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Evaluation of isoniazid-oxidative reactions in mice model

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ABSTRACT

In this study the anti-tubercular drug; isoniazid (**INH**) was investigated for their adverse effect; the oxidative stress. This effect was evaluated by using mice model, at the dose of 151 mg/kg. We found that oxidative stress induced by **INH** is associated with lipid peroxidation expressed by the increase in the level of MDA from 76.9 ± 1.74 to 79.61 ± 2.67 nmol/g tissue. The oxidative stress of **INH** is accompanied by a decrease in reduced GSH level (from 79.9 ± 12 µmol / mg to 68.48 ± 4.28 µmol / mg compared to of the control group). After treatment with **INH** at 151 mg/kg, a decrease in CAT activities occurred compared to control (2.53 ± 0.39 U/mg Pr *vs* 5.07 ± 0.73 U/mg Pr).

Keywords: isoniazid, oxidative stress, MDA, GSH, CAT

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INTRODUCTION

Isoniazid (**INH**), chemically known as isonicotinic acid hydrazide, is a low-molecular weight and water-soluble compound that can be rapidly absorbed from the gastrointestinal tract. **INH** has been a first-line drug for the treatment of tuberculosis for more than 50 years, with maximum doses for adult of 300 mg. It inhibits bacterial cell wall synthesis, thus killing *Mycobacterium tuberculosis* organisms. Due to its low efficacy towards resistant tuberculosis, it is generally used in combination with other anti-tubercular drugs such as pyrazinamide, ethambutol, and/or rifampicin [1]. In literature, **INH** is one of oxidative drugs. Three principal mechanisms for the oxidative reactions of **INH**; Immune cell products (H_2O_2 and peroxidase) activate **INH** to pro-oxidant hydrazyl, inducing oxidative stress [2]. The damage of lysosomal membrane causes ROS formation *via* Fenton reaction, which induces oxidative stress and cytotoxicity. The oxidation of **INH** leads to the formation of nitric oxide radical, causing oxidative stress [3,4]. In this study, the oxidative effect of **INH** was evaluated in vivo using mice model, which estimated by lipid peroxidation expressed the level of MDA, the activities of enzymatic antioxidants such as CAT, and non-enzymatic antioxidants such as GSH.

EXPERIMENTAL SECTION

In vivo oxidative stress parameters estimation

In this experiment, **INH** was used as an oxidizing agent that induces oxidative stress in mice. After an 18 h of fasting, **INH** was injected with intraperetenial route at 151 mg/kg [5]. The experimental mice were treated as followed:

Group I: control (untreated),

Group II: treated with, **INH** (151 mg/kg)

After 4 h, animals were scarified.

Preparation of tissue homogenates

Liver samples were stored at –4 ºC until homogenation, then an amount of 500 mg of liver of the different groups was added to 5 mL of the ice KCl buffer (0.15 M). The mixture was homogenized at 1200 rpm using electric homogenizer.

Determination of CAT activity

The CAT activity was determined in supernatant, obtained after a 15 min centrifugation of liver homogenates at 4000–5000 rpm. The CAT activities were measured as follows: 30 mM hydrogen peroxide was used as a substrate. To each sample, 1.9 mL phosphate buffer (pH 7.2) and 1 mL 30 mM H_2O_2 were added. The decrease in H_2O_2 concentration at 25 ºC was spectrophotometrically determined at 240 nm. The extinction coefficient was read exactly 1 min after H_2O_2 addition. CAT activities presented as units permg protein (Units/mg Protein).

Estimation of GSH

The GSH levels were estimated in the homogenates of liver using (5,5’-dithiobis-(2-nitrobenzoic acid), (DTNB), in which 5 mL of sodium phosphate buffer (0.1 M, pH = 8) was added to 25 µL of supernatant homogenate, then 1.5 mL of the

mixture was added to 10 µL of DTNB (0.01 M). Determination of GSH is based on the reaction of DTNB with GSH to yield a yellow colored chromophore with a maximum absorbance at 412 nm. The amount of GSH present in the tissue was calculated using its extinction coefficient (1.36×10^4 M^{-1} cm^{-1}) at 412 nm and expressed as µmoles/g tissue [5].

Estimation of MDA

The hepatic malondialdehyde (MDA) content was an indicator to determine hepatic lipid peroxidation levels. In this experiment, 125 µL of trichloric acid (TCA, 20%) and 250 µL of thiobarbituric acid (TBA, 0.67%) were added to 125 µL of tissue homogenate. The mixture was incubated at 100 °C during 20 min. After incubation, the sample was cooled. The MDA-TBA complex was extracted with 1 mL of butanol. The organic phase was separated by centrifugation at 3000 rpm for 15 min. Absorbance was measured at 530 nm. The concentration of MDA was calculated using its extinction coefficient (1.56×105 M^{-1} cm^{-1}) at 530 nm and expressed as nanomoles MDA/g tissue [6].

RESULTS AND DISCUSSION

Due to the good predicted results and the high free radical-scavenging effect and to the similar structure to indole-derivatives, which are considered potential antioxidant agents [7], the oxidative stress induced by the drug **INH**. Results of this study indicated that intraperitoneal injections of isoniazid at 151 mg/kg led to an increase in malondialdehyde (MDA) formation, decrease in the activities of enzymatic antioxidants such as CAT, and a decrease in non-enzymatic antioxidants such as GSH when compared with the control.

We found that oxidative stress induced by **INH** is associated with lipid peroxidation expressed by the increase in the level of MDA from 76.9 ± 1.74 to 79.61 ± 2.67 nmol/g tissue. This number was decreased to 68.02 ± 2.27 and 60.46 ± 4.63 nmol/g tissue. The reduced GSH is one of non-enzymatic antioxidant present in the liver which scavenges reactive toxic metabolites of anti-tubercular drugs; liver damage was observed when GSH stocks were significantly decreased [8]. The oxidative stress of **INH** is accompanied by a decrease in reduced GSH level (from 79.9 ± 12 µmol / mg to 68.48 ± 4.28 µmol / mg compared to of the control group). Results of the enzymatic antioxidant, CAT activities of treated mice are presented in **Table 1**. After treatment with **INH** at 151 mg/kg, a decrease in CAT activities occurred compared to control (2.53 ± 0.39 U/mg Pr *vs* 5.07 ± 0.73 U/mg Pr). The decreased CAT activity could be due to the enhanced generation of ROS during isoniazid metabolism [9] that is deactivated by CAT as well as to the activation of the peroxide oxidation of lipids, proteins, nucleic acids, and other macromolecules.

Table 1. Effect of **INH** on oxidative stress parameters.

Groups	CAT (U/mg protein)	MDA (nmol/g tissue)	GSH (µmol/mg protein)
Control	5.07 ± 0.19	76.9 ± 1.74	79.90 ± 12
INH 151 (mg/kg)	2.53 ± 0.63***	79.61 ± 2.67**ns**	68.48 ± 4.28***

Values expressed as mean ± SEM, n= 5 animals /group;***: $p < 0.001$, **: $p < 0.01$, **ns**: no significant differences.

CONCLUSION

Isoniazid increased the consumption of endogenous antioxidants which could be responsible for the increasing in oxidative stress during their metabolism.

ABBREVIATIONS

INH: isoniazid

ns: no significant differences

MDA: Malondialdehyde

GSH: Gluthation

CAT: Catalase

Pr: Protein

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