Impact of prolonged exposure to nicotine on the haematological parameters of male Wistar rats

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Abstract

Considering the popular usage of nicotine, the impact of prolonged exposure of nicotine on haematological parameters in male Wistar rats was evaluated by the current study. A total of thirty (30) Wistar rats were grouped into 5 groups of 6 rats each. Group 1: served as (control) and received distilled water and rat chow ad libitum. Groups 2 to 4 served as test groups; and received 250, 500, 750 and 1000 mg/kg/bw of Nicotine respectively. All treatments were administered orally using oral gavage and it lasted for 28 consecutive days. Thereafter samples were harvested and laboratory analyses done. Upon statistical analysis of data using the SPSS, there was a dependent increase in the body weight of experimental animals administered with 250 mg/kg and 500 mg/kg of the nicotine extract during week 2 and week 3. Aside from that of the 500 mg/kg treated group with marked (P < 0.05) decreased, all other nicotine treated groups compared to control group, indicated significantly raised levels of RBC. Similarly, there were non–significant (p>0.05) increases in the packed cell volume and haemoglobin concentration for the groups administered with 200 mg/kg, 750 mg/kg and 1000 mg/kg of Nicotine compared to the control. There was a significant (P < 0.05) increase in the level of neutrophils and reduction in eosinophil compared to control. In conclusion, chronic exposure to increasing doses of nicotine may have the potential to raise the levels of RBC, PCV, haemoglobin and platelet count; thus leading to a possible raised viscosity/hypercoagulable state of blood that could result in haemodynamic and other related dysfunction.

Keywords: Nicotine; Haematogical Parameters; Cardiovascular Dysfunction; Wistar Rats.

1. Introduction

Nicotine is an organic compound that is found in tobacco plants; and it is highly addictive especially when used recreationally, which is in turn associated with many health risks and problems [1]. Nicotine is a chiral alkaloid that is naturally produced in the nightshade family of plants (most predominantly in tobacco and Duboisiahopwood [1] and is widely used recreationally as a stimulant and anxiolytic. As a pharmaceutical drug, it is used for smoking cessation to relieve withdrawal symptoms [2].

Unless used in slow-release forms, nicotine use can provoke abuse [1, 3]; as have been indicated in animal models where monoamine oxidase inhibitors present in tobacco smoke revealed nicotine's addictive potency [2, 4]. Cigarette smoking is an important and independent risk factor for atherosclerosis, coronary artery disease, peripheral vascular disorders, etc and several studies provide the evidence that nicotine is strongly associated with altering the normal status of the lipid profile [5, 4].

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Although it has been reported that leukocyte count increases with the number of cigarettes smoked daily and decreases after cessation of smoking, data on smoking characteristics, such as duration of smoking, intensity of smoking, smoked pack-year and their association with leukocytes count is scanty [6]. Changes in the hematological parameters due to the inhalation of nicotine may be an important reason for various vascular diseases. Inhalation of considerable concentration of nicotine cause alternations in various hematological parameters, including white blood cells, mean corpuscular volume, hematocrit, hemoglobin, monocyte, eosinophil, neutrophil and lymphocyte counts [7].

Therefore, the various pharmacological actions of nicotine and other materials led to change the status of hematologic and hemostatic parameters. However, there are few studies to evaluate the impact of nicotine on blood cells; hence this study aimed at investigating the impact of prolonged exposure of Nicotine on haematological parameters in male Wistar rats.

2. Materials and Methods

2.1. Handling of Experimental Animals

Thirty (30) adult male Wistar rats (weighing 120-220g) were purchased from the Animal House of the Department of Pharmacology, University of Port Harcourt, Nigeria and managed under normal laboratory condition according to the University ethical guidelines. The rats were kept in clean cages and maintained at room temperature of 25°C ± 2°C with a 12hours light/dark cycle, all rats had free access to food and water during the study period. The rats were allowed to acclimatize for two weeks before the commencement of the experiment.

2.2. Experimental Design

The animals were divided into five (5) groups of six rats each. Group 1: served as (control) and received distilled water and rat chow ad libitum. Groups 2 to 4 served as test groups; and received 250 mg/kg/bw of Nicotine, 500 mg/kg/bw of Nicotine, 750 mg/kg/bw of Nicotine and 1000 mg/kg/bw of Nicotine respectively. All treatments were administered daily orally using oral gavage and lasted for 28 days.

