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EFFECT OF NICOTINE ABUSE ON OXIDATIVE STRESS AND PERCEIVED STRESS AND ITS RELATIONSHIP WITH COPING SELF EFFICACY AMONG UNIVERSITY GRADUATES

Sandeep Kumar*[1], Astha Dwivedi[1], Anuja Mishra[2], Sharmistha Singh[1], Poonam Chandra Mittal[1]

[1]Department of Biochemistry, Faculty of Science, University of Allahabad, Allahabad-211002, India
[2]Department of Biotechnology, Institute of Applied Sciences & Humanities, G.L.A. University, Mathura (U.P.), India

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Nicotine abuse

Perceived stress

Coping self-efficacy

Oxidative stress

Mediation model

ABSTRACT

An interdisciplinary review of the literature portrayed stress as an important cause for nicotine abuse among university students. Independent studies have shown nicotine to contribute perceived stress (PS) and oxidative stress (OS) but its mediation relation with PS and OS remains unclear and inspires active exploration. A prominent study on the relationship of smoking with perceived stress and coping styles in adolescents motivates to study the effect of nicotine abuse (NA) on PS, CSE, and OS indices among young adult university graduates. The study sample included 45 university graduates with 1-3 years of tobacco abuse history and 50 age-matched controls. The respondents were compared for perceived stress scale (PSS) score, coping self-efficacy (CSE) score, erythrocyte malondialdehyde (E_MDA), plasma MDA (P_MDA), erythrocyte superoxide dismutase (E_SOD), and plasma catalase (P_CAT).

The study found NA increased the PS but not the CSE. Linear regression analysis showed a strong inverse relation between the PSS Score and CSE Score in the controls. Among the biochemical indices of OS, only P_MDA showed a significant difference between the groups. Multiple regressions showed a significant positive association of E_MDA with PSS Score and a significant negative association of E_SOD with PSS Score across the groups. Further, the mediation model is used to show a significant relationship between NA and PSS Score by Combined MDA (C_MDA). The result of study suggested that nicotine increases PS and reduces CSE. P_MDA is an important biochemical marker of nicotine abuse. E_MDA and E_SOD are important predictors of PS. These findings are important for psychobiochemical interventions in the management of NA. Therefore, this study encourages an interdisciplinary discourse on nicotine abuse with psychological and biochemical measures.

* Corresponding author
E-mail: sandeep@cbcs.ac.in (Sandeep Kumar)

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1 Introduction

University students often experience stress which affects their academic and social life with negative psychological and health outcomes, subjecting them to substance abuse behaviours; predominantly alcohol, cannabis, and nicotine abuse (Tavolacci et al., 2013). College graduates are most likely to involve in nicotine abuse behaviour as they perceive nicotine helps to cope with socio-personal stress (Nichter et al., 2007; Shalaby & Soliman, 2019). A recently published article on nicotine and tobacco research reviewed the studies on youth and young adult tobacco use and illustrates the FDA's regulation of tobacco products, efforts, and education in the prevention of tobacco use by young adults (Perry et al., 2020). Nicotine has several detrimental effects on health, cancer being the most prominent (Mishra et al., 2015) and has been shown to have divergent immediate and chronic effects (Goriounova & Mansvelder, 2012). Though immediate nicotine consumption shows adrenergic activation, alleviates stress, consolidates memory (Beer et al., 2013), and act as an exogenous antioxidant (Hritcu et al., 2009), long term nicotine abuse is associated with a positive effect on oxidative stress (Supriya et al., 2017) and perceived stress and may also contribute to coping self-efficacy. There are number of theories specifying the role of stress on nicotine abuse behaviours. The conventional idea is that nicotine abuse may temporarily relieve stress but it exerts negative impacts on emotional state and coping (Hajek et al., 2010). A recent study reported an association between perceived stress and psychological distress (Hobkirk et al., 2018). Another study reported higher levels of perceived stress in chain smokers as compared to intermittent smokers (Hobkirk et al, 2018). Stress is also reviewed as a risk factor for nicotine abuse among adolescents (Leventhal et al., 2017). In the stress-coping model by Wills & Shiffman, (1985) and the self-medication model by Khantzian, (1997) of substance abuse, drugs are considered to serve the purpose of coping by facilitating the general mood regulation.

