Sub-chronic passive cigarette smoke exposures suppressed phagocytic functions and precipitated inflammatory responses in male wistar rats

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ABSTRACT

Recent evidences indicate close interplay between the exposures to passive cigarette smoke and overt signs of many degenerative diseases, such as cardiovascular diseases and cancer, etc. However, it is still enigmatic to prove scientifically the role of passive cigarette smoke in modulating the activities of immune cells in the pathology of such degenerative diseases. To investigate the effect of passive cigarette smoke on immunoactivities, a smoke chamber was fabricated and eighty male Wistar rats were picked into four groups and handled such that groups A, B, C and D received distilled water, passive smoke, passive smoke + nosbrite and nosbrite respectively for 56 days. Parameters such as haematological analyses, reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase, uric acid, ascorbic acid, protein carbonyl, malondialdehyde, protein thiol, nitric oxide, prealbumin, cortisol, carbon clearance, zinc sulphate turbidity, neutrophil adhesion, interleukin-6, C-reactive protein, tumor necrosis factor-alpha and paraoxonase and oxidized low density lipoprotein-cholesterol were determined. Exposure to passive cigarette smoke inhibited the appetite of the rats and decreased (p<0.05) the body weights of the animals, except in the nosbrite of only administered rats (p<0.05). Passive cigarette smoke offsets the oxidant-antioxidant activities, enhancing oxidative activities (p<0.05) and the administration of nosbrite did not ameliorate the oxidative status. Consequentially, chronic inflammatory responses were induced in the rats with increases (p<0.05) in the circulating levels of leukocytes, but reduced phagocytic capabilities (p<0.05). The reported chronic inflammatory responses were manifested further in microcytic hypochromic anaemia. The foregoing indicated that the sub-chronic exposure to passive cigarette smoke induced inflammatory responses, initiated immunosecretion and inhibited the systemic phagocytic activities which could be the hallmark of various degenerative diseases manifested in cigarette smokers.

Key words: Passive cigarette smoke, nosbrite, inflammatory responses, leukocytes, phagocytic activities, degenerative diseases.

INTRODUCTION

The history of smoking was dated far back to 5000 BC in many different cultures worldwide. As of 2002, a pivot survey reported that approximately 5.5 trillion cigarettes were produced globally every year that are smoked by over 1 billion people or greater than one-seventh of the overall world population (WHO, 2005). Recently, however, the occurrences of smoking recorded reductions or leveled off in some developed nations, such as the United States of America, Canada and United Kingdom etc, but escalations were recorded in many developing nations.
Smoking of cigarettes has formed one of the most common recreational drugs in the world, which is next to alcohol (West et al., 2007). The primary route of administration is by inhalation, in which the combustion releases nicotine for absorption through the lungs. The bulk of the smoke drawn from the burning cigarette is exhaled or puffed out by the smoker (mainstream smoke) through the nose and mouth and sometimes through the ears. Meanwhile, the bulk of the cigarette smoke escapes into the atmosphere during the smoke drawn cycles (sidestream smoke), which in combination with the mainstream smoke formed the passive smoke that are often inhaled by people in the surroundings.

Passive smoke is thus an involuntary act of smoking that have been shown by independent clinical studies to contain the same levels of nicotine and other toxic chemicals recorded in the main smoke. In fact, passive smoke has been shown to contain more toxins due to the increased levels of smaller molecular weight 'particulate matters' generated at lower temperatures during cigarette burning (Zhang, 2005). Therefore, the act of smoking in public places had been prohibited by legislation in many nations. However, the prohibition is limited at recreation centers, clubhouses, parks, gardens and tenement houses etc.

In Nigeria, smokers are of the habits of licking lozenges such as peppermint, tom-tom and nosbrite etc for the soothing effect, masking off the cigarette smoke and for the freshness of breathe. Although, it is known that non-cigarette smokers also do indulge in the use of some of the lozenges habitually, but the use is trending more among cigarette smoking folks. Nosbrite lozenge is a small tablet that dissolves slowly in saliva to soothe and lubricate the throat and provide a feel of oral freshness to smokers. It is alleged to prevent or alleviate cough and various cancers, such as oral, prostate, lung and breast cancer.

