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Impact of Cigarette Smoking on Serum Antioxidant Markers and Lipid Profile of Smokers and Nonsmokers in Nasarawa State University

Muhammad B. Y[1], Adam A.A[2], Mathias O.J[1] , Adamu R.M.
[1]Department of Biochemistry and Molecular Biology,
Nasarawa State University, Keffi, Nigeria.
[2]Department of Biochemistry,
Federal University Dutse, Jigawa State Nigeria.
Pharmacy Unit,
[3]Federal Medical Center Keffi Nasarawa State, Nigeria.
Email: adam.a@fud.edu.ng

Abstract
This study was carried out to investigate the effect of smoking on serum antioxidant level and lipid profile of apparently healthy student of Nasarawa state University Keffi, Nigeria. forty (40) male smokers and forty (40) male nonsmokers were included in this research. All the volunteers were males between the age of 20-25 years. Serum diagnostic kits were used to determine the levels of lipid peroxidation (MDA level), antioxidant enzymes (SOD, Gpx and catalase,) and lipid profile (total cholesterol; T-CH, triglycerides; TG, high density lipoproteins; HDL, low density lipoproteins; LDL) were also determined. The level of lipid peroxidation in smokers increased significantly with corresponding decrease in antioxidant enzymes. On the other hand, the levels of T-CH and LDL significantly increased in smokers ($P<0.05$) compared to non-smokers; while the level HDL was insignificantly higher in smokers than in non-smokers. Decrease in serum antioxidant level, with corresponding increase in the level of LDL could increase predisposition of young smokers to various diseases associated with oxidative stress and atheroma formation.

INTRODUCTION
Cigarette smoking is one of the largest single risk factor for premature death in developed and developing countries (Chełchowska *et al.*, 2018). It has been estimated that smoking will kill about 10 million by 2025 if the current trend continues (King and Graffunder, 2018). Smoking is considered as one of the common sources of oxidants, that may increase the generation of reactive oxygen radicals (ROS); beyond what the antioxidant system can handle, thus causing oxidative stress (Bizoń and Milnerowicz, 2017). Many diseases have been linked to oxidative stress as the major player in their pathogenesis (Jamal *et al.*, 2018). These diseases include: cardiovascular diseases, diabetes mellitus, leukaemia cancer, neurodegenerative diseases such as Alzheimer and Parkinson and autoimmune disorders (Jamal *et al.*, 2018) Oxidative stress is thought to be the general mechanism of aging, promote arteriosclerosis, contributing to the development of cancer and cardiovascular diseases. Metabolism of lipoprotein is assumed to occur in the artery which generate superoxide radicals, hydrogen peroxide or lipid peroxides which could lead to the oxidation of LDL (Tremellen, 2019).

The biological system has several mechanisms to neutralize ROS (Agarwal *et al.*, 2019). The most important defense mechanism is facilitated by endogenous antioxidant enzyme system, which include superoxide dismutase (SOD), glutathione peroxidase (Gpx) catalase and glutathione reductase (Tremellen, 2019). The immune system of a smoker has to work harder every day than non-smokers thus smokers blood will contain less antioxidants (King and

*Author for Correspondence

Graffunder, 2018). Despite the fact that health problems associated with smoking are documented, there is still inadequate information on health consequences of smoking among university students.

