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Hepatoprotective and antioxidant effects of fish oil on isoniazid-rifampin induced hepatotoxicity in rats

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ABSTRACT

In the present study hepatoprotective and antioxidant potential of fish oil (cod liver oil) against isoniazid and rifampin combination (INH-RMP)-induced toxicity was evaluated in rats. Administration of (50 mg INH + 100 mg RMP/kg/day, intraperitonially [i.p]) for 14 daysproducedliver injury that was evident from elevated levels of serum marker enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and histopathological changes. The drug treatment caused significant changes in the cellular redox status as reflected by about 75% decrease in the level of serum total antioxidant capacity (TAC), a marked decrease in reduced glutathione (GSH) level, an increased in lipid peroxidation (LPO) in the liver. Fish oil treatment (4 ml/kg/day, i.p.) 5–6 hrprior to (INH-RMP) dose, markedlyprevented the rise in serum enzymes levelselicited by the drugs. The histopathological alterations were also improved. Remarkably, there was a complete reversal of the changes in the levels of GSH and LPO, and partial recovery of TAC in the animals receiving fish oil along with (INH-RMP). These observations support the mechanistic role of strong antioxidant property of fish oil in the protection of hepatic injury. This study suggests the importance of fish oil as a hepatoprotective and antioxidant dietary supplement, particularly in the patients receiving anti-tubercular therapy which are at a risk of hepatotoxicity.

1. Introduction

Tuberculosis (TB), a disease caused by a bacillus *Mycobacterium tuberculosis*, is one of the oldest and deadliest diseases known to mankind. TB remains a serious public health problem among certain world populations in the developing countries even today [1]. With the advent of anti-tubercular chemotherapy, there has been a marked improvement in the natural history of the disease. Among the anti-tubercular drugs available, isoniazid (INH) and rifampin (RMP) are the first-line essential drugs and a mainstay of the anti-tubercular combination therapy along with second-line drugs, pyrazinamide, ethambutol and streptomycin.The most frequent adverse effects of anti-tubercular treatment are hepatitis, skin reactions and gastrointestinal upset [2,3].

Anti-tubercular drugs-induced hepatotoxicity causes substantial morbidity and mortality. The major deterrent to the prolonged use of both drugs is their potential to cause hepatotoxicity, which is further enhanced when the two drugs are used in combination [4,5]. Studies undertaken in the past have proposed a possible role of enhanced formation of toxic INH metabolites (acetylisoniazid, acetylhydrazine, hydrazine and their reactive products) by RMP and the resultant oxidative injury in the hepatotoxicity caused by these drugs combination [5,6].

Attempts have been made in the past to investigate the hepatoprotective potential of some herbal preparations, synthetic compounds or natural antioxidants against the toxicity of these drugs [7,24]. Silymarin, an extract from the seeds of *S. marianum,* has been shown to protect ratliver against toxic effects of anti-tubercular drugs [7,8].

Fish oil (FO) can be obtained from eating fish or taking supplements available in the market. Effectiveness of fish oil has been shown in different pathological conditions such as heart disease, stroke, neurological disorders, osteoporosis, obesity, eye diseases, inflammation and even cancer [9]. However, some clinicalstudies do not support a beneficial role for omega-3 fatty acids supplementation in preventing cardiovascular disease, stroke or cancer [10]. The benefits of fishoil (cod liver oil) have been attributed to high contents of omega-3 fatty acids (34%),

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eicosapentaenoic acid (EPA, 15%)and docosahexaenoic acid (DHA, 19%)and fat soluble vitamins A, D and E [11,12]. Long chain omega-3 fatty acids are essential as they are not synthesized by mammals and, therefore, must be supplemented in the diet for good health. Other rich sources of omega-3 fatty acids include edible seeds oils and walnut [13]. Prolonged feeding to the rats with fish oil results in omega-3 fatty acids incorporation into hepatic lipids, inhibition of *de novo*lipogenesis and change in the hepatic fatty acid profile [14].

Much less information is available on the effectiveness of fish oil in the liver diseases and in particular hepatic injury induced by drugs and other chemicals. Previous studies have shown hepatoprotective potential of fish oil againsttoxicity inducedby sodium nitrite in rats and carbon tetrachloride in rabbit [12,15]. Another study showed a decrease in paracetamol-induced hepatic lipid peroxidation in the rats receiving fish oil for 7 days [16]. The current study was therefore, designed to evaluate the hepatoprotective and antioxidant potential of fish oil against liver injury induced by INH and RMP combination in rats in order to understand the mechanism of hepatoprotective activity of fish oil.

2. Materials and methods

2.1. Drugs and chemicals

A standard cod liver oil formulation (Seacod®, marketed by Sanofi-France in India) was used. Silymarinan extract from the seeds of*S. Marianum* (marketed by Zenith Nutrition, USA in India) was commercially available. Isoniazid and rifampin (Active Pharmaceutical Ingredient) were purchased from Sisco Research Laboratories Pvt. Ltd., India. The assay kitsof ALT, AST and ALP were purchased from Span Diagnostics,Surat, India. All other chemicals used were of the analytical grade.

