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Reproductive Indices and Oxidative Stress Biomarkers of Male Wistar Rats Prenatally Exposed to Cigarette Smoke

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Summary: The negative influence of cigarette smoking on developing fetus is well documented but reports of prenatal cigarette smoking on male reproductive hormones are controversial. However, shortened anogenital distance (AGD) has been established to be an indicator of potential male infertility. We therefore investigated the effects of prenatal exposure to passive cigarette smoke on AGD, reproductive hormones and oxidative stress biomarkers of Wistar rats. Female rats were randomly divided into two groups (n=5) and cohabited with male. Group 1 was exposed to smoke from an idling cigarette from day 1 of gestation till parturition, while Group 2 served as control (no-exposure). Morphometric variables of the litters were recorded on postnatal day 1 (PND1) and at 6th week postnatal life. The male offspring were then sacrificed by cervical dislocation. Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were analysed using ELISA. Serum levels of Catalase, sodium dismutase (SOD), malondialdehyde (MDA), lipid profile and liver function biomarkers were examined spectrophotometrically. On PND1, crown rump length and total body length of rats prenatally exposed to cigarette smoke were significantly shorter. Significantly shorter AGD and crown rump length were also observed at 6th week. Testosterone, LH and FSH were not significantly affected. Cigarette smoke exposure significantly decreased Catalase and SOD while MDA increased. Liver function biomarkers, HDL and LDL were not affected but serum levels of total cholesterol and triglyceride significantly increased. The observed decline in AGD and precipitation of oxidative stress by intrauterine cigarette smoke exposure may predispose to male infertility at adulthood.

Keywords*:* Prenatal, Cigarette smoke, Anogenital distance, Sex hormones, Oxidative stress.

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INTRODUCTION

Cigarette smoke is a leading cause of avoidable death and it is a big menace to public health in the world today. According to the World Health Organization (2015), more than 1 billion people around the world smoke and about 6 million people die each year from tobacco-related illness. More than 5 million of those deaths are the result of direct tobacco use while more than 600, 000 are the result of non-smokers being exposed to second-hand smoke. That is about one person dying every six seconds. The most common reason for maintaining smoking behavior is due to nicotine addiction (Buczkowski *et al.*, 2014).

There are various documented effects of active and passive smoking on pregnancy which includes intra-uterine growth retardation, sudden infant death syndrome, neuro-developmental and behavioural problems (Wickstrom, 2007). About 15–25% of women smoke while pregnant (Coleman, 2004). Prenatal cigarette smoking is associated with a high frequency of complications during pregnancy. These include preterm birth, spontaneous abortions, premature rupture of membranes, placenta previa, ectopic pregnancies and abruptio placentae (Eastham and Gosakan, 2010). It has been established that active smoking during pregnancy induces early morphological changes of the placenta, resulting in a reduced volume of maternal intervillous space and a reduced volume and surface area of fetal capillaries (Hofhuis *et al.*, 2003). These morphological changes results in a decrease in the diffusion coefficient of oxygen across the placenta, and the outcome seem to be all-or-none, and not dose dependent effect. The fetus of pregnant smokers therefore suffers from chronic hypoxic stress. These factors may be responsible for alteration of weight, length and head circumference neonate (Deng *et al.*, 2013).

Cigarette smoke affects various physiological processes including secretion of pituitary, thyroid, adrenal and sex hormones in humans. These are mediated chiefly through pharmacological actions of nicotine and as a consequence of smoking-induced stress (Kapoor and Jones, 2005). Cigarette smoke is believed to affect testosterone levels in males and it is likely due to alterations in globulin-binding affinity

rather than a direct effect of nicotine on testosterone. It has also been established to have an effect on serum progesterone and have anti-estrogenic effect in women, which may be probably due to changes in hepatic estrogen metabolism induced by smoking. Some estrogen-dependent physiological processes such as the menstrual cycle are affected as it causes an increase in menstruation length. This leads to an increased risk of anovulation which increases with smoking intensity. These decreases fertility capability and decreases age of menopause (Kapoor and Jones, 2005).

The intra uterine development duration is a critical period for human being, as any adverse prenatal exposure and condition may affect normal growth, development and physiology of the fetus in the intra uterine life as well as postnatal health and behavior. Nicotine is generally fat soluble and of small molecular size. These characteristics help it to cross the placenta and concentrate the fetal blood and amniotic fluid. Also, it is detectable in breast milk during lactation (Lambers and Clark, 1996). Prenatal exposure to cigarette smoke can affect the fetus in utero and also after birth. The health risks include pregnancy complication and premature birth. Maternal cigarette smoking is associated with elevated prevalence of low birth weight (Suzuki et al., 2008). Older smoker women are more susceptible to the effects of maternal smoking (Zheng et al., 2016). Infants of maternal smokers weigh on average 200 g less those born by none smoker women. Also, these infants are up to three times more likely to die of sudden infant death syndrome (Bajanowski et al., 2008).