2.3. Acute Toxicity Test (LD$_{50}$ Test)

**General Toxicity Dose of Nicotine:** In experimental animals, the doses of nicotine which is lethal to 50% of the animals (LD$_{50}$) varies widely, depending on the route of administration and the species used. Therefore, the oral LD$_{50}$ dose for nicotine in rats is 50 mg/kg$^{-1}$ to 60 mg/kg$^{-1}$ [8].

2.3.1. Experimental Agent

All reagents used in the present study were analytical grade. Nicotine was purchased from United Kingdom through the license from NAFDAC to Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

The drug Nicotine solute of 0.001mg/kg was diluted with 1000ml of distilled water and properly shaken prior to constituting for administration.

2.3.2. Ethics Statement

The approval for the present study was obtained from the Ethics Unit of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. And all the study animals were handled according to the recommendations of the US National Institute of Health (NIH) guidelines for care and use of laboratory animals in experimental research [9].

2.4. Harvesting and Preparation of Samples for Laboratory Analysis

2.4.1. Body weights of experimental animals were weighed weekly and charted.

At the end of drug administration, experimental animals were anaesthetized using 80% chloroform and then sacrificed through cervical dislodge. Cardiac puncture was used for blood collection for haematological parameters (red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC), neutrophils, lymphocytes, monocyte, basophils, eosinophils and platelet count).
2.5. Statistical Analysis

Data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. Data were expressed as mean ± standard error of mean. Analysis of Variance (ANOVA) was done using Least Significant Difference (LSD) to determine the significant difference in mean at 95 percent confidence interval (P < 0.05).

3. Results

Table 1 shows the body weight of experimental animal’s administered with Nicotine. There was a duration - dependent increase in the body weight of experimental animals administered with 200 mg/kg and 500 mg/kg of the nicotine extract during week 2 and week 3. However, there was a reduction in the body weight of the animals at week 4 of the study with respect to the doses of the extracts administered. However, these changes were not statistically significant (P > 0.05).

Table 1 Body weight changes in nicotine treated male Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weights of the study animals (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td>Control</td>
<td>140.73 ± 5.55</td>
</tr>
<tr>
<td>200 mg/kg Nt.</td>
<td>143.34 ± 5.57</td>
</tr>
<tr>
<td>500 mg/kg Nt.</td>
<td>161.27 ± 2.90</td>
</tr>
<tr>
<td>750 mg/kg Nt.</td>
<td>159.45 ± 4.17</td>
</tr>
<tr>
<td>1000 mg/kg Nt.</td>
<td>180.37 ± 4.70</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n = 6; *= Significant at p < 0.05 compared to control. Nt. = nicotine

Table 2 Effects of Nicotine treatment on erythrocytes parameters and platelet count in male Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (X10¹²/L)</th>
<th>PCV (%)</th>
<th>Hb (g/dL)</th>
<th>Platelet (X10⁹ /L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.77 ± 0.02</td>
<td>41.83 ± 0.60</td>
<td>13.95 ± 0.20</td>
<td>172.50 ± 10.52</td>
</tr>
<tr>
<td>200 mg/kg Nt.</td>
<td>5.96 ± 0.04*</td>
<td>42.40 ± 0.75</td>
<td>14.14 ± 0.24</td>
<td>140.20 ± 11.46</td>
</tr>
<tr>
<td>500 mg/kg Nt.</td>
<td>5.42 ± 0.01*</td>
<td>40.83 ± 0.87</td>
<td>13.60 ± 0.29</td>
<td>186.00 ± 24.21</td>
</tr>
<tr>
<td>750 mg/kg Nt.</td>
<td>6.29 ± 0.09*</td>
<td>43.33 ± 0.92</td>
<td>14.47 ± 2.40</td>
<td>165.83 ± 8.35</td>
</tr>
<tr>
<td>1000 mg/kg Nt.</td>
<td>6.22 ± 0.08*</td>
<td>43.17 ± 1.08</td>
<td>14.40 ± 0.36</td>
<td>136.50 ± 9.92</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n = 6; *= Significant at p < 0.05 compared to control. Nt. = nicotine

The outcome on the effect of nicotine on the red blood cell count (RBC) indicated generally significant (P < 0.05) elevations across all dosages of nicotine treated groups (except that of group 4) with respect to the control. Unlike other groups, Group 4 (treated with 500 mg) showed a discordant significant (P < 0.05) decrease in their mean RBC level when compared to the control group.

