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Sub-acute administration of lower doses of nicotine caused sex-dependent improvement of renal function in Wistar rats

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ABSTRACT

The adverse and beneficial health effects of nicotine (NIC), the major alkaloid found in cigarettes and tobacco, are controversial. Most studies on NIC have focused on its effects on cardiovascular and nervous functions. This study aimed at determining dose- and sex-specific effects of sub-acute (28 days) NIC administration on some indices of kidney function in Wistar rats. Forty rats (20 males and 20 females), 8–9 weeks old (each housed in separate metabolic cage), were used for this study such that graded doses of NIC (1, 2 and 4 mg/kg i.p. for 28 days) were administered to both sexes while each control received distilled water at 0.2 mL/100 g i.p. Blood was collected under ketamine anesthesia (10 mg/kg i.m) for analyses and results obtained were compared at $p < 0.05$. The result showed beneficial alterations in plasma and urine level of creatinine, urea and uric acid ($p < 0.05$) as well as plasma and urine electrolyte level (Na^+ and K^+) in both sexes ($p < 0.05$). Also, there was significant improvement in creatinine clearance ($p < 0.05$) with no appreciable difference in their histological examination. Although these beneficial effects were more pronounced in the female than in the male ($p < 0.05$), administration at the highest dose showed potentially deleterious alterations from normal beneficial trend ($p < 0.05$) in both sexes. It was concluded that sub-acute administration of lower doses of NIC improves kidney function of Wistar rats; an effect that was more pronounced in the females than their male counterparts.

1. Introduction

Nicotine, a natural alkaloid found in the plant *Nicotiana tabacum*, is considered an important component of cigarettes [1,2]. Nicotine chewing gums and dermal patches are other ways in which nicotine is consumed through non-prescription nicotine replacement therapy [3]. Although available in synthetic forms, it also constitutes a portion of human dietary intake as it naturally occurs in small amounts in plants belonging to the Solanaceae family such as eggplants, potatoes and tomatoes [4]. However, in man it is consumed primarily through cigarettes, pipes or cigars [5]. Nicotine can also be found in electronic cigarettes (e-cig), which is a contemporary and wide spread way of smoking [6,7]. Invented and patented by a Chinese Pharmacist named Hon Lik in 2003 [8], e-cig was introduced to the market, a year later, as an alternative nicotine delivery device [9]. Simulating the operation of conventional cigarette, e-cig is a battery –operated device containing e-liquid which is composed of concentrated flavours, propylene glycol (humectants), glycerol and various amount of nicotine [10]. To date, the adverse and beneficial effects of nicotine are stranded in controversy.

Reputed to be the tobacco alkaloid that is responsible for addiction, nicotine affects the body by causing modulation in dependence and behavior resulting in repeated exposure and consumption [5,11,12]. With an elimination half-life of about two hours when absorbed in the body [12], nicotine is readily transported in the blood to various body organs where it exert its effects. More than 80% of absorbed nicotine is metabolized by the liver [13] while several of its metabolites reach the central nervous system, crossing the blood – brain barrier within 10–20 s after inhalation [12,14]. A vast body of literatures exist on its effects on the nervous system [15–20]; showing that it is both a stimulant and relaxant, as well as on the cardiovascular system [21–26]. However, there is dearth of literature on the sub-acute effects of its graded doses on the renal system.

The kidney, an important organ of the renal system, plays a

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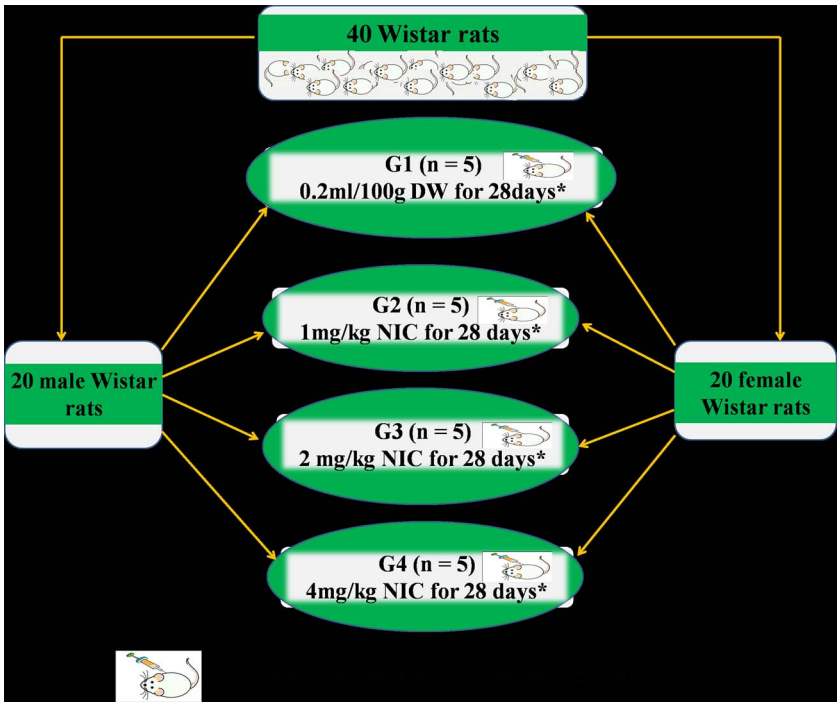