Awobajo et al (2015) reported significant reduction of sex hormones of pregnant rats exposed to cigarette smoke and the decline in hormones increased with progression of pregnancy. We reported earlier that prenatal exposure to passive cigarette smoke caused a decrease in fasting glucose level and increased the serum nitric oxide of experimental rats (Obembe et al., 2010). Since nitric oxide is an important physiological signaling molecule and its availability is a marker of oxidative stress, we undertook this study to examine the effects of prenatal exposure to passive cigarette smoke in Wistar rats on oxidative stress biomarkers. The effects on sex hormones and anogenital distance were also examined.

MATERIALS AND METHODS

Experimental animals

Wistar rats (20 male and 10 female) were obtained from and kept in well aerated cages with solid floors covered with wood shavings in the Animal House of the College of Health Sciences, Osun State University, Osogbo with a constant 12 hour light 12 hour dark cycle. Nulliparous female rats (180 – 200 g) aged 14 – 16 weeks were used for this study, while the male rats (220 – 240 g) were of proven fertility. The rats were fed with standard pellets purchased from Ladokun livestock feeds, Ibadan, which contained 21% protein, 35% fat, 30% carbohydrate, 0.8% phosphorus and 0.8% calcium and had access to water *ad libitum*. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and Use of animals (2002) as amended and were approved by the Research Ethics Committee of the College of Health Sciences, Osun State University, Osogbo Nigeria.

Female rats were allowed to acclimatize for 10 days and thereafter randomly assigned to two groups (1 and 2, n =5). Group 1 was exposed to passive smoke from an idling cigarette in an exposure chamber throughout duration of pregnancy while Group 2 served as the control (no cigarette smoke exposure during pregnancy).

Mating

Ovulation was induced by administration of a single oral dose of Stilboestrol (0.042 mg/kg bw), an orally active synthethic oestrogen (Obembe *et al*., 2010). Thereafter, the female rats were cohabited with male rats at the ratio of 1:2 (Obembe *et al*., 2012). The presence of sperm plug in vaginal of the female rats confirmed mating, and was recorded as the first day of pregnancy. Pregnancy was obvious on day 14 of pregnancy and each pregnant rat was isolated in an exclusive cage.

Cigarette Smoke Exposure

Female group 1 rats were exposed to cigarette smoke from an idling cigarette in an exposure chamber at 9.00 am, over a period of 30 minutes every day from the day 1 to day 21 of gestation. Three cigarette sticks was used consecutively for each animal group per exposure (30 mins), per day (Obembe *et al*., 2010). London king size (menthol) cigarettes produced by London tobacco company was used. Each cigarette stick contains 1.2 mg nicotine and 14.9 mg tar.

Measurement of Morphometric Variables

On postnatal day 1, pups were weighed using a digital weighing scale (EasyWay Medical, England) and morphometric variables were taken by a digital Vernier caliper (Mitutoyo, Kawasaki, Japan). The morphometric variables measured were the anogenital distance (AGD), head diameter, abdominal diameter, crown rump length and total body length. These measurements were taken by two different trained technologists who were blinded to the study design so as to avoid bias. Mean values of their records were taken**.** On PND 21, litter size of dams was standardized, so as to eliminate concerns of disparity in nutrient and milk availability to pups. Measurements were repeated at the 6th week of postnatal life (PND 42) and then sacrificed.

Animal Sacrifice

On PND 42, the dams were discarded while the male offspring were sacrificed after overnight fast by cervical dislocation under sodium pentobarbital (30 mg/kg i.p) anesthesia and blood was obtained by cardiac puncture (Institutional Animal Care and Use Committee, 2013). Blood was centrifuged at 3000 rpm for 5 minutes, the clear supernatant, that is, the serum was obtained and was stored at -20 ˚C. The reproductive organs (testis, epididymis, seminal vesicle, prostate gland) and visceral organs of the male litters were dissected and weighed (Obembe *et al*., 2014).