Based on the related literature current study was carried out to investigate the direct effect of nicotine abuse on perceived stress and oxidative stress and their interrelationship with coping self-efficacy among college graduates.

Stress can manifest in a variety of ways that require different methodologies for assessment. A common method employed in the assessment of psychological stress is the measurement of perceived stress, as "the feelings or thoughts that a person has about how much stress they are under, over a given period" (Qian et al., 2010). Oxidative stress (OS) is a biochemical stress which is defined as the state of oxidant-antioxidant system imbalance in the body, in which the oxidant species exceeds resulting in accumulation of free radicals (Yoshikawa & Yuji, 2002). Endogenous antioxidant systems include enzymes as, catalase, glutathione peroxidase, superoxide dismutase, and non-enzymatic factors as retinol, tocopherol, etc., and minerals such as selenium. Co-occurrence of physiological and emotional stresses is common, and it has been suggested by Gingrich (2005) that "oxidative stress is the new stress" and reported an association of psychological stress with higher levels of oxidative damage. Recently, de Toda et al. (2019) reported that women with a moderate or high degree of perceived stress had higher oxidative stress as compared to women with low-stress perception.

Stress and nicotine have been shown to share a disordered relationship, but stress is predominantly studied as a significant risk factor for nicotine abuse. Stress modulation has been extensively studied because of the smoking habit and nicotine action on a complex stress response system (Metcalfe et al., 2003). Nicotine is a significant source of Reactive Oxygen Species (ROS) generation, and higher consumption rates of nicotine have been closely related to higher oxidative stress (Khademi et al., 2019). High lipid peroxidation products level has been linked with nicotine addiction (Sirisha & Manohar, 2013). OS may also alter the antioxidant enzyme action and raise lipid peroxidation. For instance, elevated levels of SOD have been consistently found among chronic nicotine abusers. SOD is a key enzyme involved in the detoxification of superoxide radicals (Zhang et al., 2003). However, nicotine has also been reported to confer a constructive effect on oxidative stress acting as a potent antioxidant that scavenges the free radicals produced by monoamine Oxidase-B\ [MAO-B] (Mandel et al., 2003). For a healthy organism, any perception of stress should elicit a response to cope with the stressful situation, and an inability to cope can be expected to result in biochemical aberrations.

Coping is defined as the ability to deal successfully with a difficult situation. The involuntary stress responses do not qualify to cope, as true coping is an intentional process (Nicholls, 2013). A commonly used measure to assess a person's ability to regain control under challenging situations in coping self-efficacy that refers to a person's belief in his/her ability to cope with stress (Chesney et al., 2006) and coping can be viewed as an adaptive response to stress. Stress and coping efficacy can have an independent effect on health outcomes. Stress influences the normal physiology and hormonal homeostasis of individuals and the way an individual copes with the stress would predict an individual's health (Folkman et al., 1986).

The idea of investigating the relationship between perceived stress, coping and tobacco smoking is rooted in the pioneering work of Siqueira et al. (2000). The most commonly cited reason for nicotine use is that it helps in coping with emotional stress. The role of tobacco in coping with stress has also been highlighted because it has been pointed out that smokers have the anticipation that smoking would alleviate their negative moods (Aronson et al., 2008). Consequently, the study was aimed with the hypothesis that

chronic nicotine abuse is positively associated with oxidative stress and perceived stress. The study also hypothesized a negative correlation between perceived stress and coping self-efficacy as well as examines the effect of nicotine abuse on this correlation. The significant idea of the study is to recognize the indices of oxidative stress as the biochemical predictors of perceived stress using multiple regression analysis. Moreover, the study proposed a mediation model to observe the interaction of nicotine abuse with biochemical measures and psychological measures (Aschbacher et al., 2013). Furthermore, the study intends at testing the affectability of the psychological tools including the PS Scale and CSE Scale towards related psychobiochemical research.

