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Original Article

Protective effects of salep against isoniazid liver toxicity in wistar rats

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ABSTRACT

Introduction: Isoniazid is a drug for treatment of tuberculosis. One of the main side effects of this drug is hepatotoxicity, which is a major cause of treatment interruption in tuberculosis. This study is about the preventive effect of Salep on this side effect of isoniazid.
Materials and methods: This study is an experimental study in which the preventive effect of salep on isoniazid hepatotoxicity is evaluated. In this study 56 rats were randomly placed in 7 eight members groups including: control group, sham, isoniazid and four isoniazid/salep groups. At the end of the study the laboratory criteria and histological features of liver toxicity were compared in different mentioned groups.
Results: Significant lower serum levels of liver enzymes, billirubin, MDA and TOC; and significant higher levels of TAC and total proteins, were revealed in isoniazid/salep group in compare to isoniazid alone group.
In addition, histological studies had not showed liver injury in isoniazid/salep group, while there was significant liver injury in isoniazid alone group.
Conclusions: Orchid extract (salep), probably because of its antioxidant properties, prevent the destructive effects of isoniazid on the liver.

1. Introduction

Tuberculosis (TB), is one of the most important communicable diseases in developing countries including Iran. One of the side effects in treatment of this disease [by Isoniazid (INH), Rifampin (RMP) and Pirazinamide (PZA)] is liver toxicity and sometimes this problem may be conduce drug interruption, to save the patient from hepatotoxicity and death. On the other hand drug resistance mycobacterium is a risk which may happen with drug interruption. Prevention of liver toxicity by natural products in these patients seems to be a good strategy. We have already worked on the Salep (a plant belongs to family Orchidacea) for its protective effects on liver.[1] The results of that study were hopeful.

In most cases, during detoxification of drugs, microsomes in the liver causes production of toxic and active metabolites that can damage various tissues including the liver itself.[2]

INH, is used as a drug for treatment of active and latent tuberculosis along with other first line antituberculosis drugs. The most common side effect of this drug is elevation of liver enzymes level in blood which may be conduce to hepatitis that is a dangress complication in the patient.[3] Hepatic toxicity caused by isoniazid can appear as cellular necrosis, steatosis (accumulation of fats), or both. Metabolites of this drug also have toxic effects on liver cells.[4] Hydrazine is one of the most important metabolites of isoniazid. In animal models, a statistically significant positive correlation has been reported between plasma levels of hydrazine and severity of hepatic cell destruction caused by treatment with isoniazid.[5] Moreover, other studies have proved acetyl hydrazine is another destructive agent of hepatic cells when isoniazid is used for treatment.[6]

Fortunately, antioxidants can prevent the destructive effects of toxins and various drugs on the liver tissue.[7–9] Plants have always

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been considered one of the main options for treating poisoned livers because they are available as natural sources of antioxidants. Orchid or Dactylorhiza lancibracteata (C. Koch) Renz, formerly named Orchis maculate L. (or Salep in general dialect), belongs to the family Orchidaceae, has various species, and grows almost everywhere in the world. Its root nodules, can be harvested in early summer, and its medicinal properties may last for about two years.[10,11] This plant contains compounds including nitrogenous materials, starch, proteins, sugars, hydroxybenzaldehydes, ferulic acid, quercetin, daucosterol, cirsilineol, steroids, and glucomannan.[12,13] In traditional medicine, orchid is prescribed as ointment for chest pain, and for treating chest and intestinal disorders, tuberculosis, diarrhea, Parkinson's disease, and cancer.[14,15] Evaluation of the extracts of the roots of orchid revealed that there are numerous compounds in it; such as glucomannan and polyphenols, which possess strong antioxidant properties.

In one of our previous studies we showed that this plant has protective effects against liver toxicity overall.[1] Therefore, this research was conducted in rats; to study the preventive effects of aqueous extract of this plant against liver toxicity caused by isoniazid.

2. Materials and methods

2.1. Collection and extraction of the salep

The roots of the plant are used for providing the extract.

