

Influence of active and passive smoking on the urinary concentration of 1 hydroxyl pyrene, oxidative stress and renal activity in male subjects

ABSTRACT

Objective: The purpose of the study was to investigate the influence of active and passive smoking on the urinary concentration of 1 hydroxyl pyrene, oxidative stress and renal activity in male subjects.

Methods: Urine samples each of forty active and passive smokers including forty non-smokers were collected in a 3L specimen collection jar with volumes ranging from 1450 ml to 2600 ml and clearly labeled according to their respective group. Blood samples were also collected from each of the subjects into heparinized tubes then followed by centrifugation at 3000rpm for 10mins to get the serum. The urine samples were used for the determination of 1-hydroxyl pyrene while the serum was used for the determination of antioxidant, creatinine and urea levels.

Results: There was a significant increase ($P<0.05$) in the concentration of 1-Hydroxyl Pyrene in urine of passive and active smokers compared to non-smokers but there was no significant difference ($P>0.05$) between the concentration of 1-Hydroxyl Pyrene in urine of active smokers and passive smokers. The level of oxidative stress markers; catalase, glutathione reductase, superoxide dismutase decreased significantly ($P<0.05$) respectively and the level of lipid peroxidation marker; malondialdehyde increased significantly ($P<0.05$) in the serum of passive and active smokers compared to non-smokers. However, there was no significant difference ($P>0.05$) in the levels of oxidative stress markers; catalase, glutathione reductase, superoxide dismutase respectively and lipid peroxidation marker;

malondialdehyde between the serum of passive and active smokers. A significant increase ($P < 0.05$) in the level of creatinine and urea was recorded in the serum of passive and active smokers compared to non-smokers while there was no significant difference ($P > 0.05$) in the level of creatinine and urea between the serum of passive and active smokers.

Conclusion: In the course of active and passive smoking, oxidative stress and renal dysfunction were clearly aggravated and the dynamic equilibrium between oxidation and antioxidation was seriously disrupted. This confirms the toxicological effects of smoking on human health.

Keywords: 1-Hydroxyl pyrene, carcinogen, creatinine, urea, oxidative stress, smoking

INTRODUCTION

PAHs are a class of toxic pollutants that have accumulated in the environment due to both natural and anthropogenic activities (Saraswathy *et al.*, 2002). They are composed of two or more benzene rings and occur, depending on the type of pyrolytic process and of source material, in various compounds, but always in the form of a mixture (ATSDR, 2013). They are mainly produced from incomplete combustion of organic materials, fossil fuels, petroleum product spillage and various industrial activities, and partly also from natural processes such as forest fires and volcanic eruptions (Stefen *et al.*, 2003). The main source of PAH intake is food, on the one hand as a result of airborne PAH precipitating onto cereals, fruit and vegetables, and on the other hand as a result of PAH generated during the preparation of food (Hansen *et al.*, 2008).

Some of these PAHs are known for their recalcitrance, persistence and bioaccumulation properties. They can be highly toxic, mutagenic and/or carcinogenic especially those with

47 high molecular weight like chrysene, pyrene and fluoranthene (Stefen et al., 2003). The
 48 ubiquitous occurrence of these carcinogens represents an obvious health risk and therefore
 49 public concern as to their fate and removal from the environment is on the increase. Pyrene
 50 is present in almost all PAH mixture in relatively high concentrations and there is a good
 51 correlation between pyrene and other components in PAH mixture (Tsai *et al.*, 2004).

