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ETHAMBUTOL-INDUCED OVARIAN, UTERINE AND PLACENTAL OXIDATIVE STRESS: IMPLICATION FOR REPRODUCTIVE OUTCOME IN FEMALE WISTAR RATS

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ABSTRACT

Tuberculosis is a major global challenge, potentially infecting more individuals than other pathogens. Ethambutol, a first line drug used in tuberculosis treatment, lacks adequate research regarding its impact on female reproductive health. This study investigates ethambutol-induced oxidative stress to the ovary, uterus and placenta with implications to reproductive outcome in female Wistar rats. Twenty adult female Wistar rats weighing between 170g-190g were divided into two groups (A and B) of ten rats each. Group A served as control and received only food and water ad libitum. Group B was administered with 15 mg/kg body weight of ethambutol, orally, daily for 28 days. After 28 days, five animals from each group were sacrificed by cervical dislocation and the ovaries and uterus were harvested for oxidative stress analysis. The remaining animals from each group were mated, and ethambutol administration continued until gestational day 19 when they were sacrificed, and the placentae were harvested for oxidative stress analysis. The fetuses were used to study pregnancy outcomes. From the result, ovarian glutathione peroxidase was significantly elevated, uterine superoxide dismutase and catalase levels were significantly decreased while malondialdehyde activity was significantly elevated, placental catalase activity was significantly decreased while glutathione peroxidase and malondialdehyde activities was significantly elevated following ethambutol administration. On pregnancy outcomes, ethambutol significantly decreased crown rump length, litter weight, placental weight and fetal/placental weight ratio. In conclusion, evidence from this study suggests that ethambutol is toxic to the ovary, uterus and placenta via mechanisms that involve oxidative stress resulting in poor pregnancy outcomes.

Keywords: Ethambutol, oxidative stress, ovary, uterus, placenta.

INTRODUCTION

Tuberculosis (TB), a *Mycobacterium tuberculosis* (M. tuberculosis) remains an epidemic and it is the leading cause of illnesses and death worldwide (Sobhy *et al.*, 2017). It was the second leading cause of death from a single infectious agent in the world, after coronavirus disease (COVID-19), and caused almost twice as many deaths as HIV/AIDS (World Health Organization, 2023). The latest treatment protocol recommended by the World Health Organization (WHO) include a for a 6-month

regimen of isoniazid, rifampicin, ethambutol and pyrazinamide for people with drug-susceptible TB (both pulmonary and extrapulmonary): all four drugs for the first two months, followed by isoniazid and rifampicin for the remaining 4 months (WHO, 2022). This combination has for long remained the best for efficacy and tolerability amongst the available TB drugs and is, therefore, the mainstay "first line" therapy (Mathers, 2015).

Since tuberculosis affects people in their productive and reproductive age, there have been recent suggestions that anti-tuberculosis agents could produce adverse effects on reproductive health system (Caliskan *et al.*, 2014; Ezeuko *et al.*, 2019; Ezeuko and Ataman, 2020).There is paucity of data on the adverse drug reactions in patients and animals experiments particularly with respect to reproduction (Gwaza, 2014). There is limited data available regarding the impact of ethambutol on female reproduction and its effects on the placenta. Considering these gaps in literature, it becomes imperative to investigate the potential of ethambutol to induce oxidative stress on the ovary, uterus and placenta, with an implication of affecting female reproductive outcome.

MATERIALS AND METHOD

Experimental Animals

Wistar rats used for the study were bred at the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. They were kept in polypropylene cages under room temperature, with natural light and dark cycle photoperiodicity. The animals were fed with Topfeeds Growers Mash (manufactured by Premier feed mills Co. Ltd, Ibadan, Oyo State, Nigeria) and clean tap water. They were weighed weekly before commencement of study and throughout the duration of the experiment. Protocols for these experiments were in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council of the National Academics, 2011).

Design of Study

Twenty adult female Wistar rats weighing between 170-190g were used for this study. They were divided into two groups (A and B) with ten rats per group. Each group were further subdivided into two subgroups (A1and A2, B1 and B2) containing five rats in each. All administration was through the oral route. Group A served as the control group and received only food and water *ad libitum*. Group B was administered with 15 mg/kg body weight of ethambutol, orally, daily for 28 days. After 28 days, five animals from each group were sacrificed under chloroform anesthesia and the ovaries and uterus were harvested for oxidative stress analyses. The remaining halves of the animals from each group were mated overnight with potent male in the ratio of 1:1. Pregnancy was confirmed in the following morning (between 8 am to 9 am) by the presence of sperm plug and/or sperm cells in the vaginal fluid. The day pregnancy was confirmed was recorded as gestational day 1. Ethambutol administration continued during pregnancy until gestational day 20 when they were laparotomized under chloroform anesthesia. The uterine horns were exteriorized and incised at the greater curvature of the horns. The weights of the fetuses and placentae, placental diameter and fetal crown rump length were recorded.