2.2. Animals and treatments

All the experimental procedures involving the use of laboratory animals were approvedby the Institutional Animal Ethics Committee of Hamdard University, New Delhi. Wistar albino rats of either sex weighing 180–250 g were procured from Central Animal House Facility of the University. The animals were housed in polypropylene cages for one week to acclimatize to the standard conditions (12 h light: dark cycle; temperature, $25 \pm 2\,^{\circ}C$) and provided *ad libitum* diet.

After acclimatization, the rats were divided into six groups, each comprising of six animals (n = 6), and treated as followed. Hepatic injury in rats was produced by intraperitoneal (i.p) administration of a mixed dose of 50 mg INH + 100 mg RMP (dissolved in saline) for 14 consecutive days [7]. Animals were treated with fish oil (4 ml/kg, i.p.) 5–6 h prior to (INH-RMP) administration for 14 days [16]. Silymarin was administered orally to rats at a dose of 200 mg/kg, 5–6 h prior to (INH-RMP) administration for 14 days [7]. Control groups of animals received normal saline, fish oil or silymarin alone and proceed under similar experimental conditions.

Group 1: Control– normal saline, intraperitoneally (i.p.) for 14 consecutive days.

Group 2: Fish Oil (FO) *per se*–4 ml/kg, i.p. for 14 days.

Group 3: Silymarin (Sily) *per se*– 200 mg/kg, orally for 14 days.

Group 4: (INH-RMP)– 50 mg INH–100 mg RMP in saline/kg, i.p. daily for 14 days.

Group 5: (INH-RMP) + FO – 4 ml/kg, i.p. 5–6 h prior to 50 mg INH–100 mg RMP/kg, i.p. for 14 days.

Group 6: (INH-RMP) + Sily– 200 mg/kg, orally 5–6 h prior to 50 mg INH–100 mg RMP/kg, i.p. for 14 days.

2.3. Blood collection and tissue preparation

Blood was collected by sinocular puncture 18–20 h after the last drug treatment and centrifuged at 3,000*g* for 15 min to obtain serum for estimation of serum marker enzymes for liver function and total antioxidant capacity (TAC).

After blood collection, rats were sacrificed by decapitation and the liver of each animal was removed, washed with ice-cold normal saline and processed separately for further investigations. A small piece of liver was fixed in 10% formaldehyde for histopathological examination. Remaining tissue was used for analysis of GSH and LPO as described below.

2.4. Estimation of serum enzymes markers of hepatotoxicity

The activities of serum marker enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated spectrophotometrically by the method of Reitman and Frankel [17] using commercially available kits.

The activity ofalkaline phosphatase (ALP) was determined by following the formation of 4-nitrophenol from 4-nitrophenyl phosphate (substrate) in alkaline medium spectrophotometrical-lyat wavelength of 405 nm [18].

2.5. Estimation of markers of redox status

The total antioxidant capacity (TAC) was estimated in the serum by the method of Koracevic et al. [19] as described earlier [20]. The method was based on the principle that Fe-EDTA complex reaction with hydrogen peroxide (Fenton reaction) generates hydroxyl radical, which degrades benzoate resulting in the release of thiobarbituric acid reactive substances (TBARS). The inhibitory potential of serum against TBARS production is then determined as antioxidant activity in mmole/l usinguric acid as a standard antioxidant. The method for TAC estimation is given below.

The reaction mixture containing 0.5 ml of phosphate buffer (100 mmole/l, pH 7.4), 0.5 ml of sodium benzoate (10 mmole/l), 0.2 ml of Fe-EDTA (2 mmole/l EDTA + 2 mmole/l of $Fe[NH_4]_2SO_4$), 0.2 ml 0f H_2O_2 (10 mmole/l) and 0.01 ml of serum was incubated for 60 min at 37 °C. The reaction was stopped by addition of 1 ml of 20% acetic acid. One ml of thiobarbituric acid (TBA) solution (0.8% in 50 mmole/l NaOH) was added, and the solution was heated for 10 min at 100 °C. The absorbance of the pink color thus formed was estimated spectrophotometrically at 532 nm 0.01 ml of uric acid (1 mmole/l in 5 mmole/l NaOH) was used as the standard antioxidant for determining the antioxidant activity (AOA) of unknown samples. Proper blanks were run under similar experimental conditions. The AOA of each sample was calculated as follows:

$$\text{AOA (mmole/1)} = \frac{(CU)\,(K - A)}{(K - UA)}$$

Where K = absorbance of control; A = absorbance of sample; UA = absorbance of uric acid solution; CU = concentration of uric acid (mmole/l).

Protein was determined by the method of Lowry et al. using bovine serum albumin as standard [21]. The content of GSH was measured as non-protein sulfhydryl group using Ellman's reagent, 5, 5′-dithio (2-nitrobenzoic acid) in the liver was determined as described earlier by Sedlak and Lindsay [22]. Sulfhydryl content was measured in the supernatant obtained after deprotenization of tissue homogenate with trichloroacetic acid and detected by reacting with the Ellman's reagent.