URINARY 1-HYDROXYPYRENE CONCENTRATION AND TOXICITY BIOMARKERS IN ACTIVE AND PASSIVE MALE SMOKERS FROM LAGOS METROPOLIS, NIGERIA

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ABSTRACT

Background: *Tobacco smoking is one of the commonest addictions of modern times and it is a major source of human exposure to polycyclic aromatic hydrocarbons (PAHs).*

Objectives: *This study investigated the level of urinary 1-hydroxypyrene concentration, liver and kidney function biomarkers as well as antioxidant status in tobacco smokers and non-smokers in Lagos Metropolis, Nigeria.*

Methods: *Eighty adult males (30 Active smokers, 10 Passive smokers and 40 Non-smokers) were recruited form two different locations in Lagos and were given a standardized questionnaire. Twenty four hour urine samples were collected and used to assay for urinary 1-hydroxypyrene concentration while blood samples were used to determine haematological, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total bilirubin (TB), total protein (TP), alkaline phosphatise (ALP), creatinine, superoxide dismutase (SOD), catalase (CAT), reduced glutathione reductase (GST) and malondialdehyde (MDA) parameters.*

Results: *Urinary 1-hydoxylpyrene levels in active and passive smokers were not significantly ($p>0.05$) different but were significantly ($p\leq0.05$) higher than that of non-smokers. Similarly, AST, ALP activities; TB and creatinine levels were significantly ($p\leq0.05$) higher in active and passive smokers than non-smokers. There was also significant ($p\leq0.05$) decrease in SOD and CAT in active and passive smokers while MDA was increased in active and passive smokers compared to non-smokers.*

Conclusion: *The high levels of urinary 1-hydroxypyrene, liver enzymes and distorted antioxidant biomarkers in passive smokers are indicators that even passive smoking could predispose to adverse health effects because of spending considerable time in smoke polluted environment.*

Keywords: *Tobacco, 1-hydroxypyrene, Liver damage, Antioxidants, Smokers*

INTRODUCTION

Tobacco smoking has been implicated in the pathogenesis of many diseases, which include a variety of infections, cancers, heart diseases and respiratory illnesses (Mehta *et*

al., 2008; Zhong *et al*., 2008; Salhen and Abdalslam, 2014). Tobacco is the single greatest cause of preventable death globally and it is one of the most common addictions of modern times (WHO, 2008; Alsalhen and Abdalslam, 2014). Researches have shown that tobacco smoke is a toxic mixture of more than 5,000 chemicals, some of which are known carcinogens (*Talhout et al. 2011, Behera et al., 2014)*. Carcinogenic compounds in cigarette smoke are thought to be cause of oral, pharyngeal, lung, esophageal, pancreas, kidney, liver, bladder, stomach, and colon cancers (Bassiony *et al.* 2015).

In addition, among the constituents of tobacco smoke are polycyclic aromatic hydrocarbons (PAHs), which are formed primarily as a result of incomplete combustion of organic materials such as wood, fossil fuel, petroleum products, automobile exhaust fumes, coal, charbroiled meat and tobacco (Rengarajan *et al.,* 2015). Studies have reported that about 539 PAHs and their alkyl derivatives have been identified in tobacco smoke, some of which are potent carcinogens and tumor promoters (IARC, 2004; Zhang *et al.,* 2011; St. Helen *et al.* 2012). Polycyclic aromatic hydrocarbonshave also been shown to cause detrimental effects such as mutagenic, genotoxic, reproductive and developmental disorders (Hombach-Klonisch *et al*. 2005; Rengarajan *et al.,* 2015). However, some of the PAHs found in smoke are pro-carcinogens that are converted to carcinogens in the course of its metabolism (Hecht, 2002; Melamede, 2005).

Therefore, bio-monitoring of human exposure to PAHs from tobacco smoke is an important public health concern with the view of creating more awareness of the adverse effect of smoking, facilitating the risk assessment, formulating sustainable withdrawal approach and reducing exposure. The parent unmetabolized PAHs and their metabolic products have been explored as a means of measuring environmental and occupational exposure to PAHs (Buratti *et al.* 2007; Förster *et al.,* 2008). The conversion of PAH to hydroxylated derivatives and ultimately conjugation to glucuronides and sulfates and excretion in urine and feces is a major route of PAH metabolism (St. Helen *et al.,* 2012).These metabolite biomarkers have also been detected in other biological tissues such as blood cells, skin, placenta, fibroblasts, liver, intestinal and tracheobronchial epithelia (Pohjola *et al.,* 2003; Binková and Srám, 2004; Sexton *et al.,* 2011; Clinton *et al.* 2013; Ramesh *et al.,* 2015).

Nevertheless, there are limited data on PAHs that can be used to discriminate between smokers and non-smokers. The detection of benzo[a]pyrene (B[a]P) in tobacco smoke and its carcinogenicity has been well documented making it a potential biomarker for risk assessment. However, its concentration is low compared to other PAHs even in B[a]P exposure in some occupational settings, thus limiting its use as a PAH biomarker in tobacco smoke studies (Hecht, 2002; Ding *et al.,* 2005; Helen *et al.,* 2012). Thus excretory 1-hydroxypyene, a metabolite of pyrene, has been explored as a biological monitoring indicator of body burden of PAHs (Jongeneelen, 2001; Suzuki and Yoshinaga, 2007; Tairova *et al.,* 2009; Clinton *et al.,* 2013).

The preferential use of 1-hydroxypyene as biomarker of PAHs exposure is because of various reasons which include 1-hydroxypyrene being a product of pyrene metabolism, representing 90% of its metabolites. The half-life of 1-hydroxypyrene is about 18 to 20 hours; hence, urinary 1-hydroxypyrene represents the last 24 hours of cumulative PAH exposure (Jongeneelen, 2001; Mucha *et al.,* 2006; Clinton *et al.,* 2013). In addition, pyrene is the only known precursor of 1-hydroxypyrene,it forms a consistent proportion of higher molecular weight PAHs in the environment and tobacco smoke (IARC, 1986; Castaño-Vinyals *et al.,* 2004; Ciarrocca *et al.,* 2014). Furthermore, 1-hydroxypyrene is found to be clearly elevated in occupational settings with high PAH exposure (Chuang *et al.,* 1999; Unwin *et al.,* 2006; Leeand Byeon, 2009; Campo *et al.,* 2012).Thus, Urinary 1-hydroxypyrene (1-OHPY), a monohydroxylated metabolite of pyrene, has been suggested and used as a biomarker of total PAH uptake in smokers and non-smokers (Jongeneelen, 2001; Hu *et al.,* 2006).

