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RESEARCH ARTICLE

Protective Effects of Ferulic Acid Against Isoniazid-Induced Hepatotoxicity in Rats

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SUMMARY

Isoniazid (INH) is an antibiotic that is used for the prevention and treatment of tuberculosis. The most common side effect of INH is seemingly hepatotoxicity through the induction of oxidative damage. Ferulic acid (FA) is an organic compound with antioxidant properties that is found abundantly in plant cell walls. The aim of this study was to evaluate the hepatoprotective effects of FA against hepatotoxicity induced by INH in Wistar rats. The rats were injected with INH (100 mg/kg/d for 21 days) with and without co-administration of FA (10 and 20 mg/kg/d) or silymarin (100 mg/kg/d) from day 11 to day 21. Then, the animals were sacrificed to evaluate the serum level of aminotransferases and total bilirubin, and liver histopathology and oxidative stress parameters. Co-administration of FA prevented the hepatotoxicity of INH according to the biochemical and histology findings. FA dose-dependently decreased level of lipid peroxidation in liver tissue. The activities of catalase, superoxide dismutase, and glutathione peroxidase in liver tissues of rats treated with FA were higher than those in non-treated INH-exposed rats. Taken together, the results demonstrated that FA could be used as a hepatoprotective supplement to prevent INH-induced hepatotoxicity.

Key Words: *Isoniazid, ferulic acid, hepatotoxicity, antioxidant, oxidative stress, histopathology.*

Ferulik Asidin Sıçanlarda İzoniazidle İndüklenen Hepatotoksisiteye Karşı Koruyucu Etkileri

ÖZ

İzoniazid (INH), tüberkülozun önlenmesi ve tedavisinde kullanılan bir antibiyotiktir. INH'nin en yaygın yan etkisi, oksidatif hasarın indüksiyonu yoluyla görülen hepatotoksisitedir. Ferulik asit (FA), bitki hücre duvarlarında bol miktarda bulunan antioksidan özelliklere sahip organik bir bileşiktir. Bu çalışmanın amacı, FA'nın Wistar sıçanlarda INH'nin neden olduğu hepatotoksisiteye karşı hepatoprotektif etkilerini değerlendirmektir. Sıçanlara, 11. günden 21. güne kadar sadece INH (21 gün boyunca 100 mg / kg / gün) ya da INH ile birlikte FA (10 ve 20 mg / kg / gün) veya silimarin (100 mg / kg / gün) enjekte edilmiştir. Daha sonra hayvanlar, serum aminotransferazlar ile total bilirubinin seviyesini, karaciğer histopatolojisini ve oksidatif stres parametrelerini değerlendirmek için ötanazi edilmiştir. Biyokimya ve histoloji bulgularına göre FA'nın birlikte uygulanması INH'nin hepatotoksisitesini önlemiştir. FA, doza bağlı olarak karaciğer dokusunda lipid peroksidasyon seviyesini düşürmüştür. FA uygulanan sıçanların karaciğer dokularındaki katalaz, süperoksit dismutaz ve glutatyon peroksidaz aktiviteleri, FA uygulanmayan INH'ye maruz kalan sıçanlara göre daha yüksek bulunmuştur. Birlikte ele alındığında sonuçlar, FA'nın INH'nin neden olduğu hepatotoksisiteyi önlemek için hepatoprotektif bir destek olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler: *İzoniazid, ferulik asit, hepatotoksisite, antioksidan, oksidatif stres, histopatoloji*

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INTRODUCTION

Isoniazid, also known as isonicotinylhydrazide (INH), is one of the first-line and the most commonly prescribed drugs for the treatment of active and latent forms of tuberculosis, *Mycobacterium tuberculosis* infection (Yue, 2009a). A major concern during treatment with INH is hepatotoxicity leading to even liver failure (Chang, 2018). INH hepatotoxicity is characterized by an increase in serum aminotransferases three times the normal upper limit as well as symptoms including abdominal pain, nausea, vomiting, or jaundice (Ramappa & Aithal, 2013). INH-induced liver toxicity is related to the production of reactive metabolites including hydrazine and acetyl hydrazine through N-acetyltransferase and amidohydrolase produced by hepatic metabolism of this drug (Tasduq, 2007; Yue, 2009b; Palanisamy & Manian, 2012; Metushi, 2014; Hassan, 2015; Hassan, 2016). Toxic and reactive metabolites of INH covalently bind to the liver macromolecules and induce oxidative damages (Raghu & Karthikeyan, 2016). It has been shown that the oxidative damage caused by INH is attributed to the formation of highly active oxygen species and alteration in various protective mechanisms, including enzymatic and non-enzymatic antioxidants (Sodhi, 1998; Wang, 1998; Bhadauria, 2007). Treatments of drug-induced hepatotoxicity are mainly based on supportive care and pharmacotherapy by hepatoprotective agents (Yu, 2017).

