Songklanakarin J. Sci. Technol.
47 (6), 458–465, Nov. – Dec. 2025

http://www.sjst.psu.ac.th

Original Article

Changes in reproductive hormones, lipid profile, and antioxidants levels in male offspring of female Wistar rats exposed to nicotine administration

Abodunrin Adebayo Ojetola[1*], Dare Joshua Ogundipe[2], Ibukun Peter Oyeyipo[2], Olawale Olaleye Obembe[2], Mega Obukohwo Oyovwi[1], Muyiwa Owolabi Ladele[2, 3], Dennis Seyi Arokoyo[4], Esthinsheen Osirim[3], Oore-oluwapo Ololade Daramola[3], Grace Ayobami Fajemidagba[3], Timileyin Emmanuel Abiodun[2], Temitope Grace Awoniyi[2], and Michael Oluwaseun Ajayi[2]

[1] *Department of Physiology, Faculty of Basic Medical Sciences, Adeleke University, Ede, Nigeria*

[2] *Department of Physiology, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria*

[3] *Department of Physiology, Faculty of Basic Medical Sciences, Redeemer's University, Osogbo, Nigeria*

[4] *Department of Physiology, Faculty of Basic Medical Sciences, Federal University of Technology, Akure, Nigeria*

Received: 30 June 2024; Revised: 31 July 2025; Accepted: 8 September 2025

Abstract

A notable proportion of pregnant women are exposed to nicotine before and during pregnancy, yet little is known of its adverse consequences on their offspring. This study was therefore designed to investigate the effects of nicotine preconception and gestational exposures on male offspring. Twenty-five female Wistar rats (150-180g) were used for this study. They were grouped into 5 (n=5) treatment groups namely, Control (group 1), Nicotine 0.5mg/kg (group 2), Nicotine 1.0mg/kg (group 3), Nicotine 0.5mg/kg (group 4), Nicotine 1.0mg/kg (group 5), with respective nicotine doses administered orally. The exposed female rats were mated with normal male rats and their male offspring were correspondingly grouped. Group 1 was the control, 2 and 3 were orally administered nicotine daily for 28 days before pregnancy and during gestation, while groups 4 and 5 were only administered daily before pregnancy. The offspring were fed with normal rat chow and water for 12 weeks until sacrifice when they were euthanized, blood was collected, and organs were excised and weighed. Lipid profile, atherogenic index (AI), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assessed and data were analysed with ANOVA. There were decreases in FSH, LH and testosterone levels in offspring of the treated groups compared to control. However, increases were observed in kidney, lungs and liver weights in group 2 compared to control. Oxidative stress was observed through increase in MDA and decrease in SOD in the treated groups compared to control. Lipid profile and AI were not significantly different in the treated groups compared to control. Nicotine preconception and gestational exposures in female rats resulted in hormonal disturbances, organ weight changes, and oxidative stress in the male offspring.

Keywords: nicotine, antioxidants, reproductive hormones, offspring

*Corresponding author
Email address: bodunrin.ojetola@gmail.com

1. Introduction

Nicotine occurs naturally as an alkaloid in solanaceous plants such as tomatoes, potatoes, green pepper and tobacco. It is commonly found in hookahs, cigarettes and vaping products. In smokers, tobacco is responsible for nicotine addiction (Mishra *et al*., 2015), and both passive and active smokers are exposed to tobacco consumption. Over a billion people globally, including pregnant women, smoke tobacco according to the World Health Organization (Tarasi, Cornuz, Clair, & Baud, 2022). Smoking during pregnancy is common, and efforts to rectify this problem remain abortive. Despite awareness on the consequences associated with smoking during pregnancy, more than half of the women who smoked daily continued their smoking habit during pregnancy (Lange, Probst, Rehm, & Popova, 2018). This may be related to the widespread notion that vaping products which contain nicotine are safer, thus creating misconceptions and posing a considerable threat among youths and pregnant women that intend to quit smoking.

Growing interest in fetal programming as a potential root cause of anomalies has increased interest in exploring the adverse consequences of nicotine exposure on offspring. Nicotine intake in any form crosses the placenta and predisposes offspring to disorders such as infantile colic, respiratory infections and fetal brain disorders, bone fractures and childhood obesity (Ayubi, Safiri, & Mansori, 2021; Brand *et al*., 2020; Spindel & McEvoy, 2016; Whittington *et al*., 2018). In both male and female rats, nicotine adversely modulates reproductive hormones, sperm characteristics, fertility profile, hepatic functions, antioxidants, inflammatory responses and haematological disturbances (Iranloye & Bolarinwa, 2009; Marzouk, Awaad, Abo-Eleneen, & El-Bakry, 2022; Oyeyipo, Raji, & Bolarinwa, 2013; Oyeyipo, Raji, Emikpe, & Bolarinwa, 2011; Ray, 2019; Saad *et al*., 2018). Even smokeless tobacco during pregnancy enhances nicotine exposure, thereby resulting in low birth weight, reduced litter size, shortened gestational age, stillbirth and neonatal apnea (Gunnerbeck, Wikström, Bonamy, Wickström, & Cnattingius, 2011; Hurt *et al*., 2005). Nicotine**'**s effects have also been reported to initiate cardiovascular diseases (CVD) through elevated blood pressure, heart rate, catecholamine levels, vascular stenosis, and heart attack (Nides, Leischow, Bhatter, & Simmons, 2014; Qasim, Karim, Rivera, Khasawneh, & Alshbool, 2017; Vlachopoulos *et al*., 2016; Yan & D**'**Ruiz, 2015).