In addition, there has been growing concern on the effect of tobacco second-hand-smoke (SHS) exposure on passive smoking where individuals do not directly smoke tobacco but inhale smokes from tobacco (Bassiony *et al.* 2015). This form of exposure has been characterised as environmental tobacco polluted smoke (ETS). Therefore, the aim of this study was to investigate the influence of active and passive smoking on the urinary concentration of 1-hydroxypyrene, haematology, liver, kidney and oxidative stress biomarkers in adult male within Lagos metropolis, Nigeria.

MATERIALS AND METHODS

Questionnaire

Standard questionnaire was developed to assess eligibility of the volunteers to involve in the study. The questionnaire identified the socio-demographic parameters of the respondents such as age, smoking habit, life style habits, educational status, occupation and medical history. The enrolled subjects did not have any diagnosed critical health problem; no history of drug usage and none had donated or received blood in last 6 months. The clinical data and medical history were collected from subjects by personal interview via a questionnaire. The participants who met the inclusion criteria and volunteered to continue with the study were selected.

Participants

The subjects of this study were from two locations; Group 1: Idi-Araba Motor Park, Lagos and Group 2: Yaba College of Technology Motor Park, Lagos, Lagos State. Group 1 respondents from Idi-Araba Motor Park (n = 40), were either commercial bus drivers or conductors or vendors, they were divided into 2 groups; smokers (n = 20) and non-smokers (n = 20), the smokers group were further sub-grouped into two, passive (n=10) and active smokers (n=10).The passive smokers were those who do not smoke tobacco but spent hours in tobacco smoking area.

Group 2 respondents from Yaba College of Technology, Lagos (n = 40) were undergraduate students, they were divided into 2 groups; smokers (cigarette) (n = 20) and non-smokers (n = 20). All participants in this study willingly volunteered to donate 24-hr urine and blood samples.

Sample collection

A 24-hr urine samples were obtained from all the volunteers. The samples were collected in sterile dark bottles and kept at -4^{o}C. Urine samples were used to determine the level of urinary 1-hydroxypyrene in each subject.

Venous blood samples were collected from subjects and placed in plain and heparinized bottles respectively. The whole blood samples were used to determine haematogical parameters while samples in heparinized bottles centrifuged at 2000*x*g for 15 min, serum was collected and used to assay for liver and kidney function and antioxidant biomarkers.

Determination of 1-hydroxylpyrene in urine

Enzymatic hydrolysis

Enzymatic hydrolysis of the glucuronide-1-hydroxypyrene conjugates in the urine was accomplished with glucuronidase. Each urine sample (1 ml) was mixed with 1 ml of β-glucuronidase (2000 units) and incubated overnight for 15 hrs at 38 °C. Thereafter, the sample was shaken thoroughly to remix the precipitate with the clear solution making a homogenous solution. C_{18} cartridges SPE cartridges were activated with 2 ml of methanol, then washed with 2 ml of distilled water and used to clean-up the test sample. The sorbent was washed with 2 ml of distilled water to remove water-soluble compounds from the sample matrix. The cartridge was desorbed with 2 ml of methanol. The eluates were made up to 2 ml each with the eluting solvent. Then methanol in sample was evaporated in a 60 °C water bath with air gently blown over the sample. The concentrated solution was passed through a 0-20 pm 25 mm syringe filter into an auto sampler amber vial and sealed with a Teflon septum for HPLC analysis.

HPLC analysis of urine samples

Separation of the urine sample constituents was accomplished with reversed phase HPLC(Model 1100 WPALS G1367A; Agilent Technologies, Wilmington, DE). According to Agilent Technologies Catalogue (2007), a fully automatic sample injector was filled with a 20 µl sample loop valve under slight pressure from sealed 20 ml vials and injected subsequently into a zorbax SB C8 (750mm x 4.6mm) 5µm column. The column temperature was 30°C and the flow rate was 1.000 ml/min. Mobile phase of water: methanol (5:95%) for 5 min was used. The chromatograph was equipped with ultraviolent spectrophotometer monitored at 242nm. Each sample was injected twice with an injection volume of 20 µL.

Hematological Analysis
White blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (PDW), platelet count (PLT) and platelet distribution width (PDW) were determined. In addition, different types of white blood cells – specifically, lymphocytes, monocytes, and granulocytes – were also determined. All blood samples were analysed using a Mythic 18 (Orphée S.A., Geneva, Switzerland) autoanalyser.

Determination of Liver Function Biomarkers
The estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities was carried out according to Reitman and Frankel (1957) method using Randox laboratory test Kit (Antrim, UK). Serum albumin (ALB) was determined by the method of Doumas *et al.* (1971), serum total protein (TP) by the method of Tietz (1995) and serum bilirubin by colorimetric method based on the method described by Jendrassik and Grof (1938) using Randox laboratory test kit (Antrim, UK).

Kidney Function Biomarkers
Serum creatinine was determined according to method described by Fabiny and Ertinshausen (1971).

Antioxidant Status
Determination of Superoxide dismutase activity (SOD): The method of Sun and Sigma (1978) was adopted. An extinction coefficient for epinephrine at 480 nm of 4020 $M^{-1}cm^{-1}$ was used in calculating enzyme activity. One unit of enzyme activity was defined as the amount of enzyme needed to inhibit 50 % epinephrine per minute per milligram protein.

Determination of Catalase activity (CAT): Serum catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al.* (2005). An extinction coefficient for H_2O_2 at 240nm of 40.0 $M^{-1}cm^{-1}$ was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Determination of lipid peroxidation index (LPO): Lipid peroxidation was estimated based on the formation of TBARS (thiobarbituric acid reactive substances) as an index of lipid peroxidation according to the method of Niehaus and Samuelson as described by Usoh *et al*. (2005). Malonaldehyde which is an index of LPO was calculated with extinction coefficient of 1.5×10^5 $M^{-1}cm^{-1}$.

