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Research Article

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Supplementation of Quercetin Nutraceutical Ameliorates Stress among Female College Students

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Abstract: Restricted information regarding the protective effect of quercetin against stress-effectuated depression, anxiety and hypertension in students has been evaluated. Quercetin, king of flavonoids offers a great promise to reduce depression that negatively affects human mental and physical health which ultimately proliferates cardiovascular risks. This study aimed to enlighten the propitious effect of oral consumption of quercetin at a dose of 200 mg/day for 30 days on healthy college students prone to academic stress to explore its beneficial effects on body composition, blood pressure, oxidative stress and inflammatory markers in a randomised, double-blinded, placebo-controlled cross-over trial (n=100). In contrast to placebo, quercetin-rich supplementation significantly reduced ($p<0.05$) psychological stress scale, body fat ratio (BFR), pulse rate, systolic pressure (SP) and tumour necrosis factor-alpha (TNFα) from their baseline values. Furthermore, a significant increase ($p<0.05$) in oxidative and (anti) inflammatory marker i.e., superoxide dismutase (SOD) and interleukin 10 (IL10) were observed but did not affect catalase (CAT) and reduced glutathione (GSH) when compared with placebo. Thus, daily quercetin-rich supplementation supervenes antidepressant effects by attenuating inflammatory response, rehabilitating the activity of antioxidant enzymes, declining oxidative stress markers and hence improving alterations of the HPA axis which ultimately reduces blood pressure. These data are the first to our knowledge to show that quercetin supplementation could be a supportive therapy for improving stress and physical fitness in depressed subjects. This indicates the effectiveness of the flavonoid as a nutraceutical compound against the expansion of behavioural perturbation induced by psychological stress in a cost-effective, biofriendly manner. We explored a non-pharmacological but decent supra nutritional dose of quercetin since these data should provide a rational basis for the development of functional foods. The interminable effectiveness of quercetin supplementation for better improvement of stress remains to be investigated.

Keywords: Quercetin, Psychological stress, Depression, Cardiovascular risks, Blood pressure, Oxidative stress, Inflammation.

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1. INTRODUCTION

Stress can be defined as "a stimulus derived from physical or psychological indices that produce reactions either mental or physiological which leads to illness".[1] A college student in his or her own life interacts with several physical and psychological stresses. Among college students, stress is becoming more prevalent.[2] Many students who wish to attend a post-secondary school by moving away from their home town itself causes an atmosphere of stress, anxiety and depression. Academic performances concerning high workloads, grade requirements, time management etc. among college/university students' cognizance's about bio-psycho-behavioural responses to a naturalistic stressor.[3-7] Cognitive functions such as coordination, reasoning, attention, memory, the perception that consists of various skills can be negatively affected due to higher levels of stress. Finally, the overall academic performance and self-esteem undergo a huge downfall among students.[8] Continuous psychological stress exposure can modulate the response of primary antibody and thus permanent increase in stress levels can lead to psychosomatic disease, pathological organ changes and physiological alterations.[9] Self-performance and cognitive tasks can be improved during the mild stressful condition by achieving the academic goal but continuous influx of high academic stress cause neuropsychiatric disease entities.[10] Studies have further shown that academic life leads to anxiety, stress and depression among college students which is directly associated with pernicious behaviours that can be detrimental.[11] Lack of sleep, which is affiliated with pernicious behaviour among college students shows negative feedback on academic performance.[12] Behavioural alerting response induced by stress shows a direct relationship with an increase in sympathetic cardiac activity and a decrease in the parasympathetic activity of the heart. Psychological and physiological stress leads to poor HRV components. These changes are determinable to a conclusion that psychological stress is directly linked to an increased cardiovascular threat of hypertension.[13-15] A relationship has been investigated among psychological stress and destructive footprints of reactive oxygen species (ROS) leading to oxidative stress.[16] Continuous inflow to stress leads to intensive manufacturing of free radicals and oxidative burden.[17] ROS production is generally controlled by antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase (CAT) which scavenges free radicals and prevents biological membranes from oxidative damage.[18] This damage may evolve due to overexpression of ROS or due to exhaustion of the antioxidants.[19] Depletion of various free radical detoxifying enzymes i.e.; SOD, CAT & GSH occurs due to stress.[20] Academic examinations are also associated with changes in the level of immune activity, cortisol and catecholamine which have been reported in the literature.[21-23] Therefore, there is a huge concern regarding the health condition among college/university students of various age groups because of the highly stressful period which affects the physiological, psychological and oxidative stress wellbeing.[24] Quercetin (Q-DIP) is generally referred to as a polyphenolic flavonoid compound that is found in diversified fruits and vegetables.[25] No support in favour of toxicity of quercetin is found and recent reviews support quercetin's dietary intake.[26] It is considered to have distinctive biological properties that may boost or ameliorate psychological and physical performance and also diminish the risk of infection.[27] Specific activities of Q-DIP have been aligned with a decreased risk of cardiovascular diseases (CVD) along with anti-oxidative, anti-inflammatory properties. Q-DIP fortifies cellular complex molecules against oxidative stress damage by scavenging oxygen free radicals, lipid peroxidation inhibition and lowers 8-hydroxydeoxyguanosine formation by UV light irradiation.[28-30] In vivo studies have shown that Q-DIP elevates the antioxidant capacity by maintaining the levels of GSH because SOD rapidly engulfs O_2^- and converts it into H_2O_2 which further catalyses into nontoxic H_2O. This entire phenomenon requires GSH as a hydrogen donor. Studies derived from animals and cells found that Q-DIP induces GSH synthesis. Effects of Q-DIP on heart diseases show an increase in SOD, CAT activity which effectively protects against myocardial infractions. Elsewhere it has been shown that the effect of Q-DIP on depression lowers oxidative and inflammatory stress. However, quercetin supplementation among humans have not shown any conclusive results but conflicting results were found based on the type of compound (pure or food source), duration and dosage of study.[31-34] Moreover, very little information is there regarding the effects of daily acute supplementation of quercetin among stressful college students on physiological (pulse, systolic pressure, diastolic pressure) and biochemical (SOD, CAT, GSH, TNF α & IL 10) parameters. This present novel study was hypothesised to resolve studies regarding the role of quercetin in oxidative stress profile & stress markers among healthy stressful college students which has rarely been reported. In addition to this, the effect of quercetin on stress revealed degenerative changes in psychological stress, cardiometabolic risk factor, and oxidant-antioxidant defence mechanism that has not been demonstrated previously.