It is plausible that interplay exists between the exposures to passive cigarette smoke and the activities of immune cells, which could be implicated in the overt signs of degenerative diseases, such as cardiovascular diseases, inflammatory disorders and cancer. However, there is limited scientific information on the effect of passive cigarette smoke on immune functions in the pathology of the inflammatory disorders and diseases and cancer. Therefore, this work aimed to elucidate the effect(s) of sub-chronic exposure to passive cigarette smoke and nosbrite on the functionality of the immune cells in mediating inflammatory responses in male Wistar rats.

**MATERIALS AND METHODS**

**Materials**

*Experimental animals*

Eighty male Wistar rats that weighed between 117 and 132 g were purchased from the animal care facility, Ladoke Akintola University of Technology, Osogbo, Nigeria.

*Cigarette*

Benson and Hedges cigarette was a product of products of Philip Morris International, a subsidiary of British-American Tobacco Company, 1 Tobacco Road Oluyole, Ibadan, Oyo State, Nigeria.

*Lozenge*

Nosbrite was a product of Nosak Pharmaceutical Company, 2 Ramat Cresent Ogudu, G.R.A, Lagos State, Nigeria.

*Reagent kits*

Quantitative assay kits for uric acid, superoxide dismutase, glutathione peroxidase, catalase and pre-albumin were products of Fortress Diagnostic Laboratory, unit 2c, Antrim Technology Park, AntrimBT41, United Kingdom. The assay kits for interleukin 6, tumour necrosis factor-α and C-reactive protein were products of RayBio Technology, Incorporated, USA. Oxidized low density lipoprotein-cholesterol was a product of Cusabio Biotech Company Limited and paroxygenase was produced by Cloud-Clone Corp -Usan Life Sciences Incorporated, 11271 Richmond Avenue, Suite H104, Houston, TX77082, USA.

*Carbon ink suspension*

The carbon ink suspension was a product of Pelica AG, Germany.

*Carbon monoxide analyser*

Handheld carbon monoxide analyzer with model number 1205A was a product of Dwyer Instruments International.

*Drugs and chemicals*

All other chemicals and reagents were of analytical grade, either a product of the British Drug House (BDH) Poole England, or Sigma Aldrich, Wisconsin, U.S.A.

**Methods**

*The smoke chamber*

An enclosed box was constructed using a hard paper board.
and flat head pins to obtain a dimension of 20 by 60 inches. The box was divided into two compartments (the cigarette burning chamber and the passive smoke chamber) with the dividing paper board carefully perforated. Provisions were made for cross ventilations of 3 by 5 inches on each chamber that were well secured with metal wires. An opening was made on each compartment of the box to serve as the door with dimension of 6 by 6 inches. In the cigarette burning chamber, a small electric fan was fixed to help circulate the smoke from the burning cigarette into the passive smoke chamber.

Animal grouping, handling and administration of passive smoke and nosbrite

The eighty male rats were kept in wooden cages and allotted into four groups of twenty rats each. They were allowed access to standard feed and tap water ad libitum and acclimatized for 10 days. The rats were kept under condition of uniform humidity and temperature on a 12 h light-dark cycle and the cages cleaned daily, during which, the rats were monitored closely for any changes in their behaviour and activities. The handling was done such that:

- Group A received distilled water;
- Group B was exposed to passive smoke;
- Group C was exposed to passive smoke and nosbrite;
- Group D received nosbrite.

Exposure to the cigarette smoke was done by mimicking the model passive smoke exposure in which the rats were placed in the smoking chamber of the box five minutes prior to the burning of the cigarette sticks. The box was placed such that the burning chamber always faced the direction of the wind (east). Exposure to the passive smoke was done at 16:30 h ± 45 min GMT. A cigarette stick burnt completely to the gold ring in an average of 10 min. Three cigarette sticks were burnt per day at an interval of 10 min in between. A hand held carbon monoxide analyzer was always placed in the smoking chamber to quantify the particulate of CO₂ during exposures to the passive smoke.