Determination of Glutathione (GSH): The reduced glutathione (GSH) content of sample as non-protein sulphydryls was estimated according to the method of Sedlak and Lindsay (1968).

Determination of Lipid peroxidation:Lipid peroxidation (LPO) was estimated based on the formation of TBARS (thiobarbituric acid reactive substances) as an index of lipid peroxidation according to the method of Niehaus and Samuelson as described by Usoh *et al*. (2005). LPO index, malonaldehyde (MDA) was calculated with extinction coefficient of 1.5×10^5 $M^{-1}cm^{-1}$.

Statistical analysis

Statistical analysis was carried out using SPSS version 20.0. One way ANOVA and Student's t- test were applied to test the significance of variance ($p \leq 0.05$) of the parameters under study. All values are expressed as mean ± standard error of mean (SEM).

RESULTS

General characteristic of study participants

The general characteristics of the smokers and non-smokers are shown in the Table 1.The mean age of smokers (active and passive) from group 1 was 45.12± and 45.22±1.11 years respectively, age of group 2 was 28.12±2.16 while control subjects was 36.11±9.25. The mean body weight of smoking subjects from group 1 was 79.82±14.20 and 77.22±10.51 kg for the active and passive smokers respectively whereas; the mean body weight of those from group 2 was 71.82±11.20 while mean weight of control subjects was 73.13±13.16 kg. All the smokers took at least 10 wraps of cigarette per day while the passive smokers spent at least 10hr per day in environmental tobacco polluted smoke (ETS).

Urinary 1-Hydroxypyrene level

The standard calibration plot of 1-hydroxypyrene was linear with a correlation coefficient (R^2) of 0.993. Urinary 1-hydoxylpyrene (1-OHP) was detected in all the subjects investigated. In group 1, the level of urinary 1-hydroxypyrene detected in the active smokers ranged from 35.61 µg/mol to 252.35µg/mol, while in passive smokers, it ranged from 25.30 µg/mol to 166.45µg/mol. The average 1-OHP level was 118.15 ± 72.23 and 100.05 ± 45.48 µg/mol in the active and passive smokers respectively (Fig. 1). In group 2, the level of urinary 1-OHP detected in the smokers ranged from 34.11 µg/mol to 209.614 µg/mol with average level of 94.06 ± 50.52 µg/mol. The control had urinary 1-OHP ranged from 11.68 µg/mol to 142.02 µg/mol with average level of 49.45 ± 31.43 µg/mol. There was no significant ($p>0.05$) difference in the level of urinary 1-OHP between the active and passive smokers, however, these values were significantly ($p \leq 0.05$) higher than the control.

Haematological Parameters

The effect of tobacco smoking on haematological parameters is shown in Table 2. The analysis showed variation in the parameters evaluated amongst the subjects. WBC, LYM,

MVC and PLT were elevated in all the smokers compared to control. WBC and PLT were significantly ($p \leq 0.05$) higher in the active smokers from group 1 compared to all the other subjects.

Liver and Kidney function Biomarkers

The active and passive smokers in group 1 and smokers in group 2 had ASTactivities significantly ($p<0.05$) higher than the control. The ALT activities of active smokers and passive (group 1) were significantly ($p<0.05$) higher than the control. while ALP activities of active smokers (groups 1 and 2) were significantly ($p<0.05$) higher than the control (Table 3). In addition, total protein (TP) of the group 1 and 2smokers were significantly ($p>0.05$) higher than the control and were also in the upper human limit of. Similarly, total bilirubin (TB) level of active and passive smokers were significantly ($p>0.05$) higher than the control. There was a significant ($p>0.05$) increase in the concentration of creatinine in the groups 1 and 2 subjects compared to the control (Table 4).

Antioxidants biomarkers and lipid peroxidation status

Generally, there was suppression of superoxide dismutase (SOD), catalase (CAT) activities and reduced glutathione (GSH) level in the active smokers of group 1. In the group 2 subjects, SOD, CAT and GSH were not significantly ($p \leq 0.05$) different from the control. Malondialdehyde (MDA) levels of the active and passive smokers from group 1 were significantly ($p \leq 0.05$) higher compared to those of group 2 and control subjects (Table 5).

DISCUSSION

Tobacco consumption has remained a significant threat to public health worldwide, causing 1 in 10 deaths among adults (WHO, 2008). The adverse effect of cigarette smoke has been attributed to the presence of a large variety of toxic compounds in the main and side stream smoke (Adam *et al.* 2010; Slaughter *et al.* 2011). Mainstream and side-stream smoke are qualitatively similar in chemical composition, but may differ in the quantities of their constituents due to differences in the heat of combustion, tobacco content, additives present, and the type of filter material used (Kane *et al.,* 2010; Sahu *et al.,* 2013)**.** In the current study, the observed higher significant mean level of urinary 1-hydroxypyrene in the active and passive smokers compared to the control suggests that polycyclic aromatic hydrocarbons present in cigarette smoke may have contributed to the elevation. This corroborates previous findings which indicated that the level of 1-hydroxypyrene in urine of tobacco smokers was higher than the levels found in non-smokers (Hecht, 2002; Carmella *et al.,* 2009; Leeand Byeon, 2010). Studies have also shown reduction in urinary 1-OHP by reducing the number of cigarettes per day (Hecht *et al*., 2004; Ichiba *et al.,* 2006).

Furthermore, the non-significant difference in urinary 1-OHP concentration between active and passive smokers but were significantly higher than control non-smokers indicated that cigarette smoking contributed to ETS. National Toxicology Program has stated ETS as 9th on the list of known human carcinogens (US National Toxicology Program, 2000) and International Agency for Research on Cancer (IARC) has suggested that there is sufficient evidence that passive smoking causes lung cancer in humans (IARC, 2004). Researchers have also shown that passive smoking may cause diverse health challenges which include endothelial dysfunction of the coronary circulation in nonsmokers (Otsuka *et al.,* 2001), acute effect on heart rate and blood pressure in young healthy females (Yarlioglues *et al.,* 2010) and increased incidence of wheeze and asthma in children and young people by at least 20% (Burke *et al.,* 2012). In addition, exposure of pregnant women to ETS during the third trimester was positively associated with asthmaandallergy-related symptoms in their preschool age children (Xepapadaki *et al.,* 2009).