Nicotine adversely affects reproductive hormones, sperm characteristics, fertility profile, hepatic functions, and haematological profile (Iranloye & Bolarinwa, 2009; Marzouk *et al*., 2022; Oyeyipo *et al*., 2013; Oyeyipo *et al*., 2011; Ray, 2019; Saad *et al*., 2018). Also, nicotine consumption has been reported to induce oxidative stress and inflammatory responses (Lerner *et al*., 2015).

Despite vast reports on the adverse effects of nicotine on direct users and in animal models, studies are sparse regarding the influence of nicotine on the offspring of parents exposed to these products before or during pregnancy. This study was designed to investigate the effects of nicotine on reproductive hormones and biochemical variables in male offspring of female rats exposed during and/or before pregnancy.

2. Methods

2.1 Animals

Twenty-five female Wistar rats (weight range: 150 – 180 g) used in this study were procured at the vivarium of College of Health Sciences, Osun State University, Osogbo, Nigeria. They were kept under standard environmental conditions in plastic cages. Water and rodent pellets were provided *ad-libitum* to the animals. Animal care and experimental procedures were performed according to the ARRIVE guidelines and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

The twenty-five female rats were divided into five groups (n=5). Group 1 (Control group) was orally administered 1 ml/kg of normal saline solution daily throughout the gestation period. Groups 2 and 3 were orally administered 0.5 mg/kg and 1 mg/kg of nicotine daily for 28 days before pregnancy and throughout gestation, respectively. Groups 4 and 5 were orally given 0.5 mg/kg and 1 mg/kg of nicotine daily for 28 days before pregnancy, respectively. At the time of mating, the rats were paired separately with male animals of equal weights at a ratio of 1:1. After delivery, male offspring (n=5) were selected from each corresponding maternal group and fed with rodent pellets and water for 12 weeks.

2.2 Termination of the experiment

At week 12, an infusion of ketamine (60 mg/kg) and xylazine (10 mg/kg) was injected to anaesthetise the animals. Blood was collected via cardiac puncture and centrifuged at 1,372 x g for 15 min, at 4 ºC with a cold centrifuge (Centurion Scientific Ltd, West Sussex, United Kingdom), and supernatants were taken. Assays for biochemical markers were carried out through diagnostic kits. Organs such as testes, epididymis, prostate glands, spleen, kidneys, lungs, heart, and liver were excised and weighed, and the organ-to-body weight ratios were calculated.

2.3 Drugs and chemicals

Nicotine hydrogen tartrate (95% Nicotine) (BDH Chemicals Ltd., Poole, England) was used in the study. With normal saline, nicotine was freshly prepared and orally administered at 0.5 mg/kg and 1.0 mg/kg bw. It was stored for at most three days at 4°C in a foil-wrapped glass bottle. Determination of triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were assayed through kits purchased from Fortress Diagnostics kits Limited, Antrim, United Kingdom. However, kits for estimation of testosterone, luteinising hormone (LH), and follicle-stimulating hormone (FSH) were procured from Elabscience Biotechnology Company, Ltd., Wuhan, Hubei, China. Kits used for superoxide dismutase, glutathione peroxidase, catalase, and lipid peroxidation (malondialdehyde) assays were obtained from Oxford Biomedical Research, Inc. (USA). Ellman reagent [5, 5-dithiobis- (2- nitrobenzoate) DTNB] and thiobarbituric acid (TBA) were from Sigma–Aldrich (St. Louis, USA).

2.4 Sperm analysis

Following Zakrzewska *et al*. (Zakrzewska, Udala, & Blaszczyk, 2002), sperm motility and viability were assessed with 5% Nigrosin and 1% Eosin in a solution containing 3% sodium citrate dehydrate. Spermatozoa counts were done with a haemocytometer's improved Neubauer (Labart, Germany).

2.5 Reproductive hormones and electrolytes

The serum levels of luteinising hormone (LH), Follicle-stimulating hormone (FSH), and testosterone were measured using an assay kit (Elabscience). Serum Sodium (Na^+) and Potassium (K^+) were done through flame photometry (Corning model 410C), while Calcium (Ca^{2+}) was measured by spectrophotometry as previously described (Gindler & King, 1972)

2.6 Atherogenic index (AI), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (LDL-c) estimation

As previously described (Ojetola, Adedeji, & Fasanmade, 2021), LDL-c was calculated, VLDL estimated by this formula: VLDL = TG/5 (mg/dl), and AI expressed as log (TG/HDL).

2.7 Malondialdehyde (MDA) estimation and determination of antioxidants

MDA was determined based on thiobarbituric acid (TBA) and MDA reaction principle as previously described (Draper & Hadley, 1990). Analytical kits for the determination of glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD) were procured from Fortress Diagnostic (Antrim, UK).

2.8 Statistical analysis

Data analysis was carried out through one-way analysis of variance (ANOVA) and the data are presented as mean ± standard error of the mean (SEM). ANOVA was followed by Tukey *post hoc* test for multiple comparisons. The significance threshold was $p \leq 0.05$.