The dose of nosbrite administered was calculated assuming the use of two tablets daily by a 60 kg adult. The tablet was dissolved in water and administered to the rats orally using a metal cannular attached to a 1.0 ml syringe at 18:30 h ± 30 min GMT. Administrations of the passive smoke and nosbrite lasted for 56 days during which the rats were observed closely for any behavioural and morphological changes and the feed, water intake and body weights were recorded. Four rats from each group were fasted overnight and sacrificed by mild anaesthesia with diethyl ether after 28 and 56 days respectively. The chest region was quickly opened and blood was drawn by puncturing the heart using a syringe. Plasma and serum were prepared from the blood, labelled and refrigerated immediately. The organs of interest, such as the livers, heart, kidney and brain were harvested, cleansed of blood and rinsed in normal saline solution and weighed, respectively. The organs were minced and stored in physiological buffer and kept frozen or in fixing solution as appropriate. Three rats in each group were used respectively for carbon clearance and neutrophil adhesion tests at 28 and 56 days, following standard documented methods.

This research was conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH, 1985).

Bioassays

The concentrations of various biological parameters were determined by the methods, such as reduced glutathione (Sedlak and Lindsay, 1968), glutathione peroxidase (Thomas et al., 1990), superoxide dismutase (Arthur and Boyne, 1985), catalase (Sinha, 1971), uric acid (Kim et al., 1971), ascorbic acid (Omaye et al., 1979), protein carbonyl (Levine et al., 1994), malondialdehyde (Varshney and Kale, 1990), protein thiol (Sedlak and Lindsay, 1968), nitric oxide (Green et al., 1982), prealbumin (Guder et al., 1996), cortisol (Clark, 1955), carbon clearance test (Gokhale et al., 2003), zinc sulphate turbidity test (Pfeiffer et al., 1977), neutrophil adhesion test (Fulzele et al., 2003), interleukin-6, C-reactive protein, tumor necrosis factor-alpha, paraoxonase and oxidized low density lipoprotein-cholesterol (Sandwich ELISA as contained on the instruction manuals in RAYbiotech, Cloud-Clone Corp-Uscn Life Sciences Incorporated and Cusabio Biotech reagent kits, respectively).

Hematological parameters

The blood parameters were analyzed using the Haematology Autoanalyzer (Sysmex KX - 2IN) for the differential counts (Ike et al., 2010).

Statistical analysis

The design of this research study was a Completely Randomised Design (CRD) and the results were expressed as mean ± standard error of mean (S.E.M.) of at least three determinations. The results were subjected to ANOVA at p<0.05 and the Duncan Multiple Range Test utilized to identify the variation(s) within treatment groups and also at p<0.05.

RESULTS

Appearance, behaviour and mortality

The rats that were exposed to the passive smoke had the
scrotal sac enlarged and reddish in colour and the eyeballs were also bugged and reddish. After 19 days of exposure to the smoke, the rats were very alert, aggressive and less tolerant, inciting bite wounds on one another.

**Feed and water intake and body weight**

The exposures of the rats to passive cigarette smoke resulted in a decrease (p<0.05) in the feed intake (Table 1). However, the groups of rats that received nosbrite (groups C and D) indicated significant increases (p<0.05) in the feed intake that were not consistent in the smoke and nosbrite administered rats (group C). The pattern obtained in the water intake indicated no variation (p>0.05) in the water consumptions in all the rats (Table 1). In Figure 1, the patterns obtained in the body weights of the rats depicted significant increases (p<0.05) in the rats administered with nosbrite after 21 days of administration till the 56th day (groups C and D), while the exposures to only passive smoke gave decreases (p<0.05) in the body weight of the rats, respectively.

**Immonomodulatory indices**

**Oxidative stress indices**

Table 2 shows the activities of glutathione peroxidase in the liver and plasma were reduced (p<0.05) in the group of rats administered the passive smoke and nosbrite (groups B to D), which were time dependent in the liver in smoke exposed rats. Superoxide dismutase activities in liver and plasma decreased (p<0.05) in the smoke exposed rats, but not time dependently, while catalase activities were only altered in the liver (p<0.05), except on the 56th day in the nosbrite only administered rats. However, catalase activities and ascorbic acid levels were not altered (p>0.05) in the serum in all the rats (Table 2).

