H. FARAMARZI, J. SAFFARI-CHALESHTORF, S. ZOLGHADRF, M. BEHESHTROO, A. FARAMARZI, S. M. SHAFIEE

1Department of Community Medicine, Faculty of Medicine, Shiraz University of Medical Sciences, Iran;
2Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran;
3Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran;
4Department of Biochemistry, Shiraz Branch, Islamic Azad University, Shiraz, Iran;
5Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran;
6Autophagy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

E-mail: shafieem@sums.ac.ir

Received: 08 November 2021; Revised: 27 June 2021; Accepted: 29 September 2022

Isoniazid is one of the anti-tuberculosis therapeutic agents capable of causing side effects such as oxidative stress, brain tissue damage and mental disorders. This study aimed to investigate the effect of ferric oxide (Fe$_2$O$_3$) nanoparticles administration on isoniazid-induced oxidative stress parameters in rat brain tissue. Forty adult male Wistar rats (200–250 g) were randomly divided into a group with no treatment as control and four experimental groups. Animals of experimental groups received intraperitoneally for 12 days daily saline, 50 mg/kg of isoniazid, 50 mg/kg of isoniazid and 0.2 or 0.4 mg/kg Fe$_2$O$_3$ nanoparticles accordingly.

The activity of catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST), the level of glutathione (GSH), malondialdehyde (MDA) and total protein were determined in brain tissue homogenates by spectrophotometric methods. It was shown that CAT and GST activities, as well as GSH and MDA levels in the brain tissue of animals in the isoniazid-treated group were increased compared with the control untreated group, while following the treatment with 0.2 or 0.4 mg/kg Fe$_2$O$_3$ nanoparticles the studied oxidative stress parameters returned to the control level (P < 0.05). No changes in SOD activity in any of the treated groups were observed compared to the control. This study showed that the administration of ferric oxide nanoparticles can suppress isoniazid-induced oxidative stress in the brain tissue of rats mentally damaged by isoniazid.

**Keywords:** isoniazid, ferric oxide nanoparticles, oxidative stress parameters, brain tissue.

Isoniazid, as a well-known anti-tuberculosis therapeutic agent, has toxic properties which induce some metabolic and morphologic disorders [1]. Heart, liver, and kidney disorders are the most common side effects of treatments with isoniazid [2-4]. However, brain disorders induced by the inflammation and apoptosis in cerebral cortex tissue cause severe cognitive impairment [5].

Oxidative damage, as the worst effect of isoniazid on tissues, occurs from reactive oxygen species (ROS) [6]. These ROSs result in the formation of lipids peroxide that leads to cell membrane damage [7]. Oxidative stress, which is induced by releasing the ROSs, increases the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST), to moderate the ROSs [8, 9]. Moreover, the ROSs led to an increase in malondialdehyde (MDA) and glutathione (GSH) concentrations by inducing lipid peroxidation [10, 11]. Considerable evidence suggests that lipid peroxidation contributes to synaptic dysfunction and neuronal degeneration in brain tissue [12]. In addition, oxidative stress plays an important role in brain injury and tissue damage. The generation of oxidative stress leads to mitochondrial dysfunction, lipid peroxidation, and oxidation of pro-
teins and DNA in brain tissue. As a result, neuronal cell death takes place and people start facing some serious disabilities [13].

There are many antioxidants such as vitamins, medicinal plants, and nutritional supplements that ameliorate oxidative stress [13-16]. Nowadays, nanoparticles have been shown as new important interferers in biological systems [17]. Although, in some cases and depending on their structures, nanoparticles have demonstrated cell toxicity, most of them with antioxidant properties may improve vascular dysfunction associated with hypertension, diabetes mellitus, or atherosclerosis [18]. Some studies demonstrated the potential cytotoxicity of iron-based nanoparticles but they have potential implications in medicine, chemistry, and biology [19, 20]. Ferric oxide (Fe2O3) nanoparticles enhance hydrogenase enzyme activity and electron transfer. Therefore, they facilitate the growth of hydrogen-producing bacteria and richness [21]. Nevertheless, some Fe2O3 nanoparticles cause hemolysis inhibition of mammalian erythrocytes via their antioxidant effects [22].

It seems that the antioxidant properties of Fe2O3 nanoparticles can induce protective effects against oxidative stress [23, 24]. This study investigated the neuroprotective and antioxidant effects of Fe2O3 nanoparticles, on isoniazid-induced neurotoxicity rats.

