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# POSSIBLE PROTECTION AGAINST ISONIAZID-INDUCED HEPATOTOXICITY BY CERTAIN NATURAL AGENTS

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## Abstract

Isoniazid (INH), which is a first-line antimicrobial for tuberculosis, is adversely associated with hepatotoxicity. The aim of the current study was to evaluate the protective effect of α-LA, l-carnitine, ginseng, and $CoQ_{10}$ against isoniazid induced hepatotoxicity in rats. Thirty six male Sprague-Dawley (SD) rats were allocated into different groups and over a 3-week period, they were treated intraperitoneal (I.P) either with INH alone (100mg/kg b.w) or with INH after daily pretreatment orally with l-carnitine (250mg/kg b.w), α-LA (50mg/kg b.w), $CoQ_{10}$ (15mg/kg b.w) or I.P with ginseng (20mg/kg b.w). Biochemical and histopathological evaluations were done and INH-treated groups were compared with rats receiving no treatment and with rats given l-carnitine, α-LA, ginseng and $CoQ_{10}$.The results indicated that I.P administration of INH induced severe hepatic injury associated with oxidative stress. The combined treatment with INH plus one of the foregoing antioxidants resulted in a significant improvement in all evaluated parameters. It could be concluded that any of the previous antioxidants protect SD rats against the severe INH-induced hepatic toxic effects.

**Introduction**

Isoniazid was the first effective bactericidal drug used to treat tuberculosis and till far, an important part of most antitubercular drug regimens (Girling, 1978). It is never used on its own to treat active tuberculosis because resistance quickly develops. Rifampicin, which is another effective bactericidal drug, was added to the regimen in 1962 and has remained the most effective antitubercular combination along with isoniazid (Snider et al., 1984). Although both these drugs are very potent against the tuberculosis bacillus, both are well-known hepatotoxic drugs (Steele et al., 1991).

Isoniazid, known as isonicotinyl hydrazine (INH), is an organic compound that also has an antidepressant effect, and it was one of the first antidepressants discovered. The compound was first synthesized in the early 20th century, (Meyer and Mally, 1912) but its activity against tuberculosis was first reported in the early 1950s (Hans, 2009).

The administration of isoniazid produces many metabolic and morphological aberrations in liver. Isoniazid can cause mild to moderate elevation of serum transaminases in approximately 10-20% of patients and severe hepatotoxicity in approximately 0.5-2% (Nolan et.al. 1999). The isoniazid-induced hepatotoxicity is initiated by CYP-450-mediated metabolism to toxic metabolites such as acetylhydrazine and hydrazine. Hydrazine reacts with sulfhydryl group, which results in glutathione (GSH) depletion within the hepatocytes leading to cell death (Sarich et.al.1999). These bioactive metabolites are produced by a series of enzymes and induction of oxidative stress. CYP2E1 is involved in isoniazid-induced hepatotoxicity in human and animals by generation of free radicals (Huang et al, 2003).

Antioxidants are natural agents that protect the body from harmful free radicals. These are atoms or groups of atoms that can cause damage to cells, impairing the immune system and leading to infections and various degenerative diseases. Therefore antioxidants are intimately involved in the prevention of cellular damage which is the common pathway for cancer, aging, and a variety of diseases. (Trevelyan; 1993).

Alpha-lipoic acid (α-LA) has been identified as an ideal antioxidant that can scavenge number of free radicals and therefore used in the prevention or treatment of several pathological conditions mediated via oxidative stress (Bustamante et al; 1998). Within the body α-LA is often reduced to the more active metabolite dihydrolipoic acid (DHLA). α- LA and DHLA are some of the most powerful biological antioxidant systems (Moini et al., 2002). α-LA has a further positive effect in that it is known to be able to re-synthesize vitamins C, E, and glutathionine, and therefore enhances the effectiveness of these antioxidant systems (Kagan et al., 1992) leading to protection of cell membrane and thereby helping to limit lipid peroxidation (Biewenga et al., 1997).