Regarding the role of oxidative stress in the hepatotoxicity of INH, dietary supplementation with antioxidants is proposed to prevent and treat liver toxicity (Viswanatha Swamy, 2010; Sankar, 2015; Raghu & Karthikeyan, 2016; Kargar Jahromi, 2018). Herbal medicine has a better acceptance than chemical drugs, especially in developing countries, for its better compatibility with the human body and fewer side effects (Pal & Shukla, 2003). Ferulic acid (FA, 4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound found abundantly in plant cell walls (Graf, 1992; Mathew & Abraham, 2004; Prakash, 2011), commonly in fruits and vegetables (Zhao & Moghadasian, 2008; Prakash, 2011). This active ingredient can trap free radicals and is considered as one of the promising antioxidants (Kanski, 2002; Mathew & Abraham, 2004). This study aimed at investigating the effects of FA on INH-induced hepatotoxicity with an emphasis on its antioxidant properties.

MATERIALS AND METHODS

Chemicals

Silymarin was purchased from SEDICO Pharmaceuticals Company, 6th of October City, Egypt. All the other chemicals were bought from Sigma-Aldrich Company, St. Louis, MO, USA.

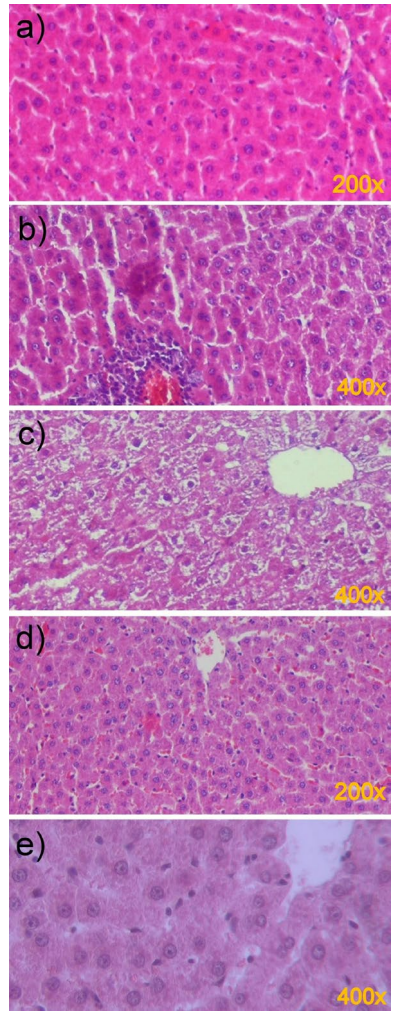

Figure 1. Histopathological analysis of liver tissue.

a) Negative (-) control group received distilled water showing normal hepatocytes; b) Positive (+) control group received isoniazid (INH, 100 mg/kg/d) showing moderate portal inflammation and congestion and lobular necrosis; c) INH-treated group supplemented with 10 mg/kg/d ferulic acid showing mild portal inflammation and moderate congestion of portal tract; d) INH-treated group supplemented with 20 mg/kg/d ferulic acid showing mild portal inflammation; e) INH-treated group supplemented with 100 mg/kg/d silymarin showing mild portal edema.

Animals

Male 8-week old Wistar rats with the mean weight of 200.0±1.5 g were obtained from the Animal House of Neuroscience Research Center, Kerman, Iran. The animals were kept in a conditioned environment (12-hour dark-light cycle, room temperature 25±1°C) with free access to food and water *ad libitum*. All the procedures were in full accord with the Ethical Committee of the Kerman Neuroscience Research Center (ethical code: ir-79kmu.rec.1395-79).