The ovaries, uterus and placenta were, immediately after harvesting, blotted free of blood and weighed immediately using an electronic weighing. The relative organ weights were evaluated as the percentage of absolute organ weight divided by the final body weight and recorded as ovariosomatic index and uterosomatic index respectively.

Antioxidant Enzymes and Lipid Peroxidation assessment

The tissues were then washed twice in cold phosphate buffered saline (PBS) after which they were homogenized using acid-washed

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sand and PBS in porcelain mortar and pestle. The tissue homogenate was centrifuged at 10000 rpm for 10 minutes at 4^{o}C. The supernatant was immediately processed for analysis of endogenous antioxidants enzymes (Ezeuko *et al.,* 2019; Ezeuko and Ataman 2020).Malondialdehyde activity was determined according to the method of Gutteridge and Wilkins (1982). Superoxide dismutase activity was determined according the method of Misra and Fridovich (1972). Catalase activity was determined according the method of Cohen *et al.* (1970). Glutathione peroxidase activity was determined according the method of Nyman (1959).

Statistical analysis

Data were analyzed using IBM statistical package for social sciences. Results were presented as mean ± standard error of mean (mean ± SEM). The parameters for all the groups were compared using students't-test. P<0.05 was considered significant.

RESULTS

There was no statistically significant effect (P>0.05) on initial body weight, final body weight, ovarian weight, ovariosomatic index and uterine weight in the ethambutol-treated group compared to the control group (table 1). However, uterosomatic index was significantly lower (P<0.05) in the ethambutol-treated group compared to the control group (table 1). Furthermore, whereas ethambutol administration showed no statistically significant (P>0.05) effect on the ovarian catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) levels, ovarian glutathione peroxidase (GPx) was significantly elevated (P<0.05) following ethambutol administration compared to the control group (table 2). Uterine SOD and CAT levels were significantly decreased (P<0.05) while MDA was significantly elevated (P<0.05) following ethambutol administration compared to control group but there was no statistically significant effect on GPx level (P>0.05) compared to the control group (table 3). On pregnancy outcomes, ethambutol administration significantly decreased (P<0.05) crown rump length, litter weight, placental weight and fetal/placental weight ratio compared to the control group without significantly (P>0.05) affecting the placental diameter (table 4). Finally, placental CAT activity were significantly decreased (P<0.05) while GPx and MDA activities was significantly elevated (P<0.05) following ethambutol administration compared to control group but there was no statistically significant effect on SOD activity (P>0.05) compared to the control group (table 5).

Table 1: Effects of Ethambutol on Body Weight, Ovarian and uterine weights

Parameters	Control	Ethambutol	P
Initial body weight (g)	179.667±2.667	175.200±4.532	0.871
Final body weight (g)	227.400±8.346	214.400±1.778	0.084
Body weight change (g)	47.733±5.132	39.200±3.441	0.127
Ovary weight (g)	0.083±0.003	0.082±0.009	0.924
Ovariosomatic index (%)	0.052±0.001	0.038±0.004	0.064
Uterine weight (g)	0.36±0.044	0.36±0.009	1.000
Uterosomatic index (%)	0.224±0.03	0.168±0.004	0.025*

* significant difference (<0.05) between the control group and the ethambutol-treated group

Table 2: Effects of Ethambutol on Ovarian Oxidative Stress

Parameters	Control	Ethambutol	P
Catalase(K/min)	0.103±0.005	0.115±0.013	0.172
Superoxide dismutase (Units/g tissue)	0.275±0.011	0.270±0.011	0.776
Glutathione peroxidase (Units/mg tissue)	0.382±0.024	0.475±0.021	0.044*
Malondialdehyde (mol/g tissue)	0.041±0.002	0.043±0.003	0.828

* significant difference (<0.05) between the control group and the ethambutol-treated group