2. MATERIALS & METHODS

A total of 117 healthy Indian female college students of Raja Narendra Lal Khan Women's College (AUTONOMOUS), West Bengal, India were randomly selected for this scientific research work after explaining to them the study protocol who gave their consent letter as per the guidelines of ICMR. Inclusive criteria include age group within 18-23 years, healthy and active female's and obtaining a high score on psychological stress scale to assess stress level. Exclusive criteria were: the existence of the disease, use of any form of medication (excluding oral contraceptives) or nutraceuticals and alcoholics, tobacco, cigar consumers. Before the study each participant was examined for body weight in kg and height in cm, basal metabolic index (BMI) in kg/m^2, blood pressure in mm/hg, pulse in beats/min and psychological stress scale has been evaluated by scorecard method. Volunteers were instructed to maintain their normal lifestyle with no heavy physical activities during the entire study. Seventeen (17) participants were excluded from the study as not found within the inclusive criteria or their unwillingness to continue further. The remaining hundred (n = 100) volunteers were randomly divided into two groups: (a) Quercetin Group (n = 50) and (b) Placebo Group (n = 50).

2.1 Ethical Consent

All procedures performed in this study were conducted in accordance with the Declaration of Helsinki (2013), National Guidelines for Biomedical and Health Research Involving Human Participants (2017). Approval for this double-blinded study has been granted by the Institutional Ethics Committee of Raja Narendra Lal Khan Women's College (Reference No: 01/IEC-ICMR/RNLKWC/2019 dated 7-12-2019) and written

informed consent was obtained from each subject as per ICMR guidelines.

2.2 Experimental Design

After obtaining the written informed consent from the selected participants, the female college students were under observation for duration of 14 days with no recent illness or injury together with a washout period of quercetin low diet food intake to avoid any impact on study results. This research work consisted of a randomized, double-blinded, placebo-controlled parallel design study along with quercetin rich supplementation, provided by Defence Food Research Laboratory (DFRL), on healthy, stressful female college students. Randomly assigned subjects were instructed to take either two placebo bars (n = 50) or two quercetin bars each day (n = 50) for a period of 30 days. Each quercetin (Q-DIP) bar consisted of 0.1g of quercetin extracted from fruits with other ingredients similar to the placebo bar such as puffed rice, oats, milk powder, sugar and honey. Assessment of psychological or stress level, anthropometric, physiological indices and oxidative-antioxidative status were estimated for each subject in the field laboratory. All the variables were recorded at baseline, before quercetin or placebo consumption (0 days) and after supplementation (30 days).

2.3 Psychological Parameters/ Stress Level

To estimate the subjective significance of academics as a means of stress, we applied a psychological stress scale to measure the existing feeling of stress, anxiety and depression among female college students. A Pre-tested Questionnaire was designed to assess various aspects of the subject's psychological status or stress level using the scoreboard method. The gradation of stress score categorized as: (i) >99 = high stress (ii) 75-99 = Medium stress (iii) 50-75.4 = low stress (iv) < 50 = No stress. The scoring system was consulted with a renowned psychiatrist, Dr Srimanti Chowdhury, DCP(Ire), MRCPSYCH(UK).

2.4 Anthropometric Parameters

Anthropometric measurements were measured with minimal clothing. Body Weight in kilograms was measured on a standard electronic weighing machine (Delmer, India) with a minimum count of 0.1kg. An anthropometric rod was used to measure the standing height in centimetres from the base of the foot up to the top of the head in an erect body position. Body mass index (BMI) was calculated by *Quetelets'* index formula.[35] Body fat percentage was assessed by following the standard formula of *Gallagher et al.*[36]

2.5 Resting Physiological Parameters

2.5.1 Resting pulse rate & blood pressure

The pulse rate of each participant were measured using a pulse oximeter. The resting blood pressure i.e., both the systolic & diastolic blood pressure (SBP & DBP) was observed and recorded using a sphygmomanometer (Doctor-R, model no: D1208) by the auscultatory method in mm/Hg in a supine position.[35]

2.6 Biochemical Estimation

2.6.1 Venous Blood Collection

Collection of blood samples from antecubital vein puncture of overnight fasting subjects in ethylene diamine tetra acetic acid (EDTA) treated vials in the morning in resting condition has taken place both at baseline (0 days) and after intervention (30 days). Collected blood samples were kept in a blood mixture roller for 5 minutes and then immediately centrifuged at 2500 x g for 10 min. at -4 ^{0}c to collect plasma. Samples were stored at -20 ^{0}c for different biochemical estimations.[35]

2. 6.2 Antioxidant Enzyme Profile

Activities of superoxide dismutase (SOD), catalase (CAT) & glutathione reductase (GSH) were assayed for antioxidant enzyme status by using standardised Elisa kits.[37-39] Analysis was read by using a microplate reader (BioRad).

2.6.3 Inflammatory markers

Activities of inflammatory markers i.e.; tumour necrosis factor-alpha and interleukin 10 (TNF alpha and IL 10) were assayed for analysis of stress levels by using standardised Elisa kits.[40] Analysis was read by using a microplate reader (BioRad).

