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The combined fixed-dose antituberculous drugs alter some reproductive functions with oxidative stress involvement in wistar rats

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ABSTRACT

The reproductive toxicity of combined fixed-dose first-line antituberculosis (CFDAT) regimen was assessed in rats. Thirty-two (32) Wistar rats weighing 168.1 ± 8.0 g were divided into four groups of eight rats per group. Two groups of male and female rats were administered oral distilled water (1.6 ml) and CFDAT drugs containing rifampicin, isoniazid, pyrazinamide and ethambutol (RIPE, 92.5 mg/m2 per body surface area) respectively for forty-five days. Serum follicle stimulating hormone, luteinizing and testosterone were reduced significantly ($p < 0.05$) in the treated male rats. Similarly, sperm count levels were decreased by 27.3% when compared with control. RIPE elevated serum oestrogen ($p < 0.05$), progesterone ($p < 0.05$) as well as prolactin ($p > 0.05$) levels in the treated females. In addition, RIPE reduced ($p < 0.05$) total proteins levels and increased ($p < 0.05$, 53%) catalase levels in male but not female animals. Superoxide dismutase, glutathione-S-transferase, glutathione peroxidase, reduced glutathione levels as well as lipid peroxidation were unaltered in all rats respectively. Histopathological studies revealed congested peritesticular vessels and no changes in the ovary when compared with control. Overall, our results demonstrate reproductive toxicity potentials of RIPE in the rat, thus, suggesting that these reproductive parameters be monitored during antituberculous chemotherapy.

1. Introduction

There have been recent suggestions that anti-tuberculosis agents would produce adverse effects on reproductive health system [1–4]. Tuberculosis (TB), a *Mycobacterium tuberculosis* (*M. tuberculosis*) remains an epidemic in many parts of the world. According to the WHO, an estimated 8.7 million new cases of TB and 1.4 million deaths from TB emerge in 2011[5]. TB is the leading cause of death from a curable infectious disease and is second only to HIV/AIDS as a cause of death from any infectious disease [7]. The rapid spread of drug-resistant TB in Asia, Eastern Europe, and Africa has limited the achievement of TB treatments goals among others [6,7]. TB incidence has been falling globally for several years and fell at an average rate of about 1.5% per year between 2000 and 2014. For instance, the TB mortality rate has fallen by an estimated 47% between 1990 and 2015 [8]. It poses significant challenges to developing economies as it primarily affects people during their most productive years. The treatment protocol for drug-sensitive TB, however, varies slightly in different parts of the world, but they are based on a combination of three, or more typically, four drugs, i.e. isoniazid, rifampin, pyrazinamide, and ethambutol. This combination has been adjudged best for efficacy and tolerability amongst the available TB drugs and is, therefore, the mainstay "first line" therapy [8,9]. There are historical and clinical proofs that these first-line antituberculosis agents are the most potent oral antituberculous medications [10–12]. In addition, in-vitro and in-vivo clinical data support the use of such individual agent [13,14]. This may be because the combination has been found to be a beneficial and cost-effective treatment for TB [15], but, this is not without some systemic toxicity from the results from human data [1,3,5]. A study from Sharma [16] suggests that the high inci-

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dence of antituberculous drugs inducing toxicities may indicate difficulties with systematic steps in the prevention and management of tuberculosis. Evidence abound that most of the adverse effects due to the antituberculous and antiretroviral drugs are experienced during the first months of therapy [17]. For instance, two authors at different times have reported nephritis associated with rifampicin [18,19]. Still, there exist cross adverse event among rifampicin, isoniazid, and pyrazinamide which is manifested as liver injury [20–23] while ethambutol is widely known for its ocular toxicity [24]. Extensions of their adverse effects include skin rashes, gastrointestinal intolerance, central nervous system symptoms, peripheral neuropathy, and blood dyscrasias. In respect to the aforementioned, the concern about adverse drug effects of antituberculous agents has been advocated [25,26] because of their negative impacts on the sustainability of public health system [6,7]. Since tuberculosis affects people in their productive and reproductive age, but available data on adverse drug reactions in patients and animals experiments particularly with respect to reproduction are limited [27]. Although our previous studies showed synergy of fixed-dose combined antituberculous drugs in liver injury, its teratogenic potential in a first filial generation of animals model as well as the activity of antioxidants against the toxicity of rifampicin and the fixed-dose combined antituberculous [25,28]. There is still a paucity of data on the reproductive toxicity of fixed-dose combined antituberculous drugs.

Therefore, in this present study, we assessed the reproductive toxicity of combined fixed-dose first-line anti-tuberculosis (CFDAT) drugs in rats.

