Chemoprevention of Silymarin and Vitamin C on Isoniazid-Induced Hepatotoxicity in Experimental Rat Model

Keywords: Isonicotinic acid hydrazide; Hepatotoxicity; Tuberculosis; Chemoprevention; Silymarin; Vitamin C

Abstract

Isoniazid, anti-mycobacterial agent employed clinically in the treatment of bacterial infections (tuberculosis), is known to cause a number of biochemical dysfunctions and suspected to induce hepatic damage to animals and humans. However, co-administration of antioxidants to ameliorate the possible ill effect of anti-tuberculosis medications should be advocated. The present study therefore evaluated its toxicity in liver cells of male rats and the chemo-preventive effect of Silymarin (SIL) and Vitamin C. Administration of Isoniazid caused a significant (p<0.05) increase in the activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST). While SIL and Vitamin C significantly reversed the toxicity effect induced by an antibiotic drug. Collectively, the results suggest that therapeutic dose of Silymarin elicits hepatoprotective effect in male rats. The chemoprotection effects of SIL and Vitamin C during Isoniazid treatment suggest their clinical applications in hepatic damage and may serve as adjunct in tuberculosis therapy.

Introduction

Isoniazid or isonicotinic acid hydrazide (INH) is a potent anti-mycobacterial agent which acts by inhibition of lipid and DNA synthesis of Mycobacterium tuberculosis, thus inhibiting its cell wall synthesis [1]. INH was introduced into clinical practice in 1952 and contributed greatly to the subsequent dramatic decrease in morbidity and mortality caused by tuberculosis. At the time of introduction, Isoniazid was found to have few side effects, excellent compliance rate and highly efficacious. It rapidly became the mainstay of anti-tuberculosis therapy. However, cases of severe hepatotoxicity, skin reactions, gastrointestinal and neurological disorders due to Isoniazid soon appeared and clearly defined its toxic potentials [2]. These ill effects of INH have been well researched and induction of oxidative stress via free radicals formation and reactive oxygen species (ROS) were opined as possible mechanisms [3]. Following these dual attributes, INH usage was then either restricted or modified for administration alongside antioxidants to effectively ameliorate the insults of ROS induced cellular damage.

Silymarin is a flavonoid extracted from the seed of Silybum Marianum (milk thistle plant). Silybum marianum is a member of the aster family (Asteraceae or Compositae) which encompasses daisies and thistles [4]. Silybin (silibinin), silychristin, and silydinin have been identified as its major active ingredients [5]. This makes it as a common herbal therapy particularly for treating liver diseases partly due to its antioxidant activity [6]. It has membrane-stabilizing and antioxidant activity, promotes hepatocyte regeneration, reduces inflammatory reaction, and inhibits fibrogenesis [7-9].

Vitamin C is a six-carbon compound structurally related to glucose, found in citrus, soft fruits and leafy green vegetables [10]. It is hydrophilic in nature and had been implicated as free radical scavenger in extracellular fluids and protecting bio-membranes from peroxide damage [11-19]. Recently, since the liver plays a major role in the metabolism of drugs and consequently the primary target of most toxic responses. Research has largely concentrated on liver functions. However, co-administration of antioxidants to ameliorate the possible ill effect of anti-tuberculosis medications should be advocated. Hence, the present study seeks to substantiate the latter fact by exploring the antioxidant modulatory effects of silymarin and vitamin C on INH induced hepatotoxicity in experimental animal models.

Materials and Methods

Chemicals and reagents

Assay kits for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were products of Randox Laboratories limited, United Kingdom. Distilled water used was glass distilled. Tablets of isoniazid and silymarin were procured from Sigma-Aldrich Chemicals Company (St. Louis, Mo, USA). Vitamin C was purchased from Emzor Pharmaceutical Industries limited, Lagos, Nigeria. Other chemicals and reagents were all of analytical grade.

Experimental protocol

Wistar strain albino rats having the weight of 180.00±2.33 g were purchased from the animal house of the University of Ibadan, Nigeria. They were kept in cages in a well ventilated room maintained at a temperature of 25±2 °C with a 12 h light-dark cycle for ten days to acclimatize, and were allowed free access to food and water ad libitum. The protocol conforms to the guidelines of the National Institute of Health for laboratory animals.
Institute of Health for laboratory animal care and use [20]. Twenty five albino rats were randomized into five groups of five rats each. Group I serves as the control, animals in Groups II, III, IV and V received 28.6 mg/kg body weight of Isoniazid (INH) per day which represents the therapeutic dose in humans. Group III was co-treated with SIL at therapeutic dose of 200 mg/kg body weight per day. Group IV was co-treated with Vitamin C at dose of 50 mg/kg body weight per day. Lastly, group V was co-treated with SIL and Vitamin C at doses of 200 and 50 mg/kg body weight per day respectively, and the experiment last for 14 days. Food and water were administered ad libitum throughout the period of the experiment. After the last administration, the animals were fasted overnight and were humanely sacrificed by cardiac puncture. Blood sample was collected in heparinized bottles, centrifuged at 3000 rpm for 15 min. The resulting plasma was carefully aspirated with a Pasteur’s pipette into sample bottles for liver enzymes assay.