Hormone and Oxidative Stress Assay

One male offspring was randomly selected amongst the pups from each dam. Serum obtained from these male offspring was used for hormonal analysis. Another set of male pups were randomly selected as above from each dam and serum obtained from these was assayed for oxidative stress biomarkers. Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assayed using enzyme-linked immunoassay method (ELISA). Oxidative stress was assayed spectrophotometrically as follows - malondialdehyde (MDA) according to Uchiyama and Mihara (1978), superoxide dismutase (SOD) according to Sun and Zigman (1978) and Catalase level was done as described by Aebi (1984).

Serum Lipid Profile and Liver function Biomarkers

Total cholesterol, triglyceride, high-density lipoproteins (HDL) and low-density lipoprotein (LDL) in serum obtained were determined by enzymatic colorimetric method as described by Rifai *et al* (1999). The determination was based on the formation of colour after enzymatic hydrolysis and oxidation. The indicator quinoneimine used was formed from H_20_2 and 4-amino-antipyrine in presence of phenol. All biochemical parameters were assayed kits obtained from Diasys Diagnostic systems (Istanbul, Turkey) on a Statfax Diasys 1904 plus Biochemical Analyzer.

The serum levels of alkaline phosphatase (ALP), alanine aminotrasferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Moss and Henderson (1999), using the respective available kits.

Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). Sample size for each treatment group is stated in respective table or figures. Data obtained were analyzed using Student's t-test for comparison between means of the two groups with similar sample size and one way analysis of variance (ANOVA) using SPSS version 16 (SPSS Inc., Chicago, IL) for comparing means of unequal sample size. $P<0.05$ was considered as significant.

RESULTS

Exposure of pregnant dams to cigarette smoke had no significant effect on litter size. Dams exposed to passive cigarette smoke during pregnancy had a litter size of (7 ± 0.7) while the control dams had a litter size of (7.2 ± 0.7). Prenatal exposure to passive cigarette smoke significantly decreased crown rump length and total body length but had no effect on total body weight, head diameter and abdominal diameter on postnatal day 1 (Table 1). However, a significant decrease in total body weight and crown rump length was observed at 6th week postnatal life. Head diameter, abdominal diameter and total body length were not affected (Table 1).

Table 1: Morphometric variables of offspring at postnatal day 1 and at 6th week postnatal life.

	Treated (PND1, n = 35)	**Control (PND1, n = 36)**	**Treated (6th wk, n = 27)**	**Control (6th wk, n = 27)**
Litter Size	7.0 ± 0.7	7.2 ± 0.7	5.4 ± 0.24	5.4 ± 0.24
Total Body Weight (g)	5.13 ± 0.12	5.65 ± 0.13	75.33 ± 3.45*	91.07 ± 2.26
Head diameter (mm)	11.05 ± 0.30	11.15 ± 0.13	18.90 ± 0.28	18.68 ± 0.30
Abdominal Diameter (mm)	12.83 ± 0.37	13.72 ± 0.29	23.66 ± 0.45	24.14 ± 0.25
Crown Rump Length (mm)	41.73 ± 0.66*	47.68 ± 0.76	131.87 ± 2.83*	142.45 ± 1.32
Total Body Length (mm)	54.00 ± 1.02*	61.35 ± 0.95	240.87 ± 3.84	247.19 ± 2.66

Values are Mean ± SEM, *P<0.05.

Table 2: Anogenital distance of the male offspring at PND1 and 6th week postnatal life

	Treated (mm)	**Control (mm)**
PND1	3.31 ± 0.21	3.48 ± 0.14
6th week	22.29 ± 0.51*	28.14 ± 0.57

Values are Mean ± SEM, *P<0.05.

Intrauterine cigarette smoke exposure also had no significant effect on anogenital distance on postnatal day 1, but after 6 weeks of postnatal life, male offspring of pregnant smoker rats had a significantly shorter anogenital distance (Table 2). At 6th week, a significant decrease in total body weight and absolute organ weights of heart and kidney were observed, while absolute weights of the lungs, liver and spleen were not affected (Table 3). Also, the absolute weights of testis, epididymis and seminal vesicles of male offspring of the pregnant smoker rats were significantly lower than offspring of the control. Weight of prostate gland was not affected. However,

Table 3: Total body weight and visceral organ weights of male offspring at 6th week postnatal day