3. Results

3.1 Nicotine administration did not alter lipid indices in male offspring

Nicotine administration did not significantly alter the atherogenic index (AI) and lipid profile in the male offspring of exposed female parents (Table 1). Neither dose of nicotine showed a significant decrease or increase in the male offspring relative to the control group ($P < 0.05$).

3.2 Nicotine administration altered reproductive hormones and sperm functions in male offspring

Nicotine administration significantly decreased testosterone ($P < 0.006$), LH ($P < 0.005$) and FSH ($P < 0.001$) levels in the nicotine 0.5 mg/kg (group 2) compared to the control group respectively. Similarly, nicotine significantly decreased Testosterone ($P < 0.002$), LH ($P < 0.002$) and FSH ($P < 0.039$) levels in nicotine 1.0 mg/kg (group 3) compared to control. Testosterone level of nicotine 0.5 mg/kg (group 4) (3.18 ± 0.1) also showed significant decrease compared to the control (4.74 ± 0.2) but not to the nicotine 1.0 mg/kg (group 5) (4.22 ± 0.3) (Table 2). However, testosterone level of group 5 was significantly higher compared to group 2 (3.04 ± 0.4), group 3 (2.9 ± 0.4) and group 4 (3.18 ± 0.1), respectively. This indicates that only gestational exposure at 1.0 mg/kg had no significant effect on the testosterone level in this group of animals, as the values were similar to the control. LH and FSH of the treated groups were all significantly reduced compared to the control. Sperm viability, sperm motility and sperm counts were not significantly different in the treated groups compared with the control (Table 2).

3.3 Effects of nicotine administration on organ weights in male offspring of Wistar rats

There were observed increases ($P < 0.05$) in kidney weight (1.07 ± 0.1), lung weight (1.45 ± 0.1) and liver weight (6.90 ± 0.3) of the group 2 compared to the control group recording (0.83 ± 0.1), (1.19 ± 0.1) and (5.73 ± 0.3), respectively. Heart weight was only significantly decreased ($P < 0.05$) in the nicotine 1.0 mg/kg (group 3) (0.48 ± 0.1) compared to the control (0.68 ± 0.1). Spleen weight was significantly increased ($P < 0.05$) in group 4 (0.67 ± 0.66) and group 5 (0.73 ± 0.1) compared to the control (0.64 ± 0.1).

Regarding the reproductive organ weights, only the nicotine 0.5 mg/kg (group 2) showed significant increases ($P < 0.05$) in epididymis and prostate weights compared to the control. The other treated groups were not significantly different from the control (Table 3).

3.4 Effect of nicotine administration on antioxidants, malondialdehyde and electrolyte levels

Serum levels of SOD were significantly decreased in the nicotine 0.5 mg/kg group (group 2) ($P < 0.026$), group 3 compared to the control, respectively. MDAs of the groups 3, 4 and 5 were significantly increased at ($P < 0.019$), ($P < 0.001$) and ($P < 0.001$) compared to the control, respectively. Also, there were significant elevations in the MDA of both groups 4 and 5 compared to group 2 (Table 4). The decrease in SOD and increase in MDA levels in the offspring of the treated groups indicates the presence of oxidative stress.

Levels of serum electrolyte of Na^+ were significantly decreased in the nicotine 1.0 mg/kg (group 2) group (117.6 ± 5.4) and in group 5 (110.3 ± 3.5) compared to the control group (139.1 ± 1.6) which indicates hyponatraemia. Similarly, both 1.0 mg/kg treated groups showed significant decrease compared to the group 2 (136.2 ± 1.7). Ca^{2+} was significantly decreased ($P < 0.044$) in the nicotine 1.0 mg/kg compared to control (Table 4), which indicates hypocalcaemia. Electrolyte levels in the other treated groups were not significantly different compared to the control. ($P < 0.047$), group 4 ($P < 0.001$) and group 5 ($P < 0.001$)

Table 1. Effects of nicotine administration on lipid indices in offspring of male Wistar rats

	TC (mg⁄dL)	TG (mg⁄dL)	LDL-c (mg⁄dL)	HDL-c (mg⁄dL)	VLDL-c (mg⁄dL)	AI
Control (Group 1)	2.30 ± 0.1	1.62 ± 0.1	1.22 ± 0.2	0.68 ± 0.1	0.31 ± 0.02	0.37 ± 0.03
Nicotine 0.5mg⁄kg (Group 2)	2.72 ± 0.1	1.66 ± 0.1	1.42 ± 0.1	0.72 ± 0.1	0.33 ± 0.02	0.37 ± 0.07
Nicotine 1.0mg⁄kg (Group 3)	2.38 ± 0.3	1.5 ± 0.1	1.24 ± 0.2	0.80 ± 0.1	0.34 ± 0.02	0.27 ± 0.45
Nicotine 0.5mg⁄kg (Group 4)	2.98 ± 0.2	1.86 ± 0.1	1.42 ± 0.1	0.70 ± 0.1	0.37 ± 0.02	0.42 ± 0.02
Nicotine 1.0mg⁄kg (Group 5)	2.46 ± 0.2	1.58 ± 0.1	1.18 ± 0.2	0.80 ± 0.1	0.30 ± 0.02	0.29 ± 0.02

