

# Histomorphological effects of nicotine on the Kidney

## ABSTRACT

**Introduction:** This experimental animal study was designed to investigate the histomorphological effects of nicotine on the kidneys of matured adult Wistar rats. **Materials and Methods:** Permission for the study was obtained from the Department of Human Anatomy and Cell Biology Ethics Committee (DELSU/CHS/ANA/68/43), and it involved 36 rats which were randomly assigned into three test and one control group. While animals in the control group (Group A) received only water and feed, 2 mg/day, 4 mg/day, and 6 mg/day of pure nicotine in solution was orally administered to test Groups B, C, and D, respectively (dose: 50 mg/kg/day). At the end of 7, 21, and 42 days, one experimental animal was selected from each of the groups at random, euthanized, and the harvested kidneys processed by standard techniques. The obtained tissue samples were viewed with a digital light microscope (Scopetek DCM 500, 20.0 mega pixel) connected through a USB to an HP computer. **Results:** The control group showed normal histomorphologic features, but the test groups which were dose and time dependent showed progressive histological alterations. Glomerulosclerosis and widening of the glomerular space were observed following acute and subacute exposure unlike with the chronically exposed rats in which an intact renal structural integrity was documented. **Conclusion:** This study that prolonged exposure to orally administered nicotine within normal doses had minimal effects on the kidneys of adult Wistar rats.

**Key words:** Acute, chronic, histology, kidney, nicotine

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## INTRODUCTION

Nicotine has been defined as a potent acetyl cholinergic alkaloid found in the leaves of the nightshade family plants, *Solanaceae*.<sup>[1]</sup> The natural product of tobacco has been documented to occur in these leaves in which and constitutes about 0.6–3.0% of the dry weight of the plant *Nicotinia tobacom*.<sup>[2]</sup> It has been reported that nicotine is commonly ingested as a powdered form called “tobacco snuff” which is either inhaled (sniffed) through the nose or ingested orally<sup>[3]</sup> nicotine has been demonstrated to display stimulant functions which at high dose and chronic ingestion, may display toxic and addictive effects.<sup>[4]</sup> This agent is the active ingredient in most cigarettes which when inhaled passes from the lungs to the brain within seconds and immediately stimulates the release of many chemical messengers.<sup>[5,6]</sup>

The presence of renal nicotinic acetylcholine receptor (nAChR) has been documented to enable the actions of nicotine on the kidney with subsequent excretion in urine.<sup>[7,8]</sup> This agent has been revealed to enhance vasoconstriction thereby modulating arterial blood pressure with resultant glomerular hypertension,<sup>[9]</sup> whereas another study explained that long-term oral administration of nicotine preserved renal function and reduced inflammation in rats with progressive kidney.<sup>[10]</sup> Though some studies have however demonstrated that nicotine containing products like tobacco may have been implicated in the formation of malignant renal neoplasms,<sup>[11,12]</sup> these sources of nicotine (e.g., tobacco), however, contain complexes which may act in synergy or in antagonism with nicotine. Since the specific effect of nicotine on the kidneys have rarely been reported, the index study was directed at investigating the

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acute, subacute, and chronic histomorphological effects of nicotine on renal histoarchitecture. This study will create awareness on the possible effects of nicotine, on the kidneys, thus educating the users of this agent, which is present in several popular house hold beverages.

## MATERIALS AND METHODS

### Materials

#### Animal handling

The experiment involved 36 adult Wistar rats of both sexes (18 males and 18 females), weighing between 100 and 200 g and obtained from animal house in the Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Science, Delta State University, Abraka.

#### Ethical considerations

Approval for this study was obtained from the Ethics Committee of the Department of Human Anatomy and Cell Biology, Delta State University, Abraka, with protocol number DELSU/CHS/ANA/68/43, and it conformed to the provision of the Declaration of Helsinki.<sup>[13]</sup>

#### Pharmacological agent

About 25 grams of nicotine hydrogen salt tartrate (95% nicotine);<sup>[14]</sup> Sigma life science 614-002-00-x United Kingdom, which was purchased from Rovet Scientific shop in Benin-City, Edo State, Nigeria.