3. INCLUSION AND EXCLUSION CRITERIA

3.1 Inclusion Criteria

a) Indian female college students of 18-23 years only.
b) Must be normally healthy and physically active.
c) Submission of consent letter as per ICMR guidelines.
d) Estimated as being stressful by stress score card.
e) Low intake of quercetin rich foods, particularly citrus foods, apples, onions, grapes, and beverages.
f) Must have a normal menstrual cycle length

3.2 Exclusion Criteria

a) Existence of any kind of disease.
b) Consumption of medications, nutraceuticals and psychoactive substances.
c) The effect of intervention is difficult to interpret.
d) Unwillingness to continue further.

4. STATISTICAL ANALYSIS

The data were calculated and statistical analysis was done by using the statistical package, GraphPad Prism In-Stat version 5.00 for Windows (GraphPad Software, San Diego, California, USA) and Origin 6.1(Northampton, Mass, USA). The statistically collected data were calculated and were expressed as mean ± standard error of the mean. Comparison of normally distributed data between the groups was performed using the unpaired student t-test and within a group using a paired student t-test. Data that were not normally distributed were compared using the Mann-Whitney & Wilcoxon signed-rank test. Normality was checked by Kolmogorov-Smirnov test, D' Agostino& Pearson Ombius test and Shapiro-Wilk test. A significant difference was set up at a p-value of ‹ 0.05.

5. RESULTS

5.1 Psychological Stress Scale

In contrast to placebo, quercetin (Q-DIP) supplementation indicates a significant decrease ($p<0.05$) in stress scores from their baseline values after 30 days of intervention. After supplementation of a placebo, a significant increase ($p<0.05$) in stress score was observed. Baseline changes were not significantly different ($p<0.05$) between the groups although between treatment groups significant changes ($p<0.05$) were observed [Figure 1].

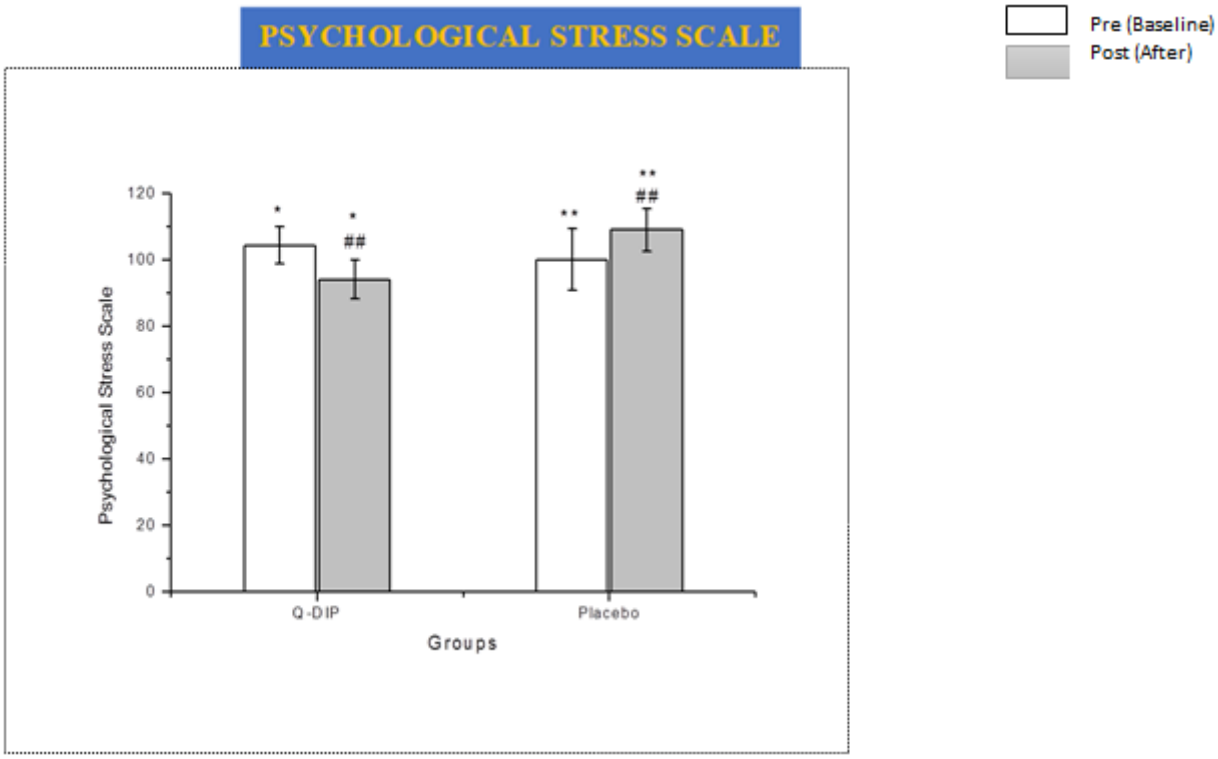

Fig 1: Graphical representation for psychological stress scale in human subjects before and after supplementation of quercetin & placebo.

Values are means with standard error of mean represented as vertical bars. *Quercetin, **Placebo mean value was significantly different from that at baseline ($p< 0.05$; intra-group comparison; Wilcoxon test or paired t test). #The two groups were significantly different with regard to any values at baseline ($p< 0.05$; independent-sample t test; Mann–Whitney U test or unpaired t test). ## The two groups were significantly different with regard to any values when compared between quercetin supplementation than during placebo intervention ($p< 0.05$; independent-sample t test; Mann–Whitney U test or unpaired t test).