In the liver, the concentration of reduced glutathione was decreased (p<0.05) in the rats administered the smoke and nosbrite in a time dependent course. The exposure of the rats to passive smoke recorded increases (p<0.05) in the serum uric acid and nitric oxide concentrations (Table 2). Likewise, the administrations of the smoke and nosbrite presented increases (p<0.05) in the levels of protein carbonyl and malondialdehyde in the liver, brain and heart of rats that were not time dependent in the nosbrite of only administered rats. In the same vein, the concentrations of protein thiol decreased (p<0.05) in the respective tissues in rats exposed to passive smoke. Exposures to passive smoke gave increases (p<0.05) in the levels of oxidized low density lipoprotein cholesterol in serum and heart, but decreases (p<0.05) in the paraoxonase activities in the serum (Figures 2 and 3).

**Acute phase proteins, immunoglobulin levels and phagocytic indices**

The concentrations of acute phase proteins, such as
prealbumin and cortisol were increased (p<0.05) in a non time dependent manner in the rats exposed to smoke, while the C-reactive protein were increased (p<0.05) time dependently (Figures 4, 5 and 8). In the same manner, increases (p<0.05) were recorded in the serum concentrations of interleukin-6 and tumour necrosis factor-alpha in the smoke and nosbrite administered rats (Figures 6 and 7).
Table 2: The trends in the oxidative stress indices in Wistar rats administered nosbrite and smoke.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Control</th>
<th>Smoke</th>
<th>Smoke and Nosbrite</th>
<th>Nosbrite</th>
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<td></td>
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<td>(A)</td>
<td>(B)</td>
<td>(A)</td>
<td>(B)</td>
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<tr>
<td>Superoxide dismutase (U/ml)</td>
<td>Liver</td>
<td>2.66 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.38 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.72 ± 0.88&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.74 ± 0.61&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td></td>
<td>Brain</td>
<td>2.00 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.66 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.39 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Heart</td>
<td>1.43 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.90 ± 0.70&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.05 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Blood</td>
<td>1.89 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.64 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.04 ± 1.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.05 ± 0.59&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Liver</td>
<td>18.02 ± 1.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.50 ± 1.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.75 ± 1.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.82 ± 1.30&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Brain</td>
<td>10.74 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.12 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.29 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.21 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Plasma</td>
<td>12.34 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.88 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.06 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Liver</td>
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<td>44.96 ± 5.34&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>24.18 ± 1.80&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Brain</td>
<td>23.04 ± 1.82&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>14.28 ± 2.00&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Liver</td>
<td>42.55 ± 2.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.90 ± 2.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>Plasma</td>
<td>66.32 ± 4.08&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>60.05 ± 5.70&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Glutathione peroxidase (µmol/ml)</td>
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<td>9.12 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Reduced glutathione in liver (mg/ml)</td>
<td>Liver</td>
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<td>56.80 ± 5.30&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Catalase (U/ml)</td>
<td>Liver</td>
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<th>Heart</th>
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<td>(B)</td>
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<td>Protein thiol (µg/mg protein)</td>
<td>Brain</td>
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<td>(A)</td>
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<td>Protein carbonyl (nmol/mg protein)</td>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(A)</td>
<td>5.36 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.01 ± 0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.54 ± 0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.98 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>(B)</td>
<td>4.89 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.55 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.85 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.71 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Ascorbate (µg/mg protein)</td>
<td></td>
<td></td>
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<tr>
<td>(A)</td>
<td>108.54 ± 5.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.70 ± 7.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.05 ± 6.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.44 ± 8.14&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>(B)</td>
<td>130.40 ± 5.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>225.90 ± 8.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>198.55 ± 11.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>122.46 ± 7.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Nitric oxide (µmol/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(A)</td>
<td>60.46 ± 4.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118.81 ± 5.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.60 ± 4.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.72 ± 5.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<td>(B)</td>
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Values are means ± SEM; n=3-4. (Mean bearing different alphabets are significantly different (p<0.05). A= 28 days and B= 56 days.

The total immunoglobulin levels were reduced (p<0.05) in the groups of rats administered the passive smoke and nosbrite in a non time course pattern (Figure 11), while the phagocytic capabilities were reduced (p<0.05) in the smoke exposed rats in a non time course pattern (Figures 9 and 10).

