

Full Length Research Paper

Biochemical changes in blood and tissues of Wistar rats following administration of anti-tuberculosis drugs

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Tuberculosis (TB) remains a major public health problem worldwide especially in resource-limited countries of Africa and Asia. The disease is curable with drugs but information on biochemical changes and oxidative indices due to anti-TB drugs is not readily available. This study was designed to investigate *in vivo* effects of anti-TB drugs in male Wistar rats. Fourteen adult male rats were randomly divided into two groups of seven animals each. The first group served as the control and was given normal saline while the second group received isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (ETB) in combination. The drugs were dissolved in normal saline and therapeutic doses [INH (5 mg/kg), RIF (10 mg/kg), PZA (15 mg/kg) and ETB (15 mg/kg)] were given by oral gavage thrice in a week for eight consecutive weeks. Estimation of serum enzymes and other biochemical parameters were assayed by standard methods. Results indicate that there was an insignificant increase in final body weight of anti-TB treated animals relative to the control ($p > 0.05$). In addition, there were significant decrease in renal and cardiac glutathione-S-transferase activities in the treated group relative to the control ($p < 0.05$) while hepatic glutathione peroxidase activity was significantly reduced in treated group when compared with the control ($p < 0.05$). Furthermore, triglyceride level was significantly increased in the treated group ($p < 0.05$). Liver marker enzymes (aspartate and alanine transaminases), renal and cardiac catalase and superoxide dismutase activities and urea levels were insignificantly increased in treated animals relative to the control ($p > 0.05$). These findings suggest that anti-TB drugs induce redox imbalance resulting in the depletion of enzymatic antioxidant parameters in rats.

Key word: Biochemical, blood, tissue, Wistar rats, Anti-TB drugs.

INTRODUCTION

Even at the 21st century, tuberculosis (TB) still remains an important public health problem worldwide despite global attention to eradicate the disease. TB causes about 2 million deaths annually among 2 billion infected individuals (WHO, 2014).

Inflammation-related oxidative stress has been implicated in the pathogenesis of infectious diseases such as malaria, HIV/AIDS and pulmonary TB (PTB) (Halliwell et al., 1999). This is mediated by activated macrophages and results in the release of a variety of

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chemicals which may damage body cells and tissues (Wiid et al., 2004). Specifically, in PTB infection, tissue inflammation secondary to release of free radicals from macrophages, poor dietary intake of micronutrients and side effects of anti-TB drugs were factors contributing to oxidative stress (Wiid et al., 2004). Availability of antioxidants to neutralise the harmful effects of the generated free radicals may be one of the factors determining severity of the disease (Alli et al., 2014). High serum concentration of lipid peroxidation (Kwiatkowska et al., 1999) and low plasma cholesterol levels were reportedly seen in PTB infection (Akiibinu et al., 2007).

Initial treatment of PTB with first-line anti-TB medications (rifampicin, isoniazid, ethambutol and pyrazinamide) is to render cases non-infectious and halt cycle of transmission in the community. Side effects of anti-TB drugs have been well investigated (Bulatovic, 2002; Awofeso, 2008) but little information is available on post-treatment biochemical changes especially in high burden countries with limited resources where the primary concern is the diagnosis and treatment of infectious cases. The contributions from infection, treatment and general condition of the host (nutritional status) and changes in dynamics of these factors during treatment are important elements in disease outcome. This study highlighted an attempt to evaluate the contribution of oxidative stress in relation to treatment changes independent of disease status and response in a controlled environment using an animal model.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing between 160-170 g were purchased from the animal house of the Departments of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. The animals were housed in well-aerated plastic cages, fed with standard mouse cubes (Ladokun Feeds, Nigeria, Ltd) and supplied with clean drinking water *ad libitum*. Handling of animals and other protocols conformed to the guidelines of the National Institutes of Health (NIH) for care of laboratory animals. The study was approved by the Animal Use Ethical Committee of the University of Ibadan, Nigeria.

Chemicals and drugs

Glutathione, hydrogen peroxide, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) and epinephrine were purchased from Sigma Chemical Co., Saint Louis, MO USA. Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were procured from British Drug House (BDH) Chemical Ltd., Poole, UK while anti-TB drugs were purchased from a pharmaceutical store in Ibadan, Nigeria. Other chemicals were of analytical grade and purest quality available.