MATERIALS AND METHODS

Experimental Design

A total number of 40 students (smokers age range 22-25 years) who have been smoking approximately 10 cigarettes per day continually for at least 4-5 years were identified; their smoking consumption (Mean ± standard error of the mean) was 18.5 ±1.3 cigarettes per day. Forty (40) nonsmokers (age range 21-25 years) were identified who reported no previous smoking experience. The subjects for this study were male student of Nasarawa State University, Keffi. All subjects were healthy and reported no use of illegal drugs (Except smoking in case of smoker). Subjects were interviewed for tobacco use and questioned on number of cigarette smoked on average per day and when they started smoking. The subjects were asked to attend the Laboratory of the NSUK Medical Center (SANPETRO), between 8.00am -9.00am, where blood samples were drawn from smokers and non-smokers respectively. Samples were drawn from the antecubital vein into some un-heparinized and heparinized containers, for evaluation of antioxidant enzymes and lipid profile respectively. To obtain serum, the blood was allowed to clot for 15 minutes and then centrifuged at 2000*g* for 15 minutes at 4°C. The serum was then transferred into 2-mL polyethylene storage containers by means of a pipette, and quickly frozen on dry ice. Serum was stored at −4°C for a period of < 1 months; to ensure accuracy of level of MDA and other biochemical parameters. A second aliquot of blood was separated and frozen for lipid profile determination. Serum lipids were measured in a Hitachi 704 chemistry analyzer. HDL cholesterol was measured on the chemistry analyzer by use of the magnetic HDL method (Polymedico). The LDL cholesterol level was calculated by the equation LDL Cholesterol = (Total Cholesterol−HDL Cholesterol) − (Triglycerides/5). All triglyceride levels were <400 mg/dL.

Ethical clearance

Ethical clearance was obtained from Nasarawa State University's Medical Center (SANPETRO), Nasarawa State University Keffi. The participants were notified about the processes and procedure of the research in order to seek their consent. A questionnaire was used to obtain the demographic information of the participants.

STATISTICAL ANALYSIS

The results of this research was analyzed using the Statistical Package for Social Sciences (SPSS), version 20. The results was expressed as Mean ± Standard deviation (Mean ± SD) for the significant differences between groups. t-test was used to compare the differences of mean of lipid profiles between smokers and non-smokers. In addition, ANOVA test was performed to find the differences of lipid profiles, lipid –peroxide and endogenous enzymes (SOD, CAT, GPX). Also, the Chi-square test was used to evaluate the association between variables. All statistical tests were considered significant in p-value of <0.05 with a confidence level of 95 %.

RESULTS

Table 1: Lipid profile analysis in smokers and non-smokers.

Parameters (mmol/L)	Smokers	Non Smokers	P – value
TG	1.2350 ± 0.4017*	1.0200 ± 0.1881*	<0.05
TC	4.1900 ± 0.3712	3.8100 ± 0.4587	< 0.05
HDLC	1.1050 ± 0.3170*	1.0850 ± 0.2323*	> 0.05
LDL-C	2.8100 ± 0.4103*	2.5250 ± 0.2673*	< 0.05

All results are expressed as Mean ± SD; n= 40

* ($p<0.05$), statistically significant.

Lipid peroxidation

The level of Lipid peroxidation in terms of MDA in smoker and non-smoker are shown in figure 1, below:

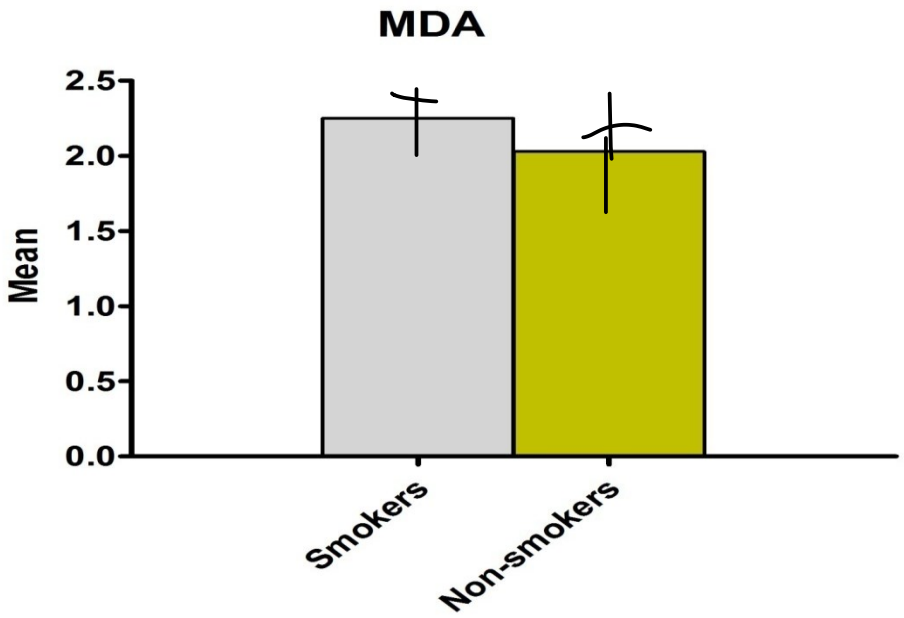

Figure 1: The MDA level in smokers increased significantly ($p<0.05$) compared to non smokers. Data are expressed as Mean ± SD, n= 40

CATALASE

The level of peroxidation in terms of CAT in smoker and non-smoker are shown in figure 2, below

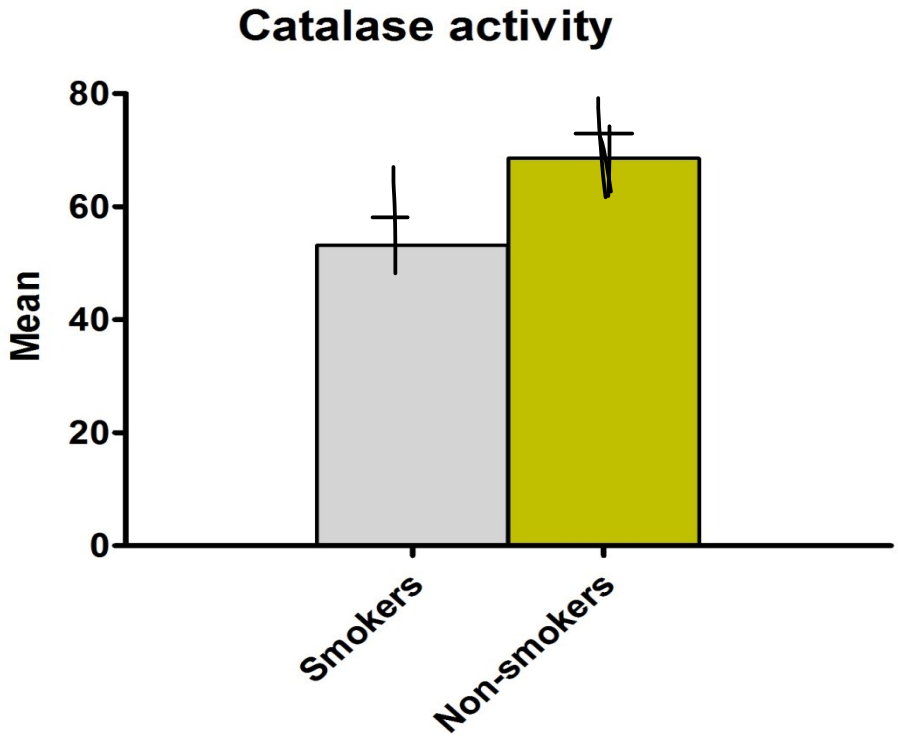

Fig. 2 catalase activity in serum of smoker and non- smoker. Data are expressed as mean±, n=20

SUPEROXIDE DISMUTASE

The level of peroxidation in terms of SOD in smoker and non-smoker are shown in figure 3. Below