2 Materials and Methods

2.1 Study Population and Design:

In a cross-sectional case study design, male young adult college graduates (age 18-35 years) were recruited through simple random sampling from the University of Allahabad and its constituent colleges, enrolled either in Master's or in PhD. programmes. The respondents were screened through a health history questionnaire to rule out any mental illness including depression, anxiety, and other medical ailments as diabetes, cardiovascular diseases, as well as medication regime that might respond to some circadian, metabolic, or redox imbalances. Respondents were screened for at least one year of nicotine abuse. Accordingly, they were divided into two matched age-groups, namely those who never consumed any tobacco, form the control group, and the regular consumers of tobacco at least for 1-3 years form the Nicotine abuse group. The NA group respondents reported consuming nicotine in the form of cigarettes and smokeless tobacco (with a habit of smoking at least 1-5 cigarettes or chewing 1-5 bouts of tobacco everyday). The respondents with informed consent were requested for 4 ml of their blood sample and to perform pre-decided psychological questionnaire-tests. The body weight in kg and height in cm of all respondents were obtained using standard techniques and groups were matched for age and Body Mass Index (BMI=Body weight in kg/ [height in m] ^{2}).

2.2 Psychological Measures

2.2.1 *Assessment of Perceived Stress*

PS was measured using the Perceived Stress Scale (PSS) as described by Cohen et al. (1983). PSS is a tool to measure the degree to which situations in one's life are appraised as stressful. The widely used psychological instrument measures the perception of stress, using questions about frequency of feelings and thoughts during the last month. Based upon the summed PSS score (1 - 40), PS could be divided into three perceived stress categories viz., low (<13), moderate (14-26), and high (27-40).

2.2.2 *Assessment of Coping Self Efficacy*

CSE was assessed using a 26 items scale that gives the measure of perceived self-efficacy for coping in situations under challenges and threats (Midkiff et al., 2018). Respondents were asked, 'When things aren't going well for you, or when you're having problems, how confident or certain are you that you can do the following. The CSE inventory is 11-point (0-10) rating scale with anchor points 0 ('cannot do at all'), 5 ('moderately certain can do'), and 10 ('certain can do').

2.2.3 *Blood sample collection and processing*

Blood samples were collected in separate anticoagulant and plain vials and were subsequently processed to obtain packed red blood cells (erythrocytes), plasma, and serum volumes. Erythrocytes were further processed to obtain hemolysate and stored at -80ºC until analysis (Dwivedi et al., 2020).

2.3 Biochemical Measures

2.3.1 *Estimation of Erythrocyte and Plasma lipid peroxidation product Malondialdehyde*

Niehaus & Samuelsson, (1968) elucidate the estimation of E_MDA separately in hemolysate by Thiobarbituric acid (TBA) method and plasma was used instead of hemolysate for P_MDA.

2.3.2 *Estimation of Erythrocyte Superoxide Dismutase*

E_SOD was estimated in hemolysate by modification method of Marklund & Marklund, (1974) by using Tris-succinate buffer (pH-8.2, 0.1 mM) and 8mM Pyrogallol (light-sensitive).

2.3.3 *Estimation of Plasma Catalase (P_CAT)*

P_CAT activity was estimated according to the method by Sinha, (1972) with slight modifications. CAT activity is expressed as Unit/g Hb in hemolysate or Unit/g plasma protein. A detailed account of all the employed biochemical methods including hemolysate preparation and hemoglobin quantification has been illustrated as per Singh et al. (2019) biochemical quantification of oxidative stress method.

2.4 Statistical analysis of data

Group differences were analyzed using t-test with 95% confidence at the significance level of $p<0.05$. Pearson's correlation coefficient was used to assess the direct relationship between PS and CSE. All values were expressed as mean ± standard error of the mean. A simple linear regression was calculated to predict the effect of psychological characteristics on NA. Further multiple regression (Baierle et al., 2015) and mediation analysis (Lu et al., 2019) models were employed to study relations between

psychological characteristics, NA, and biochemical indices. All the analysis was done using JASP software (version 0.9.1) and R statistical software.