Similar to the fashion of the RBC level, there were non-significant (P > 0.05) increase in the packed cell volume and haemoglobin concentration for the groups administered with 200 mg/kg, 750 mg/kg and 1000 mg/kg of Nicotine compared to the control. However, the group administered with 500 mg/kg of Nicotine recorded decrease in the packed cell volume and haemoglobin concentration, but was not statistically significant (P < 0.05). There was a non-significant (P > 0.05) elevation in the level of platelet count in the group administered with 500 mg/kg on Nicotine compared with the control. However, the other groups studied, differed with a non-significant (P > 0.05) reduction in the level of platelet count when compared with the control as shown in table 2.
Effects of Nicotine on white blood cells and its differentials in Wistar rats are as shown in table 3. There was a significant (P < 0.05) increase in the serum level of neutrophils for the group administered with 200 mg/kg of Nicotine as compared with the control. The other groups studied showed a non–significant (P > 0.05) reduction in the serum level of neutrophils count. There was also a significant reduction (P < 0.05) in eosinophil count in the groups administered with 200 mg/kg and 1000 mg/kg of Nicotine. There was a non–significant (p>0.05) changes in the level other parameters compared to the control.

### Table 3 Effects of Nicotine treatment on white blood cells and its differentials in male Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (X10³/L)</th>
<th>NEU (%)</th>
<th>LYM (%)</th>
<th>MONO (%)</th>
<th>EOSINO (%)</th>
<th>BASO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.08 ± 2.39</td>
<td>21.83 ± 2.54</td>
<td>64.00 ± 5.94</td>
<td>9.00 ± 3.01</td>
<td>5.33 ± 1.56</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>200 mg/kg Nt.</td>
<td>10.60 ± 1.30</td>
<td>35.00 ± 3.87*</td>
<td>59.40 ± 3.06</td>
<td>3.60 ± 0.93</td>
<td>2.00 ± 1.05*</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>500 mg/kg Nt.</td>
<td>17.67 ± 2.34</td>
<td>20.50 ± 3.24</td>
<td>69.33 ± 2.14</td>
<td>7.50 ± 0.62</td>
<td>2.50 ± 1.23</td>
<td>0.17 ± 0.17</td>
</tr>
<tr>
<td>750 mg/kg Nt.</td>
<td>16.70 ± 2.40</td>
<td>18.83 ± 2.73</td>
<td>73.33 ± 3.70</td>
<td>4.50 ± 1.48</td>
<td>3.33 ± 0.62</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>1000 mg/kg Nt.</td>
<td>16.57 ± 3.53</td>
<td>17.17 ± 2.397</td>
<td>73.67 ± 1.91</td>
<td>8.33 ± 2.11</td>
<td>0.83 ± 0.65*</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM, n = 6. *: Significant at p < 0.05 compared to control. Nt. = nicotine

### 4. Discussion

This present study was carried out to investigate the impact of prolonged exposure of Nicotine on haematological parameters in male Wistar rats, in view of the dearth of detailed literatures [10] on the influence of nicotine on blood cells. Nicotine contains numerous chemicals and large quantities of oxidants. Many of the harmful effects of nicotine are due to oxidative damage [11]. The duration - dependent increase in the body weight of the experimental animals as recorded in this study following administration of varying doses of nicotine especially on the 2nd and 3rd weeks may be due to the inability of nicotine to impact negatively on appetite in view of acute exposure. Despite the weight-suppressive effects of nicotine being studied extensively, the mechanism by which nicotine acts to suppress body weight remains poorly understood [12, 13]. However, the reduction in the body weight of the animal models over a prolonged period of time observed in the study is consistent with the findings of Audi et al. [14] which showed that administration of nicotine to rats caused a significant decrease in the body weight and food intake. The decrease in food intake and body weight caused by nicotine administration might be due to neuroregulatory substances stimulated by nicotine which effect food intake mechanism.