Animals grouping and treatments

Thirty Wistar rats were randomized into five groups of six rats each. Hepatotoxicity was induced in rats according to the procedure described by Yue, J. (Yue, 2004). Briefly, the positive control group was intraperitoneally injected with 100 mg/kg body weight (b.w.) of INH once daily for 21 days. The animals in the negative control group received only distilled water intraperitoneally. The other groups were INH-treated groups which were co-administered with FA (10 and 20 mg/kg b.w.) (Rukkumani, 2004a) or silymarin (100 mg/kg b.w.) (Baradaran, 2019) by gavage once daily from day 11 to day 21.

Histopathological analysis of liver

The rats were anesthetized with ketamine/xylazine, and the liver specimens were removed, fixed in 10% phosphate-buffered formalin, and embedded in molten paraffin. The paraffin-embedded blocks with 5µm thickness were stained with hematoxylin and eosin (H and E), scored and graded using the Ishak score (Goodman, 2007).

Biochemical parameters

At the end of the treatment, blood samples (4 mL) of all the rats were collected by cardiac puncture and centrifuged at 3000 × g for 10 min to separate serum. The serum samples were assayed for ALT, AST, and total bilirubin using the commercial diagnostic kits of Diagnosticum Zrt Company Budapest, Hungary.

Oxidative stress parameters

The liver specimens from each group were weighed and homogenized in phosphate buffer saline (50 mM, pH 7.4) for estimation of superoxide dismutase (SOD) and catalase (CAT) enzymes activities, and in potassium phosphate buffer (10 mM, pH 7.4) for estimation of malondialdehyde (MDA) level and glutathione peroxidase (GPx) activity with an ultrasonic homogenizer. Finally, the homogenized tissues were centrifuged, and the supernatants were used for the experiments. To measure the MDA level, Wasowicz's method was used in which MDA, the end product of lipid peroxidation, produces a colored complex with thiobarbituric acid, whose absorbance is measured at 532 nm. Finally, MDA concentration was calculated according to the standard curve of MDA (Wasowicz, 1993). SOD activity was assessed based on the inhibitory effect of the xanthine-xanthine oxidase system on nitroblue tetrazolium reduction. The amount of enzyme which causes 50% inhibition in the NBT reduction rate is known as one unit (U) of SOD (Sun, 1988). GPx activity was measured by the Paglia and Valentine method by calculating the decrease in absorbance at 340 nm during the oxidation of nicotinamide adenine dinucleotide phosphate, reduced to nicotinamide adenine dinucleotide phosphate + (Paglia & Valentine, 1967). The method described by Gerin was used for measuring CAT activity (Gerin, 2016).

Protein estimation

The homogenized tissues in phosphate buffer saline (50 mM, pH 7.4) were used to estimate protein content according to the Bradford method (Noble & Bailey, 2009).

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 8 (California, USA). The obtained data were statistically analyzed using one-way analysis of variance (ANOVA) test, and post comparison was carried out using Tukey's test. P values lower than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Hepatotoxicity was successfully induced after the administration of INH for 21 days. Findings of histopathology (Figure 1.) of the livers were graded according to Table 1. The histopathology scores of the specimens in the negative control group showed normal liver tissue structure with normal liver cells and normal nuclei. However, the liver specimens in the positive control group receiving INH showed lobular necrosis, portal inflammation, and congestion of the portal tract, as well as mild hydropic degeneration and portal vein congestion. In histology examination of the liver specimens treated with 10 mg/kg/d of FA, congestion of portal tract with mild lobular necrosis was observed. However, except for mild lobular necrosis in some specimens, no pathological lesions were observed in the histological sections of the group treated with higher doses of FA, 20 mg/kg/d.

As shown in Table 2, ALT, AST, and bilirubin levels in the INH group were significantly higher than those in the negative control group, and co-administration with FA significantly reduced these biomedical biomarkers ($p < 0.001$).

As shown in Figure 2, there was a significant difference between the negative control group and INH according to the parameters of the oxidant/antioxidant system. INH group had lower CAT, SOD, and GPx activities and higher MDA levels than those in the control group ($p < 0.05$). Treatment with FA significantly increased CAT, SOD, and GPx activities and significantly reduced MDA concentration ($P < 0.05$) in a dose-dependent manner. There was no significant difference between different doses of FA and silymarin-treated groups in the reduction of oxidative stress induced by INH.

Table 1. Histopathological scores in rat liver of different groups.