Carnitine is an essential cofactor in the β-oxidation of fatty acids. It is a nutrient that is found in meat and dairy products, and it can also be synthesized endogenously from methionine and lysine (Carter, et al; 1995). Approximately 98% of carnitine is found in cardiac and skeletal muscle, whereas the remaining 2% is stored in the brain, liver, and kidney (Pons and De Vivo, 1995). Carnitine is necessary inside the cell to transport long chain fatty acids from the cytoplasm into the mitochondria, resulting in mitochondrial energy metabolism through the process of β-oxidation (Matalliotakis, et al; 2000). Another important function of carnitine is to maintain an adequate ratio of fatty acyl-CoA compounds to free CoA inside the mitochondria; therefore, a lack of carnitine results in the accumulation of acyl compounds, which may be toxic to the mitochondria (Liu, 2008).

Coenzyme $Q_{10}$ ($CoQ_{10}$) or ubiquinone is a vitamin-like substance which is found in small amounts in a wide variety of foods and is synthesized in all tissues. $CoQ_{10}$ is the coenzyme for at least three mitochondrial enzymes (complexes I, II and III). Mitochondrial enzymes of the oxidative phosphorylation pathway are essential for the production of the high-energy phosphate containing compound, adenosine triphosphate (ATP), upon which all cellular functions depend (Littarru, 1994). Coenzyme Q besides its bioenergetic function in mitochondrial respiratory chain is a powerful lipid-soluble antioxidant synthesized in the liver (Rauchova et al; 1995).

Herbal compounds obtained from plant extracts that reduce chemical activating enzymes could be considered as good candidates for protection against chemically induced toxicities (Yun et al; 2001). *Panax ginseng*, a traditional multipurpose herb in Asia, has become the World's most popular herbal supplements in recent years. Ginseng has a variety of beneficial biological processes that include anti-carcinogenic, anti-diabetic and anti-inflammatory effects, as well as cardiovascular- and neuro-protection (Joo et al; 2005; Jung et al; 2005). Most of the pharmacological actions of ginseng are attributed to a variety of ginsenosides, which are phenolic acids, flavonoids and triterpenoid saponins (Attele et al; 1999; Huang et al; 2005). Pretreatment with ginseng significantly attenuates $H_2O_2$ induced free radical production and protect against cell death (Xie et al; 2010).

Materials and Methods

*1. Drugs and chemicals*

Isoniazid obtained (from Cid Company). L-carnitine and α-LA obtained (from Eva Company). Coenzyme $Q_{10}$ and ginseng obtained (from Glaxosmithkline SAE, El Salam City, Cairo, Egypt).All kits were purchased from biodiagnostics CO. (Cairo, Egypt). Other chemicals used in the study were of analytical grade.

*2. Animals and housing*

Sexually mature Sprague-Dawley male rats weighing 120-140 g were purchased from Animal House Colony, National Research Centre, Cairo, Egypt) and maintained on standard lab diet. After an acclimatization period of 1 week, animals were divided into 6 equal groups each consists of 6 rats and housed in a temperature-controlled environment (23 ± 1ºC) and artificially illuminated (12 hr dark / light cycle). All animals received humane care in compliance with the guidelines of the Animal Care.

*3. Experimental design*

After acclimatization period, rats were divided into six groups comprising six animals in each group. Rats in group 1 (untreated control). Animals in group 2 (isoniazid-treated group) were injected with isoniazid (100mg/kg, i.p.) in distilled water (Adhvaryu et al, 2007). Rats in group 3 were given α-LA (50mg/kg, p.o.) in corn oil (Arivazhagan and Panneerselvam, 2000).