52 A very important source of PAH exposure among the general population is tobacco smoke.
 53 Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, was proposed as a biological
 54 marker of exposure to PAHs in humans and their measurements have been useful for
 55 exposure monitoring (Jongeneelen, 2001). Urinary 1-OHP was widely used as biomarker of
 56 human exposure to PAHs. Many studies have investigated urinary 1-OHP in humans (Tsai et
 57 al., 2004), such as the dietary intake of PAHs and smoking (Jongeneelen, 2001). Tobacco
 58 smoking can be a more significant source of PAHs than urban exposures or road
 59 construction exposure. Cigarette smoke is a complex mixture of chemicals. Some smoke
 60 components such as carbon monoxide (CO), hydrogen cyanide (HCN), and nitrogen oxides,
 61 are gases. Others, such as formaldehyde, acrolein, benzene, and certain N-nitrosamines, are
 62 volatile chemicals contained in the liquid- vapor portion of the smoke aerosol. Still others,
 63 such as nicotine, phenol, polycyclic aromatic hydrocarbons (PAHs), and certain tobacco-
 64 specific nitrosamines (TSNAs), are contained in the submicron-sized solid particles that are
 65 suspended in cigarette smoke (Costagliola, *et al.*, 1989). In the developing world, tobacco
 66 poses a major challenge, not just to health, but also to social and economic development,
 67 and to environmental sustainability (WHO, 1998). Active cigarette smoking is considered to
 68 increase the risk of cardiovascular disease by promoting atherosclerosis; affecting
 69 endothelial cell functioning; increasing the tendency to thrombosis; and decreasing the
 70 oxygen-carrying capacity of the blood (U.S. Department of Health and Human Services,

71 1990). These same mechanisms have been considered to be relevant to second hand smoke
72 (SHS) exposure and risk for cardiovascular disorders (U.S. Department of Health and Human
73 Services, 1984).

74 Amongst the health hazards of smoking, carcinogenesis, cardiovascular disease and lung
75 disease have attracted considerable attention. In contrast, until recently the potential
76 impact of smoking on renal function and renal disease have remained largely noticed (Orth
77 *et al.*, 1997). Admittedly, the number of patients with smoking-related renal problems is
78 considerably smaller than the number of patients with smoking-related renal problems is
79 considerably smaller than the number of patients with malignancy, cardiovascular or
80 pulmonary problems, but in view of the high cost of renal replacement therapy the problem
81 is by no means trivial. Certainly the consequences for the health budget are quite
82 considerable (Grassi *et al.*, 1994).

83 There is also convincing evidence that smoking is one causal factor in the genesis of renal
84 cell carcinoma and uroepithelial cell carcinoma. In the current study, emphasis was placed
85 on the repercussions of smoking on renal function in subjects without renal disease.

86 There has been ongoing debate as to who between the passive and active smokers are more
87 exposed to health risk. Consequently, this work aims at studying the comparative levels of
88 toxicological markers such as 1 hydroxyl pyrene in urine which are biomarkers for polycyclic
89 aromatic hydrocarbon, serum antioxidant capacity and serum creatinine levels in smokers
90 and non-smokers. Smoking is a proven cause of coronary heart disease, chronic bronchitis
91 and emphysema, lung cancer.

92 **MATERIALS**

93 **CHEMICALS AND REAGENTS**

HPLC grade methanol, acetonitrile, β -glucuronidase, 1- hydroxypyrene standard enzyme were obtained from Sigma-Aldrich, Germany. Other reagents used included 1- hydroxypyrene standard (from Sigma-Aldrich, Germany) and β -glucuronidase enzyme (from Sigma-Aldrich, Germany). All the chemical and reagents were of highest purity possible. Other reagents used included creatinine picric acid reagent, and creatinine buffer (10 mM sodium borate, 240 mM sodium hydroxide and surfactant).

DEMOGRAPHIC QUESTIONNAIRE

The questionnaire was used to identify the socio-demographic such as age, smoker, life style habits and medical history of volunteers. Questionnaire was developed to assess the subject eligibility to involve in the study. It contains four questions. The first two were concerned with the participant biosocial characteristics, including age, height, weight, smoking and educational status. Question three was concerned with medical history and question four was concerned with their life-style habits. Each participant was interviewed and asked to fill the questionnaire. The participants who met the criteria and accepted to continue with the study were selected.