Experiment

Fourteen adult male rats (Wistar strain) were randomly divided into two groups of seven animals each. Animals were given a period of

2 weeks for acclimatization before commencement of the experiment. The first group served as the control and was given normal saline while the second group received INH, RIF, PZA and ETB in combination. The drugs were dissolved in normal saline and therapeutic doses [INH (5 mg/kg), RIF (10 mg/kg), PZA (15 mg/kg) and ETB (15 mg/kg)] were given by oral gavage three times in a week for 8 consecutive weeks (WHO, 2014; Shayakhmetova et al., 2015). At the end of the experiment, rats were weighed and sacrificed under light ether anaesthesia. Blood was collected from the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 h. Serum was prepared by centrifugation at 3,000 g for 15 min in a Beckman bench centrifuge. The clear supernatant was used for the estimation of serum enzymes and other biochemical indices. Liver, kidney and heart were quickly removed and washed in ice-cold 1.15% KCl solution to remove blood stains, dried and weighed. The tissues were homogenized separately in 4 volumes of 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 min to obtain post-mitochondrial fraction (PMF). Procedures were carried out at 4°C.

Biochemical assays

Serum, kidney, heart and liver protein levels were determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard. Bilirubin levels (total and direct) were assayed by the method of Rutkowski and Debaare (1966). The indirect bilirubin (unconjugated bilirubin) was obtained by subtracting the value of direct bilirubin (conjugated bilirubin) from total bilirubin. Serum alanine aminotransferase (ALT) and aspartate aminotransferases (AST) activities were determined using a combination of the methods of Mohun and Cook (1957) and Reitman and Frankel (1957). Serum creatinine and blood urea nitrogen (BUN) levels were estimated by the methods of Jaffe (1886) and Talke and Schubert (1965) respectively. Activity of superoxide dismutase (SOD) was determined according to the method described by McCord and Fridovich (1969). Briefly, the method involves inhibition of autoxidation of epinephrine (pH 10.2) at 30°C. 20 mL of the sample and 2.5 mL of 0.05 M carbonate buffer (pH 10.2) were mixed together and allowed to equilibrate in the spectrophotometer. Freshly prepared 0.3 mL of 0.3 mM adrenaline was added and mixed by inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 150 s. The specific activity of SOD was expressed in Units/mg protein.

Catalase activity was measured using the method described by Aebi (1974) while Glutathione S-transferase (GST) activity was determined by the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Assay of glutathione peroxidase (GPx) activity was determined by the method of Rotruck et al. (1973) while reduced glutathione (GSH) was measured using a method described by Moron et al. (1979). Briefly, an aliquot of liver or kidney homogenate was deproteinized by the addition of an equal volume of 4% sulfosalicylic acid, and the resulting solution was centrifuged at 10 000 g for 15 min at 4°C. Supernatant (50 ml) was then added to 4.5 ml of DTNB. GSH was proportional to absorbance at 412 nm. Values are expressed in micromoles/ g tissue. Lipid peroxidation (LPO) assay was determined based on malondialdehyde (MDA) produced after exposure to anti-TB drugs using the method of Buege and Aust (1978).

Statistical analysis

The results were expressed as mean \pm standard deviation (SD) of seven rats per group. Data were analysed using one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range test for analysis of biochemical data using SPSS (12.0) statistical software. Values were considered statistically significant at $p < 0.05$.

Table 1. Changes in the body weight and relative weight of organs of male Wistar rats following 8 weeks administration of anti-TB drugs (INH, ETB, PZA, RIF).

Group	Body weight (g)		Relative body weight (g)					
	Initial	Final	Liver	Kidney	Heart	Liver	Kidney	Heart
Control	108.00±10.95	216.00±26.78	5.62±1.00	1.27±0.20	0.63±0.12	2.62±0.41	0.61±0.17	0.30±0.01
Treated group	185.71±13.36	228.57±33.75*	5.90±0.22*	1.32±0.08*	0.70±0.03*	2.63±0.40	0.59±0.10	0.31±0.05

Values are expressed as means ± S.D. (n= 7). *Significantly different from control (p < 0.05).