#### Methodology

The animals were divided randomly into four groups (one control and three test groups), all of which were fed with growers mash and water *ad libidum*.<sup>[15-17]</sup> The test agent was orally administered to the experimental groups, further divided into B, C, and D based on the dose of nicotine administered, each consisting of eight animals.

The lethal oral dose for nicotine in rats has been demonstrated to be 50 mg/kg per body weight<sup>[18]</sup> and the recorded experimental animal weights ranged between 100 and 200 g. Animals in Groups B, C, and D received 2 mg, 4 mg, and 6 mg of nicotine, respectively.<sup>[19]</sup> The agent was administered 12 h to achieve a regular plasma concentration. The experiment lasted for 42 days.<sup>[20]</sup>

#### Method of tissue collection

One control and two test animals were selected from test Groups B, C, and D, respectively, at the end of the 7<sup>th</sup>, 21<sup>st</sup>, and 42<sup>nd</sup> day, (to assess the effects of the studies calculated doses nicotine during each of these periods). The experimental models were weighed and euthanized by cervical dislocation. Incisions were made from the chin vertically to the pubic quadrant. The kidneys which were then carefully harvested from the retroperitoneum, weighed, and fixed in 10% formaldehyde.<sup>[21]</sup> Following cut-up, sections of kidneys were placed in tissue cassettes

and processed by standard histological procedures.<sup>[21]</sup> Slides were viewed with a digital light microscope eye piece Scopetek DCM 500, 5.0 Mega pixel connected by USB 2.0 to a computer.

## RESULTS

### Control group for days 7, 21 and 42

As displayed in Figures a1-c1, the slides of the control group in the corresponding days are shown. The micrographs show intact histo and cytoarchitectural features of the kidneys. These are features of normal renal parenchyma.

### Test group 1: Day 7

As displayed in Figures a2-c2, for rats exposed to 2 mg, 4 mg, and 6 mg of nicotine for 7 days, sections of the kidneys were composed of several variably sized renal tubules separated by a loose connective tissue stroma in which were few blood vessels. The bowman's spaces were lined by flattened epithelium. Within the intervening, vaguely lobulated glomeruli were few capillaries and mesangial cells. There was mild interstitial intraglomerular vascular congestion and glomerular collapse, but renal pelvis was intact. These features were in keeping with mild renal congestion.

### Test group 2: Day 21

As displayed in Figures a3-c3 for rats exposed to 2 mg, 4 mg, and 6 mg nicotine for 21 days, sections of the kidney were composed of a cortex and a medulla. There was marked distortion of the glomeruli with several foci of glomerulosclerosis. The medullary ducts were present but were lined predominantly by flattened epithelial cells. Majority of the blood vessels displayed moderate congestion.

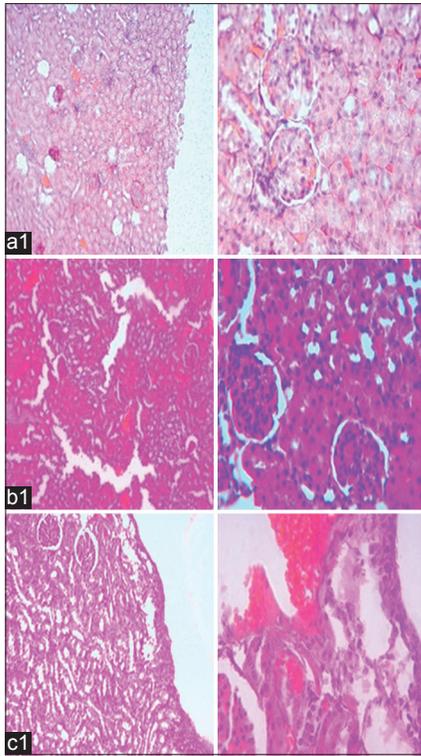
### Test group 3: Day 42

As displayed in Figures a4-c4, rats exposed to 2 mg, 4 mg, and 6 mg nicotine for 42 days showed sections of the kidney composed of a cortex, medulla, and minor calyx.