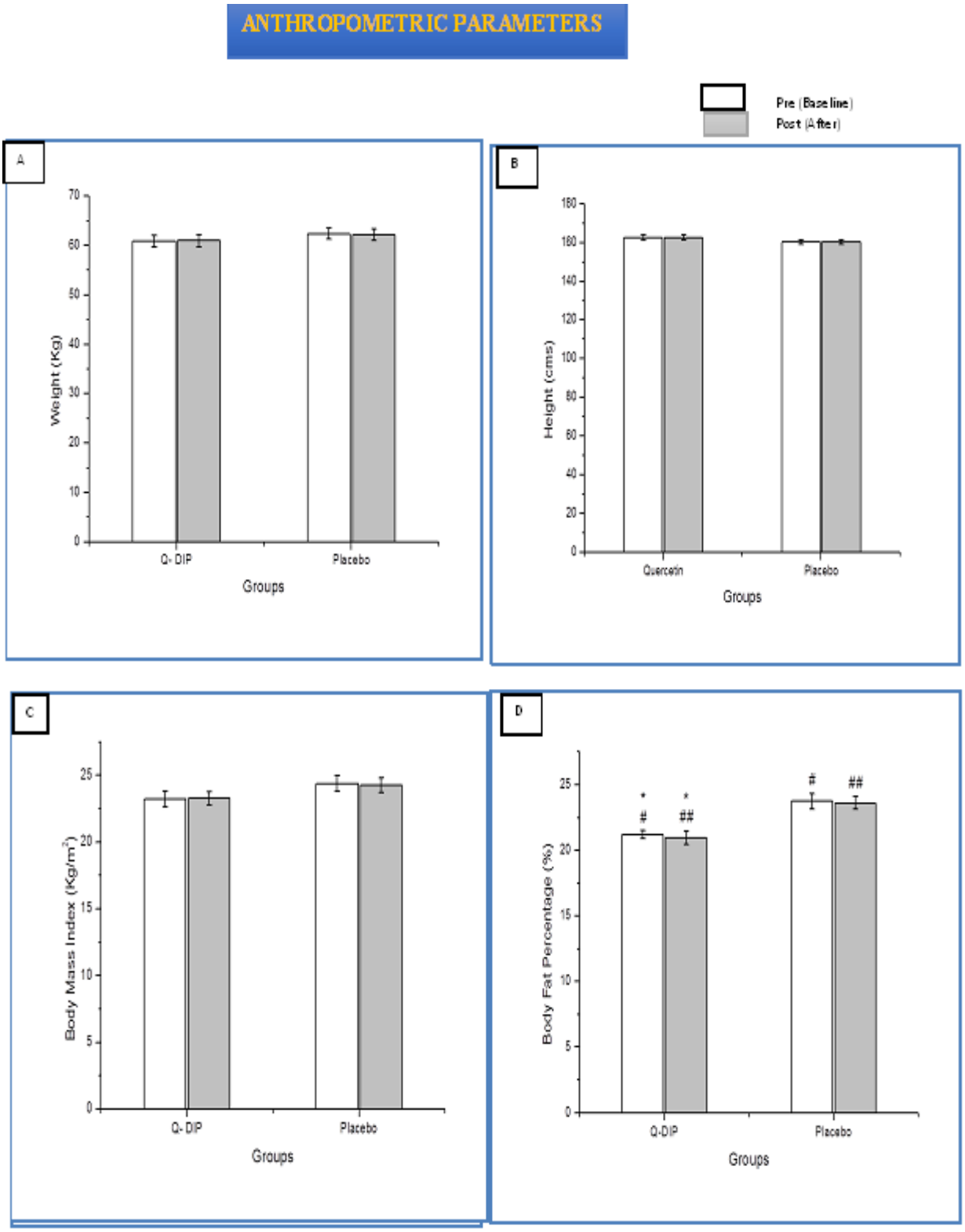

Fig 2: Graphical representation for (A) Body Weight (B) Height (C) Body Mss Index (D) Body Fat Percentage in human subjects before and after supplementation of quercetin & placebo.

Values are means with standard error of mean represented as vertical bars. *Quercetin, **Placebo mean value was significantly different from that at baseline (p< 0.05; intra-group comparison; Wilcoxon test or paired t test). # The two groups were significantly different with regard to any values at baseline (p< 0.05; independent-sample t test; Mann–Whitney U test or unpaired t test). ## The two groups were significantly different with regard to any values when compared between quercetin supplementation than during placebo intervention (p< 0.05; independent-sample t test; Mann–Whitney U test or unpaired t test).

5.2 Anthropometric Parameters

Quercetin (Q-DIP) & placebo supplementation didn't show any significant changes ($p<0.05$) in weight, height, BMI. But, a significant decrease ($p<0.05$) in BFR was observed after quercetin intervention from the baseline whereas this significant result was not shown with placebo-rich supplementation. Intergroup comparison of BFR showed significant changes ($p<0.05$) within baseline values and after intervention [Figure 2A, 2B, 2C, 2D].

5.3 Resting Physiological Variables

DP didn't show any significant reduction ($p<0.05$) after 30 days of Q-DIP and placebo supplementation. Pulse rate and SP showed a significant decline ($p<0.05$) after 30 days of Q-DIP supplementation from their baseline measurements. Intragroup comparison within the placebo group after 30 days of supplementation showed a significant increase in DP ($p<0.05$). Intergroup comparisons showed significant changes ($p<0.05$) within baseline values of pulse rate and DP [Figure 3A, 3B, 3C].