	Treated	Control
Total body weight (g)	73.09 ± 3.01*	99.68 ± 4.78
Lungs (g)	0.79 ± 0.06 (1.09± 0.08)*	0.84 ± 0.02 (0.85 ± 0.03)
Liver (g)	2.89 ± 0.16 (3.95 ± 0.18)	3.46 ± 0.54 (3.98 ± 0.11)
Heart (g)	0.33 ± 0.02* (0.46 ± 0.02)	0.55 ± 0.19 (0.40 ± 0.02)
Kidney (g)	0.37 ± 0.02* (0.50 ± 0.02)	0.46 ± 0.02 (0.47 ± 0.02)
Spleen (g)	0.39 ± 0.03 (0.53 ± 0.04)	0.55 ± 0.19 (0.54 ± 0.17)
Testis (g)	0.32 ± 0.03* (0.45 ± 0.05)	0.56 ± 0.05 (0.55 ± 0.00)
Epididymis (g)	0.06 ± 0.01* (0.09 ± 0.01)	0.11 ± 0.01 (0.11 ± 0.01)
Seminal Vesicle (g)	0.05 ± 0.01* (0.08 ± 0.01)	0.11 ± 0.02 (0.11 ± 0.01)
Prostate Gland (g)	0.04 ± 0.01 (0.06 ± 0.01)	0.06 ± 0.01 (0.07 ± 0.01)

Values are Mean ± SEM, n=13. Absolute organ weights, (% Relative organ weights). *P<0.05.

Table 4: Serum Lipid Profile of male offspring of pregnant smoker rats

	Treated	Control
Total Cholesterol (mmol/l)	2.9±0.03*	2.22±0.16
Triglyceride (mmol/l)	1.62±0.08*	1±0.14
Low density lipoprotein (mmol/l)	1.38±0.12	1.4±0.18
High density lipoprotein (mmol/l)	0.74±0.02	0.72±0.02

Values are Mean ± SEM, n = 5. *P < 0.05.

Table 5: Liver function biomarkers of male offspring of pregnant smoker rats

	Treated	Control
Total bilirubin (µmol/l)	1.76±0.09	1.34±0.10
Conjugated bilirubin (µmol/l)	0.32 ± 0.03	0.26±0.05
Albumin (g/l)	23.20 ± 2.15	20.00±1.14
ALT (U/L)	13.60 ±1.72	9.60 ± 0.92
AST (U/L)	19.80 ±1.02	17.80 ± 0.86
ALP (U/L)	87.80 ±3.49	90.20 ± 3.72

Values are Mean±SEM, n=5 (ALT= Alanine aminotransferase, AST=Aspartate aminotransferase, ALP=Alkaline phosphatase) * P < 0.05

the percentage relative organ weight of the lungs of male offspring of pregnant rats prenatally exposed to cigarette smoke was significantly higher than the control (Table 3). The relative weight of other visceral organs – liver, heart, kidney, spleen and reproductive organs- testis, epididymis, seminal vesicles, prostate gland were not statistically affected.

Testosterone, LH and FSH levels of the offspring of pregnant smoker rats were not significantly different from control (Fig 1). A significant decrease in serum sodium dismutase and catalase levels and a significant

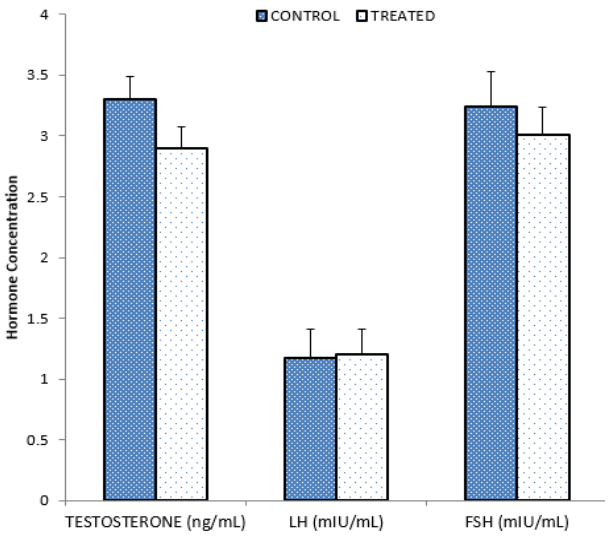

Figure 1: Reproductive hormones of male offspring at postnatal week 6. Values are expressed as Mean±SEM, n=5.

