Nicotine Addiction and Psychological Stress: a Case-control Study Among the Unemployed North Indians

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Abstract

Background: In India, nicotine addiction causes a huge socio-economic burden, mortality, and morbidity. Chronic intake develops a variety of symptoms and life-long medical complications. The study aims to examine the nicotine-induced effects on cellular oxidative stress and psychological stress and its correlations with homocysteine (Hcy) concentration in nicotine-addicted respondents.

Methods & Results: Nicotine-induced psychological stress was assessed through perceived stress scales (PSS) and coping self-efficacy (CSE) scales and oxidative stress was estimated through the markers-Malondialdehyde (MDA), superoxide dismutase (SOD), and Catalase. We found significantly higher **(p=0.0001)** perceived stress (PS) with diminished CSE in addicted respondents whereas low PS with significantly increased **(p=0.0001)** CSE were observed in non-addicted. In erythrocytes (RBCs), MDA content was found significantly increased **(p=0.0006)** in the nicotine-addicted group that may cause oxidative damage. SOD activity was significantly decreased **(p=0.0001)** in the addicted group. Similarly, plasma Catalase activity was also decreased **(p=0.02)**. Nicotine intake influences Hcy concentration, by **55%**, **27.5%**, and **7.5%** in addicted respondents categorized as moderate, intermediate, and severe Hcy categories.

Conclusions: The minimal short-term nicotine consumption has the antioxidant role that promotes positive stimulus on memory and triggers executive functions but the same gets impaired and mitigated on chronic exposure. Chronic nicotine consumption induces oxidative stress and perceived psychological stress as well as influences the Homocysteine concentration in the addicted individuals. Higher oxidative stress impairs cellular defensive mechanisms and causes the risk for severe damage to cells and tissues.

Introduction

Globally, India is the second-largest producer and consumer of tobacco (nicotine) or its related products. The most prevalent form of tobacco intake is Gutkha, Khaini, Zarda, Betel quid with tobacco, Bidi, Cigarette, and Hookah. According to the India Global Adult Tobacco Survey (2016-17), approximately 267 million adults above the age of 18 years (29% of all adults) are addicted to tobacco. Tobacco consumption causes a huge socio-economic burden, mortality, and morbidity across the world. Nearly, 1.35 million deaths were reported in India and 8 million deaths worldwide annually. The estimated economic burden in the year (2017-2018) attributed through tobacco consumption, related diseases, and mortality in the Indian population was INR 1773.4 billion or US $27.5 billion [1, 2]. The way of tobacco consumption among the rural and urban population and slum areas is diversely distributed [3]. Therefore, many policies have been constituted to manage and regulate tobacco consumption, advertising, economic burdens, and mortality in the country [4]. Despite all efforts, the ratio of tobacco consumption among unemployed youth has been raised [5]. Millions of educated adults are unemployed or under the threat of being jobless, many of them often use tobacco as a stress reliever [6, 7].

Nicotine is an addictive substance found in all tobacco products that cause poisonous effect and increase the risk for several non-communicable chronic diseases like heart disease, stroke, cancer, etc. [8]. Addiction has been identified as a brain disorder characterized by compulsive engagement in rewarding stimuli despite adverse consequences [9]. Addiction may strongly affect the way of feelings that can be euphoric and develop the tendency of its frequent use [10]. Despite the involvement of several psychosocial factors, repeated exposure to an addictive stimulus may be the core pathology to drive the development of addiction to nicotine. Resulting, neuroadaptation develops the number of binding sites on the nicotinic cholinergic receptors in the brain, probably due to the response of nicotine-mediated desensitization of binding receptors. Ligand-induced and unresponsiveness of the receptors play a vital role in nicotine tolerance and dependence; as shown in (Fig. 1) the symptoms of craving and withdrawal begin in smokers when desensitized receptors α4β2 nicotinic cholinergic become responsive during the periods of abstinence [11–13]. Chronic nicotine addiction induces psychological stress and oxidative stress; oxidative stress appears as significant biomarkers such as MDA (a product of lipid peroxidation), SOD, and Catalase. However, not much comprehensive research on nicotine's direct influence on MDA has yet been reported [14]. Several other plausible theories have arisen to explain nicotine's beneficial effects on oxidative stress as less nicotine consumption serves as a good antioxidant, scavenging the free radicals generated by monoamine oxidase-B [15, 16]. Nicotine addiction may influence the Hcy metabolism and cause Hyperhomocysteinemia (Hhcy) and Homocystinuria. These (Hhcy/Homocystinuria) are the significant risk factor for causing several diseases such as cardiovascular disease (CVD), cerebrovascular disease, diabetes, hip fracture, cognitive decline, osteoporosis, chronic kidney disease, hypothyroidism, Alzheimer’s disease, Parkinson’s disease, and cancer [17–19]. In some studies, an adverse link between smoking and Hcy metabolic abnormalities has been reported [20]. Although much collaborative research work is required to explore the influence of nicotine and oxidative stress on Hcy metabolism yet.