METHODS

EXPERIMENTAL SUBJECTS

The subjects of this study consisted of forty (40) motor park touts that smoke within/around Oshodi motor park around and forty (40) cigarette hawkers within Oshodi motor park. The non-smoking subjects were sampled from students of college of Medicine, University of Lagos, Idi-araba, Lagos State.. The subjects were divided into 3 groups; passive **smokers** (cigarette) (**n = 40**), active smokers (cigarette) (**n=40**) and **non-smokers** (**n = 40**). Subjects were eligible for the smoking sample study if they are smokers and/or are constantly

exposed to people who smoke. The criteria of the selection of active smoking subjects is that they smoke at least ten sticks of cigarette in a day while for passive smoking subjects, they are constantly exposed to a smoking environment. All male subjects included in the present study are the normal healthy subjects as their medical histories were ascertained from the completed questionnaires given to each of them respectively.

SAMPLE COLLECTION

The sample collection was done in two phases, including;

- Blood
- Urine

1. URINE SAMPLE COLLECTION

24 hour urine (50 to 80 mL) samples were obtained from active smoking, passive smoking and non-smoking volunteers. The samples were collected in sterile containers and kept at 5°C. The urine samples were collected in a 3 L specimen collection jar with volumes ranging from 1450 mL to 2600 mL. Extraction efficiency of the solid phase extraction (SPE) sorbent used prior to the extraction of 1-hydroxypyrene from urine samples, the extraction efficiency of C18 SPE cartridge with different solvents was determined. The different solvents used included methanol, acetonitrile, and a mixture of both. Urine analysis was done to determine the level of polycyclic aromatic hydrocarbon in each subject

2. BLOOD SAMPLE COLLECTION AND PREPARATION OF SERUM

Venous blood samples were taken from subjects for determination of serum levels of creatinine including the serum levels of antioxidant/lipid peroxidation enzymes Glutathione Reductase (**GSH**), Superoxide Dismutase (**SOD**), Catalase (**CAT**) and Malondialdehyde (**MDA**). Blood samples were collected by pulling up the sleeves of subjects so the phlebotomist

could find a visible vein. Mostly, a vein on the inside of the subjects elbows were used and blood was collected by inserting a needle into their veins to collect the blood. After the removal of the needle, the site of blood draw was put some cotton wool soaked in methylated spirit. Blood were collected into heparinized tubes and centrifuged at 3000 r.p.m for collection of serum for creatinine and natural antioxidant analyses.

ANALYTICAL METHODS

1.DETERMINATION OF 1-HYDROXYLPYRENE IN URINE

✓ PREPARATION OF β -GLUCURONIDASE

36.8 mg of β -glucuronidase enzyme was dissolved in 68.00ml of distilled water. Following this, 1 mL (2000 units of β -glucuronidase) was added to each of the 30 samples of urine collected from both smokers and non-smokers, and these were incubated overnight.

✓ β -GLUCURONIDASE HYDROLYSIS URINE SAMPLES

The urine samples were analyzed with high performance liquid chromatography (HPLC) using 1-Hydroxypyrene as standard. Measured into 68 clean test-tubes was 1 mL of each of the urine samples. Added to each of the samples was 1 mL of beta Glucuronidase (2000 units) in order to hydrolyze the conjugated 1-hydroxypyrene. Samples were stored in a freezer at - 20°C in opaque plastic containers and prepared for analysis by slowly thawing, stirring, and delivering a 55 ml aliquot to a pyrex Erlenmeyer flask. Urine samples were adjusted to pH 5-0 with concentrated hydrochloric acid and buffered with 5 ml of acetate buffer, pH 5-0.