**Hematological parameters**

Table 3 shows the concentrations of haemoglobin decreased (p<0.05) in the smoke and nosbrite administered rats non time dependent, while the erythrocytes, hematocrit and reticulocyte levels increased (p<0.05) only on the 56<sup>th</sup> day in the same groups of rat. Anaemia indices, such as mean corpuscular volume and mean corpuscular haemoglobin all increased (p<0.05) in the rats that were exposed to smoke in a non time course manner. In addition, the neutrophil and monocytes levels in the blood were reduced in a non time course pattern (p<0.05) in the groups of rats administered passive smoke and nosbrite. However, the platelets, lymphocytes and total leukocytes increased (p<0.05) in the same groups of rats and also, in a time non-dependent manner (Table 3).

**DISCUSSION**

The health risks associated with cigarette smoke have been a matter of scientific consensus. In fact, various concerns about the exposures to passive smoke have played a central role in many public debates over the impeding harms and regulation of...
the use of tobacco products, especially in public places (Kessler and Mckee, 2006). The patterns obtained in the feed intake of the male Wistar rats indicated a probable loss of appetite following exposures to passive cigarette smoke and stimulation of appetite in the nosbrite administered rats. This might be due to the effect of the total aerosol residue and nicotine which are a major component of cigarette smoke that could desensitize the gum and the taste bud, thereby inhibiting appetite (IARC, 1986; Orsini, 2001).
The exposures to passive smoke and nosbrite had no effect on the tissue hydration in the male Wistar rats. A close examination of the pattern presented in the body weight of the rats supported the trends in the feed intake (Figure 1). Phosphatidyl choline must have stimulated triacylglycerol biosynthesis in the tissues of the rats administered nosbrite. A probable increase in fat deposition in the tissues could lead to corresponding increases in weights (Ali et al., 2005).

The existence of all mammals requires oxygen for the vast metabolic activities that end up contributing to the pool of oxygen reactive e molecules kept in check by the complex network of antioxidant systems. An imbalance in the oxygen reactive molecules and the antioxidant system...
Table 3: Hematological indices in male rats exposed to passive smoke and nosbrite.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Smoke</th>
<th>Smoke and Nosbrite</th>
<th>Nosbrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>(A) 41.43 ± 2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 42.64 ± 1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 43.07 ± 2.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>(B) 42.17 ± 2.11&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Eyethrocyte (10&lt;sup&gt;12&lt;/sup&gt; L)</td>
<td>(A) 7.07 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 7.22 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 7.91 ± 0.37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>(B) 7.70 ± 0.76&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>(A) 13.30 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 13.08 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 13.92 ± 0.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>(B) 13.23 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Reticulocyte (10&lt;sup&gt;12&lt;/sup&gt; L)</td>
<td>(A) 2.57 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 2.72 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 3.55 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(B) 3.03 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>(A) 51.10 ± 1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 52.00 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 55.30 ± 1.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(B) 53.10 ± 1.45&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>(A) 16.37 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 17.17 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 17.97 ± 0.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>(B) 17.23 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>(A) 32.80 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 32.57 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 32.07 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(B) 32.27 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Platelets (10&lt;sup&gt;3&lt;/sup&gt; µl)</td>
<td>(A) 539.30 ± 18.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 506.00 ± 15.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 765.30 ± 19.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>(B) 581.00 ± 19.90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Leukocyte (10&lt;sup&gt;9&lt;/sup&gt; L)</td>
<td>(A) 6.33 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 6.51 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 7.70 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(B) 5.47 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Monocytes (%)</td>
<td>(A) 5.91 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 6.07 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 3.73 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(B) 4.86 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>(A) 60.60 ± 3.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 58.85 ± 4.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 70.85 ± 4.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(B) 53.70 ± 4.70&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Neutrophil (%)</td>
<td>(A) 30.65 ± 2.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(B) 33.76 ± 1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(A) 19.87 ± 2.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(B) 34.99 ± 2.18&lt;sup&gt;ab&lt;/sup&gt;</td>
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</table>

Values are means ± SEM; n=4. (Mean bearing different alphabets are significantly different (p<0.05). A= 28 days and B= 56 days.

is the hallmark of oxidative stress, which is a major contributory factor in inflammatory disorders and diseases and, or death (Oyewo et al., 2013). The result presented in the concentrations of reduced glutathione following exposures of the rats to passive smoke and nosbrite indicated a call up in the antioxidant reservoirs by activated neutrophils, macrophages and dendritic cells (Table 2).