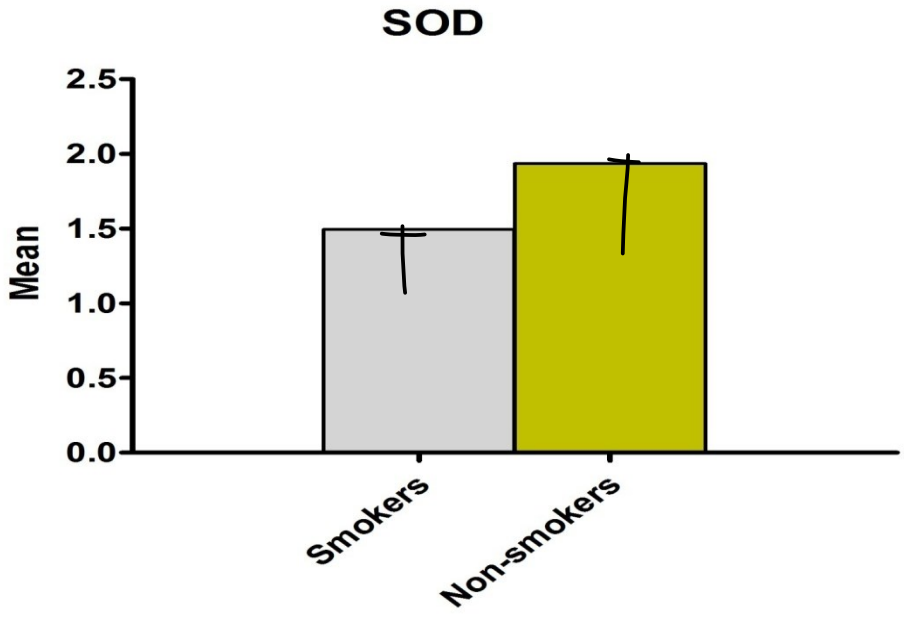

Fig. 3 In smoker student, SOD activity was decreased significantly ($p<0.05$) compared to non-smoker. Data are expressed as mean ±SD, n=20

GLUTATHIONE PEROXIDASE

The level of peroxidation in terms of GPx in smoker and non-smoker are shown in figure 4. Below

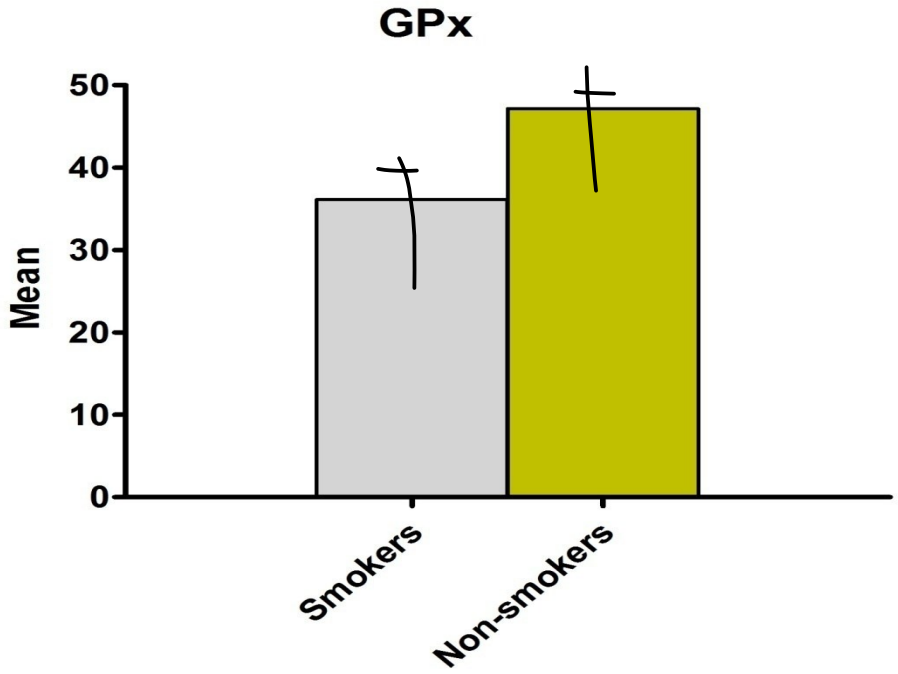

Fig.4 GPx activity of smoker and non-smoker Data are expressed as mean± SD, n=40.

From Table 1, the results showed that there was a significant difference between smokers and non-smokers in the mean values of total triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, Smokers had higher levels of total triglycerides, cholesterol, LDL-cholesterol and lower levels of HDL-cholesterol and non-HDL-cholesterol compared to the non-smokers group. Also, from the figures above, there is significant difference between smokers and non-smokers in mean values of malondialdehyde (MDA), SOD, CAT and GPX respectively.