The goal of the study is to examine the interaction of chronic nicotine consumption with cellular defensive enzymes, lipid peroxidation, and Hcy abnormalities in selected population groups. The study also focused to estimate perceived psychological stress and coping measures in the affected individuals.

Materials & Methods

Selection of Volunteers:

This study was carried out on 156 healthy unemployed literate youth volunteers (age range; 20 to 40 years), randomly recruited in Prayagraj, Uttar Pradesh, India. All the participants were surveyed for nicotine (tobacco) addiction or consumption either smoking form (Bidi, Cigarette, and Hookah) or non-smoking form (Gutkha, Khaini, Zarda, and Betel quid with tobacco). The enrolled participants were divided into two groups viz., 1. Control: never consumed nicotine; 2. Cases: consumed nicotine at least (4 times/day) in any of the available forms for two years before their enrollment. All the study participants (cases and controls) were screened through a pre-listed health history questionnaire for any medical complications such as Diabetes, Asthma, CVD, Depression, Anxiety, and other mental disorders. The

inclusion criteria also ensured that volunteers were not on any medication regime. The study objective was clearly explained to the volunteers and their consent was taken to prior recruiting in the study.

This study has been approved by the population resource and research center, institutional ethics committee, Praygaraj, UP, India, with reference number IERB19/9.42.

Anthropometric Measures:

For anthropometric measurements, the body weight in kilogram (kg), and height in centimeter (cm) of all the volunteers were measured. Body mass index (BMI, kg/m^2) was calculated. BMI values in the range of 18 to 25 were considered normal; 25 to 30 – overweight; >30 as obese class I, II, and III (30-35, 35-40, >40 respectively) [21].

Blood Sample Collection, Storage, and Processing:

Blood samples were collected from the enrolled subjects (both non-addicted and nicotine-addicted) at the registered pathology center by a well-experienced pathologist in two different vials - 1. EDTA vials for plasma and RBCs separations, 2. Plain vials for serum separation. RBCs, plasma, and serum were separated at 4 ºC temperature, collected in required volume, and frozen at minus 80 ºC temperature for further use [22].

Preparation of Hemolysate:

Hemolysate was prepared by the destruction of packed RBCs and release of its content like hemoglobin and others. A 20 µL RBCs was taken out and washed with 0.9% NaCl solution 2-3 times repeatedly, centrifuged at 3000 rpm (revolution per minute) for 5 min, supernatant was discarded and pellets were obtained carefully. A 380 µL of stabilizing solution was added to it and kept the final volume 400 µL. It was stored at -80°C for the estimation of MDA and SOD [23].

Biochemical Measures

Estimation of Hemoglobin:

Hemoglobin concentration was measured in all blood samples by Drabkin's method. A standard hemoglobin solution was used for calibration. The absorbance was measured in a UV-Vis double beam spectrophotometer at 540 nm [24].

Estimation of Plasma Protein:

Measurement of protein quantity in each plasma sample was done by the Folin-Lowry method. A standard protein solution of Bovine Serum Albumin (BSA) was used to calibrate. Absorbance was recorded in UV-Vis double beam spectrophotometer at 660 nm wavelength [25].

Estimation of Homocysteine:

Plasma Hcy levels were measured in all samples by employing a method given by Pfeiffer et al., 1999 with minor modification [26]. This estimation was performed on high-performance liquid chromatography (HPLC). Various chemicals, standard solutions, and reagents were used. Hcy concentrations are expressed as µmol/L, the concentrations between 5 to 15 µmol/L are considered to be normal, and >15 µmol/L is considered to be elevated in the body's fluids.

Estimation of lipid peroxidation product (MDA) in RBCs:

Erythrocyte's MDA was measured with a slight modification of the method given by Senthilkumar et al., 2021. MDA was considered as an index for lipid peroxidation. For estimation, hemolysate was mixed with 0.2 M phosphate buffer (pH 7.4) and TBA-TCA-HCl reagents. Incubated in boiling water bath for 30 min at 100^0C, and then centrifuged to obtain clear supernatant. The absorbance of the supernatant was measured in UV-Vis double beam spectrophotometer at 532 nm wavelength. The MDA content was calculated by using extinction coefficient (=156,000) and expressed as nmol/g of Hb or nmol/mL of RBCs [27].

Estimation of MDA in Plasma:

Plasma MDA was measured as the same method employed for erythrocyte's MDA estimation.

Estimation of Superoxide Dismutase (SOD):

Erythrocyte's SOD was measured with a slight modification of the method given by Marklund & Marklund, 1974. Tris-succinate buffer (pH 8.2) and Pyrogallol (light-sensitive) are used for the estimation of SOD activity in RBCs. The blank tube constituted only 3 mL of tris-succinate buffer whereas the reference tube constituted the solution mixture (2.9 mL buffer + 50 µL distilled water + 100 µL Pyrogallol) and in the sample solution mixture (2.9mL buffer + 50 µL hemolysate + 100 µL Pyrogallol). Absorbance was recorded in a UV-Vis double beam spectrophotometer at 420 nm for 4 min at the interval of 30 seconds. The catalytic activity of SOD was expressed as (nmol/min/g of Hb) [28].