Fig. 1. Experimental Protocol and Dose Regimen.
G1 = Group 1; **G2** = Group 2; **G3** = Group 3; **G4** = Group 4; **n** = number of rats in the group; **DW** = Distilled water; **NIC**= Nicotine; * = Point at which rats were sacrificed.

principal role in homeostasis by excreting urine [27,28]. It achieves this by filtering waste products from the blood stream and converting the ultra filtrate to urine. Therefore, any compromise of kidney function can cause deleterious health effects due to build up of unwanted substances in the body [29]. Measurements of some biological (biochemical) markers can demonstrate beneficial or harmful changes in kidney function, thereby serving as indices of renal function. Variations in these biochemical markers play an important role in accurate diagnosis and also in assessing risk and adopting therapy that improves clinical outcomes [30]. Important biochemical markers that are often used for routine analysis of kidney function include creatinine, urea, uric acid as well as some important electrolytes such as sodium and potassium [30,31]. These biochemical markers were assayed for in the plasma to assess kidney function after a sub-acute duration of nicotine study.

Sub-acute, a condition between acute and chronic, study implies a study between 2 and 29 days while *acute study* is less than 2 days to within few minutes or hours [32]. Between 30–90 days comprises *sub-chronic* study while a study beyond 90 days is considered as *chronic* [33–35].

A comprehensive understanding of nicotine's effects on renal function can point towards novel approach to its use or disuse in both social and clinical settings with regards to renal implications. This study aimed at contributing to the body of existing knowledge by providing information on the renal implication of dose- and sex-specific effects of sub-acute nicotine administration using Wistar rat model.

2. Materials and methods

2.1. Metabolic cages

Metabolic cages used for this study were fabricated by Central Technological Laboratory and Workshops (CTLW), OAU, Ile-Ife, Osun State, Nigeria.

2.2. Chemicals and assay kits

Nicotine hydrogen tartarate (04441416) ≥98% was purchased from Sigma-Aldrich Company while the standard laboratory kits for the assay of urea and uric acid were purchased from Randox Laboratories Limited, United Kingdom.

Creatinine was assayed using laboratory protocol as described Jaffe [36], Na^+ and K^- by Flame Photometry while HCO_3^- was determined by titrimetry.

2.3. Preparation of stock solution for different concentration of nicotine

Creatinine clearance was determined using conventional formula as follows;

Clearance = $\mathbf{U_cV/P_c}$ (mL/min)

Where $\mathbf{U_c}$ = concentration of creatinine in urine; **V** = urine flow rate = amount of urine/time (secs); $\mathbf{P_c}$ = concentration of creatinine in plasma.

2.4. Animal management and experimental design

Both distilled water and the different nicotine concentration were administered at 0.2 mL/100 g of rat. Different concentration of nicotine at 1 mg/kg, 2 mg/kg and 4 mg/kg were prepared by dissolving 10 mg, 20 mg and 40 mg of nicotine salt in 20 mL, each, of distilled water respectively. Eventually, the entire groups received 0.2 mL/100 g of both distilled water and nicotine.

2.5. Measurement of body and organ weight

All experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (Guide for the care and use of laboratory animals, 2011) [37] and approved by local Institutional Research Committee. Forty Wistar rats comprising 20 males and 20 females (8–9 weeks old), between 120 and 150 g, were used for this study. They were purchased from the Animal Holdings of the College of Health Sciences, OAU, Ile – Ife, Osun State, Nigeria where the study was carried out. Each rat was housed in separate metabolic cage under natural light and dark cycle and allowed to have access to food and water *ad libitum*. Each sex was divided into 4 groups of 5 rats each as follows; Group 1 (control) received 0.2 mL/100 g of distilled water (i.p. for 28 days) after which they were sacrificed, while groups 2, 3 and 4