The resulting mixtures were incubated overnight (for about 15 hours) at 38 °C. The incubated samples were thoroughly shaken to remix the precipitate with the clear solution making them homogenous solutions. The C18 SPE cartridges were activated with 2 mL of

methanol to condition and open up the SPE pores. The cartridges were washed with 2 mL of water to allow for easy passage of liquid denser than methanol through the SPE pores. Next, 1 mL of the homogenized incubated urine samples were transferred to the SPE sorbents. The sorbents were washed with 2 mL of water to remove water-soluble compounds from the sample matrix. The SPE cartridges were then desorbed with 2 mL of methanol. The eluates were made up to 2 mL each with the eluting solvent. Methanol solution was evaporated down to 2.5 mL in a 60°C water bath with air gently blown over the sample. The concentrated solution was passed through a 0.20 µm 25 mm syringe filter into an autosampler 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. A Waters' 600E multi-solvent delivery system equipped with a 712 WISP autosampler, and a MAXIMA software computer interface was used for data acquisition. The system delivered a methanol: water linear gradient (0-8 mL/min) starting at 46:54 for 5 minutes to 94:6 over 35 minutes holding for 10 minutes. Methanol and water reservoirs were purged with helium before and during sample runs. A C18 reverse phase 150 x 4mm column with 5 µm packing equipped with a guard column (Separations Group, Hesperia, CA) was maintained at 40°C in a column oven. A Perkin Elmer 650S fluorescence detector with a flow through microcuvette was used to detect 1-OHPY. The fluorimeter was set for an excitation wavelength of 242 nm and an emission of 388 nm with both slits adjusted to 5 nm. Peak area was used for quantification. A volume of 30 µL was injected on to the column. 1-Hydroxypyrene standards were prepared from material reported to be 99% pure by HPLC and ultraviolet analysis (Chemsyn Science Laboratories) in methanol at concentrations of 0.538, 1.07, 3.23, 6.46, 10.78, and 16.93 ng/mL. Only low energy red lights were used to weigh and prepare standards,

which were then stored refrigerated in amber glass. A linear calibration curve ($r^2 > 0.99$) was obtained and the intercept was not significantly different from zero ($p = 0.05$). A limit of detection of 0.023 ng/ml was established. A recovery study was conducted at four concentrations on duplicate spiked samples. A large urine sample composite was mixed and split into nine 55 ml sample aliquots. One ml of standard solution at concentrations of 0, 3, 23, 64, and 1078 ng/ml was added to each sample. The first three were prepared in duplicate and the fourth was prepared in triplicate. Recoveries for the concentrations greater than zero were 100.5 (SD 24.1%), 94.6 (SD 2.2%), and 93.7 (SD 2.5%) respectively, which exceeded the average recovery of 85% reported by Jongeneelen *et al.*¹⁹ A test was conducted to ensure that the 1- β -glucuronidase was being added to the sample in excess. A urine sample previously determined to contain high concentrations of 1-OHPY was treated with varying amounts of enzyme. For samples with > 6.3 mg of enzyme there was no difference in the 1-OHPY concentration.

HPLC analysis was carried out on an Agilent 1100 HPLC system. The mobile phase was water: methanol (12:88 v/v). The stationary phase was an XBridge C18 (150 $\times$ 4.6 mm) 5 μ m column. The wavelength was 250 nm at a flow rate of 1.2 mL/min. The temperature of the column was 30 $^{\circ}$ C. The injection volume was 20 μ L, and the run time was 3 minutes.

2. KIDNEY BIOMARKER DETERMINATION

✓ CREATININE DETERMINATION

The method used was as described by Fabiny and Ertingshausen, 1971. The reagent solution consists of R1; sodium hydroxide (0.20 mol/L) and R2; picric acid (25 mmol/L).

Creatinine + picric acid $\xrightarrow{\text{alkaline pH}}$ yellow-orange complex

212 In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye
213 formation is proportional to the creatinine concentration in the specimen. The assay uses
214 rate-blanking to minimize interference by bilirubin. To correct for non-specific reaction
215 caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones,
216 the results for serum or plasma are corrected by $-26 \mu\text{mol/L}$ (-0.3 mg/dl).

