

ORIGINAL COMMUNIATION

EFFECT OF PRENATAL CIGARETTE SMOKE EXPOSURE ON THE ARCHITECTURE OF THE HEART IN JUVENILE WISTAR RATS

*OMOTOSO Gabriel Olaiya, ADEYEMI Henry Adebiji

Correspondence to Gabriel Omotoso, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria. gabrielolaiya@yahoo.com

ABSTRACT

This study aimed at determining the effects of cigarette smoke exposure during pregnancy on the morphology of the developing heart. Twelve adult female Wistar rats were used for the study. The animals were time mated and grouped into three: a control (A) and two treatment groups (B and C). The treatment groups were exposed to smoke from 2 sticks of cigarette (St. Moritz®) on days 8 – 14 (Group B) and days 15-21 (Group C) of the gestation. Each stick of cigarette contained an average of 0.8375 g of tobacco. The pregnant rats were allowed to litter, and at postnatal day 15, pups from all the groups were sacrificed by cervical dislocation, the chest wall was dissected and the heart was excised and either placed in 10% formal saline fixative for histological preparation using Haematoxylin and eosin staining techniques, or 0.25 M cold sucrose solution and homogenised for enzyme study on the activity of lactate dehydrogenase using the colorimetric method. The pups exposed to cigarette smoke *in utero* had low birth and body weights, and markedly reduced cardiac weight. Considerable disruptions of the architecture of the heart were also seen, with poorly stained and reduced sizes of cardiac myocytes. Prenatal cigarette smoke exposure impairs the normal development of the heart with subsequent possibility of postnatal suboptimal functionality.

Keywords: Gestational cigarette exposure, heart, histology, lactate dehydrogenase

INTRODUCTION

Cigarette smoking still continues to be a major public health concern (Amos *et al.*, 2012), despite the awareness of the numerous health implications associated with it. As the incidence of tobacco use gradually declines in the West, the rate increases in the third World (Pampel *et al.*, 2011). In some parts of the Globe, the percentage of women smokers is quite high (Hernández-Martínez *et al.*, 2012), and about 25% of women of childbearing age use tobacco (Cornelius *et al.*, 2012).

Numerous studies have reported the adverse effects of cigarette smoking on body organs and systems, as well as the

developing organs, when used by mothers during pregnancy. Maternal tobacco use has been implicated in the causation of foetal malformations including increased risk of congenital heart defects (Deng *et al.*, 2013), including cardiac ventricular abnormalities, such as thickened septum and the appearance of an additional ventricle (Woods and Raju, 2001; Brandini *et al.*, 2005; Malik *et al.*, 2008;). Moreover, occurrence of conotruncal heart defects, septal defects and left ventricular outflow tract obstructions has been observed in association with paternal smoking (Deng *et al.*, 2013). There are however few studies on the effect of smoking on the

histomorphology of the heart.

The current study was aimed at determining the effect of cigarette smoke on the histology of the developing heart.

MATERIALS AND METHODS

Laboratory Animals and Grouping

Twelve (12) adult female Wistar rats (*Rattus norvegicus*) of mean weight 147.67 ± 4.33 g were used. They were housed in the Animal House of the Department of Anatomy, University of Ilorin, at room temperature. Standard rat pellets and water were given throughout the period of the experiment. The oestrous cycle of the rats was determined through vaginal smear method and mating was carried out at the proestrous phase.

The rats were grouped into 3- Group A: Control, and Treatment Groups B and C.

Administration of Cigarette Smoke

Smoking chambers were constructed indigenously made of a cylindrical bucket of 9 litres capacity with about 1cm diameter hole on the lid. St. Moritz® cigarette brand was used, and each stick contained 0.8375 g of tobacco.

Each rat in the treatment groups was exposed to 2 sticks of cigarette once daily for 7 days on days 8-14 (Group B; signifying the 2nd week of gestation) or days 15-21 (Group C; signifying the 3rd week) of gestation.

Animal Sacrifice and Sample Collection

After the pregnant rats littered, the pups were allowed to grow till postnatal day 15 (P15) before they were sacrificed by cervical dislocation. The tissues were harvested, and those for histological studies were fixed in 10% formal saline, while those for enzyme studies were placed in sucrose solution for homogenisation.

