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Effects of Local Tobacco Snuff Ingestion during Pregnancy on Renal Functions and Histology Architecture of Female Wistar Rats and on the Birth Weight of Their Pups

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Abstract **Background**: Local tobacco snuff is consumed by rural women due to the stimulating effect of its nicotine content. **Aim:** To investigate the effects of ingesting local tobacco snuff by pregnant female Wistar rats on their renal function and histology, as well as on the birth weight of their pups. **Materials and methods:** 21 female Wistar rats used in this research were randomly separated into three groups and were monitored to confirm pregnancy after mating. Pregnant rats in group II (GII) and group III (GIII) received 20% and 40% respectively of the lethal dose (1.6g/kg/day) of the stock solution (tobacco snuff) via oral gavage while pregnant rats in the control group (GI) did not receive the stock solution. The pregnant rats were weighed during gestation (pregnancy), and on the delivery days (21^{st}/22^{nd}days of gestation), the pups (newborns of the female wistar rats) were also weighed. Blood samples were collected from the mother rats for creatinine and urea evaluations using Jaffe's method and Urease-Berthelots colorimetric method respectively. The harvested kidneys were stained with Hematoxylin and Eosin stains to examine the histological architecture. **Results:** An average of 5 pups per litter was recorded and the average pup's birth weight was low in groups II (4.39±0.30g) and III (4.33±0.29g) but not significantly different ($p>0.05$) from pups' birth weight in group I (4.50±0.38g). Using Tukey's post hoc test for analysis, the maternal kidney function tests showed that serum creatinine (1.27±0.12 mg/dL) and urea (16.77±1.65 mg/dL) levels for group II were not significantly different ($p>0.05$) from the respective values in group I (creatinine1.05±0.07 mg/dL; urea 16.77±1.65 mg/dL), but there was a significant increase in these values for group III (creatinine 1.68±0.10 mg/dL; urea 21.95±1.37 mg/dL). Histological examination showed that the kidneys of mother rats in groups II and III had distorted histological architecture. **Conclusion**: Consumption of tobacco during pregnancy causes weight loss, severely damages the kidneys and significantly inhibits fetal growth.

Keywords: *snuff, tobacco, nicotine, pregnancy, kidney, pup*

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1. Introduction

Tobacco plant is an herb, glandular-pubescent and perennial plant that grows erect [1]. The variety *Nicotiana tabacum* is basically of American origin but is grown worldwide as a cash crop [1]. This plant is readily available in both rural and urban markets in natural, dried and cured forms. *Nicotiana tabacum*, one of the varieties of the tobacco plant contains six alkaloids: nicotine, nornicotine, anatabine, anabasine, cotinine and myosmine [2,3]. Of these alkaloids, nicotine accounts for 96-98% of the total alkaloid content [2,3], it causes high addiction to tobacco [4], and it has the most immediate pharmacological action [5].

Aside from alkaloids, tobacco contains other dangerous components like Propionic acid, 2- Naphylamine, Choline, Tabacinine, Tabacine, 4-(methylnitrosamino)-1-(3-pyridyl) -1-butanone), 4-(methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA), Mercury (Hg), Cadmium (Cd), 2,3,6-Trimethyl-1,4-naphthoquinone,2-Methylquinone, N-nitrosonornicotine, Pyrene, Nicotelline and Cembrene [1]. Tobacco-Specific Nitrosamines (TSNAs) are harmful carcinogens in cigarette smoke, along with combustion products like tar and carbon monoxide [6], but in smokeless tobacco, TSNAs are the most harmful carcinogens, especially in chewed tobacco [7].

In 2007, the International Agency for Research on Cancer (IARC) documented that no consumer product is as lethal as tobacco [8]. To buttress more, the World Health Organization (WHO) in 2022 documented that 22.3% of the world's population used tobacco as of 2020, 36.7% of the world's men and 7.8% of the world's women [9]. WHO in 2022 also mentioned that tobacco kills up to half of its users, it kills more than 8 million people yearly of which around 1.2 million of this number are non-smokers who are exposed to second-hand smoke [9].