217 ✓ UREA DETERMINATION

218 A simple spectrophotometric method for the determination of urea in blood and urine by
219 With TK et al.,1961 was used for the serum urea determination in experimental subjects.
220 Twenty-four milliliters of reagent Crystalline zinc sulphate, 12.5 g., dissolved in 125 ml.0.25N
221 sulphuric acid and diluted to 1,000 ml with distilled water are run from a burette into a 100
222 ml Erlenmeyer bottle. To this is added 3ml.whole blood (stabilized by oxalate), the bottle is
223 shaken to obtain complete haemolysis, and 3ml of reagent 0.75N Sodium Hydroxide added
224 and the mixture shaken again. It is then Filtered through a special filter (Schleicher and
225 Schull No.5893, Blauband) to remove the precipitated proteins.(It is important to use a
226 suitable filter paper because the usual laboratory filters may give falsely increased values.)
227 Of the clear and colourless filtrate 10 ml is pipette into a 25 ml measuring flask and 10 ml p-
228 Dimethylamino-benzaldehyde (analytic), 20g, dissolved into 1,000ml 96% alcohol. To this
229 solution is added 100 ml. concentrated hydrochloric acid (analytic)and mixed reagent-
230 urea,100mg.,is dissolved in distilled water,100 ml. in a measuring flask added. After mixing
231 dilute with distilled water to the mark and renew mixing. After 10 minutes the mixture is
232 ready for reading. The colour is stable, according to Watt and Chrisp, for 11 days.

233 The reactions are read in a spectrophotometer at 420 nm with a blank prepared in the same
234 way as the reaction mixture but with 3 ml. of distilled water instead of 3 ml. of blood. The

235 urea standard (100mg of urea dissolved in 100ml of distilled water in a measuring flask) is
 236 treated in the same way as the blood. Cuvettes of 1 cm. thickness are suitable. If p, s, and b
 237 are the extinctions of the sample, the standard, and the blank respectively, the urea
 238 concentration is $100(p-b)/(s-b)\text{mg./100ml.}$

239 **3.DETERMINATION OF ANTIOXIDANT STATUS**

240 **✓ DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY**

241 Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of
 242 epinephrine determined by the increase in absorbance at 480nm as described by Sun and
 243 Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate
 244 buffer pH 10.2, 0.02 ml of the tissue homogenate and 0.03 ml of epinephrine in 0.005 N HCL
 245 was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of
 246 substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring
 247 the change in absorbance at 480 nm for 5 min. $\Sigma = 4020\text{m}^{-1}\text{cm}^{-1}$

248 **✓ CATALASE ACTIVITY DETERMINATION**

249 Catalase activity was determined according to Luck (1971). It was assayed colorimetrically at
 250 620nm and expressed as $\mu\text{moles of H}_2\text{O}_2\text{ consumed/min/mg protein at } 25^\circ\text{C.}$ The reaction
 251 mixture (1.5ml) contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of tissue
 252 homogenate and 0.4ml of 2M H_2O_2 . The reaction was stopped by the addition of 2.0ml of
 253 dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were
 254 mixed in 1:3 ratio). $\Sigma = 40\text{m}^{-1}\text{cm}^{-1}$

255 **✓ REDUCED GLUTATHIONE DETERMINATION**

256 The reduced glutathione (GSH) content of liver tissue as non-protein sulphydryls was
 257 estimated according to the method described by Sedlak and Lindsay (1968). To the

homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm. $\Sigma = 1.34 \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}$

✓ DETERMINATION OF LIPID PEROXIDATION

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of $1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$.

STATISTICAL ANALYSIS

All results were expressed as mean $\pm$ standard error of measurement (SEM) and analyzed statistically using Analysis of Variance (ANOVA) with Prism GraphPad statistical software (version 6.0). Differences at $P < 0.05$ were considered to be significant.