2. Materials and methods

2.1. Drugs and chemicals

A Combined fixed-dose antituberculous drugs consisting of rifampicin (150 mg), isoniazid (75 mg), Pyrazinamide (400 mg) and ethambutol (275 mg) per tablet, was obtained from the DOTS clinic of Akerele Primary Healthcare Centre, Nigeria. Thiobarbituric acid (TBA) was purchased from Sigma Chemical Company (USA). Metaphosphoric acid, Reduced glutathione (GSH), and Trichloroacetic acid (TCA) were purchased from J.I. Baker (USA). Rat Follicle Stimulating Hormone (FSH) (Cat. No.: Rshakrfs-010R) and Luteinizing Hormone (LH) ELISA (Rshakrlh-010SR) kits were purchased from (Biovendor, Shibayagi Co., Ltd. (Japan). RAT Testosterone (RTC001R) and Prolactin ELISA were obtained from Biovendor, Laboratorni, medicina a.s Karasek (Czech Republic). Rat oestrogen ELISA (CSB-E07279r) (Cusabio Biotech CO., LTD.) and Progesterone (RTC008R) kit was purchased from Biovendor, Laboratorni, medicina a.s Karasek (Czech Republic) and Sodium hydroxide from MERCK (Germany). All other chemicals and reagents used were of analytical grades. Atomic UV/Visible Spectrophotometer obtained from JENWAY, Bibby Scientific (Model 7300 and 7305) (USA).

2.2. Animals

Albino rats of the Wistar strain weighing between 120 and 250 g were purchased from inbreeding animal house of the Redeemers University, Mowe, Ogun State, Nigeria. The rats were housed in the experimental animal handling facility of the University of Lagos, College of Medicine, Idi-Araba Campus, Lagos State, Nigeria, at temperature of the experimental animal rooms of $22 \pm 3\,^{\circ}C$, under controlled conditions with a 12 h light/12 h dark schedule and fed with commercially available rat pelleted diet (Ladoke Akintola Growers Mash) and water ad libitum throughout the period of the experiment. Animals were acclimatized for a period of two weeks before the experiment. The experimental protocols were approved by the Institutional Animal Care and Use Committee, Department of Pharmacology, Therapeutic and Toxicology, College of Medicine, University of Lagos, and all of the experimental procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The institution animal health officers certify the animals fit for the experiment. The beddings of the animals were changed on alternate days and the animals were sacrificed in a humane manner at the end of the experiment by cervical dislocation. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication No. 85-23, revised 1996)" for studies involving experimental animals and the procedures as documented by Kilkenny et al. [29] for reporting animal research.

2.3. Sub-chronic study

Thirty-two (32) (n: male or female = 8) Wistar rats weighing 168.1 ± 8.0 g were divided into 4 groups of 8 rats per group for each sex and treatment group. The control groups were administered 1.6 ml distilled water by oral administration daily. A combined fixed-dose anti-TB drugs (92.5 mg/m2 per body surface area, p.o) [30] was administered to the test groups (male and female separately). The rats were weighed weekly and treatments lasted for Forty-five (45)-days.

2.4. Collection of blood samples and tissues

All treatments were terminated on day 45 and Twenty-four (24) hours following the last administration, blood samples were obtained via ocular puncture into lithium heparinized bottles and animals were sacrificed via cervical dislocation. The blood samples containing anticoagulant were centrifuged at 4200 rpm for 5 min to obtain the clear supernatant (i.e. serum) from which all biochemical analyses were carried out. In addition, the reproductive organs (testis, epididymis and ovaries) were removed, weighed and stored for histological examinations.

2.5. Sperm function analysis

The testes from each rat were carefully exposed and removed along with its adjoining epididymis. The left testis was separated from the epididymis, and the caudal epididymal tissue was removed and placed in a petri dish containing 1 ml normal saline solution. An incision of about 1 mm was made in the caudal epididymis to liberate its spermatozoa into the saline solution. Progressive sperm motility, sperm count, and sperm viability were then examined under the microscope attached to a Celestron® digital microscope imager (Torrance, CA 90503) and viewed under X40 objective according to the method described by Raji et al. [31] Epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percentage motility. Epididymal sperm counts were made using the improved Neubauer hemocytometer and were expressed as million/ml of suspension. The sperm viability was also determined using Eosin/Nigrosin stain. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and unstained sperm cells were counted and an average value for each was recorded from which percentage viability was calculated. Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewa stain after they were air-dried. The slides were examined under the microscope under oil immersion with X 100 objectives.