Animals in group 4 were given L-carnitine (250mg/kg, p.o.) in distilled water (Lheureux and Hantson, 2009). Rats in group 5 were injected with ginseng (20mg/kg, i.p.) in distilled water (Chang et al, 2007). Animals in group 6 were given $Coq_{10}$ (15mg/kg, p.o.) in corn oil (Littarru and Langsjoen, 2007). Rats in groups 3,4,5 and 6 received the treatment drug one hr prior to injection of isoniazid (100mg/kg, i.p.).

Rats in each of the foregoing groups received the drugs for 21 consecutive days.

*4. Blood sampling and analysis*

At the end of experimental period, rats were anaesthetized with ether according to the method described by (Cocchetto and Bjornsson, 1983). Blood samples were collected from orbital *venus plexus* in nonheparinized tubes, centrifuged at 3000 rpm for 15 minutes, and blood sera were then collected and stored at -20º C freezer before they were analyzed. The sera were used for the determination of Alanineaminotransferase (ALT), aspartateaminotransferase (AST), total bilirubin, total proteins and lipid peroxidation biomarker (MDA).

*5. Histopathological Examination*

Samples of the liver from all animals were fixed in 10% neutral formalin and paraffin embedded. Sections (4µm thickness) were stained with hematoxyline and eosin and examination was done through the light electric microscope (Banchroft et al; 1996).

*6. Statistical analysis*

The results were expressed as mean ± standard error of the means (SEM). The results were analyzed statistically using one-way analysis of variance (ANOVA). Duncan's multiple comparison test (Waller and Duncan, 1969) was used to evaluate the significance between individual groups at $P \leq 0.05$.

Results

1. Effect of INH on the serum biochemical analysis alone or pretreated with l-carnitine, α-LA, ginseng or $CoQ_{10}$.

INH treatment induced hepatotoxicity as reflected by elevated serum AST, ALT, total bilirubin level and reduced serum total proteins. Co-administration of either L-carnitine, α-LA, ginseng or $CoQ_{10}$ and INH

had significantly ($p<0.05$) increased serum total protein level and lowered serum AST, ALT, total bilirubin levels compared with INH-treated group as shown in (Table 1).

Malondialdehyde (MDA) was significantly ($p<0.05$) increased in INH-treated rats as compared to control group. Concomitant treatment either with l-carnitine, α-lipoic acid, ginseng or $CoQ_{10}$ and INH resulted in a significant decrease in MDA level compared to INH-treated group alone.

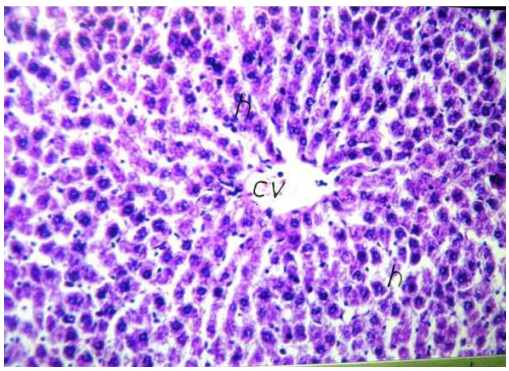
Values are expressed as mean ± SEM.

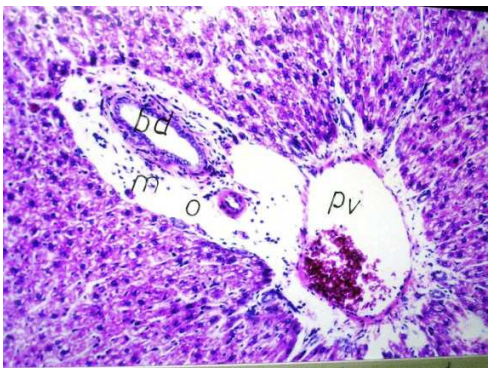
The same letters mean that there is no significant difference from control group at the level of significance $P < 0.05$.