Estimation of Catalase:

The plasma Catalase activity was measured as described by Sinha 1972 with slight modification. A 0.01M phosphate buffer (pH 7) was used to make the dilution of H_2O_2. As for the blank solution, 2.5mL of unitary phosphate buffer (PB) solution was used while a solution mixture (1.45 mL of PB + 50 µL of H_2O + 1 mL of diluted H_2O_2) was taken as a reference analytes, and in the test sample (1.45 mL of PB + 50 µL of plasma sample + 1 mL of diluted H_2O_2) was used. Absorbance was recorded at 240 nm wavelength for 3 min at the interval of 30 seconds. Due to the activity of H_2O_2 with the test sample mixture, the absorbance was recorded decreased with time. Catalase activity was expressed as IU (International Unit; denotes one unit decomposes 1µmole of H_2O_2 per min at pH-7 and 25ºC) [29].

Psychological Assessment

Perceived Stress (PS):

PSS is one of the validated and widely accepted instruments used for psychological assessment. In general, it measures the degree to which situations in one s life is appraised as stressful. A questionnaire of 10 items based on the frequency of emotional feelings and thoughts during the past month was given to volunteers and asked them to respond. According to the overall individual's score, all volunteers were categorized into three stress categories as low stress (<13), moderate stress (14-26), and high stress (27-40) [30].

Coping Self-Efficacy (CSF):

CSE scale is one of the validated and standardized lists of 26 items given by (Chesney et al., 2006) used to assess someone's coping efficacy against life's challenges and threats when the conditions aren't going well, or are difficult. All volunteers were asked for rating each item (0 to 10) carefully according to their existing perceived self-efficacy. The entire rating points for each item have been categorized as 0 points = cannot do all, 1 to 5 points= moderately can do and 6 to 10 points = certain can do [31].

Statistical Analysis of Data

The statistical data processing of the results was carried out by using Microsoft excel 2019 and GraphPad prism version 9 program. Pearson's correlation coefficient was also calculated among the groups. The result has been presented as the mean and standard error of the mean (SEM). The statistical significance of the difference was verified using the t-test at 95% confidence and p-value at ($p<0.05$). The comparisons were made between the socio-demographic, clinical, and laboratory measurements, using appropriate parametric tests where data were normally distributed.

Results

Anthropometric comparison

Non-addicted and nicotine-addicted respondents were enrolled and assessed individually by their age, height, weight, and BMI, and the addiction history was recorded. As shown in (Table 1), the two groups were matched for age, height, weight, and BMI.

Table 1
Age and anthropometric measures of enrolled volunteers

Anthropometric measures	Non-addicted males (control) n=76		Addicted males (cases) n=80	
Age (years)	22.92	±0.30	24.00	±0.54
Height (m)	01.67	±0.007	01.65	±0.007
Weight (kg)	59.16	±0.86	59.60	±1.05
BMI	21.27	±0.26	21.73	±0.28
Table1: All values are expressed as Mean and ±SEM (standard error of the mean)				

Effect on perceived psychological stress

Enrolled volunteers were divided into three stress categories Low, Moderate, and High corresponding to the ranges- 0-13, 14-26, and 27-40 on the PS scale. As represented in (Table 2 & Supplementary Fig. 1) the non-addicted volunteers were grouped either in the low or moderate PS category whereas in the addicted group only **5%** show low stress, over half (**60%**) show moderate, and a fairly **35%** proportion show high perceived stress. Observations indicate nicotine influence perceived stress level in addicted.

Table 2
The proportions of non-addicted and nicotine-addicted respondents in low, moderate, and high PS categories

PS Category	Range of scores	Non-addicted males (control) n=76		Addicted males (cases) n=80	
Low	0-13	42	(55.27%)	04	(05%)
Moderate	14-26	34	(44.73%)	48	(60%)
High	27-40	00	(00.00%)	28	(35%)
Table 2: Values in parenthesis represent the value of the cell as a percent of the total n of the column.					

Effect on Homocysteine concentration

As given in (Table 3 & Supplementary Fig. 2), approximately **55%** of nicotine-addicted express moderate, **27.5%** express intermediate, and **7.5%** express severe Hcy concentration whereas, in the non-addicted, nearly **55.3%** volunteers were placed in low, **36.8%** in moderate, and **7.8%** in intermediate Hcy categories respectively,

Table 3
Measurements of Homocysteine level in the blood plasma of non-addicted and nicotine-addicted respondents

Homocysteine Measures (in Plasma)	**Non-addicted males (control) n=76**		**Addicted males (cases) n=80**	
Low (<16 µmol/L)	42	(55.26%)	08	(10.00%)
Moderate (16 to 30 µmol/L)	28	(36.84%)	44	(55.00%)
Intermediate (31 to 100 µmol/L)	06	(07.80%)	22	(27.50%)
Severe (>100µmol/L)	00	(00.00%)	06	(07.50%)

Table 3: Values in parenthesis represent the value of the cell as a percent of the total n of the column.