Activation of the innate immune cells would increase \( \text{H}_2\text{O}_2 \), NO and NADPH requirements for ‘respiratory burst’ during apoptosis (Lorsbach et al., 1993; Clementi et al., 1998; Kelly and O’Neill, 2015). When the need for NADPH by the NADPH oxidase is overwhelming, then, the reduction of glutathione from the oxidized form might be impaired.

Glutathione in the reduced state is the required active form of continued antioxidant processes by the peroxidases and other reductive processes in the microsomes (Soon and Tan, 2002). A persistent decrease in reduced glutathione concentrations as reported in this study had been implicated in probable overt signs of a challenge in oxygen reactive molecules (pro-oxidant) production and mop up in the system. The patterns obtained in the glutathione peroxidase activities supported
the trends reported in the reduced glutathione concentration. It is plausible that the rate at which glutathione peroxidase was ‘called up to keep in check the pro-oxidants’ in the rats exposed to passive smoke and nosbrite were far beyond the glutathione replenishing process, which is mediated by the availability of reduced and oxidized glutathione.

The trends obtained in the activities of another set of endogenous antioxidant systems, such as superoxide dismutase and catalase in this study are very akin to those of the reduced glutathione and glutathione peroxidase, although, the nosbrite only administered rats gave a contrary pattern in the livers (Table 2). Cigarette smoke was reported to contain oxidants, such as volatile aldehydes, hydroperoxide and nicotine (nitrosamine formation, etc), which are indicated to be inducers of oxidative stress (Giuka et al., 2010). Superoxide dismutase performs a similar action like glutathione peroxidase, except that it requires catalase to complete the neutralization of the reactive oxygen species (ROS) unlike

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**Figure 7:** Concentration of tumor necrosis factor-α in the serum of rat exposed to passive smoke and nosbrite. (Values are means ± SEM; n=4 and mean values bearing different alphabets are significantly different (p<0.05).

**Figure 8:** Levels of C-reactive protein the plasma of rat exposed to passive smoke and nosbrite. (Values are means ± SEM; n=4 and mean values bearing different alphabets are significantly different (p<0.05).
in the glutathione peroxidase system. However, the trend in catalase activities in the plasma might not be physiologically too important. This is because human with genetic deficiencies in catalase or mice with knock out catalase genes were reported to suffer fewer health issues due to lack of catalase activities (Parsonage et al., 2005).

Products of peroxidative damages, such as malondialdehyde, protein thiol and carbonyl etc are vital markers of accumulated damage from reactive oxygen species. The result obtained in the plasma nitric oxide concentrations indicated that exposures to passive smoke triggered the induction of nitric oxide in the rats. The activation of macrophages, neutrophils and dendritic cells triggered the release of nitric oxide through the inducible nitric oxide synthase (iNOS). Excess nitric oxide would precipitate peroxynitrite formation and the most potent reactive oxygen species, which causes oxidation of lipopolysaccharide and protein and nitration reactions.
The oxidants in the inhaled passive smoke could have reacted further with the induced nitric oxide produced to form more peroxynitrite (ONOO\textsuperscript{2}), which is a disastrous oxidant than the predecessors.

In addition, NO is notorious in the shutdown of the electron transfer processes involving the Fe-S centers, such as cytochrome oxidase and NADH-Q dehydrogenase enzyme, thereby, inhibiting oxidative phosphorylation. The resultant of the foregoing is tissue hypoxia. Choudhary et al. (2016) implicated increases in nitric oxide production in the progression of vascular oxidative stress.

The result obtained in the concentrations of malondialdehyde in the liver, heart, brain and blood supported the indicated predisposition to oxidative stress by the exposures to passive cigarette smoke in the rats (Table 2). Increased formation of peroxidants, such as NO would have led to the oxidation of membrane lipids that were elucidated in the increases in the concentrations of a product of lipid peroxidation and malondialdehyde. The increases in malondialdehyde could lead to the formation of cross linkages with the disulphide bridges in enzymes, thereby inactivating the enzymes (Clementi et al., 1998; Choudhary et al., 2016).