Nevertheless, the detection of 1-OHP in non-smokers in this study may be attributed to environmental exposure to PAHs. Studies have indicated that individuals may be exposed to occupational and environmental PAHs pollution (Pośniak, 2005; Srogi, 2007; Ayi-Fanou *et al.,* 2011). As such, the non-smokers may have been exposed to environmental PAHs pollution from vehicular exhaust fume, transient cigarette smoke, smokes from power generators, and industrial fumes that are very common and predominant in urban areas like Lagos State, Nigeria. This corroborates previous findings on PAHs pollution to urban inhabitants and its environs (Xiao *et al.,* 2014; Mishra *et al.,* 2016).

The observed higher level of WBC and LYMP in smokers compared to non-smokers in this study corroborates previous studies (Kume *et al.,* 2009; Raval and Paula, 2010). Asif *et al.* (2013) reported that alteration in the level of RBC, WBC and Hematocrit may be associated with a greater risk for developing atherosclerosis, polycythemia vera, chronic obstructive pulmonary disease and/or cardiovascular diseases as well as be associated with blood viscosity and clotting in smokers (Ho, 2004). Moreover, smoking accelerates atherosclerosis, which leads to cardiovascular disease (CVD), by affecting endothelial functions, cholesterol metabolism and platelet functions and increasing inflammation and oxidative stress (Lüdicke *et al.,* 2015). It has also been suggested that nicotine may induce an increase in blood lymphocyte counts by inducing the release of catecholamine (Calapai *et al.,* 2009; Chang *et al.,* 2010).

The observed significantly higher activities of AST, ALT and ALP in the active and passive smokers compared to the control suggests deleterious effects of cigarette smoking on the liver tissue (Wannamethee *et al.,* 2005; Alsalhen and Abdalslam, 2014). Cigarette smoke propagates lipid peroxidation, which damages the biological cell membrane of the liver (Yousif and Amal, 2012). Similarly, the observed significantly higher level of total bilirubin in the smokers compared to the non-smokers could be due to inhalation of large

amounts of PAHs, which may have possibly resulted in heme catabolism and thus lead to bilirubin production (Jee *et al.*, 2004).This is also supported by the findings of Srogi (2007), who reported that breathing or swallowing large amounts of naphthalene could cause the breakdown of red blood cells.

Among the health risks associated with exposure to PAHs in tobacco smokers, is the consequence of the disturbance of the antioxidants defence system. This may result in oxidative stress (Pasupathi N *et al.*,2009a; Kuang *et al.,* 2013; Domej *et al.,* 2014), which is one of the important mechanisms behind the initiation of cancer (Burlakova *et al.*, 2010; Goldkorn *et al.,* 2014). Large amount of reactive oxygen species and many electrophiles are generated during the activation of PAHs by CYP450, which bind covalently with DNA, and disturb the homeostasis of the cell (Henkler *et al.,* 2012; Shimada *et al.,* 2013). The suppressed level of SOD, CAT and GSH in the active and passive smokers from group 1 (Idi Araba motor park) compared to the control suggest that they may be undergoing oxidative stress (Alberg *et al*., 2000; Dietrich *et al*., 2003; Pasupathi *et al.*, 2009b). This may be linked to the high number of years of smoking and duration spent ETS area. Research has suggested that smokers need more antioxidant vitamins for the scavenging of potential harmful free radicals produced by cigarette smoke (Kelly, 2003). In addition, malondialdehyde which was increased in all the smokers compared to the control indicate lipid peroxidation, this is in agreement with previous studies (Nagamma *et al.* 2011; Ferrari, 2012; Fahang and Fikry, 2013).

CONCLUSION

This present study showed that both active and passive smokers have higher levels of urinary 1-hydroxypyrene compared with non-smokers; this suggest that exposure to environmental tobacco polluted smoke (ETS) could have adverse health effects. Moreso, smoking was associated with significant rise in the levels of some haematological, liver and kidney function biomarkers while there was suppression of antioxidant biomarkers.

Recommendations

There should be awareness campaign on the adverse effect on passive smoking. Laws regulating public smoking should be promulgated and enacted so as to curb the increasing rate of smoking-associated diseases. In addition, disciplinary actions should be meted out to defiant persons that indulge in public smoking.

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REFERENCES

Adam, T., McAughey, J., Mocker, C. and McGrath, C. and Zimmermann, R. (2010).Influence of filter ventilation on the chemical composition of cigarette mainstream smoke. *Analyt. Chim. Acta*. 657:36-44.

Alberg, A. J., Chen, J. C., Zhao, H., Hoffman, S. C., Comstock, G. W. and Helzlsouer, K. J. (2000).

Household exposure to passive cigarette smoking and serum micronutrient concentrations. *Am. J. Clin. Nutr*.**72**:1576-82.

Alsalhen, K.S and Abdalsalam, R. D. (2014). Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya. *Int. Curr. Pharm. J*.**3**: 291-295.

Asif, M., Karim, S., Umar, Z., Malik, A., Ismail, T., Chaudhary, A., Alqahtani, M. H. and Rasool, M. (2013). Effect of cigarette smoking based on hematological parameters: comparison between male smokers and non-smokers. *Turk. J. Biochem*.**38**: 75-80.

Ayi-Fanou, L., Avogbe, P. H., Fayomi, B., Keith, G., Hountondji, C., Creppy, E. E., Autrup, H., Rihn, B. H. and Sanni, A. (2011). DNA-adducts in subjects exposed to urban air pollution by benzene and polycyclic aromatic hydrocarbons (PAHs) in Cotonou, Benin. *Environ. Toxicol*.**26**: 93-102.

Bassiony, M. A., Aqil, M., Khalili, M., Radosevich, J. A. and Elsabaa, H. M. (2015).Tobacco Consumption and Oral, Pharyngeal and Lung Cancers. *The Open cancer J*.**8**: 1-11.

Behera, S. N., Xian, H. and Balasubramanian, R. (2014). Human health risk associated with exposure to toxic elements in mainstream and side stream cigarette smoke.*Sci. Total Environ.***15**: 947-56.

Binková, B. and Srám, R. J. (2004). The genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM) on human diploid lung fibroblasts. Mutat. Res.**547**: 109-21.