Values are expressed as mean ± SEM. NB: TC – Total Cholesterol; TG – Triglycerides; LDL-c – Low Density Lipoprotein Cholesterol; HDL-c – High Density Lipoprotein Cholesterol; VLDL-c – Very Low Density Lipoprotein Cholesterol; AI – Atherogenic Index

Table 2. Effects of nicotine administration on hormonal level and reproductive organ weights in male offspring Wistar rats

	Testosterone (ng⁄mL)	LH (mIU⁄mL)	FSH (mIU⁄mL)	Sperm motility (%)	Sperm viability (%)	Sperm Count ($\times 10^6$⁄ml)
Control (Group 1)	4.74 ± 0.2	1.78 ± 0.1	1.66 ± 0.1	63.40 ± 7.2	51.20 ± 8.9	40.40 ± 6.8
Nicotine 0.5mg⁄kg (Group 2)	3.04 ± 0.2*	1.38 ± 0.1*	1.38 ± 0.1*	80.20 ± 2.1	62.0 ± 2.7	49.60 ± 2.7
Nicotine 1.0mg⁄kg (Group 3)	2.9 ± 0.4*	1.24 ± 0.1*	1.48 ± 0.1*	73.0 ± 2.5	50.80 ± 6.4	47.80 ± 2.3
Nicotine 0.5mg⁄kg (Group 4)	3.18 ± 0.1*	1.32 ± 0.1*	1.38 ± 0.1*	63.20 ± 4.9	44.40 ± 6.7	46.60 ± 2.3
Nicotine 1.0mg⁄kg (Group 5)	4.22 ± 0.3$^{\#ab}$	1.44 ± 0.1*	1.44 ± 0.1*	75.60 ± 1.9	63.40 ± 1.7	47.20 ± 2.2

Values are expressed as mean ± SEM. $^{*}p < 0.05$ is significant compared to control group; $^{\#}p < 0.05$ is significant compared to Nicotine 0.5 mg⁄kg group; $^{a}p < 0.05$ is significant compared to Nicotine 1.0 mg⁄kg group; $^{b}p < 0.05$ is significant compared to Nicotine 0.5 mg⁄kg recovery group. NB: LH – Luteinizing Hormone; FSH – Follicle Stimulating Hormone

Table 3. Effects of nicotine administration on organ weights in male offspring Wistar rats

	Kidney weight (g)	Spleen weight (g)	Heart weight (g)	Lungs weight (g)	Liver weight (g)	Testis weight (g)	Epididymis weight (g)	Prostate weight (g)
Control (Group 1)	0.83 ± 0.1	0.64±0.1	0.68±0.1	1.19±0.1	5.73 ± 0.3	3.01 ± 0.1	2.72 ± 0.1	2.79 ± 0.2
Nicotine 0.5mg⁄kg (Group 2)	1.07 ± 0.1*	0.62 ± 0.1	0.64±0.1	1.45±0.1*	6.90 ± 0.3*	3.72 ± 0.1	3.81 ± 0.1*	4.02 ± 0.2*
Nicotine 1.0mg⁄kg (Group3)	0.79 ± 0.1	0.62 ± 0.1	0.48±0.1*	1.15±0.1	5.19 ± 0.4	2.91 ± 0.1	2.41 ± 0.1	2.17 ± 0.2
Nicotine 0.5mg⁄kg (Group 4)	0.88 ± 0.1	0.67 ± 0.66*	0.52 ± 0.1	1.24±0.1	5.54 ± 0.2	2.62 ± 0.1	2.50 ± 0.1	2.96 ± 0.2
Nicotine 1.0mg⁄kg (Group 5)	0.86 ± 0.1	0.73 ± 0.1*	0.54 ± 0.1	1.08 ± 0.1	5.63 ± 0.5	3.60 ± 0.1	2.39 ± 0.1	2.99 ± 0.2

Values are expressed as mean ± SEM. $^{*}p < 0.05$ is significant compared to control group.

Table 4. Effects of nicotine administration on electrolyte levels, antioxidants and pro-oxidant status in male offspring Wistar rats

	GPx (U⁄L)	SOD (U⁄mL)	CAT (µmol⁄min⁄mL)	MDA (µM)	Na^+ (mmol⁄L)	K^+ (mmol⁄L)	Ca^{2+} (mmol⁄L)
Control (Group 1)	1.77 ± 0.2	2.46 ± 0.2	25.86 ± 1.5	27.80 ± 2.5	139.1 ± 1.6	6.48 ± 0.5	2.18 ± 0.2
Nicotine 0.5mg⁄kg (Group 2)	1.83± 0.1	1.83 ± 0.1*	25.14 ± 1.1	33.90 ± 1.3	136.2 ± 1.7	5.78 ± 0.2	1.82 ± 0.3
Nicotine 1.0mg⁄kg (Group 3)	1.67 ± 0.2	1.84 ± 0.1*	21.44 ± 1.1	37.98 ± 2.1*	117.6 ± 5.4$^{*\#}$	5.84 ± 0.3	1.28 ± 0.1*
Nicotine 0.5mg⁄kg (Group 4)	2.16 ± 0.3	1.60 ± 0.1*	20.76 ± 1.69	47.62 ± 2.6$^{*a\#}$	126.2 ± 3.1	5.8 ± 0.2	1.88 ± 0.3
Nicotine 1.0mg⁄kg (Group 5)	1.85 ± 0.1	1.74 ± 0.1*	24.90 ± 1.0	46.28 ± 1.6$^{*\#}$	110.3 ± 3.5$^{*\#b}$	5.38 ± 0.2	1.42 ± 0.1