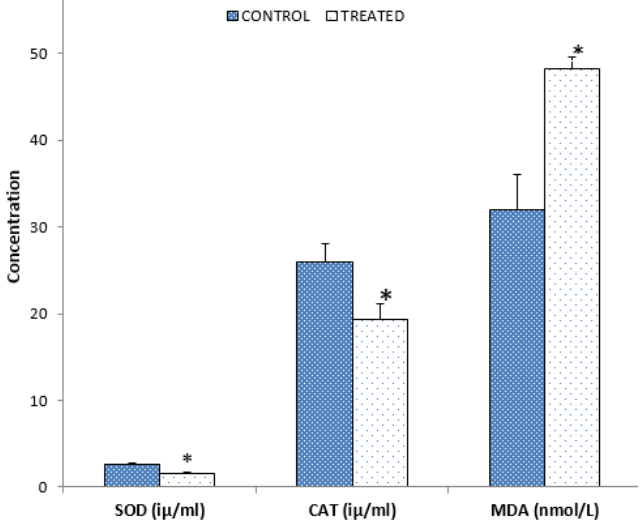

Figure 2: Oxidative stress biomarkers of the male offspring after six weeks. SOD – superoxide dismutase, CAT – Catalase, MDA – Malondialdehyde. Values are expressed as Mean ± SEM, n=5. *P<0.05.

increase in malondialdehyde levels were however observed (Fig 2). Also, serum total cholesterol and triglyceride levels were significantly higher in offspring of treated rats. However, HDL, LDL, conjugated and total bilirubin, albumin, ALT, AST and ALP were not affected (Table 4 and 5).

DISCUSSION

Cigarette smoke has been established to have several adverse effects on reproductive health of humans, though it is still unclear if these detrimental effects are entirely due to nicotine alone. In women, cigarette smoke has been reported to have anti estrogenic effects, probably due to changes in hepatic oestrogen metabolism induced by smoking. These anti estrogenic effects cause problems with the menstrual cycle, anovulation, early menopause, problems with the

placenta and adverse effects on fetal growth and development (Windham *et al*., 2005).

Several studies have reported low birth weight as an effect of intrauterine cigarette smoke. However, the degree of low birth weight associated with gestational cigarette smoking is dependent on the exposure duration and sticks of cigarette used (Omotoso, Adeyemi, 2014). The lack of effect of prenatal cigarette smoke on birth weight on postnatal day 1 observed in this study could therefore be ascribed to the comparatively low exposure duration of 30 minutes only. However, at the end of 6th week (PND 42), the body weight of offspring of smoker rats was significantly lower than the corresponding control (Table 1).

Prenatal cigarette smoke had no effect on head diameter and abdominal length of pups, both at postnatal day 1 and at six weeks after birth. Menounou (2011) reported that measuring neonatal head size can serve as a surrogate measurement of brain size and brain growth. Also, Meldere *et al* (2015) reported that measuring neonatal abdominal size serves as an important diagnostic tool in evaluating diseases of the abdominal cavity most especially necrotizing enterocolitis. These could be inferred to mean that intrauterine cigarette smoke at dose administered had no effect on brain size, brain growth nor induce development of abnormalities of abdominal structures. However, crown rump length and total body length of treated rats were significantly reduced on PND 1 and crown rump length was also shorter than control rats after six weeks of postnatal life. Dwivedi and Verma (2015) reported that length at birth may have an effect on the future height of a baby. The shorter crown rump length at 6th week therefore means that prenatal exposure to cigarette might affect height of the neonate in later life.

Swan *et al* (2005) reported a shorter AGD in the male infants of women exposed to increasing levels of known endocrine disruptors, suggesting an impairment of *in utero* male genital development. Furthermore, Einsenberg *et al* (2011) correlated AGD with a male human's fertility potential and reported a significantly lower sperm density, motile sperm count and sperm motility in men with shorter AGD. Also, males with short AGD were reported to have seven times the chance of being sub-fertile than those with a longer AGD as it is linked to both semen volume and sperm count and may give rise to conditions like cryptorchidism, hypospadiasis and testicular tumors in adulthood (Hsieh *et al.*, 2008). Shorter male AGD observed in this study at 6th week postnatal life therefore indicates that prenatal exposure to cigarette smoke predisposes to male infertility. The potential male infertility observed as short AGD in this study was not accompanied by hormonal derangement as no significant effect was observed on the testosterone, LH and FSH. This corroborates the work of Parra *et al* (2016) who reported lack of correlation between decline in AGD and testosterone level of undergraduate Spanish men. Also Eisenberg *et al* (2011) earlier reported lack of association between serum levels of male sex hormones (testosterone, LH and FSH) and shorter AGD and penile length observed in infertile men of stratified race. They noted that though AGD was significantly shorter in the infertile men, no significant difference was observed in FSH, LH and testosterone when compared with fertile men.

The reproductive functions are controlled by complex interactions between the reproductive organs and sex hormones. These interactions are however important for processes such as spermatogenesis, development of sex and accessory sex organs, sexual performance and male fertility in general. Though prenatal smoking reduced absolute testicular weight, it caused an apparent decline in serum levels of testosterone and FSH, however this was statistically insignificant. The decline in testicular weight may therefore not be solely caused by decreased testosterone level. The apparent decline in testosterone appeared to be transiently compensated for by an apparent negative feedback increase in LH.