PS and CSE relation

As represented in (Table 4), a significantly higher PS was found in the nicotine-addicted group (**p=0.0001**). Contrarily the nicotine-addicted group expressed significantly lower/reduced CSE than the non-addicted (**p=0.0001**). The data of significant differences between the groups for PS and CSE has been depicted in (Fig. 2 & 3).

Table 4
Data of Psychological stress measures and Oxidative stress measures in non-addicted and nicotine-addicted groups with their respective p-values

Measures	**Non-addicted males (control)** **n=76**		**Addicted males (cases)** **n=80**		**p-value at (<0.05)**
Psychological Stress Measures					
PS Scores	15.05	±0.58	21.75	±0.53	**0.0001*** **
CSE Scores	180.37	±4.36	154.85	±3.47	**0.0001*** **
Oxidative Stress Measures					
MDA in RBCs (nmol/g of Hb)	0.197	±0.007 .	0.229	±0.006	**0.0006*** **
MDA in Plasma (nmol/mL of Protein)	0.183	±0.01	0.183	±0.007	**1.0000**
Antioxidant Enzyme's Activity Measures					
SOD in RBCs (nmol/min/g of Hb)	0.221	±0.006	0.172	±0.004	**0.0001*** **
Plasma Catalase (IU)	0.289	±0.06	0.121	±0.04	**0.02***

Table 4: All values are expressed as Mean and ±SEM, ***indicates extremely statistically significance, **indicates the good statistically significant, and *indicates the statistical significance at p<0.05. IU denotes one unit decomposing 1µmole of H_2O_2 per minute at pH-7 and 25 ºC.

Effect on MDA and Cellular defensive mechanism

From (Table 4), we measured significantly increased MDA **(0.229 ±0.006)** content in the RBCs of the nicotine-addicted group **(p=0.0006)**, and the comparison has been shown in (Fig. 4.a). A significantly decreased **(0.172 ±0.004)** SOD activity in the RBCs of the addicted group (**p=0.0001**) was measured and comparisons have been shown in (Fig. 4.b).

Similarly, plasma Catalase activity was also found significantly decreased **(0.121 ±0.04)** in the addicted respondents **(p=0.02)** and its comparison has been depicted in (Fig. 5).

Discussion

Nicotine addiction is extensively employed as a stress reliever in today's contemporary lifestyle but long-term consumption may be hazardous for physical and mental health [32]. This may also affect brain

activity, producing neurodegenerative illnesses and degrading the nervous system's potential [33]. In the survey, we observed that nicotine users often report that their consumption helps to relieve the feeling of stress, divert the way of thinking and improve work performance. However, the stress levels of adult tobacco users have been seen adversely higher than non-tobacco users [34]. As given the possible involvement of nicotine addiction in oxidative stress, the goal of this study was to look into the long-term effects of nicotine on psychological stress and Hcy metabolism. We primarily attempted to examine the interaction of nicotine addiction on perceived stress, perceived coping efficacy, oxidative stress markers (MDA, SOD, and Catalase); and then observed its correlation with Hcy concentration among enrolled volunteers. PS is adversely associated with coping efficacy in the whole addicted group which supports hypothesis and consistency with the findings [35]. Individuals' coping strategy under stressful situations was seen lower in the nicotine-addicted group. Researchers have also explored a negative correlation between PS and CSE, implying that coping efficacy worsens with greater perceived stress [36]. Several previous studies focused on nicotine addiction that promotes oxidative stress both in vivo and in vitro which causes peroxide/antioxidant imbalance in blood cells, blood plasma, and tissues [37]. Concomitant to a study [38], we also found nicotine addiction substantially stimulates MDA levels in the current study. Significantly higher MDA content was detected in RBCs of the addicted group, although the same has not been found consistently in plasma samples. Higher MDA content in the RBCs reveals an important clue for oxidative damage in the nicotine-addicted respondents [39]. SOD is a well-known primary antioxidant enzyme that counteracts superoxide radicals produced in the cells, serves as a powerful defensive system of cells [40]. The steady-state concentration of superoxide radicals in blood tissue may fluctuate directly with the rate of superoxide synthesis and inversely with the body's tissue concentration of scavenging enzymes [41]. We examined the effect of nicotine on the activity of SOD and Catalase, SOD activity was shown to be substantially decreased in the RBCs of nicotine-addicted than non-addicted and similar findings were also noted down for the plasma Catalase of nicotine-addicted group. These measurements suggest that the non-addicted respondents have developed the good potential to defend effectively against oxidative damage [42].