Histological and Enzyme Studies

The tissues fixed in 10% formal saline were used for histological preparations. They were appropriately processed, and tissue staining was carried out using the Haematoxylin and eosin technique (Junqueira and Carneiro, 2006). Tissues for enzyme studies were placed in 0.25 M cold sucrose solution and homogenised using a homogeniser, and centrifuged at a speed of 5,000 revolutions per minute for 5 minutes, and the supernatant was collected using the Pauster's pipettes. Standard biochemical diagnostic kits was used to spectrophotometrically assay the level of activity of lactate dehydrogenase (LDH). Data were statistically analysed by student's t-test using the SPSS software.

RESULTS

Low birth weights were recorded in the treatment groups, with the least seen in Group C exposed to cigarette smoke in the 3rd week of gestation (Figure 1). However, at P15 before sacrifice of the pups, the body weights of Group C animals have surpassed those of Group B, exposed to

cigarette in the 2nd week of gestation; although both treatment groups still had lower body weights compared to the Control (Figure 1).

The weight of the heart was markedly reduced in the treatment groups. However, the pups exposed to cigarette smoke in the

2nd week of gestation had as much as twice less than the weight of those exposed in the 3rd gestational week, with statistically significant difference [$p < 0.05$] (Figure 2).

The level of activity of LDH in both treatment groups markedly increased compared to the Control, with a higher activity recorded in animals exposed to cigarette smoke in the 2nd gestational week (Figure 3).

Histological Observation of Cardiac Musculature

The control showed normochromic cells and a regular pattern of arrangement of cardiac muscle fibres. Rats exposed to cigarette smoke in the 2nd week of gestation had moderately hypochromic cardiac myocytes, smaller in size compared with the control, with some degree of disruption of arrangement of the muscle fibre, while rats exposed in the 3rd week of gestation had considerable disruption in the microarchitecture of the heart and irregular fibrillar network; cardiac myocytes were also normochromic, small-sized and indistinct.