Tobacco is either smoked in the forms of cigars, cigarettes and pipes or taken in other smokeless forms (snuffing, chewing and dipping tobacco) and its consumption is practised by over 1.3 billion people [9]. The consumption of smokeless tobacco among women in different regions of the world has been documented [10], and some studies have also reviewed the smoking rate in women [11]. In third-world countries, it has also been shown that tobacco consumption during pregnancy is common [12,13,14]. A number of these pregnant women that consume tobacco shy away from the smoking form of tobacco either because of the carcinogenic tar and carbon monoxide present while smoking or because of socio-cultural stigma, therefore pushing them to either swallow or sniff the locally made snuff just to get the stimulating effect. Pregnant women that consume tobacco do not consider its adverse effect on the health of their fetus [11,15], thus the aim of this study was to investigate the effects of tobacco ingestion by pregnant Wistar rats on their renal function and histology of their kidneys, their weight during the course of pregnancy and the birth weight of their pups (newborns).

2. Materials and Methods

2.1. Experimental Animals

Twenty-one female albino Wistar rats (*Rattus novegicus*) with an average age of 3 months and an average weight of 145g - 160g were used for this study. The animals were gotten from the animal house of the Department of Biotechnology, Ebonyi State University (EBSU), Abakaliki, Nigeria. The rats were allowed to acclimatize for a week and were fed with standard rat feed and clean water *ad libitum*. In the well-ventilated laboratory, the rats witnessed 12 hours of darkness and 12 hours of light alternatively and a room temperature of 25±5°C. Ethical approval with reference number EBSU/REC/MPC/1706/02/002 was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences, EBSU.

2.2. Animal Mating

The female rats used for this research and the male rats meant to be used for mating were isolated for 7 days. On the 8th day, the rats were placed in mating cages at a ratio of 2 females to 1 male. Mating was confirmed with the aid of vaginal plugs in the females' vagina clearly seen with the help of a ×5 magnifying hand lens.

2.3. Substance of Study and Its Preparation

Dry tobacco leaves (*Nicotiana tabacum*) were purchased from Abakaliki Main Market and identified at the herbarium of the Plant Science and Biotechnology Department of EBSU. The leaves were blended into fine snuff (powdery form) and were stored in an air-tight container. The stock solution for this research was prepared by mixing 30g of the snuff into a 300ml of clean water. The lethal dose$_{50}$ (LD_{50}) oral of the stock solution was 2.4ml which contains 0.24g of tobacco. The LD_{50} oral of tobacco per kilogram of the Wistar rats was calculated as 1.6g/kg.

2.4. Experimental Design

There were 21 pregnant female rats in total. 3 different groups (I, II and III) were created, each containing randomly selected seven pregnant rats (n=7). Rats in group I only got rat feed and drinking water, and constituted the control group (GI). Group II received 20% (0.32g/kg/day) of the lethal dose of the stock solution for 23 days. Group III received 40% (0.64g/kg/day) of the lethal dose for 23 days. The administration tool was an oral gavage. On the 21st and the 22nd day (days of delivery), the pups in every group was weighed using an electronic weighing scale made by Denver Company, USA, 2003. On the 24th day, blood samples (3-5ml per rat) were collected by ocular puncture into labeled plain bottles. The blood samples were allowed to clot after which the blood sera were carefully separated from the clots. The sera were later centrifuged at 3,000 revolutions per minute (RPM) for 10 minutes to let any minute clot sediment. These sera were refrigerated at 5^{0}C and the sera creatinine and urea levels were analyzed afterwards using Jaffe's method [16] and Urease-Berthelots colorimetric method [17] respectively in the Department of Physiology, EBSU. The mother rats were later sacrificed by cervical dislocation and the kidneys were dissected and fixed immediately in 10% formal saline. Histological technique on the tissue was done in the histology laboratory of the Department of Anatomy, EBSU. The 5-7 µm thick sectioned tissues were stained with hematoxylin and eosin to be viewed under a light microscope. Photomicrographs were snapped for all the viewed tissue slides.

2.5. Statistical Analysis

Data gotten were statistically analyzed using SPSS (version 22) and all the values taken were expressed in mean±standard deviation. Using the Analysis of Variance (ANOVA) Tukey post hoc test, multiple comparisons between the three groups were made and the level of significance was deduced at $p<0.05$.

3. Results and Discussion

Numerous studies have been carried out to check the effects of nicotine or tobacco in humans, yet these studies still prove unending. Tobacco ingestion either by means of smoking or smokeless forms has been documented by some authors to be dangerous but the oral consumption of

blended dry tobacco leaves by pregnant women is yet to get enough attention from researchers, more so, to check its effect on both the pregnant woman and her baby before and after birth.