Values are expressed as mean ± SEM. $^{*}p < 0.05$ is significant compared to control group; $^{\#}p < 0.05$ is significant compared to Nicotine 0.5 mg⁄kg group; $^{a}p < 0.05$ is significant compared to Nicotine 1.0 mg⁄kg group; $^{b}p < 0.05$ is significant compared to Nicotine 0.5 mg⁄kg recovery group. NB: SOD – GPx – Glutathione Peroxidase; Superoxide Dismutase; CAT – Catalase; MDA – Malondialdehyde; Na – Sodium; K – Potassium; Ca – Calcium

4. Discussion

In female rats, nicotine exposure before and during pregnancy did not significantly affect lipid profile or atherogenic indices of their male offspring. While nicotine use in direct users has been associated with increased cardiovascular disease risk (Bakker & Jaddoe, 2011; Schaller *et al*., 2013), findings across studies remain inconsistent, likely due to variations in experimental design (Qasim *et al*., 2017). Influence of nicotine cessation, withdrawal and periods of exposure play significant roles on the incidence of cardiovascular diseases. In direct users, nicotine use increases the risk of atherosclerosis (Mitchell, Sobel, & Alexander, 1999) through elevation in the amounts of triglycerides, low-density lipoprotein, and total cholesterol; and a decrease in high-density lipoprotein cholesterol (Mitchell *et al*., 1999), thereby promoting the incidence of coronary heart diseases and ischaemic stroke (Hussain, Ibrahim, & Ibrahem, 2009). In

this study, there was no incidence of dyslipidaemia in the offspring of the animals exposed before pregnancy and during pregnancy. The duration of exposure in parents and dosage might have prevented distortion in lipid profile indices in the offspring. It should be noted that metabolic and cardiovascular diseases are blends of genetic and environmental influences with more drift to environmental predispositions as diet and lifestyle modulate gene expressions (Phillips, 2013; Phillips, Tierney, & Roche, 2008). Thus, the normal lipid profile and atherogenic index seen in the offspring might be attributed to influences of the normal dietary and nicotine-free lifestyle the offspring were exposed to. In addition, since metabolic anomalies are largely age-dependent, development of dyslipidaemia and cardiovascular diseases might not show up until a later period in the offspring's life. Also, nicotine exposure was predominantly maternal whereas paternal genetic influence might also play a significant role.

Furthermore, the observed decrease in heart weight may be associated with increased energy expenditure, mass tissue, and anorexia, that has been reported to be linked with chronic nicotine use and reversed after cessation (Chiolero, Faeh, Paccaud, & Cornuz, 2008). Evidence on electrolyte balance in smokers is conflicting. Sodium (Na^+) is essential for maintaining homeostasis, blood pressure, and blood volume, while potassium (K^+) and calcium (Ca^{2+}) regulate cardiac electrical activity. A study (Rebat, Al-Sabbagh, Habeeb, Al-Khafaji, & Jawad, 2020) reported no significant differences in serum Na^+, K^+, and Ca^{2+} levels among healthy adults who smoked 10–40 cigarettes daily for three years, whereas other studies observed decreased Na^+ and K^+ levels in men who smoked at least four cigarettes per day. Erdemir and Erdemir (Erdemir & Erdemir, 2006) reported no differences in saliva electrolyte contents when it was checked in active smokers. In this study, only 1 mg/kg nicotine administration decreased Ca^{2+} and Na^+ concentrations in the serum. Preconceptional exposure interfered with Na^+ and was responsible for decrease compared to the control. Implications of this could include a decline in electrical stimulation, conduction of nerve impulses, defect in muscular contractions, and transport of substances through cellular membranes (Pohl, Wheeler, & Murray, 2013). Also, depletion in Ca^{2+} stores reflects that nicotine exposure before and during pregnancy contributes to the depletion of inositol – 1, 4, 5 – triphosphate sensitive Ca^{2+} stores (de Lores Arnaiz & Ordieres, 2014; Rebat *et al*., 2020).

Generally, alterations in electrolyte levels affect the activities of the cardiomyocytes, but changes in K^+ and Ca^{2+} levels have a more profound effect on the heart because of the association with arrhythmia. In this study, the K^+ was unchanged in treated groups and hypocalcaemia was observed in the group exposed to 1mg/kg nicotine administration pre-pregnancy and during pregnancy. This corroborates the decrease in heart weight observed in the offspring.

Nicotine significantly reduced LH and FSH serum levels in all the treated groups. These are essential hormones that influence reproductive functions. FSH is necessary for spermatogenesis, and LH stimulates the production of testosterone. There was observed a decrease in testosterone level of the offspring of animals with both preconceptionally and gestationally administered nicotine, indicating that nicotine administration regardless of cessation before or during pregnancy served as a key endocrine disruptor with adverse consequences in offspring.