Most antioxidant enzymes are known to be rich in disulphide bridges and the increases that might have occurred in peroxynitrite production in the rats exposed to passive cigarette smoke could be responsible for the decreases recorded in the antioxidant enzyme activities. The plausible mechanism of protein inactivation could have led to the trends obtained in the concentrations of protein thiol in the liver, brain and heart of the rats exposed to passive smoke (Table 2). This can not be farfetched, as the formation of thiol groups are the result of the sulphydryl groups. In addition, it could have led to the indiscriminate inactivation of various membrane bound enzymes, even ATPases due to the rich sulphydryl group components that might progress into critical cytotoxocities in the rats.

The patterns in the uric acid concentrations in the serum supported the predisposition to oxidative stress by the exposure to passive cigarette smoke (Table 2). Hyperuricemia was reported in individuals that consume diets rich in fructose (Nakagaw et al., 2005). Fructose is a constituent of cigarette, alleged by the manufacturers for the appealing smell during cigarette smoking and this could be responsible for the reported hyperuricemia in the passive smoke exposed rats. Hyperuricemia was reported to be tightly regulated with reduced glutathione concentration in the liver (Oyewo et al., 2013) and also to aggravate damage in the endothelial cells in various independent studies (Daniel et al., 2008). In the nosbrite administered rats, the reduction in serum uric acid concentration might be due to the inhibitory roles of the menthol on xanthine oxidase. George and Struthers (2008) accounted for the inhibitory roles of menthol in uric acid biosynthesis.

Paraoxonase is an important enzyme component in the high density lipoprotein cholesterol, whose primary activity is meant to discourage the oxidation of low density lipoprotein cholesterol (Litvinov et al., 2012). In Figure 2, the exposures of the rats to passive smoke indicated an increase in the call up of the paraoxonase enzyme, the
inhibition of the biosynthesis of the enzyme in situ and or an interference with the metabolism of high density lipoprotein cholesterol. The recorded trend in the paraoxanase activity was responsible for the patterns obtained in the concentrations of oxidized low density lipoprotein cholesterol in the heart and plasma in rats exposed to passive cigarette smoke (Figure 3). This is not surprising as a decrease in the activity of paraoxanase would increase the chances of pro-oxidation of low density lipoprotein cholesterol, which is the hallmark of the formation of 'atheroma' in the endothelial cells (Kurban and Mehmetoglu, 2010). This further strengthened the indicated pro-oxidative capabilities of the particulate matters in the inhaled passive cigarette smoke in this study.

The patterns obtained following the exposures of the rats to passive smoke and nosbrite indicated the induction of inflammatory responses that could have disastrous manifestation in the rats. This is plausible in parts due to the concentrations of the acute phase proteins, such as prealbumin, C-reactive protein, interleukin 6, tumour necrosis factor-alpha and cortisol reported in our study (Figures 4 to 8). The chronic induction of these acute phase proteins was linked to various inflammatory disorders and compromised immune functions in various scientific studies (Kallo and Biswadev, 2009; Oyewo et al., 2013; Kelly and O’Neill, 2015; Choudhary et al., 2016). These proteins stimulate the clonal selection of immune cells and the chemotaxis of the immune cells (especially phagocytes) to the sites of inflammation, except prealbumin that is, a liver stressor protein.

The administration of nosbrite only in rats partly supported the induction of inflammatory responses in the trends recorded in serum levels of interleukin 6 and tumour necrosis factor-alpha concentration (Figures 6 and 7). This might be due to the action of intestinal microbes on phosphatidyl choline to form trimethyl amine which could be oxidized further to trimethyl amine-N-oxide (TMAO), a potent stimulator of low density lipoprotein cholesterol uptake in the endothelial cells (Wang et al., 2011; Bennett et al., 2013).