**Material and Method**

*Animals and reagents.* The Wistar rats were obtained from Pasteur Institute of Iran, Isoniazid and Fe2O3 nanoparticles were purchased from (Sigma-Aldrich Corp., MI, USA), Ethylenediaminetetraacetic acid (EDTA), sodium cyanide, nitro blue tetrazolium (NBT) was obtained from (Merck, Germany).

*Treatment the animals.* In this study, 40 adult male Wistar rats weighing 200–250 g were housed in a temperature of 22 ± 2°C and the humidity of 50 ± 5% controlled room with 12 h of light/dark cycle. They were kept for a week for adaptation to the environment before the start of the experiments. A standard diet and tap water were provided *ad libitum.*

All tests and manipulations were conducted according to the guidelines of Institutional Animal Care and the Medical Ethics Committee of Shiraz University of Medical Sciences. There are 5 groups and each group contains 8 rats;

Negative control group: animals (*n* = 8) received distilled water daily and normal food for 12 days. Sham group: animals (*n* = 8) received normal saline (isoniazid solvent) intraperitoneally daily for 12 days. Positive control group: animals (*n* = 8) received 50 mg/kg isoniazid intraperitoneally daily for 12 days. Treated group 1: animals (*n* = 8) received 50 mg/kg isoniazid intraperitoneally daily and Fe2O3 nanoparticle at a dose of 0.2 mg/kg BW for 12 days. Treated group 2: animals (*n* = 8) received 50 mg/kg isoniazid intraperitoneally daily and Fe2O3 nanoparticle at a dose of 0.4 mg/kg BW for 12 days.

In this study, dead animals were excluded. All animals were sacrificed 24 h after the last INH treatment, and their brain tissue was removed quickly and washed out with ice-cold 0.9% saline solution. The tissues were homogenized and powdered by liquid nitrogen (LN2) and stored at -70°C. Then, 50 mg of the powdered brain tissue was homogenized in ice-cold phosphate buffer pH = 7.2, 10 mM and centrifuged at 20,000 g for 10 min at 4°C. The supernatant solution was used immediately for the evaluation of biochemical parameters.

* Determination of glutathione-S-transferase (GST) activity.* To determine the GST activity, we used the spectrophotometric method that has been described by Habig et al [25].

Briefly, 0.015 g of the powdered tissue in 1 ml of normal saline was added to 150 µl of 1-chloro-2,4-dinitrobenzene (CDNB) (20 µM) as the substrate, 150 µl of glutathione (20 mM), and 2.5 ml of NaH2PO4 (0.1 M) buffer. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase was directly proportional to the GST activity in the sample.

* Determination of catalase (CAT) activity.* Catalase (CAT) enzyme activity was determined according to the Aebi method [26]. The reaction mixture contained enzyme extract (0.015 g tissue powdered and 1 ml normal saline), 100 mM potassium phosphate buffer (pH 7.0), and 10 ml of 30% H2O2. The reaction was initiated by the addition of H2O2 and the absorbance was measured at 240 nm. One unit of CAT was defined as the amount of enzyme catalyzing the decomposition of 1 mmol of H2O2 per minute. The specific activity was calculated based on the units per mg (unit/mg) of protein [26].

* Determination of superoxide dismutase (SOD) activity.* SOD converts the superoxide anion into hydrogen peroxide and oxygen. The SOD activity was measured according to the Worthington method. Briefly, 0.1 M of EDTA in 0.3 mM of sodium cyanide and 1.5 mM of nitroblue tetrazolium (NBT) was added to the sample in a tube and vortexed for 5 min.
on ice. Then, 0.12 mM of riboflavin in 0.067 M potassium phosphate buffer (pH = 7.0-8.0) was added and placed at room temperature for 30 min. Absorption at a λ 560 nm was read within 5 min, and specific activity was calculated based on the units per μg (unit/μg) of protein [27].

Determination of total protein. The protein content was measured using the Bradford method [28]. Briefly, 150 µl of the sample (0.015 g tissue powdered in 2.5 ml normal saline) was added to 600 µl of Bradford solution. Then, 2.25 ml of normal saline was added and incubated for 10 min at room temperature. Subsequently, the absorbance of the solution was measured at λ 595 nm. A series of different dilutions i.e. 30, 60, 90, 120, and 150 µg/ml of BSA was prepared as a standard calibration curve.

Determination of glutathione (GSH). Glutathione is an antioxidant that protects cell components from ROS was determined by the Ellman method [29].