Apart from other defects of nicotine addictions it also affects the way of Hcy metabolism either the remethylation pathway or transsulfuration pathway, elevating Hcy concentration in the addicted respondents [43]. We found, about 55.0%, 27.5%, and 7.5% of nicotine-addicted respondents are suffering from moderate, intermediate, and severe Hcy levels respectively. Nicotine addiction and oxidative stress are found linked to the defective process of Hcy metabolism, elevating Hcy concentration in the body [44]. Elevated Hcy may act as an independent risk factor for a variety of diseases/disorders like Osteoporosis, Hip fracture, Cognitive decline, Diabetes, CVD, Chronic kidney disorder, Hypothyroidism, Alzheimer's, Parkinson's, Cancer, etc. [45,46]. However, much comprehensive and collaborative research is required to explore the association of nicotine and oxidative stress on Hcy metabolism yet [47]. To control tobacco consumption and other associated burdens many effective and aggressive anti-tobacco campaigns are needed to promote awareness of its harm. A well-planned strategy is very essentials to provide jobs/works to needy individuals and to manage unemployment in society. Because recent data highlights

that illiteracy and unemployment are the two significant factors for nicotine consumption among the youth population.

Declarations

Funding Information:

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Competing Interests:

“The authors have no any relevant financial or non-financial interests to disclose.”

Conflict of Interests:

“The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.”

Author Contributions:

All authors listed on the title page have contributed to the study's conception and design. Material preparation, sample collection, and data analysis were nicely performed by Anurag Mishra, Satya Narayan Mishra, Rishabh Kumar, and Dr. Munish Kumar. The first draft of the manuscript was written by Anurag Mishra and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

Data Availability Statements:

“*The data that support the findings of this study are available from the corresponding author upon reasonable request.”*

Ethics Approval:

“*The study has been approved by the population resource and research center, institutional ethics committee, Prayagraj, UP, India, with reference number IERB19/9.42.”*

Consent to Participate:

“Informed consent was obtained from all individual participants included in the study.”