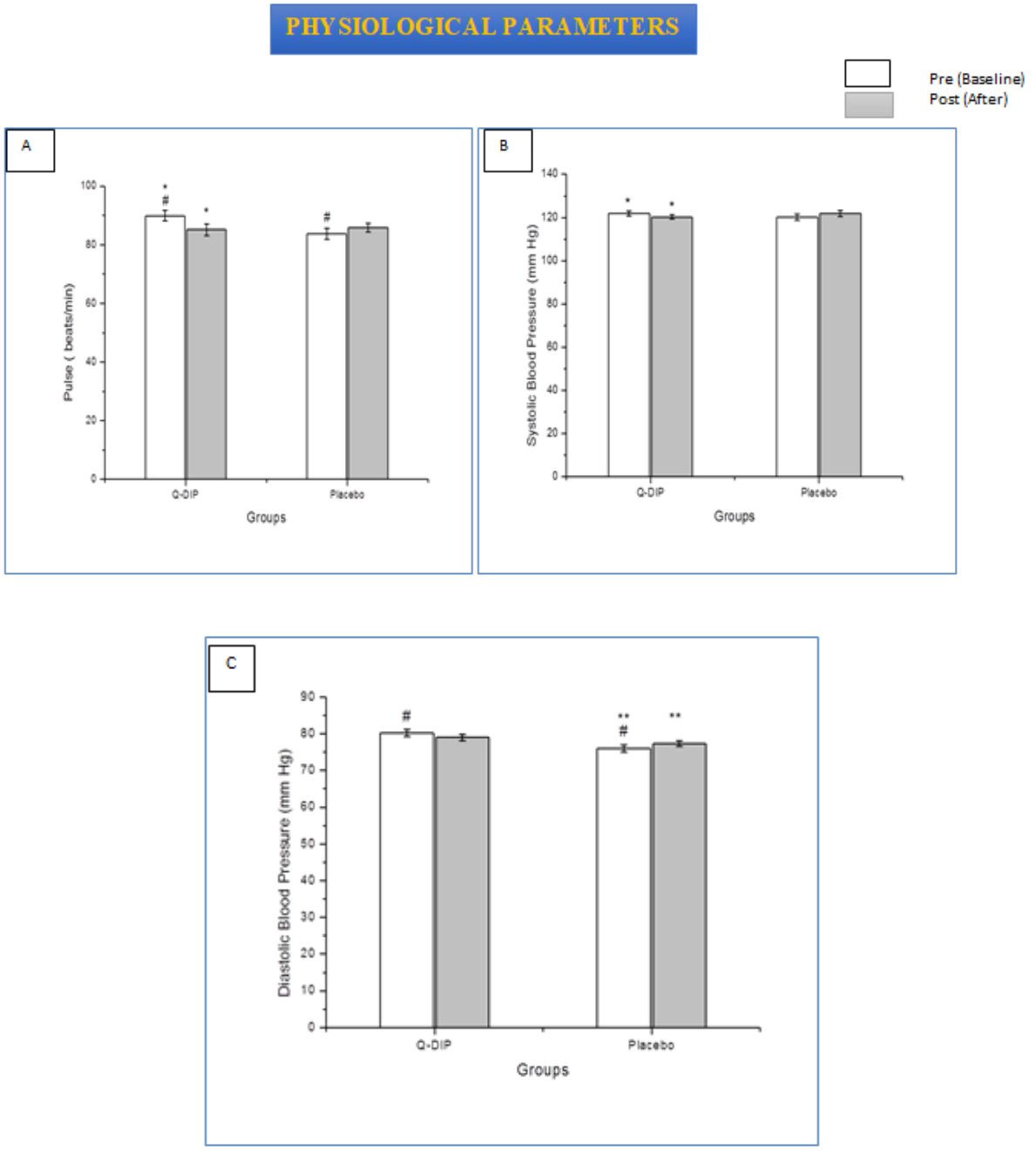

Fig 3: Graphical representation for (A) Pulse (B) Systolic Blood Pressure (C) Diastolic Blood Pressure in human subjects before and after supplementation of quercetin & placebo.

Values are means with standard error of mean represented as vertical bars. *Quercetin, **Placebo mean value was significantly different from that at baseline ($p< 0.05$; intra-group comparison; Wilcoxon test or paired t test). #The two groups were significantly different with regard to any values at baseline ($p< 0.05$; independent-sample t test; Mann–Whitney U test or unpaired t test). ## The two groups were significantly different with regard to any values when compared between quercetin supplementation than during placebo intervention ($p< 0.05$; independent-sample t test; Mann–Whitney U test or unpaired t test).

5.4 Antioxidant Levels

After 30 days of quercetin supplementation, the levels of SOD were increased significantly ($p<0.05$) in female college students dealing with psychological stress. Catalase & GSH tends to increase non-significantly. Significant reduction ($p<0.05$) in catalase (CAT) level between pre-post variables of the placebo group was estimated. Before intervention levels of CAT and GSH reported significant changes within inter-groups. SOD, CAT and GSH levels after quercetin and placebo supplementation differ significantly when analysed for inter-groups [Figure 4A, 4B, 4C].