Briefly, 0.02 g of the powdered tissue in 1 ml KH₂PO₄, 0.1 M was added to 200 µl of TCA 10% and was centrifuged at 5000 g for 10 min. Subsequently, 100 µl of supernatant was added to 100 µl of acid benzoic nitro- 2 dithiobis (DTNB) (30 mM). The absorbance was measured at λ 240 nm after 10 min. A series of five different dilutions i.e. 10-20 mM GSH was prepared as a standard calibration curve.

Determination of malondialdehyde (MDA). Lipid peroxidation was assessed using MDA level measurement by the Satoh method [30]. According to this method, 0.015 g of the powdered tissue in 2.5 ml normal saline was added to 2.4 ml of TCA (20%) and was centrifuged at 4000 g for 10 min. Afterward, 2 ml of supernatant was added to 2.7 ml of TBA (%0.86) and stored at 100°C for 30 min. Subsequently, 2.4 n-butanol was added and centrifuged at 4000 g for 15 min. The absorbance of the supernatant was measured at 532 nm. A series of different dilutions i.e. 2.5, 5, 10, 20, 30, 40, 50, and 60 μM of 1,1,3,3 Tetra Epoxy Propane (TEP) was prepared as a standard calibration curve.

Statistical analysis. All data were analyzed by SPSS software version 20 (SPSS Inc., Chicago, IL, USA). The data were analyzed between the groups by One way ANOVA and Tukey’s post hoc multi-comparison. All results were shown as means ± SD. The P-value of less than 0.05 was considered significant.

Results

As shown in Fig. 1 (I), brain GST enzyme activity in the treated rats with isoniazid (50 mg/kg) has increased significantly (P < 0.05) in comparison with control and sham group. This significant increase has also been observed in the treated group by 50 mg/kg isoniazid along with 0.2 mg/kg Fe₂O₃ nanoparticle. The brain GST enzyme activity decreased in the treated group by 50 mg/kg isoniazid with 0.4 mg/kg Fe₂O₃ nanoparticle in comparison with the treated group by 50 mg/kg isoniazid and the treated group by isoniazid (50 mg/kg) with 0.2 mg/kg Fe₂O₃ nanoparticle. Nevertheless, the brain GST enzyme activity increased in the treated group by 50 mg/kg isoniazid with 0.4 mg/kg Fe₂O₃ nanoparticle in comparison with the control and sham groups.

Isoniazid dose of 50 mg/kg led to an increase in the brain CAT enzyme activity significantly (P < 0.05) in comparison with control and sham groups (Fig. 1(II)). However, brain CAT activity has decreased significantly in treated rats with 50 mg/kg isoniazid, 0.2, and 0.4 mg/kg Fe₂O₃ nanoparticle in comparison with treated rats with 50 mg/kg isoniazid (P < 0.05). Moreover, the brain CAT enzyme activity had no significant changes in treated groups with 50 mg/kg isoniazid with 0.2, and 0.4 mg/kg Fe₂O₃ nanoparticle compared with treated rats with 50 mg/kg isoniazid. However, brain CAT activity decreased significantly (P < 0.05) in the treated group with 50 mg/kg isoniazid with 0.2, and 0.4 mg/kg Fe₂O₃ nanoparticle compared with control and sham groups. Actually, brain CAT activity decreased significantly (P < 0.05) in comparison with control and sham group. This significant increase has also been observed in the treated group by 50 mg/kg isoniazid along with 0.2 mg/kg Fe₂O₃ nanoparticle.

As shown in Fig. 2 (I), SOD activity did not increase significantly in treated rats with 50 mg/kg isoniazid in comparison with control and sham groups. SOD activity has not changed in treated rats with 50 mg/kg isoniazid and 0.2 mg/kg Fe₂O₃ nanoparticle compared with treated rats with 50 mg/kg isoniazid. However, SOD activity has decreased in treated rats with 50 mg/kg isoniazid and 0.2 mg/kg Fe₂O₃ nanoparticle in comparison with treated rats with 50 mg/kg isoniazid.