3 Results

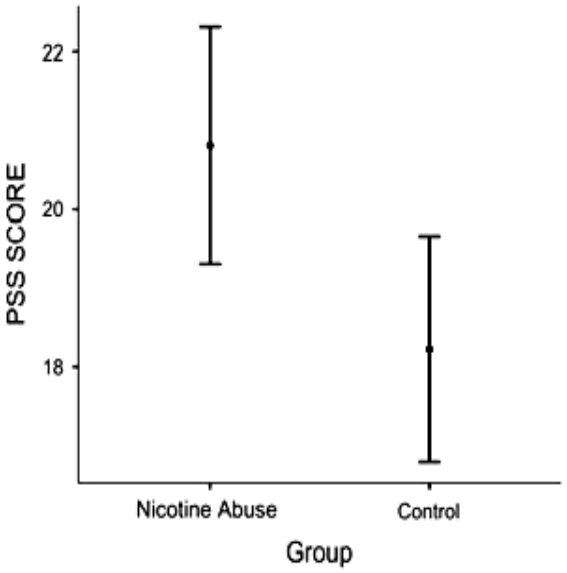
The mean PSS Score was significantly higher *(p=0.016)*, and the CSE Score, significantly lower *(p=0.001*) in the NA group as compared to the control group **(Table 1),** signifying the direct effect of NA on PS and inverse effect on CSE.

Simple linear regression analysis is further performed to predict the effect of NA on psychological characteristics. Firstly, by controlling the BMI, the regression analysis predicts the effect of NA on PSS Score in the two groups. A significant regression equation was found *[F (1, 92) =3.0, (p<0.055)]* with a R^2 of 0.0612. Based on Cohen's interpretation of regression R^2=0.0612, a medium effect of NA on PSS score was inferred. Consequently, NA shows an average increase of PSS Score by 2.5825 units **(Figure 1a).** Secondly, by controlling BMI we fitted a simple linear regression model to predict whether NA leads to differences in CSE Score. A significant regression equation was found *[F (2, 92) =9.21, (p<0.001)]* with a R^2 of 0.167. Consequently, NA shows an average decrease of CSE Score by 29.32 units **(Figure 1b).**

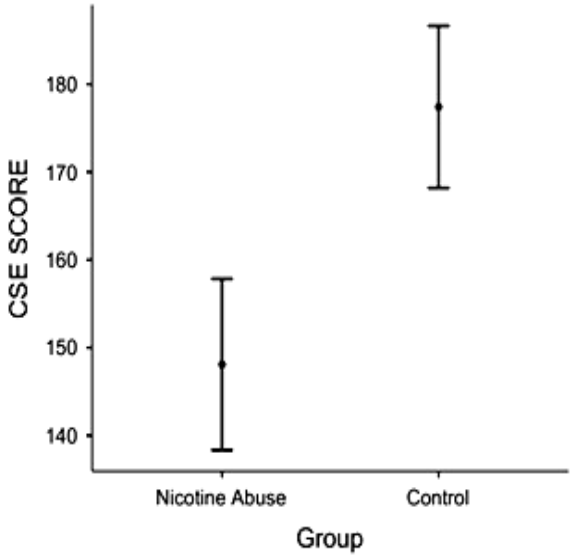
Further, the interrelationship between the PSS Score and CSE Score of the NA and control group were analyzed by the simple linear regression model. Both show strong inverse relation *(r^2= -0.172; p=0.002)* in the controls **(Figure 2a).** Interestingly, there was a nonsignificant relation between the two in the NA group *(r^2= -0.002; p=0.761)* **(Figure 2b).**

Regarding biochemical indices of OS only significant difference was found in the plasma concentration of MDA (nanomoles per milligram protein) *(p<0.05)* **(Table 2; Figure 3A)**. No significant differences were reported in the concentration of erythrocyte MDA (nanomoles per milligram hemoglobin) although the results showed a trend of increase in the NA group. Among the antioxidant enzymes, the SOD and CAT showed nonsignificant differences between these two groups, however, the SOD activity tends to decrease in the NA group **(Table 2, Figure 3C)**.

Furthermore, a multiple regression model was employed in this study, to predict which biochemical indices of the OS affect the relation between NA and PS. A significant regression equation was found



1A.