Buratti, M., Campo, L., Fustinoni, S., Cirla, P. E., Martinotti, I., Cavallo, D. and Foa, V. (2007). Urinary hydroxylated metabolites of polycyclic aromatic hydrocarbons as biomarkers of exposure in asphalt workers.*Biomarkers.***12**: 221-239.

Burlakova, E. B, Zhizhina, G. P., Gurevich, S. M., Fatkullina, L. D., Kozachenko, A. I., Nagler, L. G., Zavarykina, T. M. andKashcheev, V. V. (2010). Biomarkers of oxidative stress and smoking in cancer patients. *J. Cancer Res. Ther*.**6**: 47-53.

Burke, H., Leonardi-Bee, J., Hashim, A., Pine-Abata, H., Chen, Y., Cook, D. G., Britton, J. R. and McKeever, T. M. (2012). Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics.***129**: 735-44.

Calapai, G., Caputi, A. P., Mannucci, C., Russo, A. G., Gregg, E., Puntoni R., Lowe, F., McEwan, M., Bassi, A., Morandi, S., and Nunziata, A. (2009). Cardiovascular biomarkers in groups of established smokers after a decade of smoking. *Basic Clin. Pharmacol. Toxicol.* 104: 322-328.

Campo, L., Vimercati, L., Carrus, A., Bisceglia, L., Pesatori, A. C., Bertazzi, P. A., Assennato, G. and Fustinoni, S. (2012). Environmental and biological monitoring of PAHs exposure in coke-oven workers at the Taranto plant compared to two groups from the general population of Apulia, Italy. *Med. Lav.***103**: 347-360.

Carmella, S. G., Chen, M., Han, S., Briggs, A., Jensen, J., Hatsukami, D. K. and Hecht, S. S. (2009). Effects of Smoking Cessation on Eight Urinary Tobacco Carcinogen and Toxicant Biomarkers. *Chem. Res. Toxicol.* **22**: 734-741.

Castaño-Vinyals, G., D'Errico, A., Malats, N. and Kogevinas, M. (2004). Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution.*Occup. Environ. Med.***61**: e12.

Chang E., Forsberg, E. C., Wu, J., Bingyin, W., Prohaska, S. S., Allsopp, R., Weissman, I. L. and Cooke, J. P. (2010). Cholinergic activation of hematopoietic stem cells: Role in tobacco-related disease?.*Vasc. Med.***15**: 375-385.

Chuang, J. C., Callahan, P. J., Lyu, C. W. and Wilson, N. K. (1999). Polycyclic aromatic hydrocarbon exposures of children in low-income families. *J. Expo. Anal. Environ. Epidemiol.***9**:85-98.

Ciarrocca, M., Rosati, M. V., Tomei, F., Capozzella, A., Andreozzi, G., Tomei, G., Bacaloni, A., Casale, T., Andrè, J. C., Fioravanti, M., Cuartas, M. F. and Caciari, T. (2014). Is urinary 1-hydroxypyrene a valid biomarker for exposure to air pollution in outdoor workers? A meta-analysis. *J. Expo. Sci. Environ. Epidemiol.***24**: 17-26.

Dietrich, M., Block, G., Norkus, E. P., Hudes, M., Traber, M. G., Cross, C. E. and Packer, L. (2003). Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. *Am. J. Clin. Nutr.***77**:160-166.

Ding, Y. S., Ashley, D. L. and Watson, C. H. (2007). Determination of 10 Carcinogenic Polycyclic Aromatic Hydrocarbons in Mainstream Cigarette Smoke. *J. Agric. Food Chem.***55**: 5966-5973.

Domej, W., Oettl, K. and Renner, W.(2014).Oxidative stress and free radicals in COPD-implications and relevance for treatment. *Int. J. Chron. Obstruct. Pulmon. Dis.***9**: 1207-1224.

Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bomocresol green. *Clin. Chim. Acta.***31**: 87-96.

Elhassaneen Y. A. E and El-Badawy A. M. (2012). Influence of Charcoal Broiled Meat Consumption on the Liver Functions and Non-Enzymatic Antioxidants in Human Blood. *Food Nutr. Sc.***4**: 90-99.

Fabiny, D. L. and Ertinshausen, G. (1971). Automated reaction rate method for determination of serum creatinine with centrifichem. *Clin. chem.* **17**: 696-700.

Fahang A.A. and Fikry A. Q. (2013). Effects cigarette smoking on some immunological and hematological parameters in male smoker in Erbil city. *Jordan J. Bol. Sc.***6**: 159-166.

Ferrari, C. K. (2012). Effects of xenobiotics on total antioxidant capacity.*Interdiscip Toxicol.***5**: 117-122.

Förster, K., Preuss, R., Roßbach, B., Brüning, T., Angerer, J. and Simon, P. (2008). 3-Hydroxybenzo[a]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. *Occup. Environ. Med.* **65**: 224-229.

Goldkorn, T., Filosto, S., and Chung, S. (2014). Lung injury and lung cancer caused by cigarette smoke-induced oxidative stress: Molecular mechanisms and therapeutic opportunities involving the ceramide-generating machinery and epidermal growth factor receptor. *Antioxid. Redox. Signal.***21**:2149-74.

Hecht, S. S. (2002). Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. Carcinogenesis. **23**:907-922.

Hecht, S. S., Carmella, S. G., Le, K-A., Murphy, S. E., Li, Y. S., Le, C., Jensen, J. and Hatsukami, D. K. (2004). Effects of reduced cigarette smoking on levels of 1-hydroxypyrene in urine. Cancer Epidemiol. *Biomarkers Prev.***13**: 834-842.

Henkler, F., Stolpmann, K. and Luch, A. (2012). Exposure to polycyclic aromatic hydrocarbons: bulky DNA adducts and cellular responses.*EXS.***101**:107-31.

Ho, C. H. (2004). White blood cell and platelet counts could affect whole blood viscosity. *J. Chin. Med. Assoc.***67**: 394-397.

Hombach-Klonisch, S., Pocar P., Kietz S. and Klonisch T. (2005). Molecular actions of polyhalogenated arylhydrocarbons (PAHs) in female reproduction. Curr. Med. Chem.**12**:599-616.

Hu Y., Zhou, Z., Xue, X., Li, X., Fu, J., Cohen, B., Melikian, A. A., Desai, M., Tang, M., Huang, X., Roy, N., Sun, J., Nan, P. and Qu, Q. (2006). Sensitive biomarker of polycyclic aromatic hydrocarbons (PAHs): urinary 1-hydroxyprene glucuronide in relation to smoking and low ambient levels of exposure.*Biomarkers.***11**: 306-318.