GSH concentration (μM) has increased in the treated group by isoniazid (50 mg/kg) significantly (P < 0.05) (Fig. 3 (I)). However, GSH concentration decreased in the treated group by 50 mg/kg isoniazid with 0.2 mg/kg Fe₂O₃ nanoparticle in comparison
Fig. 1. I) Brain GST activity. II) Brain catalase activity. A) The negative group. B) The sham group. C) The positive group that is the group treated with 50 mg/kg isoniazid, D) The group treated with 50 mg/kg isoniazid and 0.2 mg/kg Fe$_2$O$_3$ nanoparticle, and E) The group treated with 50 mg/kg isoniazid and 0.4 mg/kg Fe$_2$O$_3$ nanoparticle. All data are shown as means ± SD. The groups with the same letter (a, b) are not significant (P < 0.05)

Fig. 2. I) Brain SOD activity. II) Total protein. A) The negative group. B) The sham group. C) The positive group that is the group treated with 50 mg/kg isoniazid, D) The group treated with 50 mg/kg isoniazid and 0.2 mg/kg Fe$_2$O$_3$ nanoparticle, and E) The group treated with 50 mg/kg isoniazid and 0.4 mg/kg Fe$_2$O$_3$ nanoparticle. All data are shown as means ± SD. The groups with the same letter (a) are not significant (P < 0.05)

with the treated group by 50 mg/kg isoniazid significantly (P < 0.05). Nevertheless, elevation of the GSH concentration in the treated group by 50 mg/kg isoniazid with 0.2 mg/kg Fe$_2$O$_3$ nanoparticle was significant (P < 0.05) in comparison with the control and sham groups. Conversely, GSH concentration decreased in the treated group by 50 mg/kg isoniazid with 0.4 mg/kg Fe$_2$O$_3$ nanoparticle and was equal to the control and sham groups.

MDA, as the most common criterion to determine the amount of lipid peroxidation, has increased in the treated group by 50 mg/kg isoniazid significantly (P < 0.05) in comparison with the control and sham groups. Fig. 3 (II), shows that the amount of lipid peroxidation (MDA) has decreased in the treated groups by 50 mg/kg isoniazid with 0.2 and 0.4 mg/kg Fe$_2$O$_3$ nanoparticle significantly (P < 0.05) in comparison with the treated group by 50 mg/kg isoniazid.

**Discussion**

The brain, by low antioxidant capacity and high oxygen consumption, is exposed to severe oxidative stress damage [31, 32]. Some studies have revealed that some drugs or other compounds can induce oxidative stress and brain injury [33]. Isoniazid, as the first step in the treatment and prophylaxis of tuberculosis, induces oxidative stress and tissue dama-
ge [34]. Previous studies have shown that isoniazid leads to mitochondrial dysfunction in the liver and brain by induction of oxidative stress [35, 36]. Actually, there is ample evidence that isoniazid increases the susceptibility of Mycobacterium tuberculosis by inhibiting the enzyme enoyl [acyl carrier protein] reductase and oxidative stress enhancement [37, 38]. Therefore, most of the neurotoxicity effects of isoniazid induce via increasing oxidative stress [39].

The results of this study showed that the administration of 50 mg/kg isoniazid can induce oxidative stress in rats leading to brain damage. The recent studies have demonstrated that antioxidants and some natural products have the potential to be the best defense against tissues that are damaged by inhibitory effects on oxidative stress [40], Abrahams et al. showed the antioxidant effects of curcumin on models of neurodegeneration, aging, and oxidative stress [41] and Samarghandian et al. showed that carnosol has protective effects against oxidative stress that induces brain damage in rats [42].

In recent years, nanoparticles are known as novel antioxidants that protect against some oxidative stress damage [43]. Metal-based nanoparticles including iron oxide have beneficial roles in management of diseases regarding their antioxidant effects [44, 45]. In the present study the antioxidant role of ferric oxide nanoparticles against isoniazid induced neurotoxicity was studied via the evaluation of several markers of the oxidative stress.

The results of this study showed that brain GST enzyme activity increases after treating the rats with 50 mg/kg isoniazid though it moderates by treating 0.2 and 0.4 mg/kg FeO nanoparticle (Fig. 1(I)). Mohan et al. showed that crude sulfated polysaccharide has the protective effect against oxidative stress caused by isoniazid in the liver, kidney, and brain of adult Swiss albino rats by the moderation of GST [46]. Previous studies showed that some nanoparticles such as zinc oxide nanoparticles improved the effect of vitamin E and C on GST in liver cells [47].

Increased CAT activity in the treated rats by 50 mg/kg isoniazid (Fig. 1 (II)) confirms that isoniazid can induce oxidative stress that is in line with previous studies such as Sunarsih et al [48]. However, decreased CAT activity in the treated rats by 50 mg/kg isoniazid with 0.2 and 0.4 mg/kg FeO nanoparticle indicates that ferric oxide treatment leads to the ROSs accumulation which subsequently leads to decreasing of the CAT activity. Chirra et al. showed that the carbodiimide chemistry-based bioconjugation leads to decrease in catalase activity, while the carbodiimide chemistry-based bioconjugation with gold nanoparticles can lead to more catalase activity [49]. Also Zhang et al. showed that TiO2 nanoparticles can affect the structure of catalase enzyme directly and increase its activity [50].