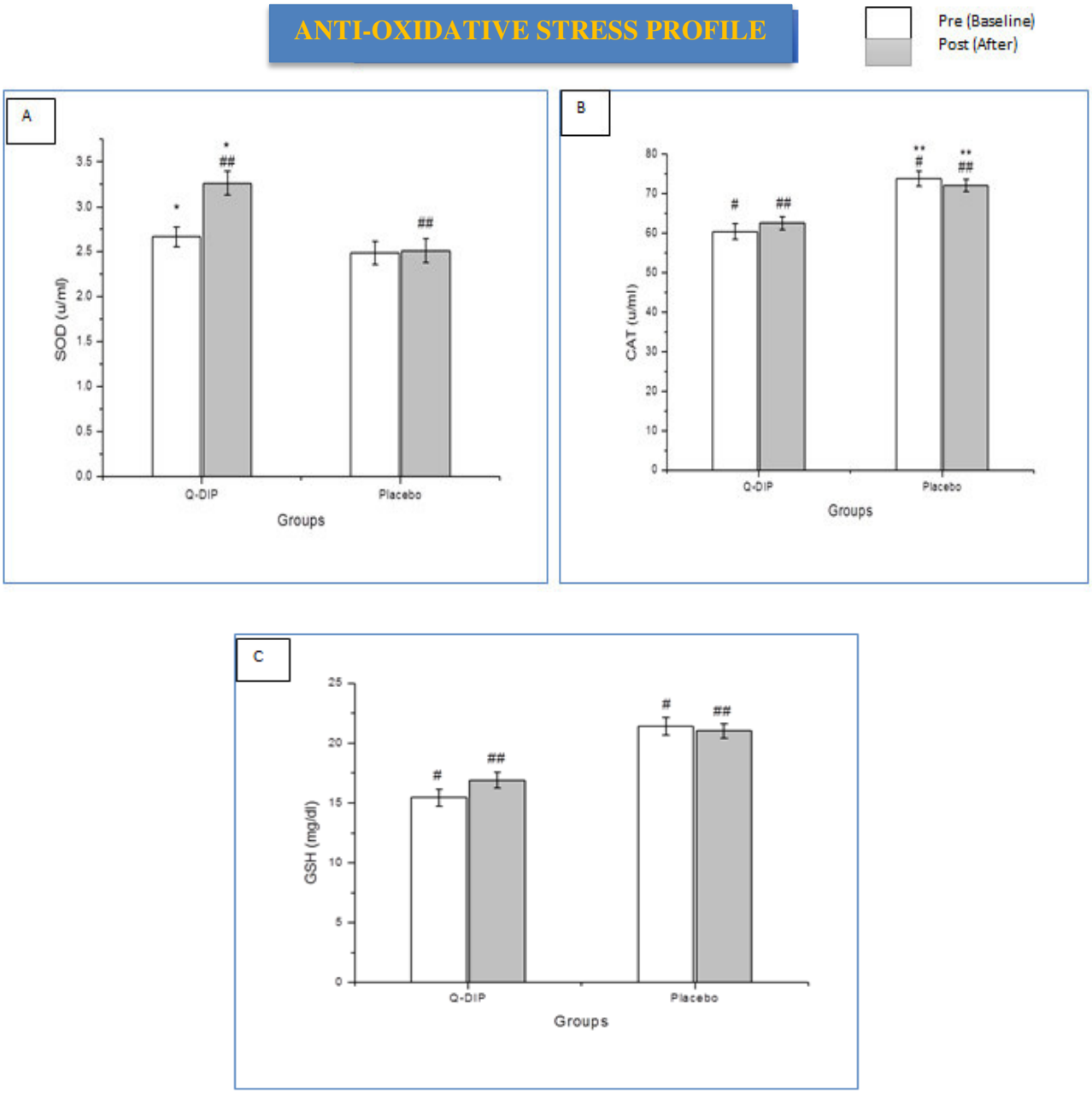

Fig 4: Graphical representation for (A) SOD (B) CAT (C) GSH in human subjects before and after supplementation of quercetin& placebo.

Values are means with standard error of mean represented as vertical bars. *Quercetin, **Placebo mean value was significantly different from that at baseline (p< 0.05; intra-group comparison; Wilcoxon test or paired t test). #The two groups were significantly different with regard to any values at baseline (p< 0.05; independent-sample t test; Mann–Whitney U test or unpaired t test). ## The two groups were significantly different with regard to any values when compared between quercetin supplementation than during placebo intervention (p< 0.05; independent-sample t test; Mann–Whitney U test or unpaired t test).

5.5 Inflammatory Markers

Changes from baseline were significantly reduced ($p<0.05$) within quercetin groups for pro-inflammatory level markers i.e.; TNF alpha. Significant changes ($p<0.05$) were observed at baseline in TNF alpha between Q-DIP and Placebo. IL-10 also showed a significant increase ($p<0.05$) in concentration after supplementation of quercetin [Figure 5A, 5B].