IARC. (2004). Tobacco smoke and involuntary smoking. *IARC Monogr. Eval. Carcinog. Risks Hum.***83**: 1-1438.

IARC (1986) *Tobacco Smoking. IARC Monogr. Eval. Carcinog. Risks Chem. Hum.***38**: 35-394.

Ichiba, M., Matsumoto, A., Kondoh, T., Horita, M. and Tomokuni, K. (2006). Decreasing urinary PAH metabolites and 7-methylguanine after smoking cessation. *Int. Arch. Occup. Environ. Health.* **79**: 545-549.

Jee, J-H., Kim, S-G. and Kang, J-C. (2004). Effects of phenanthrene on growth and basic
physiological functions of the olive flounder, Paralichthys olivaceus.*J. Exp. Mar. Biol. Ecol.***304**: 123-136.

Jendrassik, L. and Grof, P. (1938). Colorimetric Method of Determination of bilirubin. *Biochem Z.***297**: 81-82.

Jongeneelen, F. J. (2001). Benchmark guideline for urinary 1-hydroxypyrene as bio-marker of occupational exposure to polycyclic aromatic hydrocarbons. *Ann. Occup. Hyg.***45**:3-13.

Kane, D. B., Asgharian, B., Price, O. T., Rostami, A. and Oldham, M. J. (2010). Effect of smoking parameters on the particle size distribution and predicted airway deposition of mainstream cigarette smoke. *Inhal. Toxicol.***22**:199-209.

Kelly, G. (2003). The interaction of cigarette *smoking* and *antioxidants*. Part III: ascorbic acid. *Altern. Med. Rev*.**8**: 43-54.

Kuang, D., Zhang, W., Deng, Q., Zhang, X., Huang, K., Guan, L., Hu, D., Wu, T. and Guo, H. (2013). Dose-Response Relationships of Polycyclic Aromatic Hydrocarbons Exposure and Oxidative Damage to DNA and Lipid in Coke Oven Workers. E*nviron. Sci. Technol.* **47**: 7446-7456.

Kume, A., Kume, T., Masuda, K., Shibuya, F. and Yamzaki, H. (2009). Dose-dependent effect of cigarette smoke on blood biomarkers in healthy volunteers: Observations from smoking and non-smoking. *J. Health Sc.***55**: 259-264.

Lee, K. H.and Byeon, S. H. (2010).The biological monitoring of urinary 1-hydroxypyrene by PAH exposure among smokers. *Int. J. Environ. Res.***4**: 439-442.

Lüdicke, F., Magnette, J., Baker G. and Weitkunat R. (2015). A Japanese cross-sectional multicentre study of biomarkers associated with cardiovascular disease in smokers and non-smokers.*Biomarkers.***20**: 411-421.

Mehta, H., Nazzal, K. and Sadikot, R. T. (2008). Cigarette smoking and innate immunity. *Inflamm. Res*. **57**: 497-503.

Melamede, R. (2005). Cannabis and tobacco smoke are not equally carcinogenic. *Harm. Reduct. J.* 2: 21.

Mishra, N., Ayoko, G. A. and Morawska, L. (2016). Atmospheric polycyclic aromatic hydrocarbons in the urban environment: Occurrence, toxicity and source apportionment. *Environ Pollut.* **208**:110-117.

Mucha, A. P., Hryhorczuk, D., Serdyuk, A., Nakonechny, J., Zvinchuk, A., Erdal, S., Caudill, M., Scheff, P., Lukyanova, E., Shkiryak-Nyzhnyk, Z. and Chislovska, N. (2006). Urinary1-hydroxypyrene as a biomarker of PAHexposure in 3-year-oldUkrainianchildren. *Environ Health Perspect.***114**: 603-609.

Nagamma, T., Anjaneyulu, K., Baxi, J., Dayaram, P. and Singh, P. (2011). Effects of cigarette smoking on lipid peroxidation and antioxidant status in cancer patients from Western Nepal. *Asian Pac. J. Cancer Prev*.**12**: 313-216.

Otsuka, R., Watanabe, H., Hirata, K., Tokai, K., Muro, T., Yoshiyama, M., Takeuchi, K. and Yoshikawa, J. (2001). Acute effects of passive smoking on the coronary circulation in healthy young adults. *JAMA.***286**: 436-441.

Pasupathi, P., Saravanan, G. and Farook, J. (2009a). Oxidative Stress BioMarkers and Antioxidant Status in Cigarette Smokers Compared to Nonsmokers. *J. Pharm. Sci. Res.***1**: 55-62.

Pasupathi, P., Saravanan, G., Chinnaswamy, P. and Bakthavathsalam, G. (2009b). Effect of chronic smoking on lipid peroxidation and antioxidant status in gastric carcinoma patients. *Indian J Gastroenterol*. **28**: 65-67.

Pohjola, S. K.,Lappi, M., Honkanen, M.,Rantanen, L. and Savela, K. (2003). DNA binding of polycyclic aromatic hydrocarbons in a human bronchial epithelial cell

line treated with diesel and gasoline particulate extracts and benzo[*a*]pyrene. *Mutagenesis*. **18**: 429-438.

Pośniak, M. (2005). Polycyclic Aromatic Hydrocarbons in the Occupational Environment during Exposure to Bitumen Fumes. Pol.J. Environ. Stu. **14**: 809-815.

Ramesh, A., Kumar, A., Aramandla, M. P. and Nyanda, A. M. (2015). Polycyclic Aromatic Hydrocarbon Residues in Serum Samples of Autopsied Individuals from Tennessee. *Int. J. Environ. Res. Public Health.* **12**: 322-334.

Raval, M. and Paul, A. (2010). Cerebral venous thrombosis and venous infarction: Case report of a rare initial presentation of smoker's polycythemia. *Case Rep. Neurol.***2**: 150-156.

Reitman, S. and Frankel, S. (1957). Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.***28**: 56-63.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P. and Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pac. J. Trop. Biomed.* **5**: 182-189.