The weight of reproductive organs usually provides a useful reproductive risk assessment in experimental studies (Raji *et al*., 2005). Prenatal cigarette smoke exposure reduced the absolute weight of the testis, epididymis and seminal vesicle. This further buttress the risk to fertility capability of male offspring of pregnant smokers. However, no effect was observed on prostate gland weight. The absolute weights of the lung, liver and spleen were also not affected but it significantly reduced weight of heart and kidney. The reduction in heart weight is consistent with the research carried out by Omotoso and Adeyemi (2014) who reported that intra uterine cigarette smoke exposure resulted in significant decline in the heart weight of juvenile rats, coupled with

alterations in the cardiac muscle which could affect cardiac functions and lead to cardiac disorder. These changes were suggested to be due to apoptosis or premature exit of cardiomyocytes from cell cycle, as occurs in hypoxia to compensate for reduced oxygenation. The reduction in kidney weight is not surprising as maternal smoke exposure leads to developmental abnormalities in the kidney in early life and functional deterioration in adulthood though the mechanism underlying this is still unclear as reported by Al-Odat *et al* (2014). The observed increase in relative lung weight of treated rats may be due to emphysema or otherwise. Future studies will include histopathological examination of the lungs amongst others in other to account for this. Metabolism may not have been affected, as no significant effect was observed on liver function biomarkers.

Cigarette smoke contains a wide range of xenobiotics, including oxidants and oxygen free radicals such as superoxide radicals, nitric oxide and hydroquinones that can increase lipid peroxidation and promote oxidative damage. Its toxicity is enhanced by the stimulation of reactive oxygen species production by neutrophil. Cigarette smoke has been calculated to contain 10^{17} oxidant molecules per puff, of which 10^{14} are reactive oxygen species and some of these have been documented to cross the placental barrier (Church and Pryor, 1985; Pryor and Stone, 1993; Perera *et al*., 2004). These reactive oxygen species or oxidants are cleared off by antioxidants such as catalases and superoxide dismutases. Oxidative stress results from the imbalance between the generation of reactive oxygen species and the antioxidant defense system in favor of the former. Prenatal exposure to cigarette smoke decreased the serum catalase activity and sodium dismutase (SOD) activity thereby promoting oxidative stress (Fig 4). This result correlates with reports of Kar *et al* (2008) on smoking induced oxidative stress in the serum and neutrophil. Catalase acts as a preventive antioxidant and SOD is a chain breaking antioxidant and they both play key role in the protection against the injurious effects of lipid peroxidation (Dinvoko-Kotsova, 2002). Where SOD stops its function, catalase exerts its function. The primary role of catalase is to scavenge hydrogen peroxide (H_2O_2) that has been generated by free radicals or by SOD in removal of superoxide anions and convert it to water (Ribbiere *et al*., 1992). In this study, the decrease in catalase activity is suggested to be due to excess H_2O_2 produced by smoking or SOD inhibition.

Due to oxidative stress, the ROS causes a progressive damage to lipid macromolecules in a process called lipid peroxidation. Peroxidation of membrane of lipids leads to loss of membrane fluidity and elasticity, impaired cellular functioning and even cell rupture. Malonialdehyde (MDA) is the terminal product of lipid peroxidation and serves as its index. This biomarker of oxidative stress was significantly increased ($p<0.05$) due to prenatal cigarette smoke exposure. Lipid peroxidation can indirectly reflect the status of the metabolism of free radicals, the degree to which the cells are attacked by free radicals and the degree to which lipid is peroxidated (Mirela *et al*., 2012). The lower levels of catalase and SOD antioxidants and higher levels of MDA due to prenatal cigarette smoke signifies increased oxidative stress and an increased risk of the development of chronic diseases. Kummerow *et al* (2000) demonstrated that circulating levels of lipid oxidation products correlated with the degree of coronary artery stenosis. In several studies, lipid oxidation was linked to cardiac disease and atherosclerosis, the primary cause of heart disease. Detrimental effects on offspring vascular function were also demonstrated in 4 week old rats exposed to sidestream smoke *in utero,* and they had increased aortic ring sensitivity to phenylephrine-induced vasoconstriction and reduced maximum endothelium-dependent acetylcholine-induced relaxation (Sheung *et al*., 2009).