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Figures

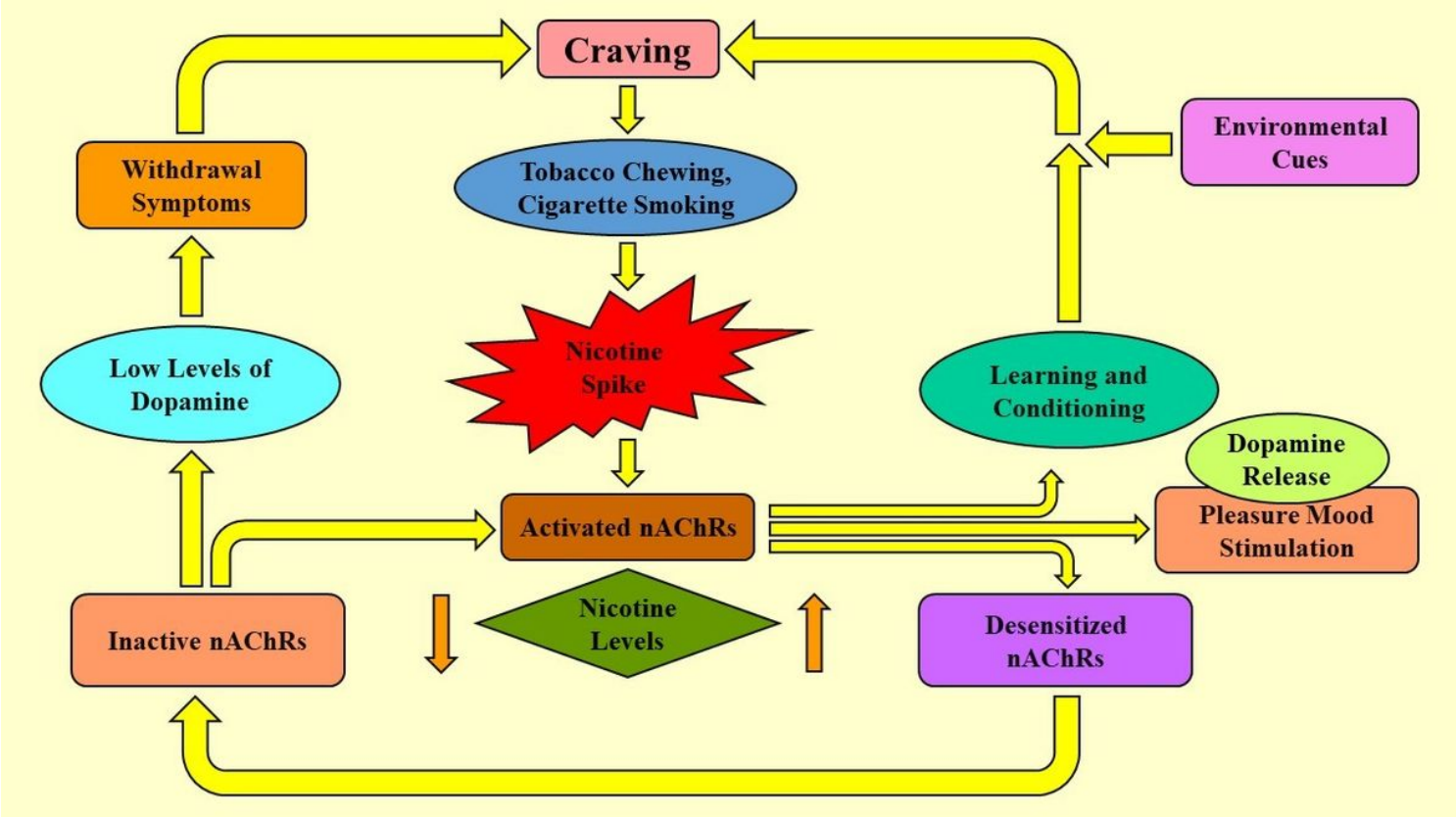


Figure 1

Schematic diagram of the mechanism of nicotine action; the symptoms of craving and withdrawal begin in smokers when desensitized receptors nicotinic cholinergic become responsive during the periods of abstinence. Structurally, nicotine is similar to acetylcholine (Ach) that is an important class of neurotransmitters involved in systems concerned with physical and mental arousal, learning memory, and several aspects of emotions. Ach receptors are traditionally classified as nicotine receptors and muscarine receptors. Primarily, nicotine stimulates the central nicotinic cholinergic receptors inducing the release of several neurotransmitters in the brain like dopamine, norepinephrine, serotonin, γ-aminobutyric acid (GABA), etc. these lead to arousal, mood modulation, performance enhancement, analgesic, and weight-loss effects.

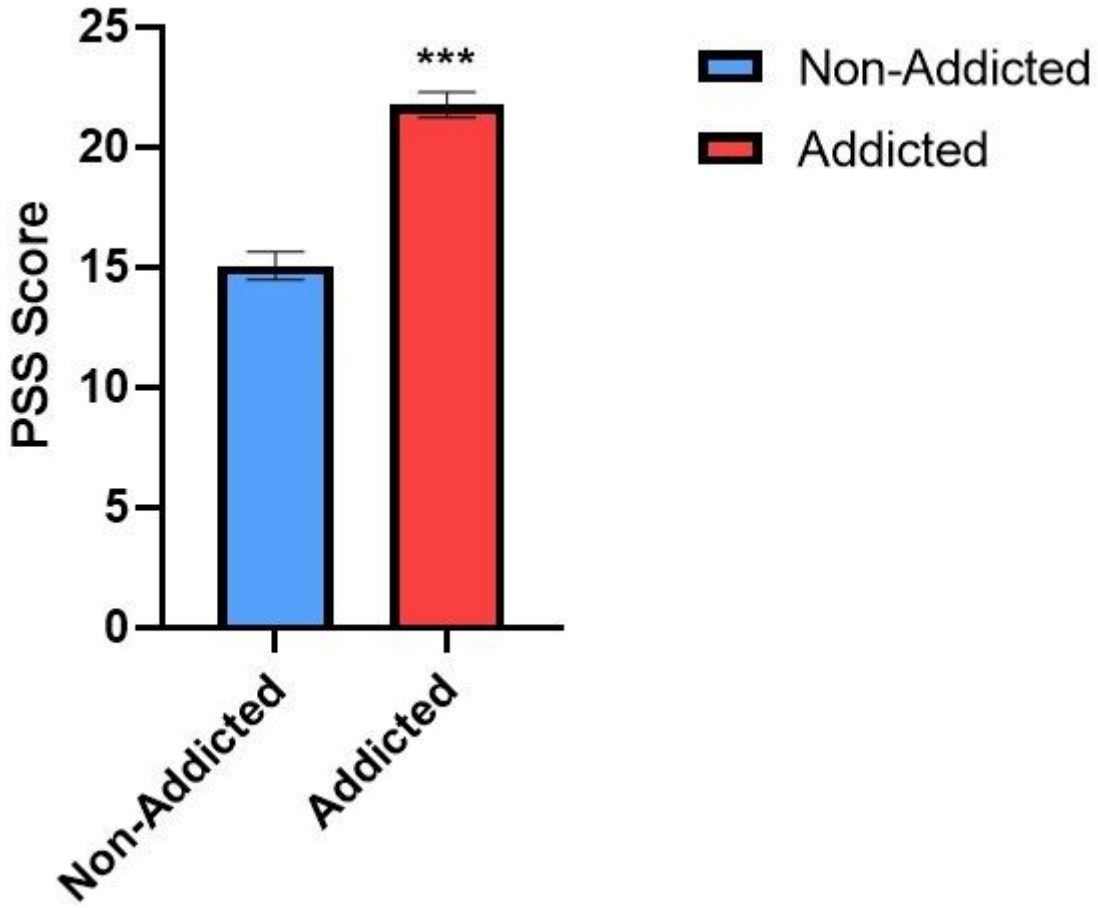


Figure 2

The PS scores of addicted and non-addicted were calculated and expressed as the mean and standard error of the mean (±SEM), and differences were obtained at (**$p<0.05$**). Due to the effects of nicotine addiction, we have observed higher PS scores in nicotine-addicted respondents, and the differences were found statistically significant (**$p=0.0001$**).