Sahu, S. K., Tiwari, M., Bhangare, R.C., Pandit, G. G. (2013). Particle Size Distribution of Mainstream and Exhaled Cigarette Smoke and Predictive Deposition in Human Respiratory Tract. *Aerosol Air Qual. Res.***13**: 324-332.

Salhen, A and Abdalslam, R. D. (2014). Effects of cigarette smoking on hematological parameters in male smokers in Al-Bayda city, Libya. *Al. Mukhtar. J. Sc.***29**: 40-57.

Sedlak, J. and Lindsay, R. H. (1968). Estimation of Total Protein-Bound, and Nonprotein Sulfhydryl Groups in Tissue with Ellman's Reagent. *Analyt. Biochem.* **25**: 1192-1205.

Sexton, K., Salinas, J. J., McDonald, T. J., Gowen, R. M. Z., Miller, R. P., McCormick, J. B. and Fisher-Hoch, S. P. (2011). Polycyclic Aromatic Hydrocarbons in Maternal and Umbilical Cord Blood from Pregnant Hispanic Women Living in Brownsville, Texas. *Int. J. Environ. Res. Public Health.* 8: 3365-3379.

Shimada, T., Murayama, N., Yamazaki, H., Tanaka, K., Takenaka, S., Komori, M., Kim, D., Guengerich, F. P. (2013). Metabolic Activation of Polycyclic Aromatic Hydrocarbons and Aryl and Heterocyclic Amines by Human Cytochromes P450 2A13 and 2A6.*Chem. Res. Toxicol.,* **26**: 529-537.

Slaughter E., Gersberg, R. M., Watanabe**,** K., Rudolphs, J., Stransky, C., and Novotny, T. E. (2011). Toxicity of cigarette butts and their chemical components to marine and freshwater fish. *Tob. Control.* **20**: 25-29.

St. Helen, G., Goniewicz, M. L., Dempsey, D., Wilson, M., Jacob, P. III. and Benowitz,N. L. (2012). Exposure and Kinetics of Polycyclic Aromatic Hydrocarbons (PAHs) in Cigarette Smokers. *Chem. Res. Toxicol.***25**: 952-964.

Srogi, K. (2007). Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environ. Chem. Lett.***5**: 169-195.

Sun, M., and Zigman, S., (1978): An improved spectrophotometric assay for Superoxide

Dismutase based on Epinephrine autoxidation. *Analyt. Biochem.***90**: 81-89.

Suzuki, K. and Yoshinaga, J. (2007). Inhalation and dietary exposure to polycyclic aromatic hydrocarbons and urinary 1-hydroxypyrene in non-smoking university students. *Int. Arch. Occup. Environ. Health.***81**:115-121.

Talhout, R., Schulz, T., Florek, E., Van Benthem, J., Wester, P. and Opperhuizen, A., (2011). Hazardous compounds in tobacco smoke. *Int. J. Environ. Res. Public Health*. **8**: 613-628.

Tairova, Z. M., Anders, M. B., Giessing, H. R. and Andersen, O. (2009). 1-Hydroxypyrene as a biomarker of PAH exposure in the marine polychaete *Nereis diversicolor*. *Mar. Environ. Res.***67**: 38-46.

Tietz, N. W. (1995). Clinical guide to laboratory tests. 3rd Edition. WB Saunders Company. Philadelphia, P. A. pp. 518-519.

Unwin, J., Cocker, J., Scobbie, E. and Chambers, H. (2006). An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK.*Ann. Occup. Hyg.***50**: 395-403.

Usoh, I. F., Akpan, E. J., Etim, E. O. and Farombi, E. O. (2005). Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa*. *Pakistan J. Nutr.***4**:135-141.

Wannamethee, S. G., Lowe, G. D., Shaper, A. G., Rumley, A., Lennon, L. and Whincup, P. H. (2005). Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *Eur. Heart J.***26**:1765-1773.

WHO Report on the Global Tobacco Epidemic, 2008. The MPOWER package: ww.who.int/tobacco/mpower/en/

Xepapadaki, P., Manios, Y., Liarigkovinos, T., Grammatikaki, E., Douladiris, N., Kortsalioudaki, C. and Papadopoulos, N. G. (2009). Association of passive exposure of pregnant women to environmental tobacco smoke with asthma symptoms in children. *Pediatr. Allergy Immunol.***20**: 423-429.

Xiao, Y., Tong, F., Kuang, Y. andChen, B. (2014). Distribution and source apportionment of polycyclic aromatic hydrocarbons (PAHs) in forest soils from urban to rural areas in the Pearl River Delta of Southern China. *Int. J. Environ. Res. Public. Health.***11**: 2642-56.

Yarlioglues, M., Kaya, M. G., Ardic, I., Calapkorur, B., Dogdu, O., Akpek, M., Ozdogru, M., Kalay, N., Dogan, A., Ozdogru, I. and Oguzhan, A. (2010). Acute effects of passive smoking on blood pressure and heart rate in healthy females. *Blood Press. Monit.***15**: 251-256.

Zhang, S. M., Chen, K. M., Aliaga, C., Sun, Y. W., Lin, J. M., Sharma, A. K., Amin, S. G. and El-Bayoumy, K. (2011). Identification and Quantification of DNA Adducts in the Oral Tissues of Mice Treated with the Environmental Carcinogen Dibenzo[a,l]pyrene by HPLC-MS/MS. *Chem. Res. Toxicol.***24**:1297-1303.

Zhong, C. Y., Zhou, Y. M. and Pinkerton, K. E. (2008). NF-kappa B inhibition is involved in tobacco smoke-induced apoptosis in the lungs of rats. *Toxicol. Appl. Pharmacol*. **230**: 150-158.