RESULTS

TABLE 1: DEMOGRAPHIC DATA BETWEEN THE GROUPS (SMOKERS AND NON SMOKERS)

PARAMETERS	NONSMOKERS	PASSIVE SMOKERS	ACTIVE SMOKERS
Sex	Male	Male	Male
Age (yr)	27.00± 1.20 ^a	32.00 ± 0.70 ^b	25.00 ± 0.50 ^a
Body weight (kg)	73.00± 2.40 ^a	71.00± 3.20 ^a	62.00± 1.70 ^b
Body height (m)	1.72.00± 0.20 ^a	1.75.00± 0.80 ^b	1.77.00± 0.50 ^b

Results represented as mean±S.E.M, n=40

Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 2: The concentration of 1-Hydroxyl Pyrene in urine of smokers (active and passive) and non-smokers

	NON SMOKERS	PASSIVE SMOKERS	ACTIVE SMOKERS
CONCENTRATION OF 1-HYDROXYL PYRENE (µg/ml)	50.04 + 7.382 ^a	115.10 + 22.84 ^b	118.10 + 14.38 ^b

Results represented as mean±S.E.M, n=40

Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 2 shows the concentration of 1-Hydroxyl Pyrene in urine of smokers (active and passive) and non-smokers. There was a significant increase ($P<0.05$) in the concentration of 1-Hydroxyl Pyrene in urine of passive and active smokers compared to non-smokers but there was no significant difference ($P>0.05$) between the concentration of 1-Hydroxyl Pyrene in urine of active smokers and passive smokers (Table 2).

Table 3: Serum antioxidant activity of smokers (active and passive) and non-smokers

SERUM ANTIOXIDANT ACTIVITY	NON SMOKERS	PASSIVE SMOKERS	ACTIVE SMOKERS
CATALASE ($\mu\text{mol/ml}$)	480.80 $\pm$ 10.49 ^a	175.10 $\pm$ 22.84 ^b	177.1 $\pm$ 15.29 ^b
GLUTATHIONE REDUCTASE ($\mu\text{mol/ml}$)	60.23 $\pm$ 2.12 ^a	38.32 $\pm$ 1.55 ^b	39.90 $\pm$ 1.07 ^b
SUPEROXIDE DISMUTASE ($\mu\text{mol/ml}$)	109.39 $\pm$ 2.34 ^a	77.85 $\pm$ 1.88 ^b	79.08 $\pm$ 4.23 ^b
MALONDIALDEHYDE ($\mu\text{mol/ml}$)	0.45 $\pm$ 0.06 ^a	1.01 $\pm$ 0.02 ^b	1.06 $\pm$ 0.07 ^b

Results represented as mean $\pm$ S.E.M, n=40

Values with the same superscripts across the same row are not significantly different ($P>0.05$)

Table 3 shows the serum antioxidant activity of smokers (active and passive) and non-smokers. There was a significant decrease ($P<0.05$) in the level of oxidative stress markers; catalase, glutathione reductase, superoxide dismutase respectively and a significant increase ($P<0.05$) in the level of lipid peroxidation marker; malondialdehyde in the serum of passive and active smokers compared to non-smokers. However, there was no significant difference ($P>0.05$) in the level of oxidative stress markers; catalase, glutathione reductase, superoxide dismutase respectively and lipid peroxidation marker; malondialdehyde between the serum of passive and active smokers (Table 3).

Table 4: Serum (renal activity) of smokers (active and passive) and non-smokers

SERUM ANTIOXIDANT ACTIVITY	NON SMOKERS	PASSIVE SMOKERS	ACTIVE SMOKERS
CREATININE ($\mu\text{mol/ml}$)	72.54 $\pm$ 2.20 ^a	102.7 $\pm$ 1.59 ^b	101.08 $\pm$ 3.08 ^b
UREA($\mu\text{mol/ml}$)	10.06 $\pm$ 0.50 ^a	25.01 $\pm$ 0.5 ^b	27.03 $\pm$ 0.5 ^b

Results represented as mean $\pm$ S.E.M, n=40

Values with the same superscripts across the same row are not significantly different ($P>0.05$)

Table 4 shows the serum renal activity of smokers (active and passive) and non-smokers. There was a significant increase ($P<0.05$) in the levels of creatinine and urea in the serum of passive and active smokers compared to non-smokers. However, there was no significant difference ($P>0.05$) in the level of creatinine and urea between the serum of passive and active smokers (Table 4).