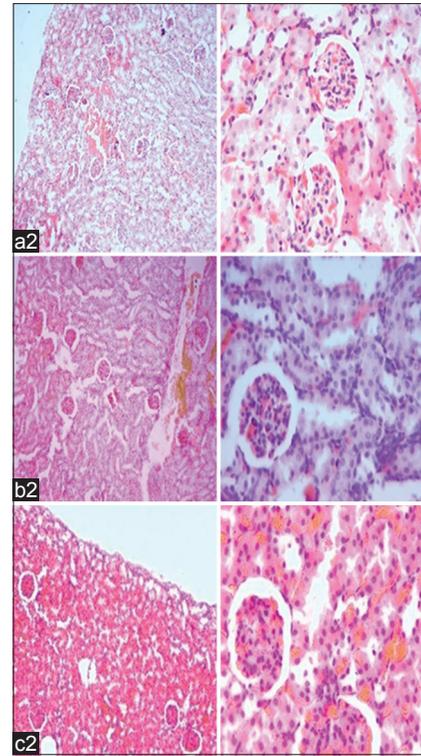
Several glomeruli were present within the cortex intermixed with renal tubules both of which had normal histo- and cyto-architectural features. The medullary ducts were separated by a thin connective tissue stroma in which were thin walled blood vessels. The calyces were lined by a transitional epithelium. The observed findings were in keeping with normal renal parenchyma.

## DISCUSSION

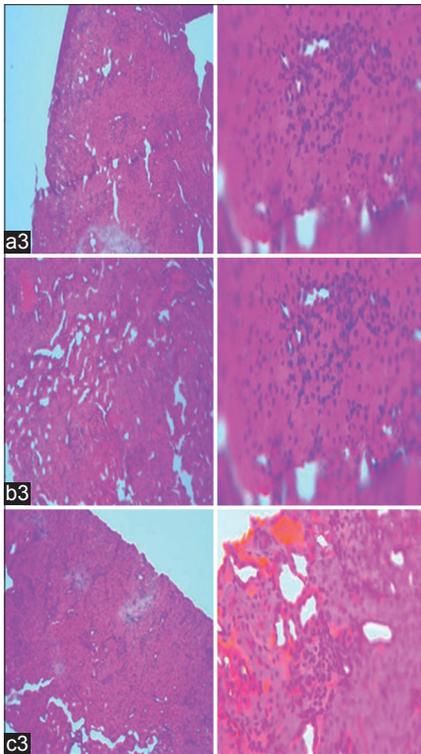
The index study has demonstrated that acute (7 day) oral administration of nicotine mildly disrupted the histological and cytological organization of the kidney. There was a dose-dependent mild increase in bowman's space and vascular congestion when compared with the



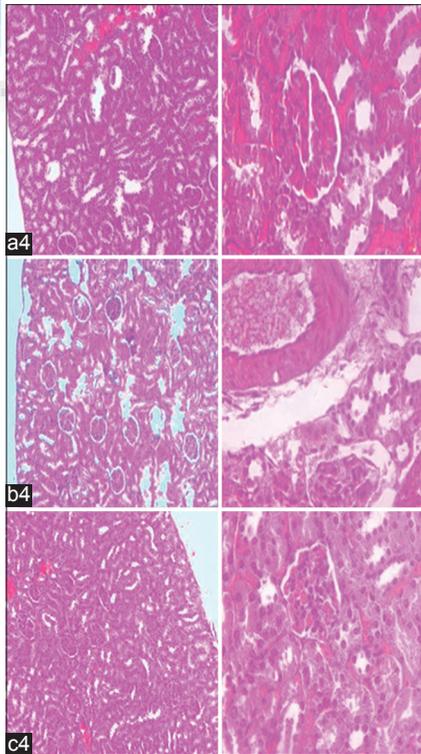
**a1:** Sections of the Kidneys control 7 days ( $\times 100$ ,  $\times 400$ )  
**b1:** Sections of the Kidneys control 21 days ( $\times 100$ ,  $\times 400$ )  
**c1:** Sections of the Kidneys control 42 days ( $\times 100$ ,  $\times 400$ )