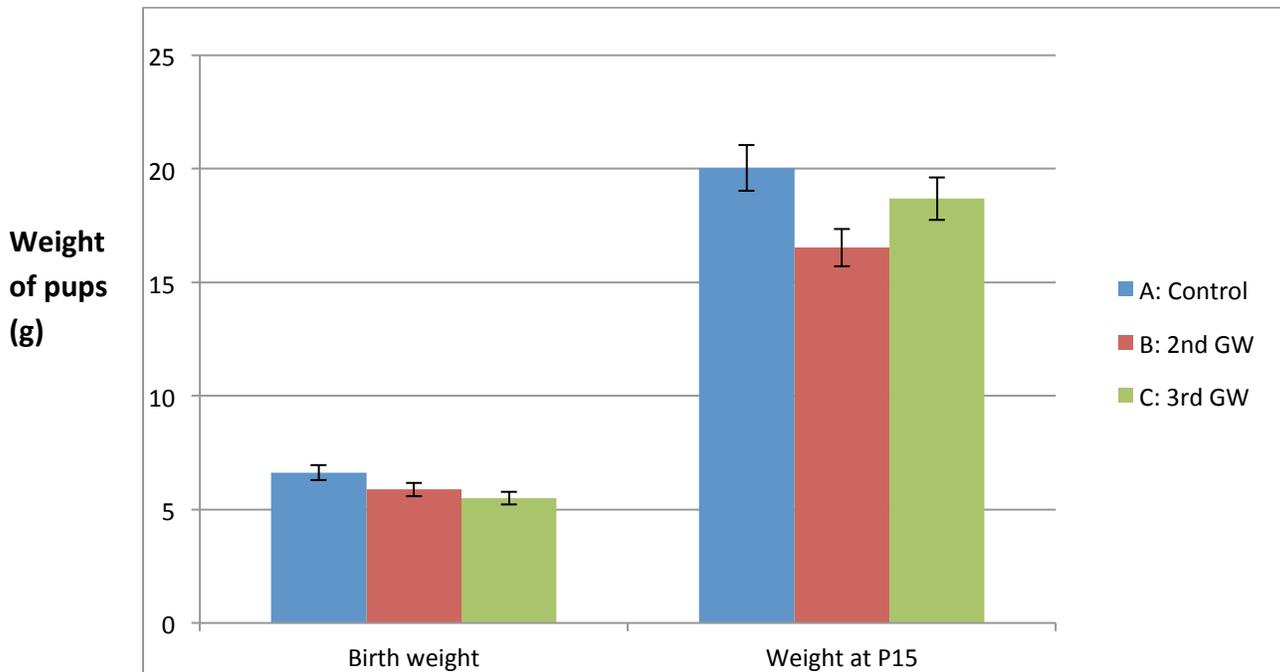


Figure 1: Weight of pups at birth and postnatal day 15 (P15) before sacrifice.

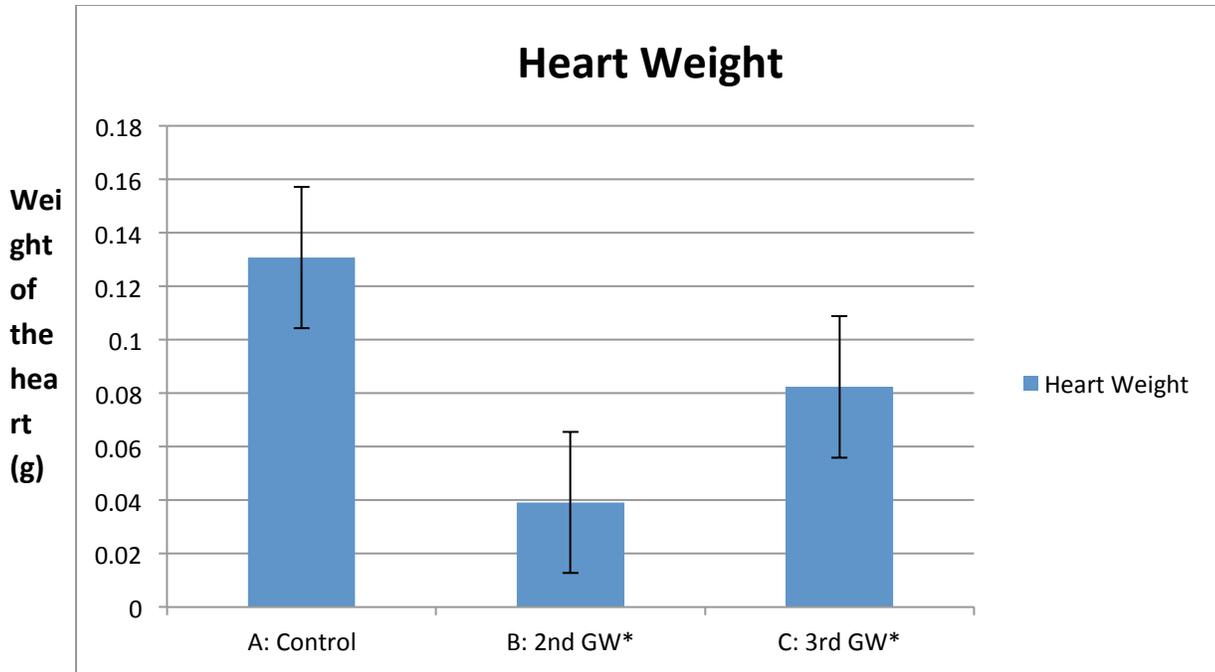


Figure 2: Weight of cardiac tissue at P15

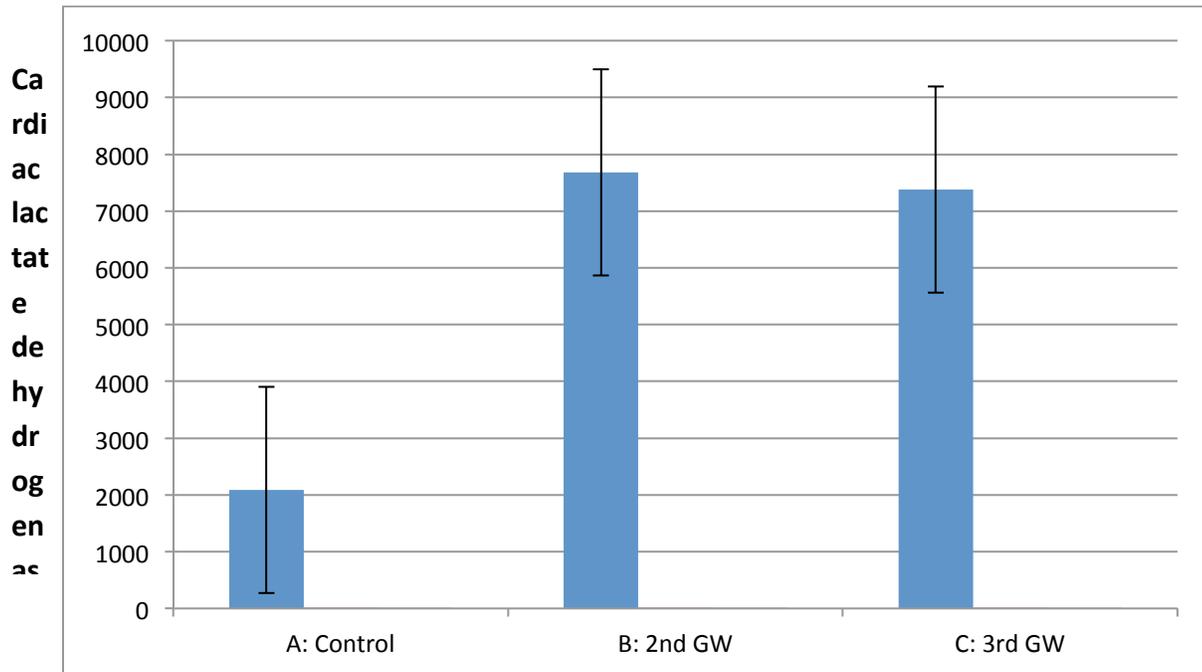


Figure 3: Level of activity of lactate dehydrogenase in cardiac tissue

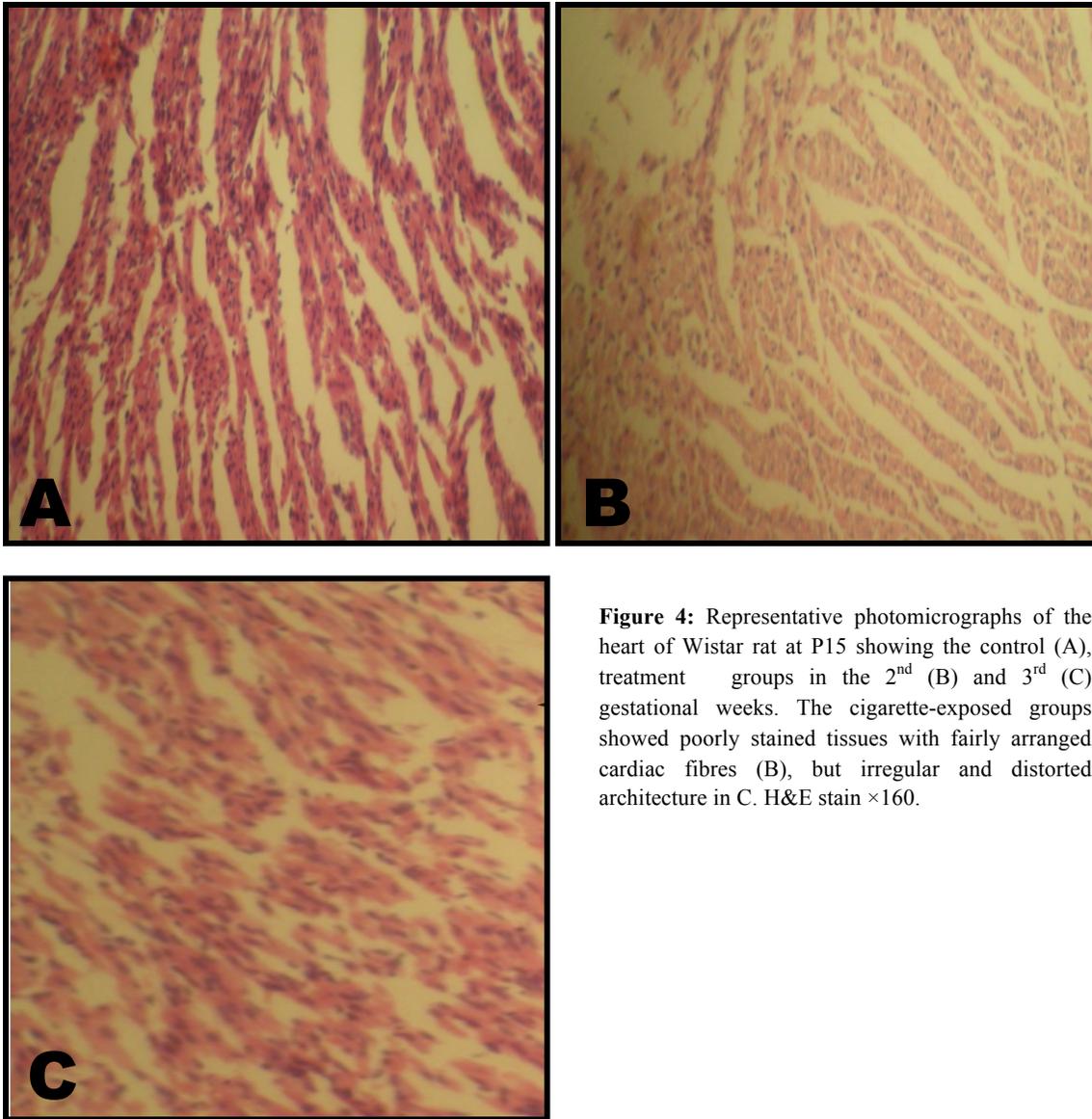


Figure 4: Representative photomicrographs of the heart of Wistar rat at P15 showing the control (A), treatment groups in the 2nd (B) and 3rd (C) gestational weeks. The cigarette-exposed groups showed poorly stained tissues with fairly arranged cardiac fibres (B), but irregular and distorted architecture in C. H&E stain $\times 160$.

DISCUSSION

The effect of cigarette smoke on birth weight has extensively been reported. However, the degree of low birth weight associated with gestational cigarette smoking is dependent on the time of exposure (Omotoso *et al.*, 2014a; 2014b). As seen in this study, exposure of pregnant Wistar rats to two sticks of cigarette in the 3rd gestational week led to a much lower birth weight in the pups, compared to those exposed in the 2nd gestational week. However, the rate of postnatal growth in prenatally exposed rats, as measured by

body weight increase, was higher in animals exposed to cigarette smoke in the 3rd gestational week.

Prenatal cigarette smoke exposure caused a significant reduction in the weight of heart of juvenile rats, as well as alterations in the cardiac musculature, and these could affect cardiac functions. These quantitative and qualitative changes are probably due to apoptosis, premature exit of cardiomyocytes from cell cycle of fetal tissue damage as occurs in hypoxia, to compensate for

reduced oxygenation (Zhang, 2005; Hutter et al., 2010). Pertinent to this suggestion are reports that smoking causes hypoxia (Socol et al., 1982; Sunuchratura et al., 2014). These changes may underlie the cardiac functional disorders in hearts of fetuses exposed to smoke (Meyer and Zhang, 2007)

Lactate dehydrogenase (LDH) is a marker frequently used in assessing cell damage (Anbarasiet al., 2005). Similar studies have observed increased levels of LDH in the serum of smokers (Padmavathiet al., 2009), due to the oxidative activities of cigarette toxicants on the plasma membrane causing lipid peroxidation, leading to cellular damage. This eventually results in the leakage of LDH from the cells into the

circulation (Anbarasiet al., 2005). Although serum LDH was not assessed in the current study, tissue LDH levels markedly increased in animals prenatally exposed to cigarette smoke, especially during the mid-gestational period. The concomitant decrease in tissue LDH earlier reported by Anbarasiet al. (2005) as opposed to the increase serum levels, cannot be ascertained in the current study. The increased activity of LDH currently reported is a reflection of the severity of necrotic damage to the myocardial membrane.

In conclusion, exposure of the foetus to cigarette smoke adversely affects the morphology of the developing heart, and could result in cardiac dysfunctions in early life.

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