Increased total cholesterol and triglyceride levels of offspring of pregnant smokers further corroborates a correlation between maternal smoking and offspring dyslipidemia. This reflects proatherogenic phenotype that may culminate in initiation and progression of atherosclerotic plaques, and therefore greater tendency towards increased risk for atherosclerosis and other cardiovascular diseases (Sheung *et al*., 2009; Glass, 2001).

In conclusion, prenatal exposure to passive cigarette smoke induces oxidative damage that may predispose to male infertility at adulthood as evidenced by congenitally short AGD. Therefore, induction of free radicals by constituents of cigarette smoke may probably explain the mechanism by which it causes deleterious toxicity to reproductive health of the fetus. Offspring of pregnant smokers are also predisposed to

increased risk of cardiovascular diseases as a result of the smoke induced dyslipidemia. Summing up, these findings further reiterate the call for cessation of cigarette smoking during pregnancy.

Disclosure

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REFERENCES

Aebi H. Catalase in vitro. *Methods in Enzymology* 1984; 105, 121-126.

Al-Odat I, Chen H, Chan YL, Amgad S, Wong MG, Gill A. The impact of maternal cigarette smoke exposure in a rodent model on renal development in the offspring. *PLoS ONE* 2014; 9 (7): e103443 doi: 10. 1371/journal.pone.0103443

Anderka M, Romitti PA, Sun L, Druschel C, Carmichael S, Shaw G. Patterns of tobacco exposure before and during pregnancy. *Acta Obstetricia et Gynecologica Scandinavica* 2010*;* 89 (4), 505-514.

Awobajo FO, Oyesola OA, Amah GO, Olakitan HA, Akinyemi TM. Cigarette smoke pollution promotes oxidative stress imbalance and hormonal changes affecting pregnancy outcome in rats. *J. Afr. Ass. Physiol. Sci.* 2015; 3 (2), 110 – 117.

Bajanowski, T, Brinkmann B, Mitchell EA et al. Nicotine and cotinine in infants dying from sudden infant death syndrome. *International Journal of Legal Medicine* 2008; 122 (1), 23–28.

Buczkowski K, Marcinowicz L, Czachowski S, Piszczek, E. Motivations toward smoking cessation, reasons for relapse, and models of quitting: results from a qualitative study among former and current smokers. *Patient preference and adherence* 2014; 8, 1353.

Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect*. 1985; 64, 111-126. 10.2307/3430003.

Coleman T, Britton J, Thorton J. Nicotine replacement therapy in pregnancy. *BMJ* 2004; 328, 965-966.

Deng K, Liu Z, Lin Y et al. Periconceptional paternal smoking and the risk of congenital heart defects: a case-control study. *Birth Defects Res A Clin Mol Teratol.* 2013; 97:210-216.

Dinvoko-Kotsova AT. Protection against cancer by plant phenyl propenoids: induction of mammalian anti-carcinogenic enzymes. *Mini Rev Med Chem*. 2002; 2, 595-610.

Dwivedi D, Verma A. Maternal and foetal factors affecting the birth length of healthy newborn babies: An observational study. *Indian J Child Health* 2015; 2(3), 109-112.

Eastham R, Gosakan R. Smoking and smoking cessation in pregnancy. *The Obstetrician & Gynaecologist* 2010; 12(2), 103-109.

Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI. The Relationship between Anogenital Distance, Fatherhood, and Fertility in Adult Men. *PLoS ONE* 2011; 6(5): e18973. doi:10.1371/journal.pone.0018973.

Glass CK, Witztum JL. Atherosclerosis, The road ahead. *Cell*. 2001; 104(4), 503–516.

Hofhuis W, De Jongste JC, Merkus PJFM. Adverse health effects of prenatal and postnatal tobacco smoke exposure on children. *Archives of disease in childhood* 2003; 88(12), 1086-1090.

Hsieh MH, Breyer BN, Eisenberg ML, Baskin LS. Associations among hypospadias, cryptorchidism, anogenital distance, and endocrine disruption. *Curr Urol Rep.* 2008; 9,137–142.

Institutional Animal Care and Use Committee (IACUC). Austin Guidelines for the use of cervical dislocation for Rodent. The University of Texas, 2013; pp 1-2.

Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. *European Journal of Endocrinology* 2005; 152, 491–499.

Kar MS, Subhasis D, Sankar KD, Somenath R. Smoking induced oxidative stress in serum and neutrophil of the university students. *Al Ameen J Med Sci*. 2008; 1(1), 20-31.

Kummerow FA, Olinescu RM, Fleischer L, Handler B, Shinkareva SV. The relationship of oxidized lipids to coronary artery stenosis. *Atherosclerosis*. 2000; 149(1), 181–190.

Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. *Seminars in Perinatology* 1996; 20(2), 115–126.