	Negative control	Positive control	Low dose FA	High dose FA	Silymarin
Portal inflammation	None	Moderate	None	None	Mild
Portal edema	None	None	None	None	None
Lobular necrosis	None	Moderate	Mild	Mild	None
Congestion of portal tract	None	Moderate	Moderate	None	None
Hydropic degeneration	None	mild	None	None	None
Congestion of central vein	None	mild	None	None	None

Table 2. Aminotransferases of total bilirubin level in serum of different groups.

	Negative control	Positive control	Low dose FA	High dose FA	Silymarin
ALT	39.89±2.57***	78.80±60.27	64.00±3.07***	55.15±1.64***	51.59±3.65***
AST	66.54±2.31***	145.6±3.94	95.31±3.34***	78.24±2.31***	69.69±1.9***
T. Billi	0.82± 0.08***	2.18± 0.05	1.55± 0.11***	1.13± 0.12***	0.92± 0.09***

Data were expressed as Mean ± SD; n = 8; *** p value < 0.001, were compared with the positive control group (one-way ANOVA followed by Tukey's multiple comparison test).

Negative (-) control group received distilled water and positive (+) control group received isoniazid (INH, 100 6 mg/kg/d) and INH-treated group supplemented with 10 and 20 mg/kg/d ferulic acid and 100 mg/kg/d silymarin. Abbreviation: aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), Total bilirubin (T. Billi, mg/L), Ferulic acid (FA).

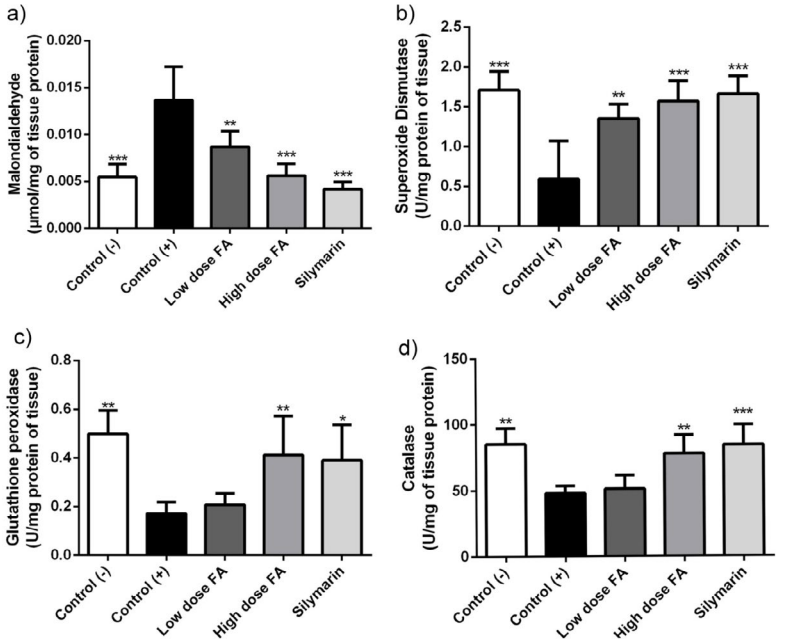

Figure 2. Oxidative stress biomarkers.

a) Lipid peroxidation as malondialdehyde level; b) Superoxide dismutase activity; c) Glutathione peroxidase activity; d) Catalase activity in liver tissue of negative (-) control group received distilled water and positive (+) control group received isoniazid (INH, 100 mg/kg/d) and INH-treated group supplemented with 10 and 20 mg/kg/d ferulic acid and 100 mg/kg/d silymarin. Values represent means ± SD; n=6. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with control (+).

DISCUSSION

The results of this study showed that the hepatoprotective effects of FA against INH-induced hepatotoxicity were similar to those of silymarin. According to the histopathology findings, FA at the dose of 20 mg/kg/d was more effective than the dose of 10 mg/kg/d, but the differences were not significant in serum biochemical findings and oxidative stress biomarkers of the liver. Sudheer also reported that FA at the dose of 20 mg/kg/d was more effective than the doses of 10 and 40 mg/kg/d in the reduction of oxidative damages (Sudheer, 2005).