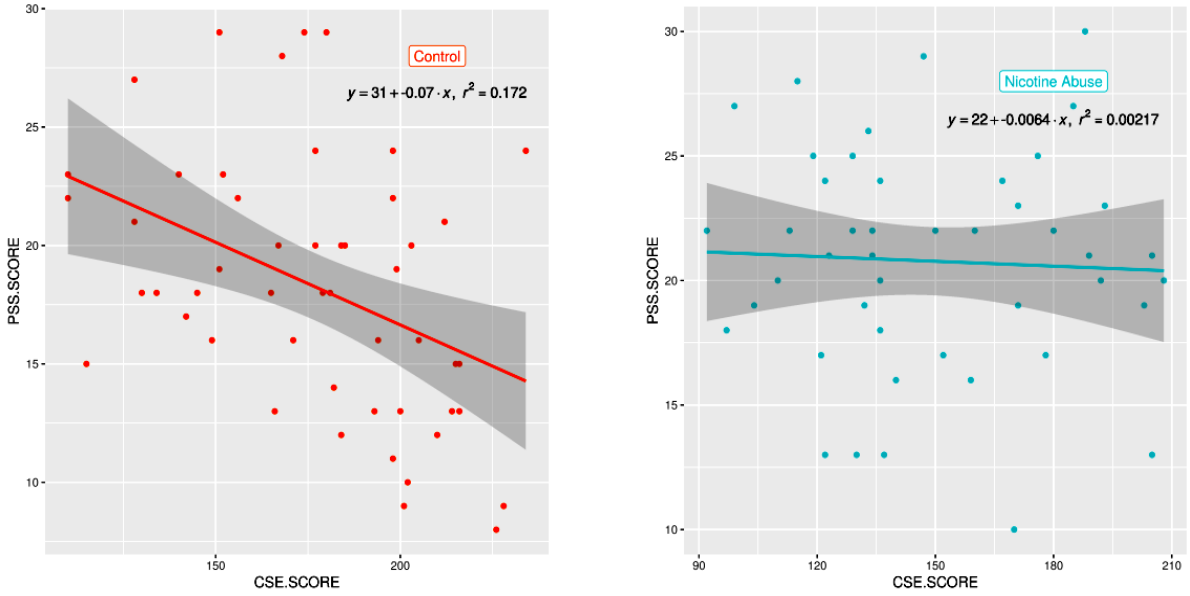
1B.

Figure 1 (A) Averaged PSS Score between groups (B) Averaged CSE Score between groups. Error bars shows the 95% CI.

Table 1 Descriptive Statistics of Nicotine abuse on Perceived Stress (PSS) Score and Coping Self Efficacy (CSE) Score

Psychological Variables	Controls n=50	Nicotine Abuse Group n=45	Significance p<0.05
PSS Score	18.26 ± 5.42	20.78±4.50	0.016
CSE Score	176.9± 32.32	148.7±32.52	0.001

PSS (Perceived Stress); CSE (Coping Self Efficacy); All values were expressed as Mean ± SD.



2A. 2B.

Figure 2 (A) Simple linear regression between PSS Score and CSE Score in Control group and (B) Nicotine Abuse group. Shaded region shows the 95% CI of regression line.