DISCUSSION

Cigarette smokes contain range of xenobiotics including oxidant and ROS which can increase lipid peroxidation (Jamal *et al.*, 2018). In this study, antioxidant status was diminished in smokers and simultaneously lipid peroxidation (increase in MDA level) was increased compared to non-smokers. The increase in the level of peroxidation may be due to the generation of ROS by smoking that lead to degradation of phospholipid bilayer indicated by the high level MDA and this indicated lower capacity to combat ROS (Bizoń and Milnerowicz, 2017). The significant decrease in the level of SOD, Gpx and catalase may be due to increased utilization of these enzymes to scavenge free radicals generated due to smoking (Bizoń and Milnerowicz, 2017).

In this study, the antioxidant status was diminished in smokers and simultaneously lipid peroxidation was increased compared with non-smokers. The increased level of peroxidation (fig.1) in smokers may be due to the generation of reactive oxygen species by smoking that leads to cell damage and also indicated the lower capacity to combat against reactive oxygen species (McAdam *et al.*, 2018). Antioxidant defense system protects our body from the

deleterious effect of reactive oxygen metabolites, catalase act as preventive antioxidants and superoxide dismutase (SOD), a chain breaking antioxidant, play an important role in protection against deleterious effect of lipid peroxidation (Tremellen, 2019). In the study, the decreased level of catalase activity (fig.2) may be related to excess H_2O_2 production from smoking or SOD inhibition. The significant decreased ($p<0.05$) in the level of Superoxide dismutase (fig.3) (Agarwal *et al.*, 2019), and Glutathione peroxidase(fig.4) in smokers of the university students may be due to increased utilization of antioxidants to scavenge free radical generation along with smoking (Agarwal *et al.*, 2019). The result shows that smoking is associated with free radical scavenging system.

Smoking has been reported to be associated with reduced HDL-C level by alteration of the critical enzymes of lipid transport lowering lecithin-cholesterol acyltransferase (LCAT) activity and altering cholesterol ester transfer protein (CETP) and hepatic lipase activity (Gebrie *et al.*, 2018). The consumption of tobacco among adolescent and youths in developing country is alarming and causes premature development and cardiovascular disorders (Singh *et al.*, 2019). The result shows that smoking is significantly and positively associated with serum total cholesterol (TC), triglycerides and low density lipoprotein cholesterol (LDL-C). In other words, total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-C) are increased by smoking. The mean serum total cholesterol in nonsmokers was 3.81± 0.46mmol/L. while it was significantly higher ($P<0.05$) in smokers i.e. 4.19 ± 0.37mmol/L. These observations are in agreement with previous findings (Singh *et al.*, 2019). The mean serum triglyceride level showed a significant difference (<0.05) between smokers and non-smokers, the mean values were 1.24 ± 0.40mmol/L and 1.02 ± 0.19mmol/L respectively. The mean values for LDL-C level in smokers and non-smokers was 2.81 ± 0.41 and 2.53 ± 0.27 respectively, showing a significant increase in smokers. It has been demonstrated that the significance of LDL-C levels strongly depends on the number of cigarette smoked per-day. Contrary report to this has also been reported, that total cholesterol (TC) and LDL-C did not vary between smokers and non-smokers (Torun *et al.*, 2019). The variations observed can be attributed to ethnic differences in the population studied. The result suggest that cigarette smoking adversely alter the lipid profile resulting in dyslipidemia in smokers (Mohammed *et al.*, (Hallit *et al.*, 2019). Moreover, some studies have shown that the changes are more pronounced as the number of cigarette smoked increases Smoking causes an increase in oxidized LDL-C which plays a key role in atherosclerosis. It has been suggested that the oxidation of LDL-C generates potent pro-artherrogenic mediator (Attanzio *et al.*, 2019).

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