The plant was obtained from farmlands around Yasouj (a city in the southwest of Iran). At first the roots of the plant washed by water, and then dried in the laboratory environment (away from direct light of sun). Then by electric mill, the dried roots ground into flour. The ethyl alcohol added to the obtained powder in 5 to 1 proportions (5 part alcohol and 1 part powder) and mixed for 24 h to yield a uniform solution. In the next stage, the solution filtered and dried for 48 h to obtain the dried extract without alcohol. At the end the extract was mixed with distilled water in 20, 40 and 80 mg/mL proportions and used for the study.[16]

2.2. Animals and their classification

First of all: With due attention to the usefulness of the results of this study for humanity specially the patients with tuberculosis the" Animal care committee" and " Medical ethics committee " in Jahrom university of medical sciences approved this study. In these committees it is emphasized that the process on animals should be done with the lowest torment for the animal.

In this research, 56 mature Wistar rats with the average weight of 180–200 g were kept in the animal breeding room at Jahrom University of Medical Sciences for one week to become acclimated to the environment. During the entire research, the rats were kept under a photoperiod of 12 h light: 12 h dark at the ambient temperature of 20–25 °C and had free access to food and water. By considering previous research and the pilot project that was carried out, it was decided to use extract of orchid at the prescribed doses of 80,[17,18] 160, and 320 mg/kg. The rats were randomly divided into 7 eight-member groups. In control group any additive material (except food and water) was given to rats, but in the sham group 1 ml of distilled water (based on weight of each rat) was injected intraperitoneally, every day for 4 weeks. The isoniazid group received 50 mg/kg of isoniazid through intraperitoneal injection, and the experimental groups 1, 2, 3, and 4 recieved 50 mg/kg of isoniazid together with 40, 80,160, and 320 mg/kg of the aqueous extract of orchid intraperitoneally every day for 4 weeks.

2.3. Blood sampling and biochemical studies

At the end of the research (on day 29), the rats were weighed and anesthetized by cervical dislocation and, blood samples were taken directly from their hearts. The blood samples were centrifuged at 3000 rpm for 15 min and the serums were kept at -20 °C for measuring concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, bilirubin,[19] malone dialdehyde (MAD), total oxidant capacity (TOC), and total antioxidant capacity (TAC).

ALT and AST were measured by the Deutsche Gesellschaft fur Klinische Chemie (DGKC) phosphate buffer method, ALP by the para-nitrohphenyl phosphate AMP method, and albumin by the bromocresol green method. Total bilirubin was measured by the diazo method using diazotized sulfanilic acid (employing commercial kits manufactured by Zist Shimi Co. in Iran), and total protein by the biuret reaction (end point) method (using commercial kits manufactured by the Pars Azmoon Co. in Iran).[20] MAD levels were determined by the ELISA method employing commercial kits manufactured by the Biospes Company in Italy, and TOC and TAC by the ELIZA method using commercial kits made by the LDN Company in Italy.

2.4. Histological studies

After taking blood samples, the rats were dissected and their livers were removed, weighed accurately, and fixed in 10% formalin. Tissue cross sections of the livers were prepared, stained, and studied under microscope.

2.5. Statistical analysis

One-way ANOVA was used to analyze the information, and Duncan's test was employed to determine the differences between the means where there were statistically significant differences between the various groups. Statistical calculations were performed using SPSS 21, and the selected significant level was ($p < 0.05$).

3. Findings

3.1. Liver enzymes

No significant differences were observed in mean serum levels of liver enzymes between the control and sham groups; but, mean concentrations of AST, ALT, and ALP was higher in isoniazid-alone group compared with control group and sham groups ($p < 0.05$).

Furthermore, significant low concentrations of ALT, and ALP are seen in isoniazid/salep groups VI and VII (160 and 320 mg/kg) in compared with isoniazide alone group ($p < 0.05$) (Table 1).

3.2. Serum albumin (ALB), total protein (TP), and total bilirubin levels

There was not any significant difference in the serum levels of ALB and TP in control and sham groups.

However, mean serum levels of ALB and TP were significantly low in isoniazid alone group in compare to the control and sham groups ($p < 0.05$)

Average serum level of ALB in groups that received isoniazid together with 160, or 320 mg/kg of the extract (groups VI and VII) was significantly high in compare to the isoniazid alone group ($p < 0.05$).

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