Elevation in serum aminotransferases and total bilirubin level, which was observed after 21 days of exposure to INH in the current study, indicated disruption of bile production and flow and hepatocyte degradation (Tonomura, 2015). INH is a potent inducer of cytochrome P450, and also it is metabolized by 2E1 isoenzyme to toxic derivatives (Yew, 2018). Biotransformation of INH by N-acetyltransferase 2 produces active metabolites, acetyl hydrazine, and hydrazine, that bind to macromolecules of hepatocytes (Hassan, 2015). It seems that reactive oxygen species derived from the reaction of INH metabolites with oxygen result in the peroxidation of membrane lipids, which lead to the formation of lipid peroxides, the loss of integrity of the cell membrane, and ultimately damage to the liver (Sodhi, 1997). INH induces hepatotoxicity in zebrafish by overproduction of reactive oxygen species, which further leads to endoplasmic reticulum stress and apoptosis (Jia, 2019). The findings of the present study showed an increase in lipid peroxidation in the liver tissue of the INH-treated group with the reduction of antioxidant enzymes. In this regard, many studies proposed the usefulness of chemical and natural antioxidants for the prevention of hepatotoxicity induced by INH (Kumar, 2014; Yang, 2016; Bhilare, 2020).

The results also demonstrated that both doses of FA elevated the activity of antioxidant enzymes and subsequently reduced lipid peroxidation of the liver tissue. FA is a non-toxic compound with a wide range of therapeutic effects because of its strong antioxidant activity (Srinivasan, 2007). The antioxidant capacity of FA seems to depend on scavenging the radical species and reducing free radical-induced lipid peroxidation (Mancuso & Santangelo, 2014). Joshi (2006) showed that this lipophilic antioxidant phenolic compound could potentially be used in the treatment of oxidative stress-related neurodegenerative disorder (Joshi, 2006). As shown by Trombino in 2013, FA effectively inhibited lipid peroxidation induced by peroxyl radicals or peroxynitrite in rat brain microsomes (Trombino, 2013). It has been shown that FA enhances cell stress response by regulating several key enzymes that are mainly involved in counteracting free radical-induced damage, such as SOD and CAT (Rukkumani, 2004b; Wang, 2014). The study conducted by Dong (2003) reported an elevation in SOD activity and reduction in MDA level in the colon segment of a rat model of colitis by sodium ferulate (Dong, 2003). In a clinical study, FA (1g /day for 4 weeks) improved lipid profiles, oxidative stress, and inflammation in hyperlipidemic individuals, and thus reduced the risk of cardiovascular diseases (Bumrungpert, 2018). It was found that FA ameliorated lipid peroxidation and inflammation induced by methotrexate by elevation in the cellular antioxidant capacity and the activity of the antioxidant enzymes CAT, SOD, and GPx (Roghani, 2020). Another proposed hepatoprotective mechanism of FA against liver toxicity of acetaminophen is downregulation of P450 2E1 (Yuan, 2016), which 2E1 also is related to the metabolism of INH. In another study, a reduction in lipid peroxidation and improvement of liver histopathology after the administration of FA in diosbulbin B-treated mice occurred along with elevation in the activity of CAT and SOD (Wang, 2014). Also, in diosbulbin B-induced liver injury in mice, it was reported that the administration of FA at the high doses of 40 and 80 mg/kg inhibited the hepatic inflammation and apoptosis (Niu, 2016).

Hepatoprotective effects of FA was also revealed in the hepatotoxicity of formaldehyde in rats through an elevation in the activity of CAT, SOD, and GPx and also reduction of lipid peroxidation end products and serum cytokines (Gerin, 2016). The finding of the current study is in full accord with previous studies concerning the hepatoprotective effects of FA through the reduction of oxidative stress by elevation in the antioxidant capacity of the liver tissue.

CONCLUSION

We can conclude that FA could be a promising hepatoprotective supplement in hepatotoxicity induced by INH in male Wistar rats. However, very few clinical studies have been conducted regarding the effects of this compound as a drug or food supplement (Harder, 2004; Murray, 2008; Kimura, 2011; Bumrungpert, 2018). Therefore, further studies on pharmacokinetics, pharmacodynamics, and the possible toxicity of FA are needed to evaluate its effects on human health.

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CONFLICT OF INTEREST

All the authors of this article declared no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis, literature research, analysis and interpretation of the data, reviewing the text (Karami-Mohajeri, S., Sharififar, F.), literature research, experimenting, preparing the study text, analysis and interpretation of the data (Ahmadipour, A., Anani H.).

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