0.12
	<i>GL 1000 mg</i>	0.79±0.20	0.75±0.16	0.69±0.10**
SDNN (ms)	<i>PL</i>	74.96±2.44	74.73±3.31	76.32±1.27
	<i>GL 500 mg</i>	70.09±2.02	72.35±1.71	75.00±2.19
	<i>GL 1000 mg</i>	76.26±3.07	77.93±3.12	80.97±2.93
RMSSD (ms)	<i>PL</i>	65.19±4.13	63.92±3.91	60.81±3.53
	<i>GL 500 mg</i>	67.78±3.55	68.31±3.07	69.99±2.81
	<i>GL 1000 mg</i>	70.99±5.21	71.95±4.71	73.00±2.19**

Abbreviations: *PL*= Placebo, *GL*= *Ganoderma lucidum*, *HF*= high-frequency power, *LF*= low-frequency power, *LF/HF*= ratio of low frequency to high frequency, *RMSSD*= root mean square of successive differences; *SDNN*= SD of all NN interval
nu= numeric unit; *ms*= milliseconds.

Values are expressed as Mean ± SE

** $p < 0.01$ when compared between *PL* and *GL*_{1000 mg} supplementation trials.

Table 4: Lipid profile parameters

Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
HDL (mg/dl)	<i>PL</i>	64.36±3.87	64.22±1.83	64.08±3.14
	<i>GL 500 mg</i>	65.44±2.79	65.80±2.52	65.99±2.11
	<i>GL 1000 mg</i>	64.63±2.32	65.26±2.49	66.93±3.13
LDL (mg/dl)	<i>PL</i>	93.71±1.63	93.37±1.54	93.01±1.28
	<i>GL 500 mg</i>	90.13±2.52	89.92±2.27	89.32±2.28
	<i>GL 1000 mg</i>	94.76±2.26	93.99±2.36	93.35±1.95
TG (mg/dl)	<i>PL</i>	126.6±4.64	127.9±4.56	128.2±4.66
	<i>GL 500 mg</i>	123.8±2.63	123.4±2.16	122.6±2.18
	<i>GL 1000 mg</i>	125.6±2.84	124.4±4.49	123.9±3.98

Abbreviations: *PL*= Placebo, *GL*= *Ganoderma lucidum*, *HDL*= high-density lipoprotein, *LDL*= low-density lipoprotein, *TG*= triglycerides.

Values are expressed as Mean ± SE

Table 5: Anti-oxidative markers

Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
SOD (u/ml)	<i>PL</i>	2.30±0.24	2.31±0.18	2.23±0.20
	<i>GL 500 mg</i>	2.53±0.17	2.69±0.16	2.75±0.25
	<i>GL 1000 mg</i>	3.07±0.22	3.22±0.22	3.45±0.23***
CAT (u/ml)	<i>PL</i>	72.78±3.10	71.28±2.79	70.45±4.22
	<i>GL 500 mg</i>	75.57±3.02	75.63±3.05	76.30±3.06
	<i>GL 1000 mg</i>	73.83±3.69	74.95±3.61	76.75±4.03
GSH (mg/dl)	<i>PL</i>	17.86±1.09	17.76±1.47	17.37±1.09
	<i>GL 500 mg</i>	17.03±0.85	18.14±1.09	18.81±0.87
	<i>GL 1000 mg</i>	19.76±0.84	20.36±0.95	22.23±0.94*#

Abbreviations: *PL*= Placebo, *GL*= *Ganoderma lucidum*, *SOD*= superoxide dismutase, *CAT*= catalase, *GSH*= glutathione reductase.

Values are expressed as Mean ± SE

* $p < 0.05$; *** $p < 0.001$ when compared between *PL* and *GL*_{1000 mg} supplementation trials.

$p < 0.05$ when compared between *pre* and *post* supplementation trials.

Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
Cortisol (pg/ml)	<i>PL</i>	72.19±3.09	72.25±2.01	72.99±3.07
	<i>GL 500 mg</i>	70.84±2.26	70.10±1.08	69.30±2.16
	<i>GL 1000 mg</i>	74.49±2.67	73.99±2.48	70.39±2.11 [#]
Dopamine (ng/ml)	<i>PL</i>	939.7±35.39	905.5±20.69	863.0±26.49
	<i>GL 500 mg</i>	795.7±27.11	810.8±22.82	819.0±30.30
	<i>GL 1000 mg</i>	860.4±36.36	887.8±23.63	900.1±31.89

Table 6: Stress and neurotransmitter markers

Abbreviations: *PL*= Placebo, *GL*= *Ganoderma lucidum*

Values are expressed as Mean ± SE

[#]p <0.05 when compared between *pre* and *post-supplementation* trials.