Table 2. Changes in the levels of hepatic, renal and cardiac glutathione-S-transferase (GST), reduced glutathione (GSH) and glutathione peroxidase (GPx) in male Wistar rats following treatment with anti-TB drugs.

Organ	Assay			
	GPx (µg/ml)	GST (µg/ml)	GSH (µg/ml)	MDA (Umol/mg)
Liver				
Control	9.39±0.42	40.34±18.72	41.81±0.32	3.39±2.60
Treated group	4.82±0.9*	36.29±13.97	40.83±2.36	3.82±1.15
Kidney				
Control	20.21±6.09	2.41±1.41	46.65±0.32	4.04±1.51
Treated group	16.95±2.92*	1.23±0.61*	45.63±1.17	5.24±1.08
Heart				
Control	19.08±3.15	1.78±1.23	51.27±2.22	2.51±0.29
Treated group	13.78±2.26*	0.65±0.10*	49.48±1.18	3.27±0.01

Values are expressed as means ± S.D. (n= 7). *Significantly different from control (p < 0.05).

RESULTS

There was an increase in final body weight of all the experimental animals after treatment when compared with control even though the association was not significant ($p > 0.05$) (Table 1). Table 2 shows reductions in hepatic, renal and cardiac GST, GSH and GPx levels in male wistar rats following treatment with anti-TB drugs. Precisely, hepatic, renal and cardiac GST decreased by 10.1, 49.0 and 63.5% while GSH decreased by 2.3, 2.2 and 3.5% and GPx decreased by 48.7, 16.1 and 27.8%, respectively. Of the lipid profile and biochemical indices of the experimented animals, triglyceride and serum protein assays were significantly increased in the treated group compared with control ($p < 0.05$). Similarly, serum creatinine and direct bilirubin levels were significantly increased in treated group compared with the control ($p < 0.05$). Although, AST level was much higher in treated group than the control, the association was not significant ($p > 0.05$) (Table 3).

Furthermore, there was a significant increase in serum total bilirubin level in the treated group. Even though urea level was increased in treated group compared with the control, the association was not significant ($p > 0.05$) (Figure 1). Changes in the activities of hepatic catalase (CAT) and superoxide dismutase (SOD) were shown in Figure 2. Although, renal and cardiac SOD levels were

reduced in the treated group compared with the control, the association was not significant ($p > 0.05$) (Figures 3 and 4).

DISCUSSION

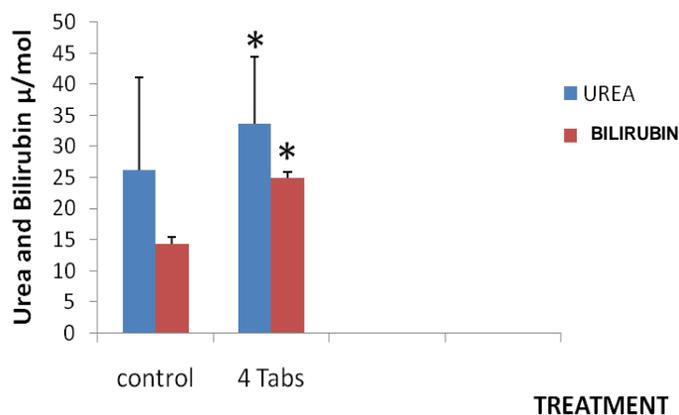
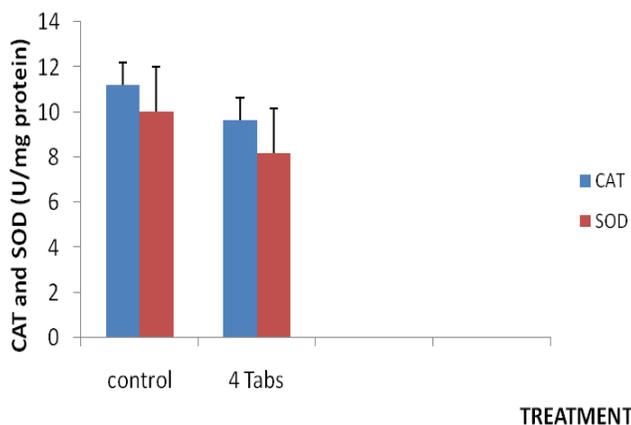
Anti-tuberculosis drug therapy is the mainstay of global TB eradication program. Oxidative stress in TB patients may be due to the disease itself (tissue inflammation leading to generation of free radicals from activated macrophages), treatment (effects of anti-TB drugs), and general condition of the host including poor dietary intake of micronutrients due to illness (Wiid et al., 2004; Mokondjimobe et al., 2012). In this study, we report the contributions of oxidative stress in relation to treatment changes independent of disease status and response in a controlled environment using an animal model.