Meldere I, Urtans V, Petersons A, Abola Z. Measurement of abdominal circumference in preterm infants. *BMC Research Notes* 2015; 8, 725.

Menounou A. Head Size: is it important? *Peadiatric Neurology ACNR*. 2011; 11:2

Mirela P, Andriana M, Ilean D. Oxidative Stress and Antioxidant Status in Hypo and Hyperthyroidism. In *Antioxidant enzyme* (eds Mohammed AE), 2012; pp. 197-236. InTech Open Access publishers. DOI: 10.5772/51018

Moss DW, Henderson AR. Clinical enzymology. In *Tietz Textbook of Clinical chemistry*, 3rd edition (eds Burtis CA, Ashwood ER), 1999; pp. 617–721. W. Saunders Company, Philadelphia.

Obembe OO, Olopade JO, Raji Y. Implication of *HongrES1* protein in Quassin-induced male reproductive abnormality. *Endocrinology and Metabolic Syndrome* 2014; 3(2), 128.

Obembe OO, Onasanwo SA, Raji Y. Preliminary study on male reproductive toxic effects of

Buchholzia coriacea seed extract in male rats. *Nigerian Journal of Physiological Sciences* 2012; 27: 165-169.

Obembe OO, Ukwenya VO, Ige AO, Oyeyipo IP, Fasanmade AA. Prenatal exposure of pregnant rats to cigarette smoke and nicotine: Effects on nitric oxide and fasting glycemia in treated rats and untreated neaonates with Vitamin C. *Electron J. of Biomed*. 2010; 3: 16-20.

Omotoso GO, Adeyemi HA (2014). Effect of Prenatal cigarette smoke exposure on the architecture of the heart in juvenile Wistar rats. *Anatomy Journal of Africa* 2014; 3 (3), 405-411.

Parra MD, Mendiola J, Jorgensen N, Swan SH, Torres-Cantero AM. Anogenital distance and reproductive parameters in young men. *Andrologia* 2016, 48, 3-10.

Perera FP, Tang D, Tu YH et al. Biomarkers in maternal and newborn blood indicate heightened fetal susceptibility to pro-carcinogenic DNA damage. *Environ Health Perspect*. 2004; 112,1133–1136.

Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann NY Acad Sci*. 1993; 686,12–27.

Raji Y, Ifabunmi SO, Akinsomisoye OS, Morakinyo AO, Oloyo AK. Gonadal response to antipsychotic drugs: chlorpromazine and thioridazone reversibly suppress testicular functions in male rats. *International J. Pharmacol*. 2005; 1(3), 287-292

Ribbiere C, Hininger I, Rouach H, Nordmannb R. Effect of chronic ethanol administration on free radical defense in rat myocardium. *Biochem Pharmacol*. 1992; 44, 1495-1500.

Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Tietz Textbook of Clinical chemistry, 3rd edition (eds Burtis CA,

Ashwood ER), 1999; pp. 809-861. W. Saunders Company, Philadelphia.

Sheung PN, Daniel JC, Aruni B, Duane DB, Jessica L, Judith TZ. Prenatal Exposure to Cigarette Smoke Induces Diet- and Sex-Dependent Dyslipidemia and Weight Gain in Adult Murine Offspring. *Environ Health Perspect*. 2009; 117(7), 1042–1048.

Sun M., Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Analytical Biochemistry* 1978; 90, 81-89.

Suzuki K, Tanaka T, Kondo N et al. Is maternal smoking during early pregnancy a risk factor for all low birth weight infants? *J Epidemiol*. 2008; 18:89–96.

Swan SH, Main KM, Liu F. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* 2005; 113, 1056–1061.

Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry* 1978; 86,271-278.

Wickstrom R. Effects of nicotine during pregnancy: human and experimental evidence. *Current neuropharmacology*, 2007; 5(3), 213-222.

Windham GC, Mitchell P, Anderson M, Lasley BL. Cigarette Smoking Effects on Hormone function in Premenopausal Women. *Environmental Health Perspectives* 2005; 113(10), 1285-1290.

World Health Organization. WHO global report on trends in prevalence of tobacco smoking. *World Health Organization* 2015; Chicago USA.

World Medical Association, American Physiological Society. Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp physiol*. 2002; 283: R281-R283.

Zheng W, Suzuki K, Tanaka T, Kohama M, Yamagata Z. Association between Maternal Smoking during Pregnancy and Low Birthweight: Effects by Maternal Age. *PLoS ONE* 2016; 11(1): e0146241. doi:10.1371/journal.pone.0146241s.