The first observation in this study was a profuse leaking of transparent, slimy saliva from the mouths of the pregnant rats immediately after each administration of the stock solution. This was observed in groups II and III. No analysis was done on the saliva but this response was suspected to be the effect of the snuff constituents on the superior and inferior salivatory nuclei of the brainstem, which in turn sends parasympathetic input to the salivary glands just as observed by Sairam, *et al* [18].

Table 1 shows the effect of tobacco ingestion on the body weight of pregnant rats. There was a significant increase in the body weight of the pregnant rats in the control group for the first seven days of gestation as they gained 11.34% of their initial body weight. The weight increased from an initial weight of 140.20±11.38g before conception to 156.10±8.34g on the 7th day of gestation. This initial weight gain was not observed in groups II and III, which was comparable to Mangubat, *et al*'s study which stated that nicotine reduces gain in body weight [19].

In group II, there was a significant 6.24% weight loss ($p<0.05$). The mean body weight for pregnant rats before conception was 157.00±9.48g and on the 7th day, the mean body weight became 147.20±8.06g.

In group III, the pregnant rats lost weight for the first 7 days of conception, but this 0.18% weight loss was not significant ($p>0.05$). These pregnant rats had a mean body weight of 166.40±1.13g before conception and 166.10±0.35g on the 7th day. From the 8th day of gestation, the body weight of the pregnant rats in all the groups increased due to fetal growth until the day of parturition after which the weight fell below their initial body weight before conception.

Table 2 shows the mean values of the renal parameters (creatinine and urea) that were investigated in this study and the statistical comparison between the biochemical indices of the experimental groups and the control group. The creatinine value in group III (1.68±0.10mg/dL) was significantly higher ($p<0.05$) than the value in the control group (1.05±0.07 mg/dL). The creatinine value in group II (1.27±0.12mg/dL) was higher than the creatinine value in the control group (1.05±0.07 mg/dL), although not significant ($p>0.05$).

For urea, the result showed that the urea level in group II (16.77±1.65mg/dL) was statistically indifferent ($p>0.05$) from the control group (14.20±0.28 mg/dL); but at a higher dose administered to pregnant rats in group III, the urea level (21.95±1.37mg/dL) showed an emphatic significant increase ($p<0.05$).

This also compares with Okonkwo, *et al*'s study as it proved that tobacco ingestion damages the kidney as it raises the levels of serum creatinine and urea [20]. Creatinine occurs naturally in chordates and primarily facilitates the recycling of adenosine triphosphate (ATP) in muscle and brain tissues [21]. It is found to build up in blood serum if the kidneys cannot naturally filter it out. Urea increases once a toxic substance is ingested. Both biomarkers increased significantly ($p<0.05$) at a rate which was dependent on dose in this study, suggesting tobacco is a toxic substance that damages the kidney and impairs the blood-clearing function of the kidneys of the pregnant Wistar rats.

The pups born in the experimental groups and the control group all appeared physically normal at birth. As such, no anomaly was recorded. The pregnant rats in the groups had a litter of 5 pups on average and these pups were closely monitored day and night. Sadly, 4 pups each from groups II and III died within 32 hours after birth because the mother could not produce breast milk to feed them. Table 3 shows the mean pups' birth weight. The mean birth weight in groups II and III were 4.39±0.30g and 4.33±0.29g respectively. These values were lower than the value of the birth weight of the pups in the control group (4.49±0.38g), although the lower birth weights were not significantly different ($p>0.05$) from the birth weight of pups in group I using the Tukey's test. This result supports Krishnamurthy & Joshi's study which stated that children of women who smoke tobacco have relatively low birth weight [22].

Table 1. Effect of snuff ingestion during gestation on body weight: weight distribution of pregnant Wistar rats

Groups	BW before conception(g) ± SD	BW at 7th day of gestation (g) ± SD	% BW difference	p-value	BW at 14th day of gestation (g) ± SD	% BW difference	BW at 1 day P.P. (g) ± SD
GI	140.20 ± 11.38	156.10 ± 8.34	+11.34	**0.001***	161.75 ± 14.78	+3.62	137.30±8.91
GII	157.00 ± 9.48	147.20 ± 8.06	-6.24	**0.005***	161.60 ± 3.68	+9.78	149.25±19.87
GIII	166.40 ± 1.13	166.10 ± 0.35	-0.18	**0.742****	169.00 ± 3.39	+1.75	157.30±6.51

BW = body weight; SD = standard deviation; g = gram; P.P. = postpartum; * = significantly different ($p<0.05$); **= not significantly different ($p>0.05$).