The SOD activity of the rats’ brain had no significant change after treating with 50 mg/kg isoniazid or treating with 50 mg/kg isoniazid with 0.2 and 0.4 mg/kg FeO nanoparticle (Fig. 2(I)). This can be related to low SOD enzyme activity in the brain compared with other tissues such as the liver [51].

A sharp increase of GSH in the treated rats by 50 mg/kg isoniazid (Fig. 3 (I)) and a significant decrease of GSH in the treated rats by 50 mg/kg isoniazid with 0.2 and 0.4 mg/kg FeO nanoparti-
A significant increase in the MDA concentration in treated rats with 50 mg/kg isoniazid (Fig. 3 (II)) shows that isoniazid is a potent oxidant that induces hyper peroxidation in biological lipids. Actually, increasing of MDA concentration in these rats indicates the higher production of free radicals [54]. In this study, ferric oxide nanoparticles caused a significant decrease of MDA in the treated rats by 50 mg/kg isoniazid with 0.2 and 0.4 mg/kg Fe$_2$O$_3$ nanoparticles (Fig. 3 (II)). Some nanoparticles such as TiO$_2$ induce lipid peroxidation [55]. In the present research ferric oxide has improved the lipid peroxidation in the treated rats by 50 mg/kg isoniazid. He et al. showed that some nanoparticles such as nano Fe$_2$O$_3$ and nano MgO can decrease lipid peroxidation and promote cell growth at low concentrations in green algae [56]. Similarly, Behera et al. indicated that nano-Fe as a feed additive can improve the hematological and immunological parameters of fish and moderate the stress oxidative factors [57]. Because of this, this study was done on brain tissues of rats and determined some antioxidant factors and related enzymes it seems that, it is necessary to conduct more studies on the expression of genes and proteins involved in oxidative stress. Altogether, further studies are needed to confirm the ameliorative effects of Fe$_2$O$_3$ nanoparticles on the neurotoxicity of isoniazid.

Conclusion. Isoniazid, as the most important anti-tuberculosis agent, induces oxidative stress and tissue damage. Because of its sensitivity and its properties, the brain tissue is more exposed to stress oxidative damage than other tissues. This study showed that ferric oxide nanoparticles can improve the oxidative stress parameters on isoniazid-induced stress oxidative in brain damaged rats. However, there is a need for more experimental studies such as in vivo and in vitro studies to confirm these results.

Acknowledgments. We would like to thank Seyed Mohammad Jafari in the Research Consultation Center of Shiraz University of Medical Sciences for editing the English language of the manuscript.

Author contributions. Hossein Faramarzi, Ali Faramarzi and Samaneh Zolghadri, performed the experiments; Javad Saffari-Chaleshtori, drafted the manuscript; Mojtaba Beheshtroo, analyzed the data; Sayed Mohammad Shafiee designed the experiments, oversaw the project and finalize the manuscript with inputs from all the authors.


Funding. This study was funded by the Research and Technology Deputy of Shiraz University of medical sciences, Shiraz, Iran, based on the research grant number: 12048.
гом 12 діб щоденно фізіологічний розчин, 50 мг/кг ізоніазиду, 50 мг/кг ізоніазиду та 0,2 або 0,4 мг/кг наночастинок Fe₂O₃ відповідно. У гомогенатах тканин головного мозку спектрофотометричними методами визначали активність каталази (CAT), супероксиддисмутази (SOD), глутатіон-S-трансферази (GST), рівень глутатіону (GSH), малонового діальдегіду (MDA) і загального протеїну. Показано, що активність CAT і GST, а також рівні GSH і MDA в тканинах мозку тварин у групі лікування ізоніазидом були підвищені порівняно з контрольною групою, тоді як після додавання 0,2 або 0,4 мг/кг наночастинок Fe₂O₃ досліджувані показники оксидативного стресу повернулися до контрольного рівня (P < 0,05). Активність SOD у жодній із оброблених груп не змінювалася порівняно з контролем. Це дослідження показало, що введення наночастинок оксиду заліза може пригнічувати індукований ізоніазидом оксидативний стрес у тканині мозку щурів, психічно пошкоджених ізоніазидом.

Ключові слова: ізоніазид, наночастинки оксиду заліза, параметри оксидативного стресу, тканина головного мозку.

References


23