Considering the myriad of adverse effects of nicotine in the biological system, it has been the focus of many earlier studies performed on humans and animals to largely examine the actions of nicotine on haematological parameters [15]. Nicotine is known to be associated with an increased risk of cardiovascular diseases, including coronary artery disease, peripheral vascular disease, ischaemic heart disease atherosclerosis, myocardial infarction [16] and stroke [17]. It is presumed that these effects are caused by abnormalities in the blood rheology, infection and inflammation, oxidative stress, and alterations of antithrombotic and fibrinolysis system.

The outcome of the present study revealed significant increases in the RBC levels of the groups administered with 200 mg/kg, 750 mg/kg and 1000 mg/kg of Nicotine compared to that of the control group. In a related manner to the changes in RBC level, the present study also found a non–significant (P < 0.05) increase in the packed cell volume and haemoglobin concentration following administration of nicotine in the study animals.

The outcomes in the present study regarding levels of RBC Count, hemoglobin concentration and PCV, are consistent with some earlier researches that also reported marked increases [18, 19, 20].

Asgary et al., [21] gave a suggestive clue to the foregoing outcome of the present study, that nicotine could have inhibited RBC hemolysis by 36.7% at higher dosages however, raised RBC hemolysis at the lower dosages. In another earlier report by Aldosari et al., [22], smoking tobacco, that is rich in nicotine, may be able to adversely alter the shape of red blood cells (RBCs) thereby lowering the blood’s ability to transport oxygen. The inference from this finding of the current study is that higher doses of nicotine treatment in the study animals may raise the PCV, Hb and RBC population, considering its deleterious effects on the morphology and flow of the cells, their functional integrity may be negatively impacted. Thus the need for great caution in the use of nicotine in mammalian models.
These increases may be as a result of the mediated response of some pro-inflammatory agents in circulation, which may have led to a possible degradation of oxygen with subsequent generation of carbon monoxide. This postulation is in agreement with the literature of Pankaj et al., [23], which suggested that increased hemoglobin level in blood of smokers could be a compensatory mechanism. Carbon monoxide binds to Hb to form carboxyhemoglobin, an inactive form of hemoglobin having no oxygen carrying capacity. This leads to a compensatory decreased oxygen delivering capacity, resulting in stimulation of erythropoiesis due to tissue hypoxia.

The group administered with 500 mg/kg of nicotine recorded a non-significant (P < 0.05) elevation in the level of platelet count compared to control. However, the other groups studied, differed with a non-significant (P < 0.05) reduction in the level of platelet count when compared with the control. There seem to be inconclusive and controversial reports on the effect of nicotine on platelet count [24]. This elevated level of platelet seen in this study is consistent with the findings of Sharif et al., [24] which reported a significant increase in platelet count amongst smokers. Nicotine have been reported to cause vascular inflammation and injury with endothelial dysfunction. These are pivotal in the process of thrombosis [25]. Platelets activation is an important mechanism in cardiovascular disease and progression [25].

Neutrophilia and eosinopenia were recorded for the experimental animal group administered with 200 mg/kg of nicotine. These findings suggest that nicotine may have a potential to alter the pathophysiology associated with allergic airways disease. This is in agreement with a recent study which reported that cigarette smoke led to alveolar macrophage apoptosis and necrosis, both of which occurred to a greater extent when the exposure contained nicotine [26].

5. Conclusion

From the foregoing, it can be concluded that chronic exposure of the studied doses of nicotine causes an increase in the levels of packed cell volume, haemoglobin and platelet count. These alterations may predispose to hypercoagulable state given the endothelial and vascular dysfunctional potentials of nicotine; which is a major risk factor for cardiovascular disease progression. It can also be concluded that the ingestion of nicotine leads to neutrophilia and eosinopenia; which highlights the potentials of nicotine as an airway proinflammatory agent.

Compliance with ethical standards

Disclosure of conflict of interest

All the authors, Sunday Ogbu Ojeka, Mpakaboari Tonye Bekinbo, Bestman Njoku and Ebibi Elizah Onwoke, declare that there are no conflicts of interest / competing interests with publication of the manuscript / institution or product that is mentioned in the manuscript.

Statement of ethical approval

The ethical approval for the present study was granted by the Research Ethics Committee of the Department of Human Physiology, Faculty of Basic Medical Science, University of Port Harcourt, Nigeria.

Reference


BRAR S. Effect of smoking on red blood cells count, hemoglobin concentration and red cell indices. Group. 2014 Jan;31:40yrs.