**a2:** Sections of the Kidneys 2 mg 7 days ( $\times 100$ ,  $\times 400$ )  
**b2:** Sections of the Kidneys 4 mg 7 days ( $\times 100$ ,  $\times 400$ )  
**c2:** Sections of the Kidneys 6 mg 7 days ( $\times 100$ ,  $\times 400$ )



**a3:** Sections of the Kidneys 2 mg 21 days ( $\times 100$ ,  $\times 400$ )  
**b3:** Sections of the Kidneys 4 mg 21 days ( $\times 100$ ,  $\times 400$ )  
**c3:** Sections of the Kidneys 6 mg 21 days ( $\times 100$ ,  $\times 400$ )



**a4:** Sections of the Kidneys 2 mg 42 days ( $\times 100$ ,  $\times 400$ )  
**b4:** Sections of the Kidneys 4 mg 42 days ( $\times 100$ ,  $\times 400$ )  
**c4:** Sections of the Kidneys 6 mg 42 days ( $\times 100$ ,  $\times 400$ )

control group. This alteration which could be attributed to the vasoconstrictive potential of nicotine on the blood vessels by suppressing the production of an important vasodilator (nitric oxide),<sup>[22]</sup> correlated positively with the report of a previous finding.<sup>[23]</sup> Increases in Bowman's space may compromise the ultra-filtrating functions of the glomeruli by simultaneous shrinking of the glomeruli resulting in a decrease in glomerular filtration rate. The findings were in tandem with a previous study which reported that persistent exposure to nicotine altered renal function ultimately progressing to renal disease.<sup>[22]</sup> This was however unlike Agarwal *et al.*'s report where nicotine, was documented to be renoprotective, albeit, following prolonged nicotine exposure.<sup>[10]</sup> The observed differences may therefore not be unrelated with the differences in duration of both studies which may further indicate the time dependent effects of the agent.

The mild interstitial intraglomerular congestion, which was observed in the experimental groups further buttressed the likelihood that nicotine affected the pressure within the renal corpuscle which was similar to a previous finding where nicotine was reported to potentiate glomerular hydrostatic pressure.<sup>[22]</sup> Previous studies have also reported that exposure to nicotine for 2–3 weeks was suitable to produce deleterious effect on the kidney.<sup>[24,25]</sup> The mechanisms by which this damage occurred were however obscure. From this study, however, it could be inferred that nicotine may act via extensive stimulation of renal stromal receptors.<sup>[26]</sup> Although another author concluded that the mechanism of action was by damage to the renal tubules and blood vessels,<sup>[27]</sup> vague understanding of the specific mechanisms responsible for this observation has however been noted by others.<sup>[27,28]</sup>

It was further revealed that animals exposed to nicotine for 42 days showed no morphological or histological alterations in the experimental group when compared with the control group. Sections from the chronically exposed experimental groups had a better histological profile when compared with those of day's 7 and day's 21. The normal histo- and cyto-architecture observed in the experimental group was an indication that prolonged oral consumption of nicotine were not nephrotoxic which corroborated reports of Agarwal *et al.* in which long-term oral nicotine were observed to improve renal morphology as well as reduce renal inflammation.<sup>[10]</sup>

The extensive glomerulosclerosis and increase in intra glomerular and interstitial space as well as vascular damages which were evident at day 7 and day 21 was however not present in day 42, it may therefore be inferred, unlike previously reported, that chronic exposure to nicotine may display scant nephrotoxicity.

## CONCLUSION

Result from this study has shown that long-term oral administration of nicotine preserved normal renal morphology.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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