The total immunoglobulin (Ig) content increased in rats administered with nosbrite and passive cigarette smoke. Figure 11 indicated upward demand in Ig secretion by recognized passive antigens in the cigarette smoke which could be allergic in nature. However, the increase in Ig in the nosbrite only administered rats might possibly be a boost in the upward regulation of the immunity. Although, Ig are secreted in response to particular antigens, but the slight increase in Ig concentration in our study might be due to immunomodulatory capability of phosphatidyl choline and or Bowman brik inhibitor, which are components of nosbrite. Surprisingly, the exposures of the rats to the passive cigarette smoke inhibited the phagocytic capabilities of the innate immune cells (Figures 9 and 10). Perhaps, this is a very interesting piece supporting the induction and progression of inflammatory processes in endothelial cells as more neutrophils and monocytes are extravasated into the cells to contain tissue invasion and, or inflammatory responses due to increases in acute phase proteins.

Neutrophils and monocytes have low half life in circulation, but are incorporated into tissue (extravasation) to perform the phagocytic functions and only about 40% of the total amount can be determined in circulation (Spleman et al., 2006). In addition, the recorded decrease in phagocytic functions might be due to nicotine, a major component in cigarette smoke. Geng et al. (1995) reported that nicotine inhibited the phagocytic action in antigen presenting cells. Thus, the reported slight increase in the total Ig in the nosbrite only administered rats might not be a cause for alarm.

The reported patterns in the hematocrit, erythrocyte, haemoglobin and reticulocyte in rats exposed to passive cigarette smoke (Table 3) might be due to the effect of heavy metals and nicotine. Traces of heavy metals, such as lead, nickel and arsenic were reported in cigarette smoke and are renowned to interfere in iron (Fe²⁺) incorporation during heme synthesis and inhibit also, the activities of δ-aminolevoninic acid synthase, the regulatory enzyme in heme synthesis (Bailar et al., 1999; Asgary et al., 2005; Champe et al., 2006).

Nicotine and oxides of carbon (CO and CO₂) were reported to interfere with haemoglobin functions by altering the exchange of gases between cellular compartments and enhanced the peroxidation of the erythrocyte membrane (Geng et al., 1995; Asgary et al., 2005). These processes would further enhance haemolysis and hypoxia in tissues that led to the reported trends in the reticulocyte counts. In addition, the chronic activation of neutrophils and monocytes would alter their cellular metabolism and hypoxia will be induced that might stimulate erythropoesis in the bone marrow.

The results of the mean corpuscular volume and mean corpuscular haemoglobin supported the enhanced haemolysis due to the inhibition of iron incorporation into heme that led to microcytic hypochromic anaemia or anaemia of chronic inflammation disorders (Oyewo and Akanji, 2011).

The increases in the platelets contents in the rats administered with nosbrite and passive cigarette smoke might be a consequence of the healing processes by the immune cells. This was supported by the reported induction of inflammatory responses in rats exposed to the passive cigarette smoke (Figures 4 to 8). However, increases in platelet concentration over a period of time could be unhealthy due to the likely arbitrary formation of foam cells as reported during chronic inflammatory disorders and diseases.

The recorded increases in the total leukocytes in the rats were due to stimulated immune responses by some components in the passive antigens in cigarette smoke as indicated by the result obtained in the lymphocyte count (Table 3) and supported by the result reported in the total.
immunoglobulin content (Figure 11). In addition, the results of the monocyte and neutrophil counts, as well as, those of the phagocytic functions of the innate immunity (Figures 9 and 10) strengthened this submission that the increases in the total leukocytes were not desirous and healthy. Marked increases in total leukocytes and lymphocytes might be due to chronic inflammatory responses or disorders (Jeremy et al., 2001; Oyewo and Akanji, 2011). Although, some of these aforementioned parameters suggested an upward modulation of the immune system in the nosbrite only administered rats, which might further enhance the ability of the rats to suppress opportunistic infection and, or manage inflammatory response. However, not all of the results were consistent in the support of the upward modulation of the immune system with the sole administration of nosbrite.

Conclusion

Our research showed that the use of nosbrite without the risk of smoke exposure could be desirable and the sub-chronic exposures to passive cigarette smoke induced oxidative stress, inflammatory responses and impaired immune functions, in which the use of nosbrite had no ameliorating effects on the conditions, except the appetite. Thus, involuntary exposure to passive cigarette smoke should be strictly regulated, especially in public places due to inherent health hazards.

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REFERENCES


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