Results represented as mean $\pm$ S.E.M

*statistically significant at $p<0.05$ compared with the control, $n=20$

DISCUSSION

Mainstream and side-stream smoke are qualitatively similar in chemical composition, but the quantities of constituents are different, due to differences in the heat of combustion, tobacco content, additives present, and the type of filter material used (Kawachi *et al.*, 1997). Cigarette smoking has been implicated in the pathogenesis of many diseases including coronary heart disease, pulmonary disease, and neoplastic disorders (Block *et al.*, 2002). Besides active cigarette smokers, passive smokers are also exposed to harmful effects of tobacco (Glantz and Parmley, 2001). The adverse action of the cigarette smoke is due to the presence of a large variety of compounds that could initiate or amplify oxidative damage (Yildiz *et al.*, 2002). In this study, the effect of PAHs exposure (from tobacco smoke) on biomarkers of oxidative stress, urinary concentration of hydroxyl 1 pyrene and serum creatinine concentration were investigated.

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds consisting of two or more fused benzene rings and/or pentacyclic molecules arranged in various structural configurations. They are highly recalcitrant molecules persisting in the environment due to their hydrophobicity nature (Cerniglia, 1992). There are a variety of important sources for the uptake of PAH in non-occupationally exposed persons, including tobacco smoke, diet, ambient air, and coal-tar-containing medications (Hattemer-Frey *et al.*, 1991).

336 In the current study, there was a significant increase ($P<0.05$) in the concentration of 1-
 337 Hydroxyl Pyrene in urine of passive and active smokers compared to non-smokers but there
 338 was no significant difference ($P>0.05$) between the concentration of 1-Hydroxyl Pyrene in
 339 urine of active smokers and passive smokers. In the present study, the smokers (passive and
 340 active) seemed to have been exposed to a significant high level of PAHs more than the non-
 341 smokers based on the urinary 1-OH Pyrene. Other studies indicated that the smoking of
 342 cigarettes significantly increase the urinary 1-OHP concentration, while alcohol consumption
 343 does not affect the urinary 1-OHP concentration (Hattemer-Frey *et al.*, 1991). Hence, the
 344 smoking life style significantly affected the urinary 1-OHP concentration in this study.

345 The health risks associated with exposure to PAHs in tobacco smokers is the consequence of
 346 the disturbance in the antioxidants defense system, resulting in significant oxidative stress
 347 (Singh *et al.*, 2008), which is one of the important mechanisms behind the initiation of
 348 cancer (Garcon *et al.*, 2001). Large amount of reactive oxygen species and many
 349 electrophiles are generated during the activation of PAHs by CYP450, which bind covalently
 350 with DNA, and disturb the homeostasis of the cell (Cavalieri *et al.*, 1995). Low antioxidants
 351 activity in exposed subject show that they suffer from oxidative stress, because PAHs are
 352 known for their potential to cause oxidative stress (Bae *et al.*, 2010). B(a)P is a carcinogenic
 353 PAHs, it can affect catalase (CAT) (Kim and Lee, 1997) and GSH activities in humans (Emre *et*
 354 *al.*, 2007). A single molecule of CAT is capable of converting up to 6 billion molecules of H_2O_2
 355 into water and molecular oxygen (Halliwell and Gutteridge, 2007). According to the present
 356 study, There was a significant decrease ($P<0.05$) in the level of oxidative stress markers;
 357 catalase, glutathione reductase, superoxide dismutase respectively and a significant
 358 increase ($P<0.05$) in the level of lipid peroxidation marker; malondialdehyde in the serum of
 359 passive and active smokers compared to non-smokers. However, there was no significant

360 difference ($P>0.05$) in the level of oxidative stress markers; catalase, glutathione reductase,
 361 superoxide dismutase respectively and lipid peroxidation marker; malondialdehyde
 362 between the serum of passive and active smokers. This effect of PAHs exposure on oxidative
 363 stress of tobacco smokers was similar with that conducted by Nagler and Reznick, 2004
 364 where the comparison of antioxidant in smokers and non-smokers was carried out.