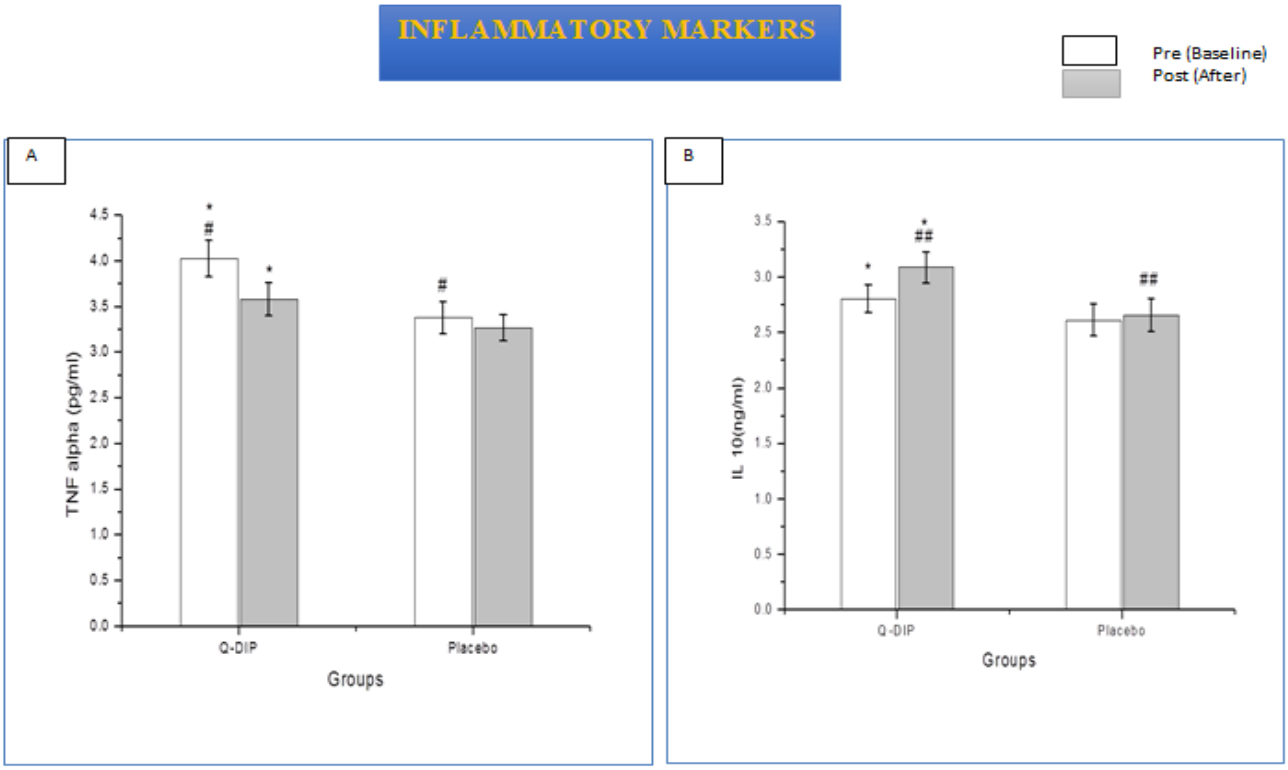

Fig 5: Graphical representation for (A) TNF alpha (B) IL-10 in human subjects before and after supplementation of quercetin& placebo.

Values are means with standard error of mean represented as vertical bars. *Quercetin, **Placebo mean value was significantly different from that at baseline ($p< 0.05$; intra-group comparison; Wilcoxon test or paired t test). #The two groups were significantly different with regard to any values at baseline ($p< 0.05$; independent-sample t test; Mann–Whitney U test or unpaired t test). ## The two groups were significantly different with regard to any values when compared between quercetin supplementation than during placebo intervention ($p< 0.05$; independent-sample t test; Mann–Whitney U test or unpaired t test).

6. DISCUSSION

Stress is controlled differently among males & females.[41] Limited studies show that cognitive performance differs in both males & females when emotionally driven or during anxiety and depression following stress.[42] The polyphenolic flavonoid quercetin tends to demonstrate a powerful anti-stress product along with its phytonutrient &bioprotective properties. However, there is a lack of information regarding the ameliorative effects of acute quercetin supplementation on the autonomic nervous system, oxidative stress and stress level markers. The centre of attention of our research is to investigate the efficacious effects of 30 days supplementation of quercetin (200 mg/day or 0.2 g/day) on anthropological measurements, physiological indices, antioxidant enzyme levels & markers of stress level in a psychosocial stress paradigm in female college students. To quantify the subjective significance of academics as a stressor we used the psychological stress scale/stress scorecard method. Escalation in anxiety, depression caused by high academic day to day schedules is a potent cause of stress. As academics are an important part of college student's life and without a persuasive objective towards an academic target, students can be troubled with destroying bursts of stress.[43] High-level psychological stress is a major cause of depression characterised by overactivity of the hypothalamus-pituitary-adrenal axis (HPA axis) and an uncontrolled regulation of the autonomic nervous system.[44] As per reports of the World Health Organisation (WHO), depression is considered as the second cause of disability most often among women.[45] Studies have shown that neuronal transporter gene *SLC6A15* in particular sites of the brain is responsible for emotional regulation but when altered becomes vulnerable to stress and depression.[46] The initial goal of this survey was to correlate psychological stress with academic performance, a burden to success, post-graduation map out's, enormous workload and expectations from family. Thus, these all concerns may cause a spike increase in stress score. Our study findings show high scores in the psychological stress scale both in the quercetin and placebo group before supplementation signifying the effects of academic loads.[47]An acute dose of quercetin for a period of one month significantly reduces the effects of stress on the HPA axis & autonomic nervous system. Several preclinical studies gauge operative properties of glycosides in quercetin in depressive-like behaviours'[48]. In our study, supplementation with quercetin caused a significant reduction in BFR. Although no study evidenced such changes in human experiments, very rare success was revealed in animal & human studies.[49-51] Stimulation of mitochondrial biogenesis by ingesting