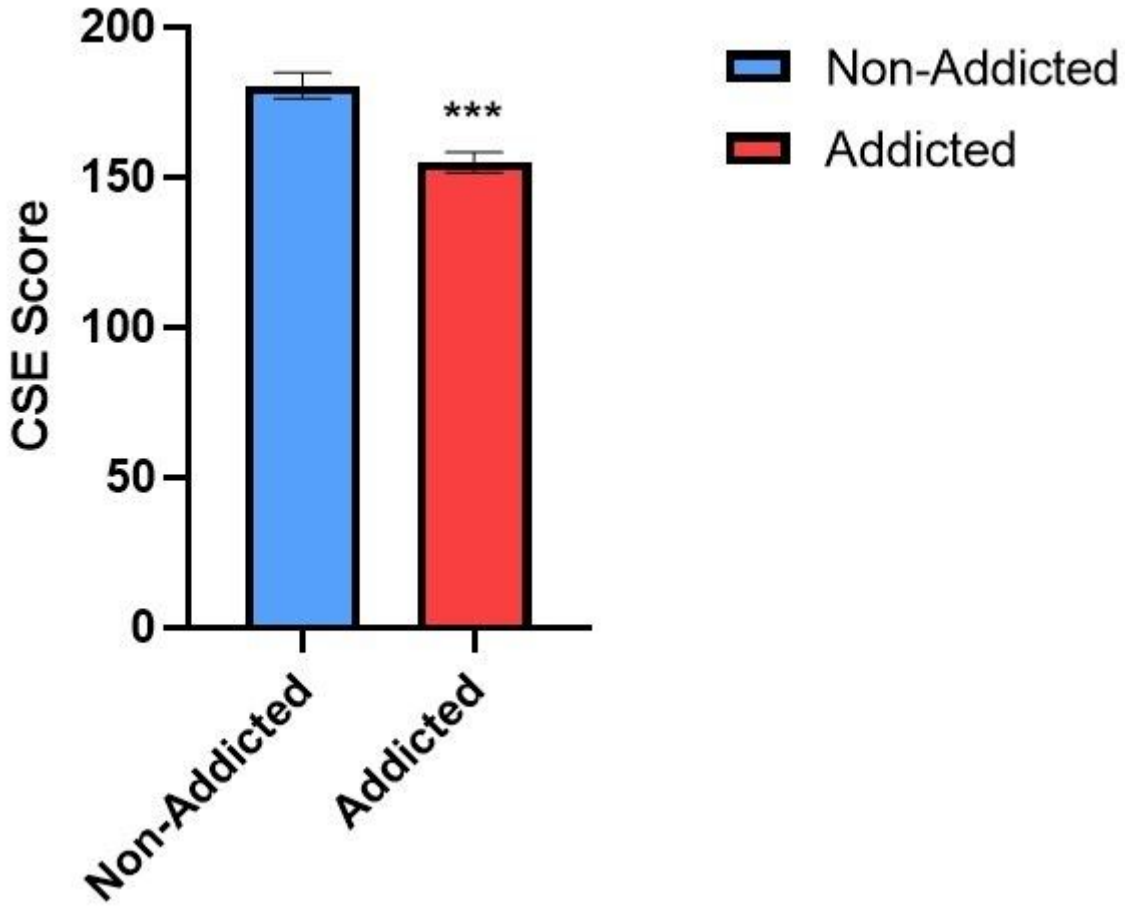
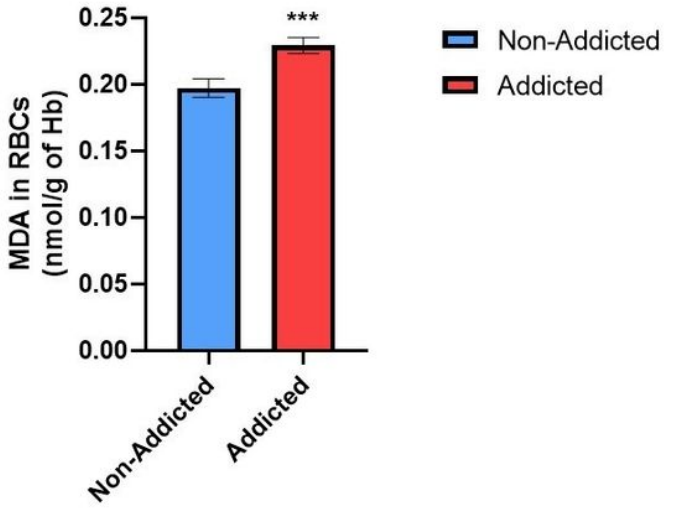
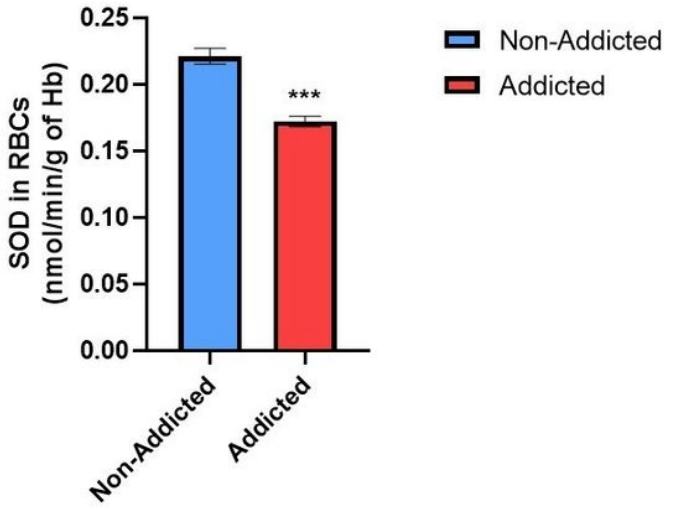


Figure 3

Distribution in CSE scores; the non-addicted respondents showed a higher value of CSE than addicted; thus differences (**p=0.0001)** were found statistically significant at (**p<0.05**). Low/poor CSE in the addicted indicates that these are unable to develop powerful coping strategies at the time of life's challenges and threats.