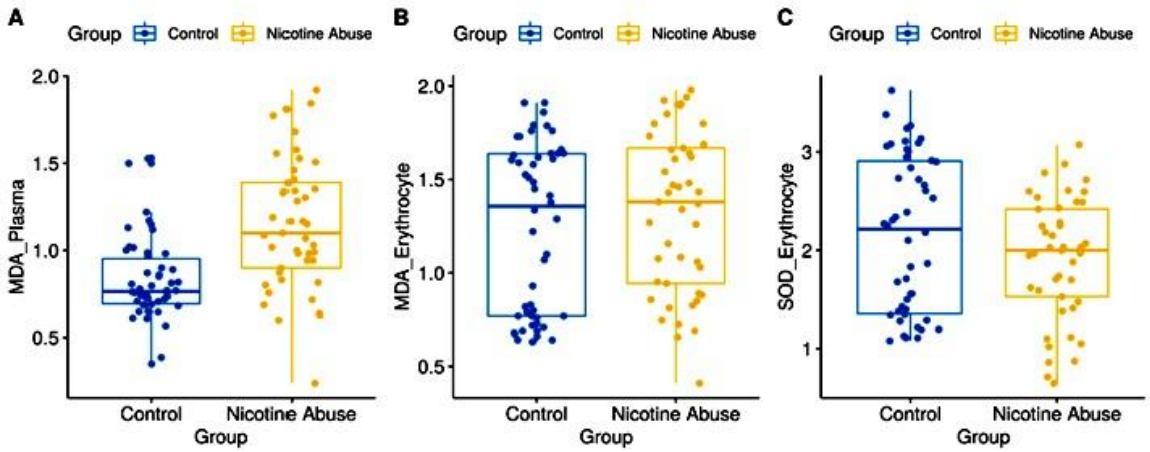
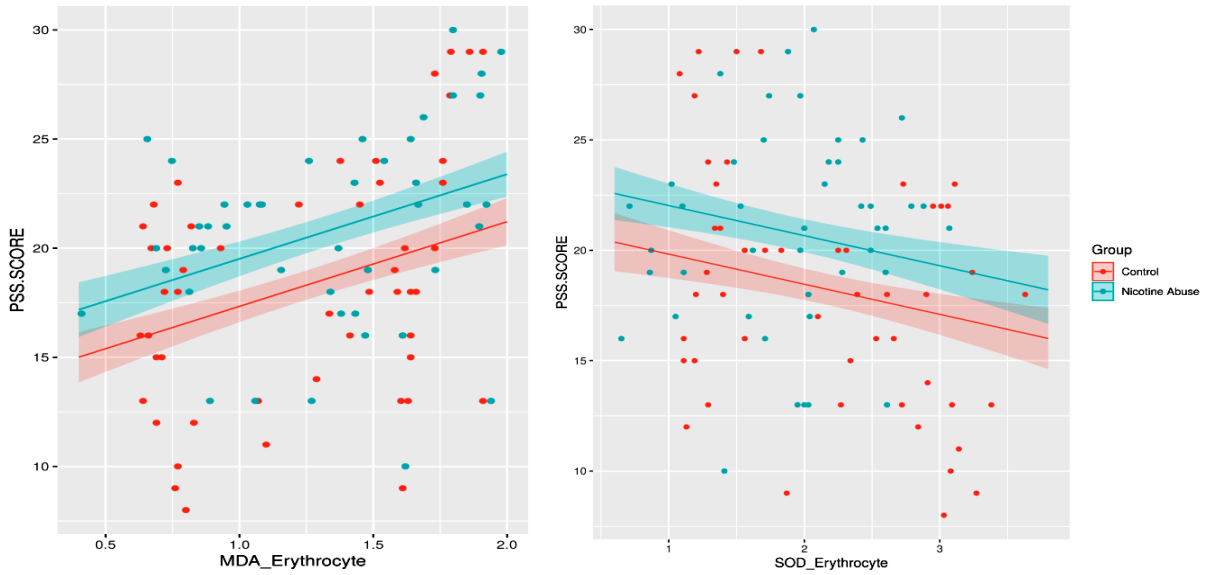


Figure 3 A, B, C - Distribution plots of oxidative stress indices between control and Nicotine Abuse group.

Table 2 Effect of nicotine abuse on indices of oxidative stress, erythrocyte and plasma MDA, and antioxidant enzymes, erythrocyte SOD and plasma Catalase.

Erythrocyte MDA (nanomoles/mg Hb)	1.23± 0.45	1.32±0.42	0.335
Plasma MDA (Nanomoles/mg protein)	0.82± 0.22	1.14±0.37	0.001*
Erythrocyte SOD (unit/mg Hb)	2.14± 0.79	1.91±0.61	0.117
Plasma Catalase (unit/mg protein)	1.10± 0.25	1.11±0.28	0.773

MDA (Malondialdehyde); SOD (Superoxide dismutase); *denotes statistical significance at p<0.05.



4A 4B

Figure 4 (A) Multiple linear regression model of PSS Score with Erythrocyte MDA between groups. (B) Multiple linear regression model of PSS Score with Erythrocyte SOD between groups. Shaded region shows the 95% CI of regression line.

Table 3 Mediation analysis results with C_MDA as mediator of Nicotine Abuse behaviour and PSS Score.
(a) Percentage of mediation and (b) Estimated path relation between C_MDA, Nicotine abuse and PSS Score

a) Mediation Estimates

Effect	Label	Estimate	SE	Z	P	%Mediation
Indirect	a*b	1.14	0.494	2.3	0.021	45.1
Direct	c	1.38	1.06	1.3	0.192	54.9

b) Path Estimates

Label			Estimate	SE	Z	p
Group	C_MDA	A	0.408	0.101	4.04	<.001
C_MDA	PSS SCORE	B	2.779	0.994	2.8	0.005
Group	PSS SCORE	C	1.383	1.06	1.3	0.192

[F (4, 90) =5.73, (p<0.001)] with a R^2 of 0.203. The significant predictors in this equation were NA, E_MDA, and E_SOD. The E_MDA shows significant positive relation with PSS Score *(t=3.48, p<0.059)* in both groups. NA group shows an increase of E_MDA by 3.84 units as compared to the control group **(Figure 4a)**, whereas, the P_SOD shows significant negative relation with PSS Score *(t= -1.91, p<0.001)* in both the groups. NA showed a decrease in E_SOD by 1.31 units as compared to the control group **(Figure 4b)**.