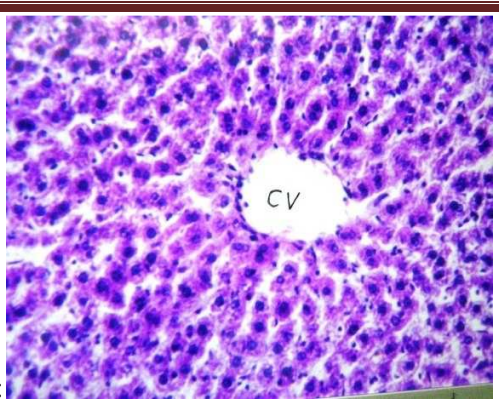
The different letters are significantly different from each other at $P < 0.05$.

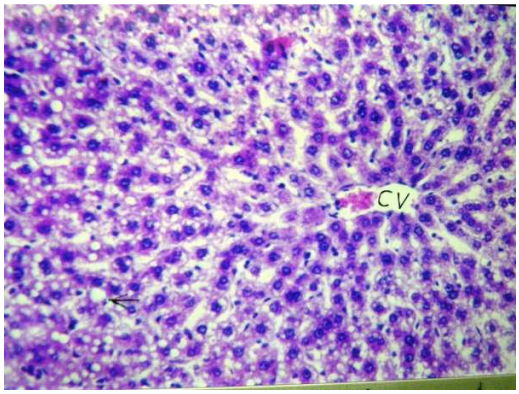
*2.* Histopathological examinations

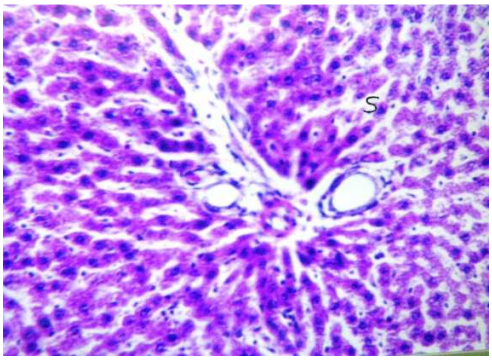
There was no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes were recorded in control liver specimens (Fig. a). Sever dilatation and congestion were observed in the portal and central vein associated with oedema and inflammatory cells infiltration surrounding the dilated bile duct in INH-treated groups (Fig.b). Combined administration of l-carnitine and INH showed intact histological structure (Fig.c). Combined administration of α-LA and INH showed fatty change in the hepatocytes of the periphery of hepatic lobules (Fig.d). Combined administration of ginseng and INH showed congestion in the hepatic sinusoids (Fig.e). Combined administration of $CoQ_{10}$ and INH showed inflammatory cells infiltration in the portal area (m) with kupffer cells proliferation in between the degenerated hepatocytes (Fig.f) .

a 


b 

c 


d 


e 

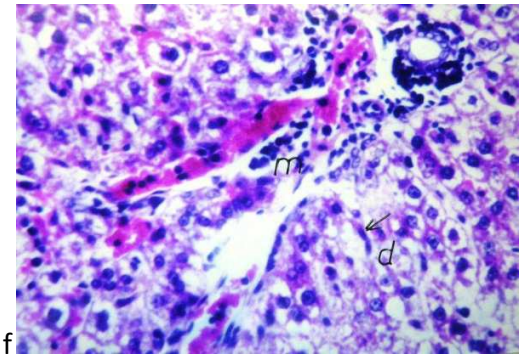

f 


Figure.6. a liver section from control rats showing normal histological structure of the central vein and surrounding hepatocytes. b liver section from rats administered INH showing Sever dilatation and congestion were observed in the portal and central vein associated with oedema and inflammatory cells infiltration surrounding the dilated bile duct. c liver section from rats treated with l-carnitine + INH showing intact histological structure. d liver section from rats treated with ALA + INH showing fatty change in the hepatocytes of the periphery of hepatic lobules. e liver section from rats treated with ginseng + INH showing Combined administration of ginseng and INH Showed congestion in the hepatic sinusoids. f liver section from rats treated with Coq10 + INH Showing inflammatory cells infiltration in the portal area (m) with kupffer cells proliferation in between the degenerated hepatocytes.

