

## HISTOPATHOLOGIC MANIFESTATIONS OF BRAIN, HEART, LUNG AND ILEUM OF MALE RATS DOSED COUNTERFEIT SILDENAFIL CITRATE

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### ABSTRACT

Sildenafil citrate is an orally administered drug used for the treatment of male impotence through inhibition of phosphodiesterase-5 in the corpus cavernosum. While drugs like analgesics, antibiotics, and anti-malarials are the most commonly found counterfeit drugs in the developing countries; the problem of counterfeit sildenafil citrate is a world-wide phenomenon. Though the liver and kidney are the sites/routes of its metabolism and excretion, the penis is the target organ of its action. With this study the impact of sildenafil citrate counterfeit on the brain, heart, lung and ileum in Wistar rats is being investigated. Twenty one male rats (250 g), randomly distributed to 3 groups of 7 rats each were used for the study. While rats in the first group were dosed with 25 mg/kg of fake sildenafil, those in the second group were dosed with 25 mg/kg BW of genuine sildenafil, and the third group served as the control and was administered with distilled water. Sections of lung, heart, brain, and ileum were processed and stained with haematoxylin and eosin (H & E). The slides were viewed under the microscope at magnification of  $\times 400$ . Results of the study revealed that while control rats and rats dosed with genuine drug manifested no visible lesions in all tissues examined, fake drug administered rats features the following histopathological manifestations brain (severe meningeal congestion and haemorrhage); heart (congestion of the coronary vessels); lung (mild proliferation of alveolar pneumocytes); and ileum (erosion and degeneration of mucosal surface). From the results of the study, it is evident that the counterfeit sildenafil citrate used for the study was toxic to tissues. Therefore, no effort should be spared in discouraging the sale, distribution and consumption of fake drugs.

**Key words:** Pathological manifestation; tissue histology; counterfeit sildenafil citrate.

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### INTRODUCTION

Pharmaceutical products which are made and offered for sale with the intent to deceptively represent its origin, authenticity or effectiveness are generally referred to as counterfeit medications [1]. This definition is in agreement with the view of World Health Organization (WHO) on counterfeit medicines. The World Health Organization defined a counterfeit medication as a therapeutic agent that is deliberately and fraudulently mislabelled with respect to identity and/or source. The term counterfeit medication, according to Jackson et al. [2] applies to both branded and generic products and

counterfeit medicines may include drugs with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredient(s) or with fake packaging' [1, 3, 4].

Globally, counterfeits make up more than 10% of the medicines made available for sale. The situation is even more precarious in the developing world since it has been reported that as much as 25% of the medicines consumed in poor countries are counterfeit or substandard, with counterfeit drugs being accountable for the death of more than 10,000 Africans yearly [5]. Drug counterfeiting also creates as much as between 2 to 5% losses for governments around the world. In Africa especially, the



government suffers huge economic loss that is usually more than the global estimate. Additionally, pharmaceutical companies and manufacturers are also confronted with brand issues and financial losses due to patent and copyright infringement. Aside the economic aspects, a number of other risks have been linked to fake drugs. For example, Wertheimer and Wang [6] identified such risks to include unexpected side effects, allergic reactions, worsening of medical conditions and even death. But when death does not occur, the possibility of slight harmful effects occurring in many tissues cannot be ruled out. Therefore the aim of this study is to investigate the possible harmful effects of fake sildenafil citrate on male Wistar rats using histologic presentation of the brain, heart, lung and ileum as markers of study.

### MATERIALS AND METHODS

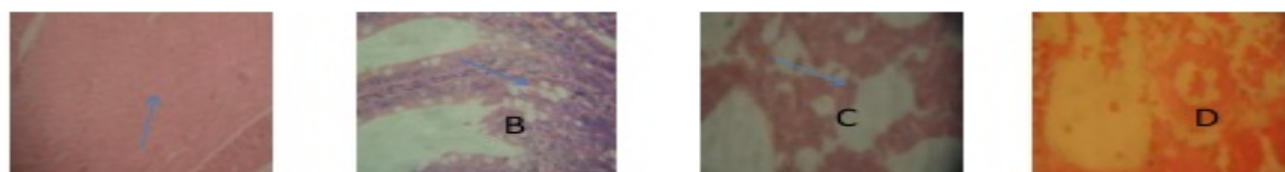
**Experimental Animals:** This animal study was carried out in accordance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). Male albino rats of average weight of 250 g were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. Subsequent to the commencement of the study the rats were left to acclimatize for two week. Animals were kept in cages at ambient temperature of  $26\pm 3^{\circ}\text{C}$  and a 12 h light, 12 h dark cycle. All the animals were supplied with feed and water without any form of restriction. The twenty-

one rats used for the study were randomly distributed into 3 groups comprising of 7 rats each. While the first group was administered with 25 mg/kg of fake sildenafil that was supplied by National Agency for Food and Drug Administration and Control (NAFDAC), Western regional office Ibadan, the second group was administered with 25 mg/kg BW of original/genuine sildenafil [7], purchased from a reputable Pharmacy. The third group was administered with distilled water served as the control. The treatment groups were exposed to this product for a period of 7 days. Drug administration took place each day (5 days per week) between 09.00 h and 11.00 h and the three treatment groups were administered orally by gastric gavage. Sildenafil supplied by NAFDAC was identified as fake based on WHO definition that states that counterfeit medicine is a drug that is deliberately and fraudulently mislabelled with respect to identity and/or source.

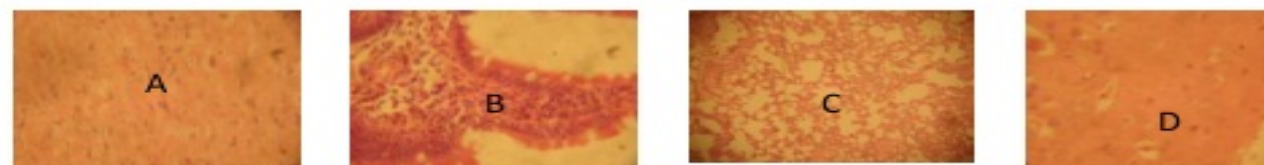
### Histopathology

Sections of lung, heart, brain, and ileum were cut and fixed in 10% neutral buffered formalin after the animals had been sacrificed. The tissues were embedded in paraffin block and cut in 5  $\mu\text{m}$  sections using motorized rotary microtome. After which they were stained with haematoxylin and eosin (H&E), slides were then examined under compound light microscope and photographed and histopathological changes were assessed. The slides were viewed under the microscope at magnification of  $\times 400$ .

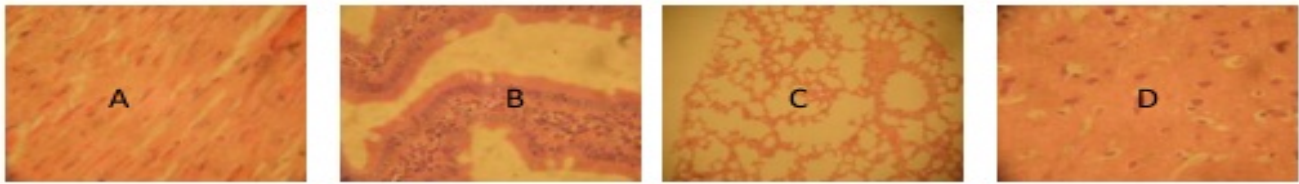
**Figure 1: A- heart, B- ileum, C- lung, D- brain; showing congestion of the coronary vessels, erosion and degeneration of mucosal surface, mild proliferation of alveolar pneumocytes and severe meningeal congestion and haemorrhage respectively in fake sildenafil citrate administered rats.**



**Figure 2: A- heart, B- ileum, C- lung, D- brain; all showing no visible lesion in genuine sildenafil citrate administered rats.**



**Figure 3: A- heart, B- ileum, C- lung, D- brain; all showing no visible lesion in control rats.**



## RESULTS

The results of the study are presented in Figures 1- 3 below. Histology results revealed that administration of male Wistar rats with fake sildenafil citrate caused abnormalities in the functional integrity of cells of all tissues examined. While the photomicrographs of the heart and ileum showed manifestations such as congestion of the coronary vessels, and erosion and degeneration of mucosal surface respectively, abnormalities of those of lung and brain were mild proliferation of alveolar pneumocytes and severe meningeal congestion and haemorrhage respectively as shown in Figure 1. On the other hand, results of rats administered with genuine drug as well as those of the control rats showed no damage as the photomicrographs for the brain, lung, heart and ileum featured no visible lesion as revealed in Figures 1-3.

## DISCUSSION

Sildenafil citrate is a drug used to treat pulmonary arterial hypertension and erectile dysfunction. Its mode of action in erectile dysfunction- (i.e. inability to sustain a satisfactory erection to complete intercourse) is through inhibition of cGMP-specific phosphodiesterase type 5, an enzyme that promotes degradation of cGMP, which regulates blood flow in the penis [8-10]. While its route of administration is oral, pharmacokinetic data include bioavailability- 40%; metabolism - hepatic (mostly CYP3A4, also CYP2C9); half-life- 3 to 4 hours and the routes of excretion are mainly fecal (80%) and renal ( $\approx$ 13%). For most patients, the recommended dose of 50 mg/kg is administered approximately 1 hour before sexual activity but may be administered anywhere from 4 hours to 0.5 hour before sexual activity. Based on effectiveness and toleration, the dose may be increased to a maximum recommended dose of 100 mg or decreased to 25 mg. The minimum dose of 25 mg/kg usually advised in conditions associated with increased plasma levels of sildenafil such as age (>65); hepatic impairment (e.g., cirrhosis), severe renal impairment (creatinine clearance <30 mL/min), and concomitant use of potent cytochrome P450 3A4 inhibitors such as ketoconazole, erythromycin, and saquinavir was employed for this study. The possibility of sildenafil citrate causing histopathological changes in some tissues e.g. testes has been established. For example, long term

administration of sildenafil citrate at 50 mg and 100 mg for 8 weeks was identified as a cause of a significant decrease and a significant increase in sperm count and sperm malformations respectively. Histopathological changes in testis of male rats treated with 50 mg and 100 mg sildenafil citrate were reported by Al-Fartosi [11]. Not only for 100 mg/kg administered rats but also 50 mg/kg treated ones, all sections of testes collected had necrosis of both somniferous tubules and the interstitial tissue, congested blood vessels, hypertrophy of the interstitial leydig cells and degeneration of the spermatogonial cells. Moreover, the basement membrane was thin compared with thickness of basement membrane in control group; there was also loss of lumen architecture and spermatogenesis in testis of rats treated with 50 mg of sildenafil citrate. In addition to these, destruction of testis and the presence of inflammatory cells in testis of male rats treated with 100 mg of Viagra were observed, whereas, control rats did not manifest these histological abnormalities.

The result of that study seems to suggest that sildenafil is capable of causing abnormal tissue histology at least at 50 and 100 mg levels. In addition Luu et al. [12] and Purvis et al. [13] also revealed that sildenafil is potentially retinotoxic due to an associated increase in retinal c-GMP, since it caused a depression of electroretinogram (ERG) functions that suggest clinical toxicity of the retina. Data obtained from the present study though showed that administration of 25 mg of genuine sildenafil did not cause histopathological changes in intestine, heart, and lung of male rats.

Histopathological changes in testis as a result of sildenafil citrate which caused sperm abnormality have been linked to changes in the expression of various receptors associated with cGMP or in the responsiveness of these receptors in the brain that eventually caused damage in tissue of testis and failure in spermatogenesis [14, 15]. Aside this, the inhibition of cGMP breakdown that produced changes in nitric oxide (NO) production via negative feedback mechanisms [15] has been suggested as alternative mechanism. This is because NO may be capable of affecting neurotransmitter activity differentially across brain systems [14]. Yet no visible lesion was observed in photomicrographs of brain tissues of rats administered 25 mg/kg with genuine drug.



Although many drugs have been known to induce oxidative stress in many tissues, the results of the study of Akintunde et al. [16] which revealed that sildenafil citrate significantly reduced MDA levels in brain tissue seem to support the result of this study of no visible lesion in brain tissue of genuine drug administered rats. They also observed a significant increase in GSH content of brain. Superoxide dismutase (SOD) and catalase (CAT) activities for the brain were increased significantly compared to their corresponding control. Furthermore, they also identified that on histological examination of the testicular tissues no visible lesions were observed. Therefore, they concluded that therapeutic dose of sildenafil citrate elicits modulatory roles by stabilizing/boosting antioxidant defense systems in male rat. Akintunde et al. [16] even suggested that the therapeutic dose of the sildenafil is not deleterious when taken for erectile dysfunction. Since according to them therapeutic dose of Viagra may be capable of protecting the brain from many assaults that may arise from reactive oxygen species. Their earlier observations can then be taken as the bases of normal histologic presentation of not only the brain but also of other examined tissues i.e. the ileum, lung and heart of genuine drug-administered rats.

Other evidence to support that sildenafil is not able to induce oxidative stress and lipid peroxidation in the brain of the experimental animals has been suggested by Akintunde et al. [16] since MDA level in brain tissue decreased significantly in a dose dependent fashion. Like the result of this study in which rats administered with 25 mg/kg featured no visible lesions in photomicrograph of brain tissue, significant reduction in MDA levels of brain tissue is an indication that the drug does not have any deleterious effects to the brain. Mohamed and Laila [17]; Devan et al. [18]; and Hong et al. [19] also revealed that sildenafil has no harmful effects on the brain rather beneficial, they reported a significant decrease in MDA levels and increase in antioxidant (GSH) levels during alloxan- induced non-insulin dependent diabetes mellitus (NIDDM) of male rats, with Akintunde et al. [16] suggesting sildenafil as a preventive therapy against oxidative stress in the brain [20].

On the other hand, Akintunde et al. [16] revealed that the physiologic mechanism of penile erection involves the release of nitric oxide (NO) in the erectile tissues during sexual stimulation. Nitric oxide (NO) that is known as endothelium derived relaxing factor [21], is produced from the amino acid L-arginine, oxygen, and a variety of cofactors, by nitric oxide synthase enzymes [22]. Its function as a gaseous chemical compound that acts as an important signaling molecule within the human body as well as facilitating a variety of critical functions by enhancing blood flow and increasing immune defense system [23] have also been noted. Nitric oxide also reacts with superoxide ion to produce peroxynitrite, a very highly

reactive nitrogen species, known to play an important role in paracetamol-induced tissue damage. Yet NO<sup>•</sup> generation that occurs with sildenafil administration seemed not to have provoked abnormal histologic changes in the lung, heart and ileum of genuine drug administered rats.

While genuine sildenafil administered rats showed no visible lesion for all tissues examined at 25 mg/kg, fake drug administered rat manifested the following histologic changes: brain (severe meningeal congestion and haemorrhage); heart (congestion of the coronary vessels); lung (mild proliferation of alveolar pneumocytes); and ileum (erosion and degeneration of mucosal surface). This may not be unassociated with an earlier observation in which high level of Cd was detected in the serum of rats administered with fake sildenafil used for this study. Cd is known to cause damage to many organs. This it does through its free radical generating property. Although formation of free radicals occurs naturally as by-products of many intracellular metabolic processes, they are capable of causing oxidative damage to a number of molecules in cells including membrane lipids, proteins and nucleic acid [24, 25].

The potential harmful effects of these species are well managed at physiologic level by the cellular antioxidant defense system [26]. Antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase play vital role in not only the scavenging process of free radicals but also in maintaining cellular stability [27]. Reduced glutathione is another predominant defense against ROS free radicals in many tissues of the body [28]. But oxidative stress occurs- as can be suspected to have taken place in fake drug administered rats- when production of ROS in cells impairs antioxidant defenses or exceeds the ability of the antioxidant defense system to eliminate them [27]. This means that lipid peroxidation occurs as a result of imbalance between the production of oxidants and antioxidants [29].

That tissue damage was observed in fake drug treated rats may be oxidative stress-induced can be deduced from the fact that serum Cd level was reported to have been significantly higher in rats administered with 25 mg/kg sildenafil compared with both control and genuine drug treated rats. Cd induced tissue damage is a well known phenomenon and is usually associated with lipid peroxidation. Therefore, further study that focuses on assessing the brain Cd and MDA levels in fake sildenafil administered rats may helpful to understand the mechanistic basis of tissue damage observed as a result of exposure to the fake sildenafil citrate used for this study.

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