There was an increase in body weight in the treated animals compared to control even though the association was not significant ($p > 0.05$) (Table 1). This increased weight gain may be as a result of improved appetite exhibited by the treated animals as an attempt to overcome the drug-induced stress. Significantly, renal and cardiac GST and also hepatic GPx levels were reduced in the treated animals compared with control (Table 2). This is in agreement with the findings of Wiid et al. (2004) who reported lower

Table 3. Effect of 4 anti-TB drugs on lipid and biochemical indices of the treated rats.

Assay	Control	Treated group
Trigs (mmol/L)	53.60 ± 3.50	169.69 ±77.36*
HDL-C (mmol/L)	97.13 ± 34.55	102.67 ± 39.34
Total-Chol (mmol/L)	37.24±1.78	40.35±5.56
Serum protein (mmol/L)	1.65±1.61	16.05±2.54*
ALT (U/L)	0.31±0.08	0.42±0.07
AST (U/L)	227.5±56.57	295.5±54.4
Creatinine (µmol/L)	1.37±0.60	4.82±1.26*
Direct bilirubin (µmol/L)	2.92±1.36	23.78±5.55*
Indirect Bilirubin (µmol/L)	13.86±2.65	14.32±12.39

Values are expressed as means ± S.D. (n= 7). *Significantly different from control (p < 0.05). Trigs= Triglycerides; HDL-C = High density Lipoprotein; Total Chol = Total cholesterol; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

**Figure 1.** Serum urea and total bilirubin assays in Wistar rats following anti-TB drugs. Values are expressed as means ± S.D. (n= 7). *Significantly different from control (p < 0.05). 4-Tabs means anti-TB drug treatment.**Figure 2.** Changes in the activities of hepatic catalase (CAT) and superoxide dismutase (SOD) in male wistar rats following 8 weeks treatment with anti-TB drugs. Values are expressed as means ± S.D. (n= 7). *Significantly different from control (p < 0.05). 4-Tabs means anti-TB drug treatment.

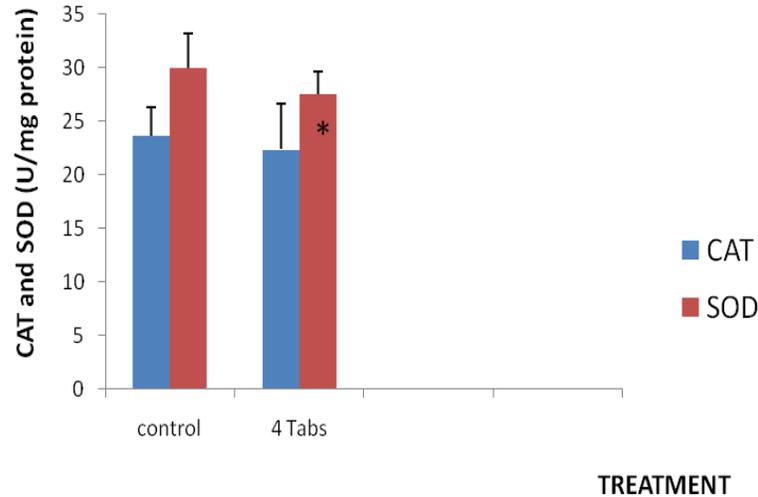


Figure 3. Changes in the activities of renal catalase (CAT) and superoxide dismutase (SOD) in wistar rats after 8 weeks treatment with anti-TB drugs. Values are expressed as means \pm S.D. (n= 7). *Significantly different from control (p < 0.05). 4-Tabs means anti-TB drug treatment.