Discussion

The present study deals with investigating the possible protective effectiveness of certain antioxidants on experimentally-induced hepatotoxicity using INH. The

extent of hepatic damage was assessed by estimating serum levels of ALT, AST, Total protein and Total bilirubin. In addition INH-induced hepatotoxicity was evaluated by determining biomarkers of oxidative stress such as MDA.

Our results revealed that isoniazid induced elevations in serum levels of AST, ALT, total bilirubin, total proteins and MDA. This due to isoniazid caused hepatocellular damage. The present results are in harmony with findings of (Yuen et al; 2003 and Tasduq et al; 2005) who improved that INH-induced liver injury in rats.

Liver function has been reported to be affected by administration of isoniazid. Naik and Panda (2008) found that the administration of isoniazid to rats significantly increased serum levels of AST, ALT, total and direct bilirubin with presence of inflammation, centrilobular necrosis, and sinus congestion as evidenced by histological examination.

According to Tasduq et.al.(2007) I.P injection of isoniazid in rats caused a significant elevation (approximately 2 fold increase) in the activities of AST and ALT. Increased activity of these enzymes is due to defect of the integrity of hepatocytes, resulting in the release of intracellular enzymes into the systemic circulation. Also, results revealed that there is a significant decrease in serum albumin, total protein and 2 fold increases in the total bilirubin levels. Steatosis and patchy necrosis were detected by histopathologic examination. Rao, (1973) demonstrated that in liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release, which was observed in isoniazid treated group.

It has been reported that INH is metabolized into the bioactive metabolites hydrazine and acetylisoniazid followed by hydrolysis to acetylhydrazin which is oxidized into hepatotoxic intermediaries by CYP 450(Boxenbaum & Riegelman, 1974). There is evidence that antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system (Attri et al, 2000). The depletion of antioxidant defenses and/or rise in free radical production deteriorates

the prooxidant-antioxidant balance, leading to oxidative stress-induced cell death. MDA was one of the main lipid peroxidation (LPO) product, its elevated levels could reflect the degrees of LPO injury in hepatocytes (Yuan et al, 2008). The increase in MDA level in INH-treated rats indicates enhanced peroxidation leading to a failure of the antioxidant defense mechanism to prevent formation of excess free radicals (Naik, 2003).Results of the present study reveal that exposure to isoniazid induced significant elevation in MDA levels. This finding is in harmony with (Santhosh et al, 2006) and (Santhosh et al, 2007).

Since the effect of INH metabolites on liver cells as indicated by increased MDA levels, is related to oxidative stress, an attempt to counteract this effect was carried out by prior administration of four known antioxidants. Thus, L-carnitine, α-LA, ginseng or coenzyme $Q_{10}$ were administered daily for 21 consecutive days before INH-treatment. Results showed that each antioxidant ameliorated INH-toxic effect.

Results of the present study are further supported by the finding of Allis et al (1990) who found that exposure to l-carnitine prior to induction of hepatotoxicity by $CCl_4$ resulted in a significantly decreased serum AST and ALT activities, significantly increased hepatic total proteins and significantly decreased hepatic LPO level. Citil et al, (2005) suggested that l-carnitine caused inhibition of lipid peroxidation by enhancing antioxidant capacity. The authors concluded that the action of l-carnitine in mitochondrial energy production is to facilitate the transfer of long-chain fatty acids from cytosol to mitochondria, thereby playing an important role in the production of ATP (Kelly, 1998).

The present study showed that α-LA reverses serum liver biomarkers in INH-treated rats. These findings are in agreement with those of previously reported investigations. According to Eidelman et al; (2002) biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. Our results are further supported by the finding of Attri et al; (2000) who found that α-LA pretreated animals showed significant reductions in the level of hepatic

peroxidative markers together with concomitant improvement in hepatic antioxidative defense system. The exact antioxidant properties of α-LA could be attributed to its protective effects on GSH levels via increasing cysteine uptake, which is the rate limiting step of GSH synthesis (Han et al; 1997).