RESULTS

Table 1: Socio-Demography of study participants

Characteristics	Group 1		Group 2	Control
	Smokers (n=20)		Smokers (n=20)	Non-smokers (n=40)
	Active	Passive		
Age	45.12±1.25	45.22±1.11	28.12±2.16	36.11±9.25
Body weight (kg)	79.82±14.20	77.22±10.51	71.82 ± 11.20	73.13 ± 13.16
Education/Occupation	Driver	Trader	Tertiary student	Tertiary student/Traders
No. of wraps per day	≥10/day	0	≥10/day	0
No. of hours spent in an ETS-Polluted space	≥6 hours/day	≥10 hours/day	≥4 hours/day	0
Length of years of smoking	≥15 years	0	≥5 years	0

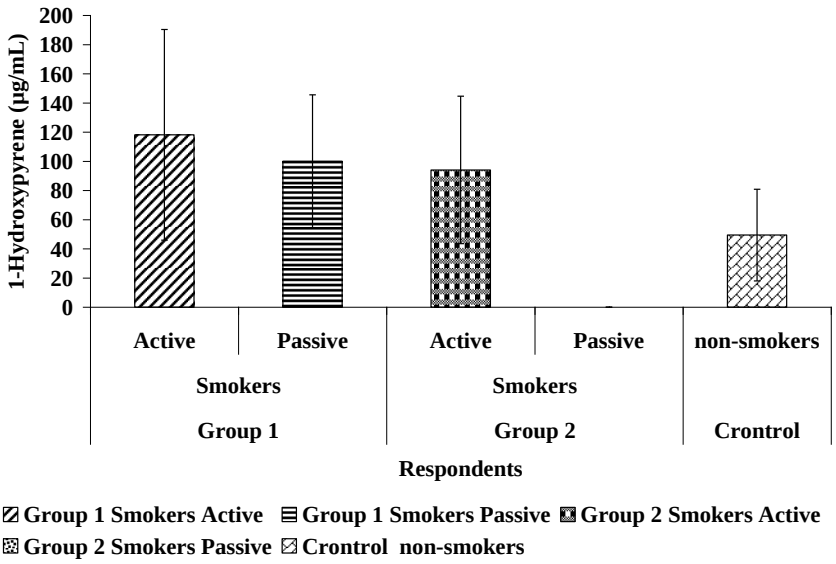

Figure 1: Level of urinary 1-hydroxypyrene in respondents

Table 2: Effect of Smoking on hematological Parameters

Parameter	Group 1		Group 2	Control
	Smokers		Smokers	Non-smokers
	Active	Passive		
WBC (10^3/µl)	5.45±1.19[a]	5.80±1.71[b]	5.89±1.06[c]	4.87±1.45[d]
LYMP (10^3/µl)	2.53±0.33[a]	2.40±0.37[b]	2.45±0.46[b]	2.04±0.64[c]
GRAN (10^3/µl)	2.38±0.81[a]	2.87±1.38[b]	3.05±0.59[c]	2.33±1.03[a]
HGB (g/dl)	11.63±1.38[a]	12.27±1.13[a]	12.84±0.96[a]	11.95±1.27[a]
RBC (10^3/µl)	4.25±0.41[a]	4.60±0.50[a]	4.92±0.35[b]	4.42±0.48[a]
HCT (%)	36.93±4.15[a]	38.88±3.18[b]	40.51±2.27[b]	37.91±3.74[a]
MCV (fL)	88.93±6.84[a]	90.04±4.57[a]	89.28±4.74[a]	86.00±5.54[a]
MCH (pg)	27.34±1.74[a]	26.74±2.60[a]	28.74±1.10[a]	27.04±2.17[a]
MCHC (g/dl)	31.43±0.62[a]	31.50±1.08[a]	32.27±0.76[a]	31.47±0.86[a]
PLT (10^3/µl)	208.60±46.62[a]	185.10±48.92[b]	145.05±1.24[c]	196.85±1.62[d]

** Values with different alphabetical superscripts for the same parameter in a row are significant with respect to each other ($p \leq 0.05$).* White Blood cells (**WBC**), lymphocytes (LYMP), granulocytes (GRAN), Hemoglobin (**HGB**), Red Blood cells (**RBC**), Hematocrit (**HCT**), Mean Corpuscular Volume (**MCV**), Mean Corpuscular Hemoglobin (**MCH**), Mean Corpuscular Hemoglobin Concentration (**MCHC**), Platelets (**PLT**).

Table 3: Effect of Smoking on Liver Function Biomarkers

Parameter	Reference range	Group 1		Group 2	Control
		Smokers		Smokers	Non-smokers
		Active	Passive		
AST (U/L)	4-36	60.28±4.72[a]	34.58±8.53[b]	42.28 ± 3.99[c]	26.20±0.93[d]
ALT (U/L)	4-32	31.93±2.04[a]	20.25±6.22[b]	14.64 ± 1.18[c]	12.10±0.46[d]
ALP (U/L)	45-115	71.92±2.56[a]	63.73±13.74[b]	76.92 ± 7.54[a]	61.14±1.55[b]
ALB (g/L)	35-55	46.01±6.23[a]	44.74±3.99[a]	46.11 ± 2.23[a]	43.55±0.85[a]
Total Protein (g/L)	60-80	79.56±6.67[a]	78.72±3.09[a]	74.65 ± 1.88[a]	69.93±1.47[b]
Total Bilirubin (µmol/L)	≤ 1.25	5.61±3.04[a]	3.51±2.33[b]	1.8 ±1.22[b]	0.61±0.03[c]

** Values with different superscripts have significant difference at $p \leq 0.05$.* Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)

Table 4: Effect of Smoking on Kidney Function Biomarkers

Parameter	Group 1		Group 2	Control
	Smokers		Smokers	Non-smokers
	Active	Passive		
Creatinine (µmol/L)	92.08±3.08[a]	102.7± 1.59[b]	96.37±2.41[a]	72.54±2.20[c]

Values with different superscripts have significant difference at $p \leq 0.05$

Table 5: Effect of smoking on antioxidants biomarkers and lipid peroxidation status

Parameter	Group 1		Group 2	Control
	Smokers		Smokers	Non-smokers
	Active	Passive		
SOD (U/mg)	1.96±0.17[a]	3.04 ±0.07[b]	4.132 ± 0.272[b]	3.886 ± 0.832[b]
CAT (U/mg)	15.38±0.66[a]	17.79±0.01[b]	19.529 ± 1.351[c]	20.699 ± 3.141[c]
GSH (U/mg)	0.83±0.03[a]	0.83±0.03[a]	1.936 ± 0.0758[b]	1.867 ± 0.103[b]
MDA (U/mg)	1.066±0.07[a]	0.81±0.02[a]	0.110 ± 0.099[b]	0.0221 ± 0.015[c]

Values with different superscripts have significant difference at $p \leq 0.05$. Superoxide dismutase activity (SOD), Catalase (CAT), Glutathione (GSH), malonaldehyde (MDA)