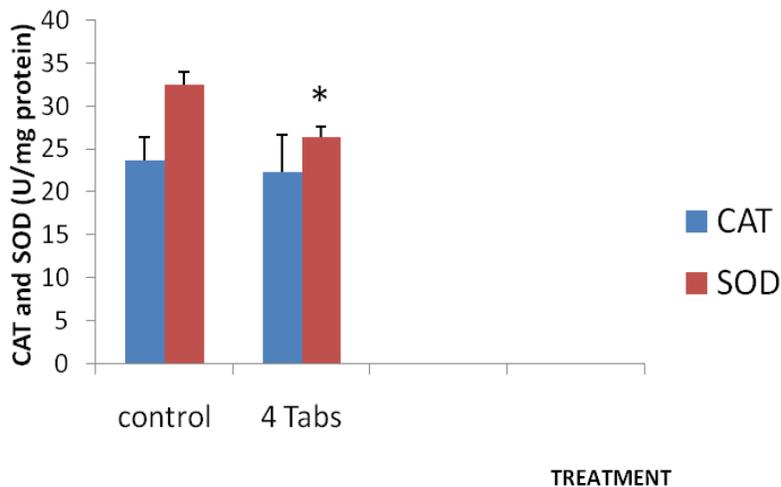


Figure 4. Changes in the activities of cardiac catalase (CAT) and superoxide dismutase (SOD) in male wistar rats after 8 weeks treatment with anti-TB drugs. Values are expressed as means \pm S.D. (n= 7). *Significantly different from control (p < 0.05). 4-Tabs means anti-TB drug treatment.

serum levels of antioxidant enzymes such as GST and GPx in human patients undergoing anti-TB therapy. This low antioxidant levels may be attributed to drug toxicities as part of pharmacokinetics of anti-TB drugs. Significantly, high levels of triglycerides and serum protein were observed in this study. This finding is not in support of the submission of Akiibinu et al. (2007), who reported low level of triglyceride, total cholesterol, low and high-density lipoprotein cholesterol and serum protein including albumin in PTB infected humans. Low

levels of triglyceride and serum proteins were also reported by other workers (Yamanaka et al., 2001). This discrepancy may be due to the fact that human subjects used in the previous studies were confirmed PTB patients while TB diagnosis was not established in this present experiment.

This is one of the limitations of this study. TB is a chronic inflammatory disease often associated with anorexia, malnutrition, malabsorption, tissue damage and may present as a wasting disease. All these may lead

to reduced triglyceride and serum protein in the body.

Serum creatinine and direct bilirubin levels were significantly elevated in the treated animals. Also hepatic transaminases (ALT and AST) were higher in the treated group compared to the control group (Table 3). This finding is in agreement to that of Mokondjimobe et al. (2012) which revealed that liver enzymes (AST and ALT) were markedly elevated in PTB patients following 2 months treatment with anti-TB drugs. This drug-induced hepatitis may further be worsened by malnutrition, excessive alcohol intake, co-morbidities such as hepatitis B and C infections, HIV and poor drug compliance (Breen et al., 2006; Tostmann et al., 2008). It is worthy to note that 3 out of the 4 anti-TB drugs used in this experiment have been documented to be hepatotoxic (Frieden et al., 2003). These drugs are isoniazide, rifampicin and pyrazinamide. Significantly, high levels of serum urea observed in this study may be due to oxidative host tissue damage. Reduced CAT and SOD levels were observed in liver, kidney and heart of the treated animals (Figures 2 to 4). This agrees with the submission of Ali et al. (2014), who reported that serum CAT and SOD levels were significantly reduced in patients with pulmonary TB including those with multi-drug resistant TB who were undergoing therapy. The depletion of CAT and SOD antioxidants may be due to an attempt to limit the inflammatory process.

In conclusion, hepatic, renal and cardiac GST, GSH, GPx, CAT, SOD levels including renal and cardiac GST levels were significantly reduced in the treated group compared to control. In the same vein, triglycerides, serum protein and liver enzymes (ALT and AST) were significant raised in the treated group when compared with the control.

Conflict of interest

Authors declare no conflict of interest.

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