Similarly, a simple linear regression was additionally calculated to predict the effect of combined MDA (Plasma + Erythrocyte) on Nicotine abuse. A significant regression equation was found *[F (1, 93) =15.97, (p=0.001)]* with an R^2=0.1466. Based on Cohen's interpretation of R^2=0.1466, a large effect of NA on C_MDA could be inferred. Thus, the participant's C_MDA values on average increased by 0.40842 units in the NA group.

Mediation analysis was performed to understand the relationship between NA and PSS Score mediated by C_MDA. A significant partial mediation of relationship was observed between NA and PSS Score by C_MDA as an indirect effect with an estimated mediation of 45.1% **(Table 3, Figure 5)**.

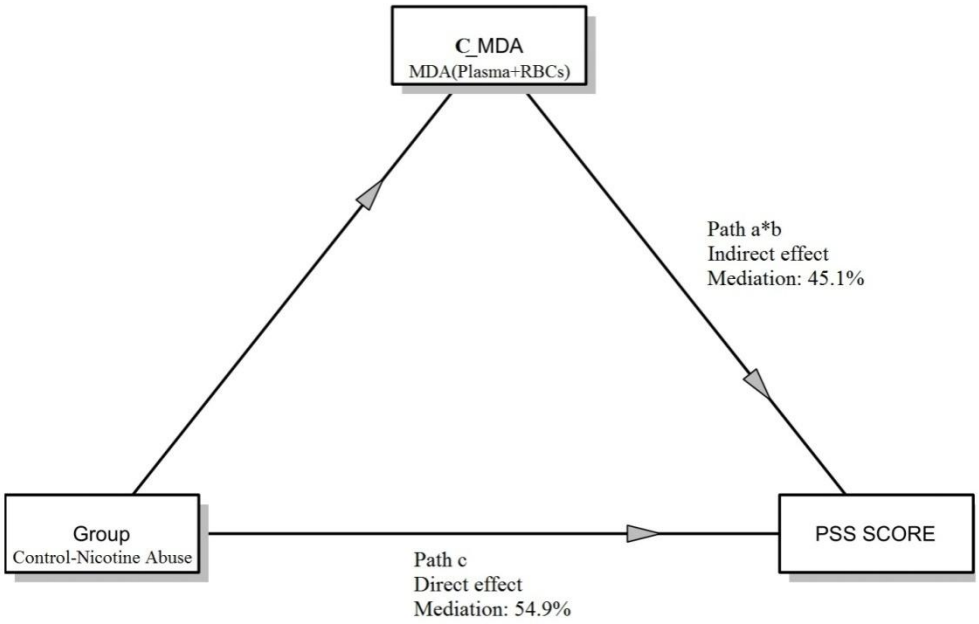


Figure 5 Mediation model depicting the path relationship between the Group and PSS Score via mediator Combined MDA.

4 Discussion

This study appears to be one of its kind in the field of psychobiology because it provides simultaneous information about the psychological and biochemical effects of NA. Earlier studies have, however, examined the role of PS with nicotine abuse, and the results of the current study consistently support the idea of Parrott (1999) that nicotine abuse does not reduce stress but elevates it. The present study found a negative effect of nicotine on CSE, consistent with a previous study on tobacco smoking in adolescence that suggests smoking interferes with the development of self-efficacy or ability to employ appropriate coping behaviours in a given situation. In a study by McGee et al., (2013) a small but direct inverse effect on adaptive coping and a direct effect on maladaptive coping was reported. Recently McGee et al. (2020) proposed a mediating model in which one interaction (Perceived Stress × Coping Self-Efficacy [CSE]; p = .043) found significant, the relationship between perceived stress and depression decreased as CSE increased. Consequently, this study proposed an inverse model proposed between PS and CSE. A negative correlation was reported between PS and CSE among the control group but interestingly, this study did not find this correlation in the NA group which underlines the inefficient coping among nicotine abusers. This is also suggestive of the fact that immediate nicotine consumption has the illusionary perception of relieving the stress and strengthening coping but in reality, exerts a worsening effect on PS and CSE.