Liver enzymes assay

Plasma of hepatic alanine aminotransaminase (ALT) and hepatic aspartate aminotransaminase (AST) were estimated by the method of Reitman and Frankel [21].

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS software package for windows (Version 16) and expressed as mean ± standard deviation (SD) (n=5). Significant difference between the treatment means was determined at 5% confidence level using Duncan’s Multiple Range Test.

Results

The present investigation revealed the chemo-protection effect of silymarin and vitamin C on isoniazid-induced hepatotoxicity in male rats. As observed (Figure 1), the activities of plasma AST was significantly (p<0.05) increased in isoniazid induced by 4762% when compared with the corresponding control. While the observed high activities of plasma AST was significantly (p<0.05) reversed in the animals co-treated with INH + SIL, INH + Vit C and INH + SIL+ Vit C by 3175%, 1935% and 235% respectively (as shown in Figure 1). Similarly, it was observed (Figure 2) that the activities of plasma ALT was significantly (p<0.05) increased in isoniazid induced by 4748% when compared with the corresponding control. The observed elevations were however significantly attenuated (P < 0.05) in the rats co-treated with INH + SIL, INH + Vit C and INH + SIL+ Vit C by 3175%, 1935% and 235% respectively (as shown in Figure 2). In addition, Vit C has a higher reversal effect than silymarin in Isoniazid induced hepatotoxicity. Collectively, as shown in this study, co-administration of Vitamin C and silymarin exhibited high efficacy in treating liver problems than when they are singly co-administered.

Discussion

The liver is susceptible to injury by xenobiotics such as drugs. One of the most serious and frequent adverse effects of anti-tuberculosis drugs is hepatotoxicity [2]. For effective administration and compliance however, co-prescription with antioxidants have been advocated over the years. This is the focus of the present investigation.

Antioxidants elicit protective potentials by interacting with biomolecules at cellular and molecular levels to induce cytoprotective enzymes or inhibits those involve in carcinogen activation. Alteration in activity of liver marker enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) suggests a possible damage to the hepatocyte membrane and thus a compromised integrity and permeability of the membrane [22]. The significant increase in plasma activity of AST and ALT following administration of INH in the treated animals may be attributed to generation of free radicals. This might trigger chains of reactions resulting in liver damage. It also suggests possible leakage of the enzymes resulting from susceptibility of the hepatocyte membrane to adverse influence of INH which could have consequential effect on the metabolism and regulation of other enzymes in the liver [23]. This agrees with the findings of Snodgress et al., Karthikeyan and Adedayo AJ et al. where INH was reported to have caused hepatic damage in experimental animals manifested by increased activities of liver marker enzymes in the serum [3,24,25]. Conversely, the significant reduction in enzyme activities of rats co-administered with either silymarin or vitamin C and both suggests that both molecules were able to ameliorate the deleterious effects of INH on the rat’s hepatocytes.
Both silymarin and vitamin C have been reported to have excellent antioxidant potentials against oxidative stress induced cellular damage [6,15,19,26,27]. Thus, the effects elicited by co-administration of the two molecules could possibly be attributed to their electron-donating capacities to form stable products and subsequently, terminating free radical chain reactions. The membrane-stabilizing and antioxidant activity which promotes hepatocyte regeneration as reported by Shalan et al. for silymarin and vitamin C might also be linked to the reduction in the enzymes activities of rats co-administered [28].

### Conclusion

The present study reveals that administration of therapeutic dose of isoniazid to male rats induced hepatotoxicity by increasing the key markers of liver damage - AST and ALT. SIL and Vitamin C exhibited similarities in their capability to mitigate the toxic responses of isoniazid, which suggests that the adverse effects of isoniazid on the liver are due to leakage of liver enzymes from mitochondrial matrix of hepatic cells. However, co-administration of Vitamin C and silymarin exhibited high efficacy in treating liver problems than when they are singly co-administered.

### References