Table 2. Mean values (with standard deviation) and statistical significance of kidney function parameters of pregnant Wistar rats in all the groups

Parameters	Groups	Mean±SD	A	B	p-value
Creatinine (mg/dL)	GI	1.05±0.07	GI	GII	**0.117****
	GII	1.27±0.12		`	
	GIII	1.68±0.10		GIII	**0.001***
Urea nitrogen (mg/dL)	GI	14.20±0.28	GI	GII	**0.179****
	GII	16.77±1.65			
	GIII	21.95±1.37		GIII	**0.001***

SD = standard deviation; A and B = indices groups; level of significance at $p<0.05$; * = significantly different; **= not significantly different.

Table 3. Mean values (with standard deviation) and statistical significance of the birth weight of pups from pregnant Wistar rats in all the groups

Parameter	Groups	Mean±SD	A	B	p-value
	GI	4.50±0.38		GII	**0.761****
Pup's birth weight (g)	GII	4.39±0.30	GI		
	GIII	4.33±0.29		GIII	**0.411****

SD = standard deviation; A and B = indices groups; level of significance at $p<0.05$; ** = not significantly different.

The histological findings in this study support the results gotten from the biochemical analysis. Figure 1 shows normal renal histology of the mother Wistar rat in the control group. The photomicrograph displays normal kidney histology which supports the result of the kidney function test of the control group. The renal histology of the mother rat was distorted as the glomeruli were clumpy in appearance; capsular space was distended and at a higher dose, there was extensive fatty change as seen in Figure 2 and Figure 3. From these histological findings, it can be concluded that the kidneys of the mother rats were affected in a dose-dependent manner.

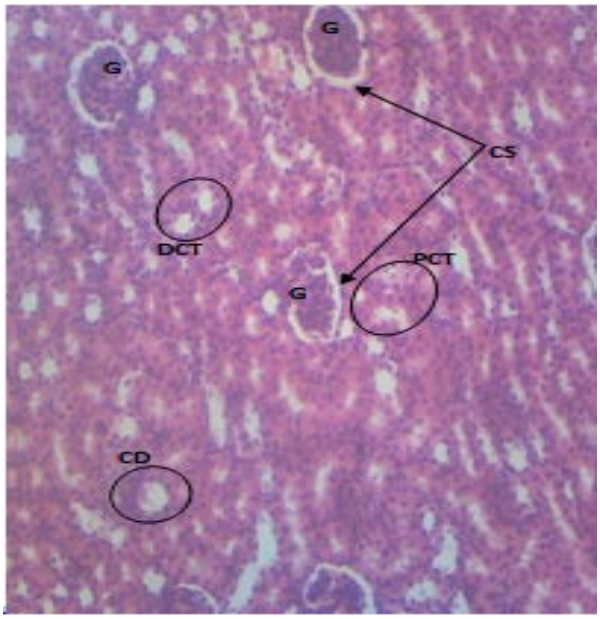

Figure 1. Photomicrograph of the kidney of mother rats in the control group that did not receive tobacco snuff. Normal kidney histology with glomeruli (G), distinct renal tubular cells, proximal convoluted tubule (PCT), distal convoluted tubule (DCT), capsular or urinary space (CS), and collecting duct (CD). Magnification: (x150)