A previous study showed that the decrease in testosterone secretion might not be associated with pituitary distortion but with testicular dysfunction (Oyeyipo *et al*., 2013). Similarly, there was a decrease in testosterone secretion in all the treated groups compared to the control, indicating that nicotine has the ability to distort testosterone secretion in male offspring of maternally exposed rats. These changes were not associated with testicular organ weight changes which might imply that nicotine could impair Leydig cell function and steroidogenic enzyme expression without significantly affecting overall testicular mass in offspring. FSH acts on Sertoli cells and facilitates, supports and nurtures developing sperm cells while LH stimulates Leydig cells to produce testosterone. Decreases in their secretion corroborate the decreased spermiogenesis and testosterone secretion even though sperm count, viability and motility were unaffected at this stage in the offspring rats. Also, nicotinic components can cross the blood-testis barrier and activate nicotinic acetylcholine receptors (nAChRs), which can cause gonadotoxic effects on male reproductive organs, particularly on testes (Condorelli *et al*., 2013; Ray, 2019). Furthermore, oxidative stress is a possible mechanism that can induce reproductive damage in rats exposed to nicotine administration leading to disrupted spermatogenesis, seminiferous tubule degeneration and abnormal sperm profile (Ray, 2019; Sepaniak *et al*., 2006). Although the sperm functions were not disrupted in the offspring at week 12, there are possibilities that sperm functions may rapidly decline as age advances because of hormonal imbalances.

Previous research has demonstrated that nicotine increases the risks of cellular damage (Gunes, Koklu, Gunes, Narin, & Koklu, 2008), and it is associated with oxidative stress (Ashakumary & Vijayammal, 1996). Maternal influence on heredity cannot be overemphasized during preconception or gestational nicotine exposure because it is known that nicotine crosses the placenta freely and rapidly (Pastrakuljic, Derewlany, & Koren, 1999), which significantly implicates the role of maternal inheritance. In this study, nicotine administration increased MDA level in the offspring of exposed maternal parents. This is an indication that there was presence of oxidative stress in the offspring with or without the influence of nicotine exposure timing. Similarly, there was also a decrease in SOD in all nicotine-treated groups. The evidence of oxidative stress in offspring even at such low doses of nicotine in parent rats indicates that nicotine can rapidly cross the placenta and increase amniotic fluid accumulation (Condorelli *et al*., 2013). Also, the depletion of SOD, unlike the catalase level, might have resulted from its role as the first line of defence against oxygen-derived free radicals.

Lung weight increase observed in the nicotine 0.5 mg group may be associated with maternal nicotine exposure during and before pregnancy. An increase in lung weight resulting from nicotine and tobacco intake is linked with pulmonary oedema, fibrosis or increased interstitial fluid emphysema and lung cancer because it interferes with alveolar development (Iranloye & Bolarinwa, 2009; Martinez, Cline, & Burrows, 1992). Studies have shown that nicotine exposure during developmental stages disrupts physiological processes in the lungs and occurs quite early (Gibbs, Collaco, & McGrath-Morrow, 2016; Maritz, 2013). The exposures during

formative and developmental periods eventually increase susceptibility to respiratory tract infections that can degenerate into chronic lung diseases.

Liver weight increase is associated with hepatomegaly. The aetiology of hepatomegaly has been traced to the accumulation of lipids in the hepatocytes. This is might be linked with the occurrence of non-alcoholic fatty liver diseases and the development of atherosclerosis (Ojetola, Asiwe, Adeyemi, Ogundipe, & Fasanmade, 2022). Nicotine administration was reported to be hepatotoxic in neonates of female rats exposed to nicotine during pregnancy in a dose-dependent manner (Marzouk *et al*., 2022) which agrees with this study. The increase in liver and lung weights was only observed in the offspring of rats pre-exposed to nicotine before and during pregnancy with only preconception administration at 0.5 mg/kg. This indicates that nicotine intake has a more propounding effect before gestation. This occurrence similarly occurred in kidney weight and weights of reproductive organs, such as the testes, prostate and epididymis.

5. Conclusions

Nicotine administration led to hormonal and electrolyte disturbances, oxidative stress, and an increase in some organ weights in male offspring. Also, exposures before and during pregnancy had more pronounced effects on testosterone levels and organ weights than just gestational exposure.

5.1 Biological plausibility

The findings of this study are biologically plausible based on established evidence that nicotine readily crosses the placenta and can interfere with fetal development, and this study confirmed disrupted endocrine signalling, organogenesis, and metabolic programming. Maternal exposure, particularly during critical windows such as preconception and gestation, affects offspring's phenotype through developmental programming pathways, establishing the plausibility of the observed and reported effects. The observed alterations in reproductive hormone levels and biochemical variables in male offspring may be linked to nicotine-induced epigenetic modifications, oxidative stress, and impaired organ development mechanisms.

5.2 Limitations

Nicotine exposure in this study was limited to maternal source and the potential contribution of paternal genetic or epigenetic influences was not assessed. Secondly, although changes in biochemical and hormonal parameters were observed, the study did not include histological evaluations or molecular analyses to confirm underlying mechanisms. Lastly, only male offspring were evaluated, leaving the potential sex-specific effects unexplored.

References

Ashakumary, L., & Vijayammal, P. L. (1996). Additive effect of alcohol and nicotine on lipid peroxidation and antioxidant defence mechanism in rats. *Journal of Applied Toxicology*, *16*(4), 305–308.

Ayubi, E., Safiri, S., & Mansori, K. (2021). Association between maternal smoking during pregnancy and risk of bone fractures in offspring: A systematic review and meta-analysis. *Clinical and Experimental Pediatrics*, *64*(3), 96.