Table 3: Effects of Ethambutol on Uterine Oxidative Stress

Parameters	Control	Ethambutol	P
Catalase (K/min)	0.097±0.002	0.015±0.008	0.004*
Superoxide dismutase (Units/g tissue)	0.273±0.011	0.194±0.037	0.018*
Glutathione peroxidase(Units/mg tissue)	0.430±0.026	0.475±0.047	0.448
Malondialdehyde(mol/g tissue)	0.048±0.002	0.149±0.005	0.000*

* significant difference (<0.05) between the control group and the ethambutol-treated group

Table 4: Effects of Ethambutol onPregnancy Outcomes

Parameters	Control	Ethambutol	P
Crown rump length (mm)	34.447±0.916	26.138±1.196	0.000*
Litter weight (g)	3.621±0.280	1.604±0.153	0.000*
Placental diameter (mm)	12.793±0.440	13.379±0.231	0.210
Placental weight (g)	0.478±0.019	0.402±0.016	0.003*
Fetal/placentalweight ratio	7.936±0.803	3.811±0.318	0.000*

* significant difference (P<0.05) between the control group and the ethambutol-treated group

Table 5: Effects of Ethambutol on Placental Oxidative Stress

Parameters	Control	Ethambutol	P
Catalase (K/min)	0.381±0.014	0.056±0.002	0.000*
Superoxide dismutase (Units/g tissue)	0.249±0.003	0.298±0.005	0.127
Glutathione peroxidase (Units/mg tissue)	0.372±0.011	0.810±0.020	0.000*
Malondialdehyde (mol/g tissue)	0.031±0.013	0.171±0.008	0.000*

* significant difference (P<0.05) between the control group and the ethambutol-treated group

DISCUSSION

Ethambutol has remained one of the four first-line drugs for the treatment of tuberculosis, especially in the first two months (WHO, 2023). Whereas concerns have been raised on the toxic potentials of antituberculosis drugs, less attention has been paid to ethambutol as a potential female reproductive toxicant. The present study attempts to expose the possibility of ethambutol to induce oxidative damage to the ovary, uterus and placenta using experimental animal model.

The present study revealed weight gain following ethambutol administration, comparable to control group. This is consistent with earlier reports by several researchers of weight gain in tuberculosis patients following treatment with anti-tuberculosis agents been pointed as a marker for response to treatment (Vasantha et al., 2009; Gler et al., 2013; Wassie et al., 2014; Phan et al., 2016; Ezeuko et al., 2019; Ezeuko and Ataman, 2020). The insignificant effect on ovarian following ethambutol administration is at variance with AL -Chalaby (2012), Adebayo et al. (2018), Ezeuko and Ataman (2020) who had all implicated other first-line antituberculosis drug in ovarian weight reduction. This, in a way exonerates ethambutol in ovarian weight reduction. Furthermore, the present study revealed no significant effect of ethambutol on ovariosomatic index. This is in agreement with Ezeuko and Ataman (2020) on insignificant effect of other first-line anti-tuberculosis agents on ovariosomatic index.

The significant decrease uterosomatic index following ethambutol administration is in agreement with Adebayo et al. (2018) and Ezeuko and Ataman (2020) who found significant decrease in uterine weight and

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relative uterine weight following administration of other first-line anti-tuberculosis agents.

One of the most studied mechanisms of anti-tuberculosis agents-induced toxicities is their ability to induce oxidative stress. Adebayo et al. (2018) and Ezeuko and Ataman (2020) had shown the capacity of first-line anti-tuberculosis agents to induce oxidative stress in the reproductive organs. This is also confirmed in this study which revealed significant increase in ovarian glutathione peroxidase activity, significant decrease in uterine superoxide dismutase and catalase activities and significant increase in malondialdehyde activity following ethambutol administration.

Furthermore, in this study, placental catalase activity was significantly decreased while glutathione peroxidase and malondialdehyde activities were significantly elevated following ethambutol administration. Wang and Walsh (1996, 2001) and Ezeuko et al. (2019) had all implicated oxidative stress in pathological placental tissues.

The present study further showed that ethambutol administration significantly decreased crown rump length, litter weight, placental weight and fetal/placental weight ratio. These findings agree with previous literature by Awodele (2013) that suggests that ethambutol lowers birth weight of fetuses.

CONCLUSION

Evidence emanating from this study suggests that Ethambutol to toxic to the ovary, uterus and placenta via mechanisms that involve oxidative stress resulting in poor pregnancy outcomes.

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