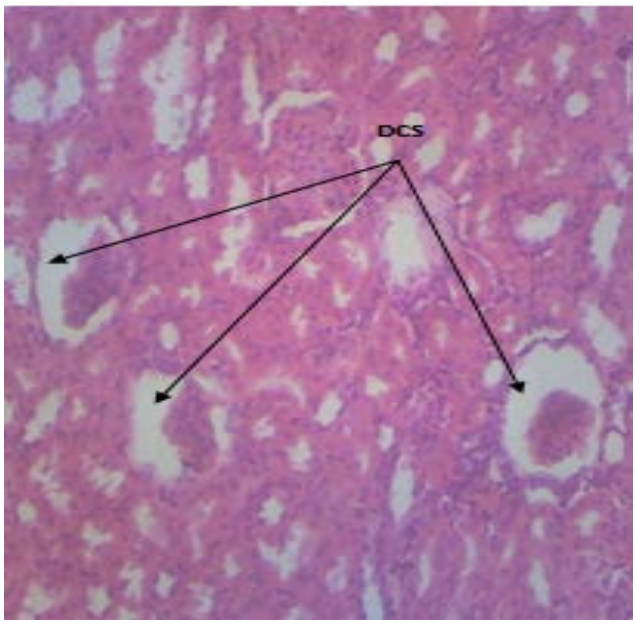

Figure 2. Photomicrograph of the kidney of mother rats in group II that received 0.32g/kg of tobacco snuff. The observable **s**tructural distortions of the kidney histology are distended capsular space (DCS) around the glomeruli, and necrotized and clumped glomeruli (NG). Magnification: (x150)

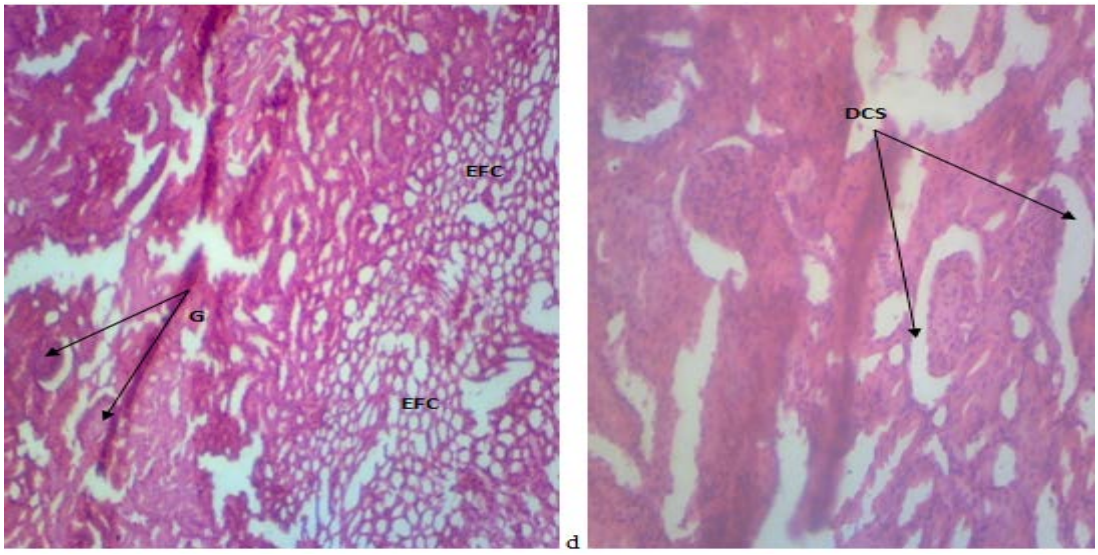

Figure 3A and 3B. Photomicrographs of the kidney of mother rats in group III that received 0.64g/kg of tobacco snuff. Observable distortions of the kidney histology include distended capsular space (DCS); clumpy and reduced glomerular size; glomeruli looking atrophic (G); extensive fatty change (EFC). Magnification: A and B (x60 and x150 respectively)

4. Conclusion

Tobacco (*Nicotiana tabacum)* is harmful to the kidneys of pregnant Wistar rats and distorts the histological architecture of the kidneys. Ingested tobacco impairs the blood-clearing function of the kidneys as serum creatinine and urea levels are elevated in the blood. In addition, tobacco ingestion by Wistar rats during gestation reduces the birth weight of their pups.

Prospects for Further Research

To ascertain the cause(s) of the absence of breast milk production by the mother rats after delivery; to analyze the effect of tobacco snuff ingestion during pregnancy on prolactin level; and to study the renal and liver functions of pups from mothers who ingested tobacco snuff during pregnancy.

Conflict of Interest

The authors guarantee responsibility for everything published in this manuscript, as well as the absence of a conflict of interest and the absence of their financial interest in performing this research and writing this manuscript. This manuscript was written from an original research work and has never been published, neither is it under consideration for publication elsewhere.

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