Table 7: Inflammatory markers

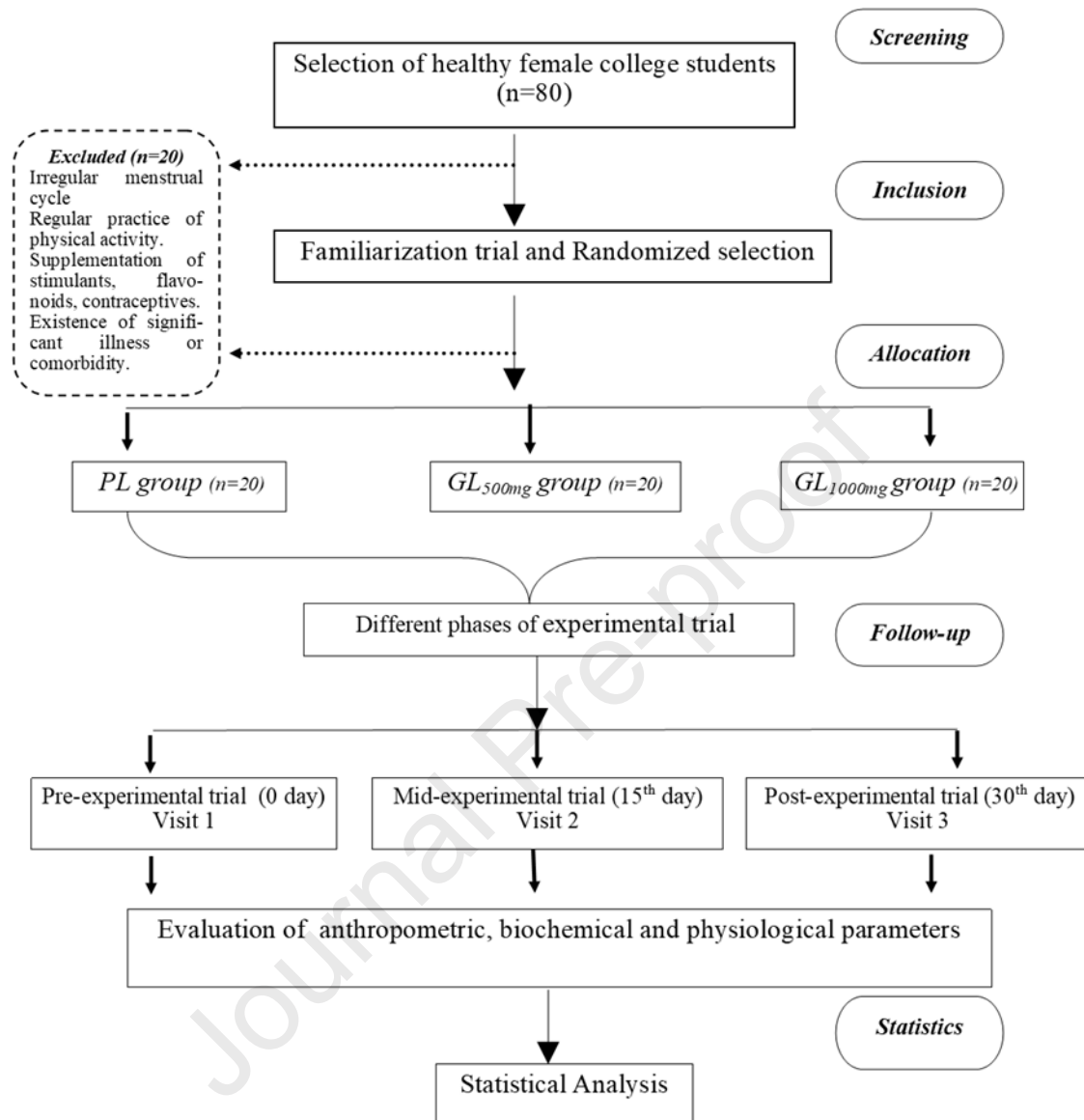
Parameters	Supplementation	Different Phases of Experimental Trial		
		Pre-experimental trial	Mid-Experimental trial	Post-experimental trial
TNF α (pg/ml)	<i>PL</i>	1.66 $\pm$ 0.26	1.79 $\pm$ 0.34	1.70 $\pm$ 0.21
	<i>GL 500 mg</i>	1.51 $\pm$ 0.11	1.50 $\pm$ 0.19	1.54 $\pm$ 0.22
	<i>GL 1000 mg</i>	1.87 $\pm$ 0.28	1.75 $\pm$ 0.31	1.51 $\pm$ 0.25 [#]
IL-6 (ng/ml)	<i>PL</i>	2.32 $\pm$ 0.17	2.38 $\pm$ 0.14	2.43 $\pm$ 0.10
	<i>GL 500 mg</i>	2.10 $\pm$ 0.36	2.08 $\pm$ 0.38	2.08 $\pm$ 0.42
	<i>GL 1000 mg</i>	1.82 $\pm$ 0.44	1.76 $\pm$ 0.37	1.70 $\pm$ 0.39 ^{**}

Abbreviations: *PL*= Placebo, *GL*= *Ganoderma lucidum*, *TNF*= Tumour necrosis factor, *IL*= Interleukin.

Values are expressed as Mean $\pm$ SE

[#]p <0.05 when compared between *pre* and *post* supplementation trials.

^{**}p <0.01 when compared between *PL* and *GL*_{1000 mg} supplementation trials.



CONSORT FLOW DIAGRAM

Fig 1: EXPERIMENTAL DESIGN

Highlights

1. Academic stress may lead to an elevated generation of free radicals causing cellular oxidative strain due to the depletion of antioxidants by an overexpression of reactive oxygen species (ROS) which causes harmful psychophysiological effects.
2. The anti-oxidative components of *GL*, were easily absorbed and swiftly dissolved in the circulation, aiding in the regulation of psychological well-being by reducing oxidative stress in healthy female college students after 30 days of 1000mg daily supplementation compared to 500mg.
3. Triterpenoids derived from *GL* delay the signalling pathway of nuclear factor- κ B (NF- κ B) and activator protein 1 (AP-1), which decreases the release of TNF- α and IL-6 (Pro-inflammatory markers), for attenuating stress response.
4. Our findings revealed that the *GL* modulated influence on both the time domain (ANS activity) and frequency domain (ANS balance) parameters of HRV for determining better psychophysiological fitness, leading to better coping skills in female college students via the predominant effect on the PNS.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: