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RESEARCH ARTICLE

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Serum levels of toxic metals in wistar rats treated with counterfeit Sildenafil Citrate

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ABSTRACT

While it is known that some counterfeit drugs contain either sub-optimal or abnormally high level of active ingredients, heavy metal contamination of fake drugs has also been recognized. The aim of the study to determine the levels of select heavy metals in serum of male Wistar rats dosed with counterfeit sildenafil citrate. Twenty-one male Wistar rats (250 g) were divided into 3 groups of 7 rats each. The route of administration was by gastric gavage. Groups 1, 2, and 3 (control) were dosed with 25 mg/kg BW of fake sildenafil, genuine drug (Vega®), and distilled water respectively. Serum levels of mercury, lead, nickel, aluminum, silicon, cadmium, and arsenic, were determined by the atomic absorption spectrometric method. Data were subjected to analysis of variance. $P \leq 0.05$ was considered significant. Results revealed that there were no significant differences in the serum levels of the metals estimated except cadmium. From the results of this study, it is evident that exposure to fake drugs may provoke a number of pathological conditions as cadmium is capable of inducing tissue damage.

Keywords: Counterfeit; sildenafil citrate; heavy metals.

INTRODUCTION

Sildenafil citrate, an oral medication used to treat male impotence by inhibition of phosphodiesterase-5 in the corpus cavernosum (so as to facilitate penile erection) has been well studied in relation to its toxicity, tolerance, efficacy and possible drug interaction [1]. It is an agent that has a worldwide appeal, therefore because of this huge demand, the problem of counterfeiting of sildenafil citrate is global in scope [2]. To buttress the universality of the problem of counterfeit sildenafil citrate is the fact that reports are available to confirm that seizure of fake sildenafil citrate takes place all over the world. While it a well established fact that counterfeit drugs are rarely efficacious, their usage may also be accompanied by serious adverse effects. The contamination of heparin by Chinese counterfeiters in 2007 and 2008 is a good example of this, an episode that resulted in the death of 149 patients in the USA [3].

In 2009, the European Union alone seized 34 million fake tablets in just 2 months, mostly drugs such as antibiotics, cancer treatments, and sildenafil citrate (Viagra). Although in high-income countries, counterfeit versions of therapeutic agent like Viagra or others like cancer medicines are more dominant than antibiotics, data are available to suggest that all forms of fake drugs are more prevalent in absolute terms in developing and middle-income markets [4-7]. In these two regions regulatory and enforcement systems for medicines are weak. In addition, assistance to many other agencies involved with eradication of the menace of counterfeit drug is also grossly

lacking. In recent times the fact that drug supply chains has increasingly become cross continental, is a known cause of substantial counterfeiting even in the developed countries [8, 9].

In the developing countries counterfeits account for more than 25% of medicines offered for sale, while it is known that some counterfeit drugs contain either sub-optimal or abnormally high level of active ingredients, the presence of adulterants/contaminants (e.g. heavy metals) in some of these fake products has also been recognized. The reason why a study of this nature is timely is because even World Health Organization (WHO) has raised the possibility that "counterfeit drugs may erode public confidence in health care systems, health care professionals, the suppliers and sellers of genuine drugs, the pharmaceutical industry and national Drug Regulatory Authorities". The aim of this study is to determine serum levels of select heavy metals in male Wistar rats since heavy metal contamination of fake drug is a possibility.

MATERIALS AND METHODS

Experimental Animals: Twenty-one male Wistar rats of average weight of 250 g were purchased from the Experimental Animal Unit of the Faculty of Veterinary Medicine of the University of Ibadan, Nigeria. Prior to the commencement of the experiment, the animals were left in their respective cages to acclimatize for about a period of two week. The animals were kept in cages at ambient temperature of 26±3°C and a 12 h light, 12 h dark cycle and were fed standard laboratory chow and supplied with water with any restriction. The rats were divided into 3 groups with each group comprising of 7 rats. The route of administration was by gastric gavage.

The first group of rats was administered with 25 mg/kg BW [10] of fake sildenafil citrate while those in the second group received genuine sildenfil citrate and the third group which served as the control and were administered with distilled water. The fake sildenafil citrate used for the study was obtained from National Agency for Food and Drug Administration and Control (NAFDAC), Western region office in Ibadan, but the original product was purchased from a reputable Pharmacy. The duration of the experiment was for a period of 7 days. This study was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985).

Preparation of serum samples & heavy metal estimation: On the 8^{th} day, blood was drawn from each rat by retro-orbital bleeding and introduced into an anticoagulant free bottle. This blood was centrifuged at 3000 g after which serum was separated and stored at -20 ºC. Levels of lead, nickel aluminum, silicon, cadmium, and arsenic in serum of rats were determined by the atomic absorption spectrometric method using Buck Scientific 205 Atomic Absorption (East Norwalk, Connecticut, USA).

Statistical analysis: Data were subjected to statistical analysis using SPSS package (version 15) to obtain mean ± SEM (standard error of mean). The level of significant difference among the three groups was determined by using analysis of variance (ANOVA). $P \leq 0.05$ was considered significant.

RESULTS

Administration of fake and genuine sildenafil citrate to male Wistar rats did not result in significant differences in the serum levels of most of the heavy metals estimated when data were subjected to analysis of variance. While the serum levels of cadmium were significantly different, the levels of others like arsenic, aluminum, silicon, nickel, and lead were comparable and not significantly different at ≤ 0.05. All the results are presented in **Table 1.**

Table 1: Serum levels of select heavy metals in fake and genuine sildenafil citrate-administered rats.

	Pb (µg/L)	As (ng/mL)	Cd (mg/dL)	Si (µg/L)	Al (µg/L)	Ni (µg/dL)
Control	0.14±0.02	0.005±0.001	0.007±0.002	0.12±0.03	0.12±0.03	0.16±0.02
Fake sildenafil citrate	0.14±0.02	0.006±0.002	0.020±0.004	0.13±0.02	0.10±0.01	0.16±0.03
Genuine sildenafil citrate	0.013±0.02	0.006±0.002	0.008±0.001	0.13±0.03	0.11±0.01	0.17±0.03
F-value	0.619	1.029	54.343	0.157	1.444	0.362
P-value	0.552	0.381	0.005	0.856	0.267	0.702

Results are expressed as mean ±standard error of mean. p <0.05 is significant using ANOVA. N=7.

DISCUSSION

The history of counterfeits is as old as the history of commodities [2]. Counterfeiting is known to be more common in those regions where regulatory and enforcement systems for medicines are weakest. In most industrialized countries there is effective regulatory systems and market control, therefore the ratio of fake drugs to genuine ones is extremely low—perhaps less than 1% of market value according to the estimates of the countries concerned. On the other hand, in many African countries and parts of Asia and Latin America, a much higher percentage of the medicines on sale may be counterfeit. While Intellectual Property Institute (IIPI) has described drug counterfeiting as not only an issue of intellectual property but also of trade problem, the significant increase in the level of toxic metal-Cd observed in rats administered with fake sildenafil citrate drug compared with two other groups, seems to suggest that it is also an unrecognized public health problem with particular consequences in the area of injury, mortality, and morbidity [2].

This public health dimension may be global in scope. Since the problem of sexual-enhancing drug counterfeiting is not limited to the developing world as can be deduced from the report of Sugita and Miyakawa [2]. They revealed that the size of the market for counterfeit phosphodiesterase type 5 inhibitor (PDE5Is) in Japan is as much as 2.5 times larger than that of genuine PDE5Is yet the price of the counterfeits is nearly equal to that of the genuine PDE5Is. While the results of our study indicate probable contamination of sildenafil citrate with heavy metals e.g. Cd, because of significant increases in the serum level of Cd in the rats administered with fake sildenafil citrate, other past studies revealed contamination of PDE51 with antidiabetic drug. In Singapore in 2008, an outbreak of severe hypoglycemia among users of counterfeit PDE5Is containing an antidiabetic was reported. Seven patients remained comatose as a result of prolonged neuroglycopenia with four of the cases resulting in death.

Cadmium contamination of sildenafil citrate is possible because Chaubey et al. [11] has raised the problem of poor quality-control measures during the manufacturing process of counterfeit Cialis. The metal Cd observed to be significantly increased in rats administered fake sildenafil citrate is known to be a very toxic heavy metal and an important environmental pollutant, present in the soil, water, air, food and in cigarette smoke. It causes poisoning in various tissues of humans and animals [12, 13]. The uptake of this heavy metal in the liver is important in its overall concept of its toxicity. As much as 50% of cadmium absorbed systemically is rapidly accumulated in the liver, which results in the reduced availability of cadmium to other organs as the kidneys and testes, which are more sensitive to its toxic effects [14].

The harmful effects of cadmium are due to its inhibition of liver metabolic enzyme systems, especially of those possessing sulphydryl groups as well as uncoupling of oxidative phosphorylation in mitochondria [15], a process which eventually leads to enhanced lipid peroxidation, hepatic congestion, ischemia and hypoxia [16]. Generation of ROS and oxidative tissue damage due to cadmium has been identified as the cause of cadmium-induced hepatocellular damage [17]. Aside this, a wide range of accompanying changes in antioxidant defense enzymes has been observed [18]. It has been shown that free radical scavengers and antioxidants are highly essential in conferring protection against cadmium toxicity. The significant increase in the serum level of Cd in the fake drug administered rats is an indication that some of these abnormal processes may manifest in humans who consume Cd contaminated fake drug.

Another element that its serum level was assessed was arsenic, a naturally occurring element and recognized human poison since ancient times that is present in low concentrations in air, soil, and water. Chronic exposure of human and animals to this element is indicated usually by its higher levels in hair nail, hoof, and urine [19, 20]. It is also an element that affects not only the mitochondrial enzymes like Cd but also impairs cellular respiration, leading to cellular toxicity. In addition, like Cd a higher level has been associated with increased lipid peroxidation levels in liver, kidney, and heart [21]. This it does through over-generation or an ineffective elimination of ROS that induces oxidative stress and causes damage to all types of molecules such as proteins, lipids, and nucleic acids [22]. This element arsenic as well as others like Ni, Si, Al, and Pb was not significantly different when all three groups were compared. Since the significant increase was peculiar to only Cd, it rules out a modulation of the metabolism of these heavy metals, rather results raise the possibility of Cd contamination of the fake sildenafil citrate used for this study.

CONCLUSION

Therapeutic agents that are formulated and administered to patients for management of pathological condition to restore the body to a position of health should not provoke adverse health condition. The unfortunate thing is that administration of sildenafil citrate that is supposed to address the problem of erectile dysfunction may provoke Cd induced events such as described above in an otherwise unhealthy person. All efforts therefore should be directed towards eradication of drug counterfeiting. It is therefore being suggested that in order to obtain genuine drugs generally, especially in the developing world, a patient must be examined and have a prescription written at a medical institution, and drugs should be bought at a dispensing pharmacy. In addition, physicians should warn their patients not to buy counterfeit sildenafil citrate, and when prescription is written for the purchase of genuine sildenafil citrate, patients should be informed of the severe health problems that may occur if exposed to fake drug.

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