Our findings suggested that ginseng returns the values of AST, ALT, total protein, total bilirubin and MDA to normal control values. Our results are supported by those of other researchers (Muller and Fellin; 1974; Kitts and Hu; 2000; Karadeniz et al; 2009; Li et al; 2010) who found that pretreatment with ginseng was found to significantly suppressed an increase in serum AST and ALT activities induced by $CCl_4$ in rats. This finding implies that ginseng challenge to protect liver tissue from $CCl_4$ injury. (Mannaa et al; 2006 and Khalil et al; 2008) Also, these studies declared that the antioxidant properties of ginsenosides (the active constituents of ginseng) those phenolic acids, flavonoids and saponins contribute to protection against hepatotoxicity in rats. These compounds may be responsible for its hepatoprotective action by scavenge and destroy lipid peroxyl radicals and reactive oxygen species like the superoxide anion ($O_2^-$), the hydrogen peroxide ($H_2O_2$) and the hydroxyl radical ($OH^-$). Abdel Wahhab and Ahmed; 2004; and Nada et al; 1996 found that inhibition of lipid peroxidation by ginseng is attributable to its free radical scavenging activity.

Our results suggested that Coq10 showed a marked decrease in the values of AST, ALT and Total bilirubin while showed a little improvement in the values of total protein and MDA. These findings are in agreement with those of previously reported investigations. Allis et al; 1990 observed that administration of Coq10 significantly decreased serum AST and ALT activities which previously increased upon exposure to $CCl_4$. Recently, it has been also evidenced that Coq10 significantly decreases the level of lipid peroxidation in vivo and in vitro (Sawicka and Dlugosz, 2008; Sena et al; 2008). This effect of Coq10 may be related to its antioxidant activity since it significantly decreased ROS generation.

## Conclusion

It could be concluded that regarding protecting effect against induced changes in

liver function, the most effective antioxidant was l-carnitine followed by ginseng, α-LA and $CoQ_{10}$ respectively.

Table (1): Effect of INH on the serum biochemical analysis alone or pretreated with l-carnitine, α-LA, ginseng or $CoQ_{10}$.

| *Parameter* | *Control* | *INH* | *INH + l-carnitine* | *INH + ALA* | *INH + Ginseng* | *INH + CoQ10* |
|---|---|---|---|---|---|---|
| *AST (IU/L)* | $10.53^{a}$±0.53 | $15.50^{b}$±0.76 | $9.8^{a}$±0.32 | $8.9^{a}$±0.23 | $11.02^{a}$±0.52 | $9.28^{a}$±0.22 |
| *ALT (IU/L)* | $5.77^{a}$±0.20 | $7.58^{b}$±0.23 | $5.8^{a}$±0.19 | $5.55^{a}$±0.19 | $6.22^{a}$±0.23 | $5.87^{a}$±0.19 |
| *Total bilirubin (mg/dl)* | $0.27^{a}$±0.02 | $0.45^{b}$±0.02 | $0.31^{a}$±0.02 | $0.39^{b}$±0.02 | $0.4^{b}$±0.02 | $0.35^{a}$±0.01 |
| *Total Protein (g/dl)* | $12.6^{a}$±0.36 | $12.48^{a}$±0.53 | $12.7^{a}$±0.21 | $11.22^{b}$±0.53 | $12.46^{a}$±0.37 | $8.62^{b}$±0.34 |
| *MDA (nmol/ml)* | $12.43^{a}$±0.40 | $24.52^{b}$±1.41 | $5.37^{c}$±0.39 | $20.42^{c}$±0.82 | $13.3^{a}$±0.94 | $23.05^{b}$±1.08 |

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