365 Cigarette smoke inhaled with normal respiration may impair the oxidant/antioxidant
 366 balance in the organism and cause the generation of free radicals (Pryor *et al.*, 1993). Free
 367 radicals in cigarette smoke or stimulated by smoking cause oxidative damage and may
 368 initiate the breakdown of lipids contained in the cell membrane (Akkuş *et al.*, 1995).

369 Malondialdehyde (MDA), as the final product of lipid peroxidation, is a chemically active
 370 molecule and may have harmful effects, particularly on proteins at the molecular level, by
 371 being diffused easily in surrounding cells and tissues (Yılmaz *et al.*, 2005). It was reported
 372 that MDA levels measured in blood samples taken from rabbits exposed to cigarette smoke
 373 (for studying the effects of smoking on MDA levels) were higher than in the control group;
 374 smoking accelerates the production of free radicals and causes oxidative cell damage
 375 (Özaras *et al.*, 1998). It was also reported that the quantity of the smoking causes oxidative
 376 damage in smokers and leads to an increase in the MDA level, and thus increases the risk of
 377 coronary artery disease (Guentsch *et al.*, 2008).

378 Creatinine, generated from muscle metabolism is produced from creatine, a molecule of
 379 major importance for energy production in muscles (Yu, 2005). Approximately 2% of the
 380 body's creatine is converted to creatinine every day. In the current study, there was a
 381 significant increase in the concentration of creatinine in serum of passive and active
 382 smokers compared to non-smokers. Likewise, there was a slight increase in the serum
 383 concentration of creatinine in passive smokers compared to the active ones. It was also

discovered that in this study, there was a significant increase ($P < 0.05$) in the levels of creatinine in the serum of passive and active smokers compared to non-smokers. However, there was no significant difference ($P > 0.05$) in the level of creatinine between the serum of passive and active smokers. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired due to tobacco smoke, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys (Yu, 2005). The increased blood level of creatinine can lead to uncontrolled or poorly controlled high blood pressure as a result of reduced flow of blood to the kidney. This can confirm that all of the subjects studied are without any renal failure, since the determination of creatinine has been reported to be useful in evaluating the renal handling of uric acid and as concentrations of this parameter are highly dependent on endogenous production as well as on renal excretion. Some of the relationships between tobacco and urea are very significant; however, they are all very weak. If these relationships have the same origin, a hypothetical renal mechanism must first be considered. In fact, the blood urea is a product of the catabolism of proteins and their amino acids. Moreover, urea while circulating in the blood, remain unlinked, either directly or by a common carrier. On the contrary, it is excreted by the kidney, and in the disease processes, it generally varies in the same way: a rise in blood urea is well known as an early sign of renal failure. An increase in the renal excretion of urea under the influence of tobacco is, therefore, a reasonable hypothesis, and it is supported by the known action of nicotine on the metabolism of catecholamines and the effect of these substances on renal function (Watts, 1960).

CONCLUSION

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408 It could be established from this study that passive and active smokers are at great health
409 risk. It is even more worrisome going by the results of this study which signified that the
410 passive smokers are more predisposable to increased health risks than the active smokers.
411 Therefore, it emphasizes that smoking outdoors where people are present should be
412 prohibited and it is required that smoking shall be avoided in any environment where
413 people younger than 18 years are present. Laws regulating the environments for smoking
414 should be considered again and required regulations should be applied so as to curb the
415 increasing rate of smoking associated disorders.

RECOMMENDATION

416

417 The impact of this study is of significance in the sensitization of populace on the inherent
418 danger in the health of both passive and active smokers. Government and relevant health
419 agencies are encouraged to enact stricter laws on enforcement of ban on outdoor smoking.
420 The present laws on outdoor smoking seem to have been lenient and not being enforced as
421 evidence by the flagrant abuse of the law in different public places.
422 Furthermore, more enlightenment programmes are needed using several health agencies
423 on the associated health risks with smoking. The use of print and visual media including
424 billboard campaigns is veritable tools to use in this aspect.

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