Bakker, H., & Jaddoe, V. W. V. (2011). Cardiovascular and metabolic influences of fetal smoke exposure. *European Journal of Epidemiology*, *26*(10), 763–770. Retrieved from https://doi.org/10.1007/s10654-011-9621-2

Brand, J. S., Hiyoshi, A., Cao, Y., Lawlor, D. A., Cnattingius, S., & Montgomery, S. (2020). Maternal smoking during pregnancy and fractures in offspring: National register based sibling comparison study. *Bmj*, *368*.

Chiolero, A., Faeh, D., Paccaud, F., & Cornuz, J. (2008). Consequences of smoking for body weight, body fat distribution, and insulin resistance1. *The American Journal of Clinical Nutrition*, *87*(4), 801–809. Retrieved from https://doi.org/10.1093/ajcn/87.4.801

Condorelli, R. A., La Vignera, S., Giacone, F., Iacoviello, L., Vicari, E., Mongioi, L., & Calogero, A. E. (2013). In vitro effects of nicotine on sperm motility and bio-functional flow cytometry sperm parameters. *International Journal of Immunopathology and Pharmacology*, *26*(3), 739–746.

de Lores Arnaiz, G. R., & Ordieres, M. G. L. (2014). Brain Na+, K+-ATPase activity in aging and disease. *International Journal of Biomedical Science*: *IJBS*, *10*(2), 85.

Draper, H. H., & Hadley, M. (1990). [43] Malondialdehyde determination as index of lipid Peroxidation. *Methods in Enzymology* (Vol. 186, pp. 421–431). Elsevier. Retrieved from https://doi.org/10.1016/0076-6879(90)86135-I

Erdemir, E. O., & Erdemir, A. (2006). The detection of salivary minerals in smokers and non-smokers with chronic periodontitis by the inductively coupled plasma-atomic emission spectrophotometry technique. *Journal of Periodontology*, *77*(6), 990–995.

Gibbs, K., Collaco, J. M., & McGrath-Morrow, S. A. (2016). Impact of tobacco smoke and nicotine exposure on lung development. *Chest*, *149*(2), 552–561.

Gindler, E. M., & King, J. D. (1972). Rapid colorimetric determination of calcium in biologic fluids with methylthymol blue. *American Journal of Clinical Pathology*, *58*(4), 376–382.

Gunes, T., Koklu, E., Gunes, I., Narin, F., & Koklu, S. S. (2008). Influence of maternal nicotine exposure on neonatal rat oxidant–antioxidant system and effect of ascorbic acid supplementation. *Human and Experimental Toxicology*, *27*(10), 781–786.

Gunnerbeck, A., Wikström, A.-K., Bonamy, A.-K. E., Wickström, R., & Cnattingius, S. (2011). Relationship of maternal snuff use and cigarette smoking with neonatal apnea. *Pediatrics*, *128*(3), 503–509.

Hurt, R. D., Renner, C. C., Patten, C. A., Ebbert, J. O., Offord, K. P., Schroeder, D. R., … Moyer, T. P. (2005).

Iqmik–a form of smokeless tobacco used by pregnant Alaska natives: Nicotine exposure in their neonates. *The Journal of Maternal-Fetal & Neonatal Medicine*, *17*(4), 281–289.

Hussain, S. M., Ibrahim, O. S., & Ibrahem, A. K. (2009). Effect of Smoking on Lipid Profile in Men in Ramadi Municipality. *Al-Anbar Medical Journal*, *7*(1).

Iranloye, B., & Bolarinwa, A. (2009). Effect Of Nicotine Administration On Weight And Histology Of Some Vital Visceral Organs In Female Albino Rats. *Nigerian Journal of Physiological Sciences*, *24*(1). Retrieved from https://doi.org/10.4314/njps.v24i1.46374

Lange, S., Probst, C., Rehm, J., & Popova, S. (2018). National, regional, and global prevalence of smoking during pregnancy in the general population: A systematic review and meta-analysis. *The Lancet Global Health*, *6*(7), e769–e776. https://doi.org/10.1016/S2214-109X(18)30223-7

Lerner, C. A., Sundar, I. K., Yao, H., Gerloff, J., Ossip, D. J., McIntosh, S., … Rahman, I. (2015). Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PloS One*, *10*(2), e0116732.

Maritz, G. S. (2013). Perinatal exposure to nicotine and implications for subsequent obstructive lung disease. *Paediatric Respiratory Reviews*, *14*(1), 3–8.

Martinez, F. D., Cline, M., & Burrows, B. (1992). Increased incidence of asthma in children of smoking mothers. *Pediatrics*, *89*(1), 21–26.

Marzouk, H. S., Awaad, A. S., Abo-Eleneen, R. E., & El-Bakry, A. M. (2022). Effects of experimentally induced nicotine on the liver of neonatal albino rat. *Adv. Anim. Vet. Sci*, *10*(1), 151–159.

Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., & Garg, A. (2015). Harmful effects of nicotine. *Indian Journal of Medical and Paediatric Oncology*, *36*(01), 24–31.

Mitchell, B. E., Sobel, H. L., & Alexander, M. H. (1999). The adverse health effects of tobacco and tobacco-related products. *Primary Care*: *Clinics in Office Practice*, *26*(3), 463–498.