quercetin may cause oxidation of fat and hence BFP may decrease. Quercetin plays an effective role in the apoptosis of adipocytes in vitro through the AMPK pathway (adenosine monophosphate-activated protein kinase pathway).[52] Our research highlights the hypothesis, fat mass can increase due to the proliferation of preadipocytes during psychosocial stress and quercetin induces apoptosis in 3T3-L1 pre-adipocytes by reducing mitochondrial membrane potential, knock downing poly (ADP ribose) polymerase (PARP) and Bcl-2 and initiating caspase 3, Bax & Bak. Further, quercetin shows rat-adipocytes to undergo lipolysis and is thus reported to be a potent PDE inhibitor at an effective dose[53-55]. Our results show that 30 days of quercetin consumption at an effective dose of 200mg/day significantly declines the baseline measurements of pulse rate & SP in the hypertensive group.[56] Different researchers have manifested, quercetin's ability to lower blood pressure in animal and human models during hypertensive conditions due to quercetin ability to regulate oxidative stress.[57] Controversial reports have been accounted for in human intervention studies while investigating the blood pressure-lowering results. Our results were very close to the findings of quercetin supplementation (150 mg/d) for 6-week reduces SBP.[58] Thus, to show ameliorative effects of quercetin certain exposure to hypertension is required in younger and middle-aged individuals to improve endothelial function by increasing endogenous NO (S-nitroso-thiols, nitrite and nitrate) and reducing endothelin-1 production[59]. Studies reviewed, proliferative levels of perceived stress to be directly proportional to an escalation of oxidative damage among women.[60] Research findings recommend that vascular and renal oxidative stress may occur in human subjects due to findings of hypertensive animals that show a local increase in vascular and renal oxidative stress. Quercetin might produce local effects.[61] This study shows after quercetin supplementation SOD activity increases significantly as compared to CAT and GSH. The placebo trial shows a significant decline in catalase concentration. Hydrogen peroxide (H_2O_2) is liberated instantly from superoxide through an expeditious reaction of dismutation that might take place impulsively or enzymatically with SOD. Thus, H_2O_2 evolution takes place simultaneously along with O_2^- generation. O_2^- and H_2O_2 are catalysed by metalloproteins i.e., SOD & CAT. SOD and CAT catalyse the formation of H_2O_2 from two molecules and oxygen & water from H_2O_2 molecules. Hike in SOD produces additional H_2O_2 molecules. Thus, hydroperoxide levels were decreased in the quercetin supplementation group. Catalase activity was non-significantly increased because consumption of quercetin catalysed more H_2O_2 and produced O_2^- and H_2O. The richest endogenous thiol i.e.; glutathione (GSH) is not influenced by 30 days of quercetin supplementation (200mg/day). This might be due to the possibility that GSH levels are appropriate to consume the ROS products of quercetin but not in placebo. [62] Egert et al reported no significant changes in oxidative stress/ antioxidative status after supplementation of 150 mg/day of quercetin for 2 weeks. Further, the potential consequences of quercetin in type 2 diabetes patients show control in oxidative stress.[63] Investigations also suggest quercetin might be carried in rat and human blood by serum albumin in the form of complexes between albumin and quercetin 3-O conjugates which still remain an issue of hypothesis.[64] Additional literature enlights the fact that absorption of quercetin with vitamin c, folate and additional flavonoids upgrades its solubility &effectivity which may be a predictable cause of our result. An increase in activity of endogenous antioxidant enzymes and inhibition of free radical generation was reported in Sprague- Dawley rat models.[65] Chronic consumption of quercetin shown to reduce erythrocyte oxidative damage after strenuous physical exercise in humans.[66] In the western population recommended dose for Q-DIP ranges from 200-1200mg/day. Our findings reflect the beneficial effects of quercetin as an antioxidant supplementation among female college students with enhanced oxidative stress. The present study describes a vital differentiator between the baseline values caused by oxidative damage and the remunerative execution of antioxidant supplementation. To the best of our understanding so far, no studies have explored the effects of pure quercetin on inflammation at an acute dose of 200 mg/day for a month. Previous experimental investigation showed inhibition in gene expression & in the production of TNF α (a pro-inflammatory cytokine) by peripheral mononuclear cells (PBMC). This mechanism occurs due to NF-κβ signal transduction cascade modulation.[67] During academic's students, tend to show advancement in oxidative stress in the body due to an increase in mental stress. Supplementation of pure flavonoid (quercetin) in pre-hypertensive women (40-80yrs) didn't show any significant change in TNF α at a dose of 160 mg/day for 4 weeks although CRP (c- reactive protein) concentration was suppressed and such similar studies conclude that dosage/duration in presence of oxidative stress may be a concern of investigation to show a significant decrease in pro-inflammatory markers in study subjects. In relation to the above statement, it has been investigated, quercetin reduces oxidative stress along with a decline in TNF α among pre-post-menopausal women.[68] Boots et al, supplemented quercetin (500 mg x 4) for one day in sarcoidosis patients to show a decline in oxidative stress markers & inflammatory markers. In murine macrophages, quercetin inhibits production in LPS- induced nitric oxide & TNF α production. In light of the above shreds of evidence, our study is the first to show anti-inflammatory & anti-oxidative effects by consumption of 200mg/day quercetin. A possible route for this mechanism might be due to the fact that ROS are instantaneously entangled with the development of oxidative stress and are capable of stimulating the transcription factors nuclear factor kappa B (NF-κB) & activator protein 1 that cause inflammation. TNF alpha is induced by these transcriptional factors. Quercetin may show scavenging properties against ROS and simultaneously weaken inflammation. As previously stated, modulation of NF-κB inhibits TNF α in human peripheral blood mononuclear cells. Simultaneously, it was noted that (NF-κB) activation will initiate radical formation. Inevitably, anti-inflammatory repercussions of quercetin decrease NF-κB activation. Hence, preventing the radical formation and reducing oxidative stress. This shows an interlink between anti-inflammatory & anti-oxidative effects of quercetin.[69]

7. LIMITATIONS

The duration of study intervention was short i.e., only for 30 days that need to be improved. An additional limitation is that this study only includes female college students without any involvement of male graduates. In our future studies, we plan to increase the sample size by incorporating both male & female students and supplementing quercetin for long-term intervention. Further, MDA study would be conducted

among college students as it's a crucial marker for estimating oxidative stress.

8. CONCLUSION

Based on the results of the present study, it can be emphasized that quercetin at an effective acute dose of 200 mg/day can detoxify the harmful effects of oxidative stress & hypertension in female college students during their academics. Normal healthy lifestyle and physical fitness can be rebuilt by regular supplementation of quercetin at 200 mg/day by decreasing body fat percentage. The findings of the study validate the efficacy of quercetin at acute dose on body composition, psychological stress scale, physiological indices, antioxidant status, and inflammatory markers in healthy stressful volunteers. Our data may be beneficial for students, parents and teachers to cope with the continuous adverse effects of academic stress. Thus, regular consumption of quercetin (200 mg/day) may be helpful to reduce stress and maintain a disease-free lifestyle.

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11. AUTHORS CONTRIBUTION STATEMENT

Sudeep Mitra, Mousumi Mitra, Dilip Kumar Nandi were responsible for participants' recruitment, physical check-up, blood sample collection & analysing answers to the questionnaires (to determine psychological stress scale). Sudeep Mitra & Mousumi Mitra had contributed to literature search, data acquisition, data analysis, manuscript preparation. Sudeep Mitra, Mantu Saha, Dilip Kumar Nandi had contributed to concept design. All authors have read and approved the manuscript for submission.

12. CONFLICT OF INTEREST

Conflict of interest declared none.

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