4a



4b

Figure 4

4.a: Effects of nicotine-addiction on MDA levels in the RBCs of addicted and non-addicted respondents. MDA levels (markers of oxidative stress) in the RBCs of nicotine-addicted were measured significantly higher (**p=0.0006**).

4.b: Effects of nicotine addiction on the antioxidant activity of superoxide dismutase (SOD) in the RBCs. Comparatively, low/reduced activity of SOD was assessed in the nicotine-addicted respondents that were found statistically significant (**p=0.0001**).

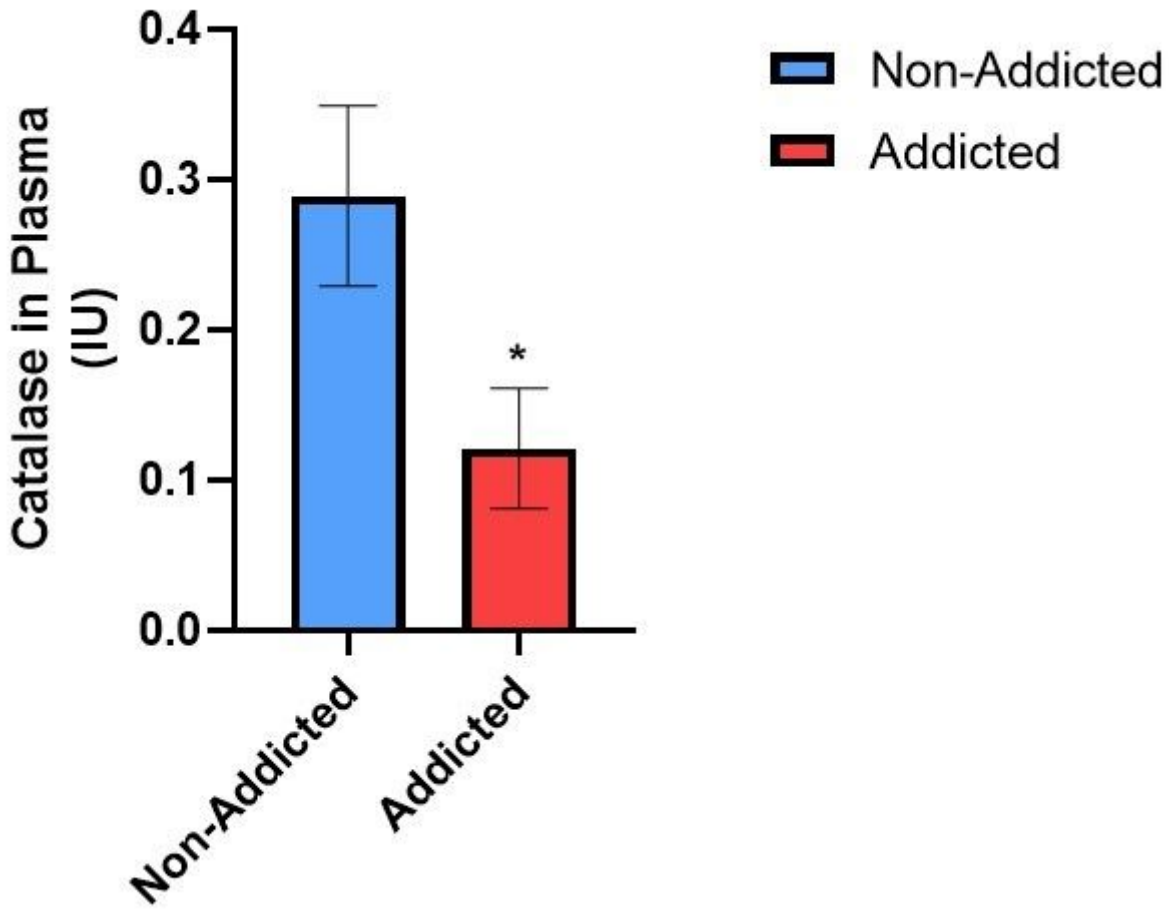


Figure 5

Effects of nicotine addiction on the antioxidant activity of plasma Catalase. Diminished Catalase activity was measured in the addicted group whereas non-addicted respondents expressed high Catalase efficacy that was found statistically significant (**p=0.02**).

Supplementary Files

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- SupplementaryMaterials.docx