Nides, M. A., Leischow, S. J., Bhatter, M., & Simmons, M. (2014). Nicotine blood levels and short-term smoking reduction with an electronic nicotine delivery system. *American Journal of Health Behavior*, *38*(2), 265–274.

Ojetola, A. A., Adedeji, T. G., & Fasanmade, A. A. (2021). Changes in antioxidants status, atherogenic index and cardiovascular variables after prolonged doses of D-ribose-L-cysteine in male Wistar rats. *Heliyon*, *7*(2).

Ojetola, A. A., Asiwe, J. N., Adeyemi, W. J., Ogundipe, D. J., & Fasanmade, A. A. (2022). Dietary supplementation with D-Ribose-L-Cysteine prevents hepatic stress and pro-inflammatory responses in Male Wistar Rats Fed a high-fructose high-fat diet. *Pathophysiology*, *29*(4), 49.

Oyeyipo, Ibukun P., Raji, Y., & Bolarinwa, A. F. (2013). Nicotine alters male reproductive hormones in male albino rats: The role of cessation. *Journal of Human Reproductive Sciences*, *6*(1), 40–44.

Oyeyipo, Ibukun Peter, Raji, Y., Emikpe, B. O., & Bolarinwa, A. F. (2011). Effects of nicotine on sperm characteristics and fertility profile in adult male rats: A possible role of cessation. *Journal of Reproduction and Infertility*, *12*(3), 201.

Pastrakuljic, A., Derewlany, L. O., & Koren, G. (1999). Maternal Cocaine Use and Cigarette Smoking in Pregnancy in Relation to Amino Acid Transport and Fetal Growth. *Placenta*, *20*(7), 499–512. https://doi.org/10.1053/plac.1999.0418

Phillips, C. M. (2013). Nutrigenetics and metabolic disease: Current status and implications for personalised nutrition. *Nutrients*, *5*(1), 32–57.

Phillips, C. M., Tierney, A. C., & Roche, H. M. (2008). Gene–nutrient interactions in the metabolic syndrome. *Lifestyle Genomics*, *1*(3), 136–151.

Pohl, H. R., Wheeler, J. S., & Murray, H. E. (2013). Sodium and potassium in health and disease. *Interrelations between Essential Metal Ions and Human Diseases*, 29–47.

Qasim, H., Karim, Z. A., Rivera, J. O., Khasawneh, F. T., & Alshbool, F. Z. (2017). Impact of electronic cigarettes on the cardiovascular system. *Journal of the American Heart Association*, *6*(9), e006353.

Ray, D. (2019). Effects of nicotine on sperm health and semen quality: A review based on animal studies. *Journal of Drug Delivery and Therapeutics*, *9*(4-A), 754–758.

Rebat, B. W., Al-Sabbagh, J. K., Habeeb, Z. T., Al-Khafaji, N. M., & Jawad, R. A. (2020). Effect of cigarette smoking on some electrolytes levels in men live in city of karbala. *AIP Conference Proceedings*, *2290*(1). AIP Publishing.

Saad, B. A., Rjeibi, I., Alimi, H., Ncib, S., Bouhamda, T., & Zouari, N. (2018). Protective effects of Mentha spicata against nicotine-induced toxicity in liver and erythrocytes of Wistar rats. *Applied Physiology, Nutrition, and Metabolism*, *43*(1), 77–83.

Schaller, K., Ruppert, L., Kahnert, S., Bethke, C., Nair, U., & Pötschke-Langer, M. (2013). Electronic cigarettes—An overview. *Tobacco Prevention Tobacco Control*, *19*, 1–39.

Sepaniak, S., Forges, T., Gerard, H., Foliguet, B., Bene, M.-C., & Monnier-Barbarino, P. (2006). The influence of cigarette smoking on human sperm quality and DNA fragmentation. *Toxicology*, *223*(1–2), 54–60.

Spindel, E. R., & McEvoy, C. T. (2016). The role of nicotine in the effects of maternal smoking during pregnancy on lung development and childhood respiratory disease. Implications for dangers of e-cigarettes. *American Journal of Respiratory and Critical Care Medicine*, *193*(5), 486–494.

Tarasi, B., Cornuz, J., Clair, C., & Baud, D. (2022). Cigarette smoking during pregnancy and adverse perinatal outcomes: A cross-sectional study over 10 years. *BMC Public Health*, *22*(1), 2403. Retrieved from https://doi.org/10.1186/s12889-022-14881-4

Vlachopoulos, C., Ioakeimidis, N., Abdelrasoul, M., Terentes-Printzios, D., Georgakopoulos, C., Pietri, P., … Tousoulis, D. (2016). Electronic cigarette smoking

increases aortic stiffness and blood pressure in young smokers. *Journal of the American College of Cardiology*, *67*(23), 2802–2803.

Whittington, J. R., Simmons, P. M., Phillips, A. M., Gammill, S. K., Cen, R., Magann, E. F., & Cardenas, V. M. (2018). The use of electronic cigarettes in pregnancy: A review of the literature. *Obstetrical and Gynecological Survey*, *73*(9), 544–549.

Yan, X. S., & D'Ruiz, C. (2015). Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regulatory Toxicology and Pharmacology*, *71*(1), 24–34.

Zakrzewska, H., Udala, J., & Blaszczyk, B. (2002). In vitro influence of sodium fluoride on ram semen quality and enzyme activities. *Fluoride*, *35*(3), 153–160.