A recent study in scientific reports on a global perspective on perceived stress and smoking suggests a significant relationship of PS with higher smoking rates (Stubbs et al., 2017). A study by Naquin & Gilbert, (1996) reported the mean perceived stress score of current smokers significantly higher than that of the students who had never smoked as consistent with the present findings. A similar association between stress and addiction as obtained in the current study on coping efficacy has been reported by several studies (Sinha, 2008; Folkman, 2010; Farooq, 2018).

Several researchers have recently studied the biochemical impact of nicotine relative to OS markers (Agarwal et al., 2019). Nicotine induces OS via peroxidant/antioxidant imbalance in blood cells, blood plasma and tissues *in vivo* and *in vitro* conditions and a decreased E_SOD activity with NA but no alteration in E_CAT activity (Suleyman et al., 2002). Lowered levels of E_SOD, CAT and Glutathione Peroxidase (GPx) were reported by Sirisha & Manohar, (2013) in nicotine abusers. Recently, more lipid peroxidation and lowered SOD was shown as the impact of nicotine (Ugochukwu et al., 2017). MDA, a key index of membrane pathology, produced from the primary and secondary decomposition of lipid peroxidation products. Trofor et al. (2019) reported a significant increase of serum malondialdehyde (MDA) concentration among 73% of smokers. The present study also shows a significant increase in P_MDA and a pattern of increase in E_MDA with NA. The increase in lipid peroxidation was accompanied by a decrease in the E_SOD, the key and primary antioxidant enzyme in the

cell which provides cellular defense against superoxide radicals. However, the present study does not find any significant decrease in the activity of enzyme SOD. P_CAT, another antioxidant enzyme, showed no difference between the two groups. To further explore the relation between oxidative stress and NA multiple regression models was used. This model showed significant positive relation of E_MDA with PSS Score *(t=3.48, p<0.059)* and a significant negative relation of P_SOD with PSS Score *(t= -1.91, p<0.001)* in both the groups. Consequently, the present study is the first to report the E_MDA, and E_SOD as important biochemical predictors of perceived stress.

The most prominent theory to explain the link between NA and PS is the hypothesis that stress may increase hypothalamus-pituitary-adrenal (HPA) axis reactivity (al'Absi, 2006). Recently, a psychosomatic connection was reported between perceived stress and the 'milieu intérieur', in healthy first-year medical students sitting for an examination through increased cortisol secretion, β-endorphin levels and induced oxidative stress (Myint et al., 2017). Because of these reports, we further explored the causality between PS and NA by mediation analyses. Surprisingly, the biochemical indices; E_MDA and Plasma MDA when analyzed collectively as combined MDA through mediation model showed mediation of relation between nicotine and perceived stress by 45.1%. This provides a mechanistic explanation for the effect of OS with nicotine abuse. Aschbacher et al. (2013) employed a similar mediation model to test the cortisol mediated relationship between perceived stress and oxidative stress among the participants subjected to chronic stress. They explained the model of 'eustress' or 'good stress' which suggests that manageable levels of life stress may enhance psychobiological resilience to oxidative damage as supported in the present study.

Conclusion

In summary, the results of this study showed that NA leads to an increase in PS and diminishes the CSE that further appears to impact the inverse relationship between the two. P_MDA is the significant biochemical marker of OS in NA. E_MDA and E_SOD are found to be important predictors of PS. Our mediation model represents C_MDA as a significant mediator of the relationship between NA and PS. Firstly, by improving the CSE as it is essential to minimize the subjective PS. Secondly, by reversing the oxidative damages to the cell via managing the antioxidant enzymes and lipoperoxidation of the cellular membranes. The study, thus suggests prospective works with more controlled assessment of C_MDA and antioxidant enzymes including SOD and CAT simultaneously studied in the milieu of NA and PS will have meaningful health interventions with nicotine abusers in both psychiatric and non-psychiatric settings.

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Conflict Of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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