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Hepato–Protective effects of vitamin C against toxicity induced by tuberculosis drug" isoniazid" on rats

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Abstract

It is known that isoniazid, an anti–mycobacterial medication used clinically to treat bacterial infections (tuberculosis), causes liver damage in both people and animals as well as metabolic malfunction. Antioxidant supplementation, however, may improve the potential adverse effects of anti–tuberculosis drugs. Thus, the purpose of this study is to assess its toxicity in male rat liver cells. Rats weighing between 250 and 300 grams were randomly assigned to three groups, each consisting of six animals: group (1) received water; group (2) received isoniazid medication orally (50 mg/kg); group (3) received vitamin C medication (100 mg/Kg) and isoniazid drug. The levels of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ALP increased significantly ($p<0.05$) after isoniazid treatment. The toxicity impact caused by the medication was greatly reversed by both the medicine and vitamin C. Histopathological findings indicated a potential protective effects of vitamin C extracts as it prevented isoniazid–induced degenerations. This was in the form of pyknosis, swelling of hepatocyte, foci of necrosis. The findings imply that rats experience hepatotoxicity at a dose of isoniazid. Vitamin C may be used as a protective in tuberculosis therapy and has potential clinical applications in hepatic damage due to its chemo–protective properties during isoniazid treatment.

Keywords: Isoniazid; liver damage; liver function, vitamin c, antioxidant.

1– **Introduction: Tuberculosis resulting from Mycobacterium affects the lungs and other vital organs of the body. It is a leading health problem worldwide, particularly in developing countries. Approximately nine million cases of tuberculosis result in two to three million fatalities worldwide, accounting for about one–third of all tuberculosis cases (Adhvaryu *et al.*, 2007).**

Two antibiotics used to treat tuberculosis (TB) include isoniazid and rifampicin (Ravi *et al.*, 2010& Tostmann *et al.*, 2008). An anti–mycobacterial drug called isoniazid, also known as isonicotinic acid hydrazine (INH), works by preventing Mycobacterium TB from synthesizing its cell walls, which in turn prevents the bacteria from synthesizing lipids and DNA (Hussain *et al.*, 2003). In 1952, INH was added to clinical practice and resulted in a significant decrease in morbidity and mortality caused by tuberculosis. But it has some side effects (Frieden *et al.*,2003& Ramachandran and Swaminathan, 2015). It quickly evolved into the cornerstone of treatment for tuberculosis. However, isoniazid's hazardous potentials were rapidly established by cases of severe hepatotoxicity, gastrointestinal, and neurological issues. The activation of oxidative stress by the production of free radicals and ROS (reactive oxygen species) is one of the well–studied side effects of this medication (Adebayo *et al.*, 2012).

In extracellular fluids, vitamin C is regarded as an important free radical scavenger. By capturing radicals like singlet oxygen, it shields the cell membranes from peroxide damage (Smirnoff and Wheeler, 2000). This water–soluble vitamin inhibits the process of lipid peroxidation and exhibits fast electron control over reactive oxygen radicals. (Padayatty *et al.*, 2003; Smirnoff *et al.*, 2003).

2– Material and method

Animals: The Adult male rats weighing 250–300 g were acquired from Theodor Bilharz Research Institute in Cairo, Egypt's Schistosoma Biological Supply Program (SBSP). randomly allocated into three groups, containing six rats each. All rats kept for two weeks for acclimatization, at 25°C +/– 2°C, 50±5% relative humidity, and 12 hours of light and dark. Ad libitum tap water and a regular rat meal were supplied to the rats.

Procedure for the experiment: Three groups (A, B, and C) of six animals each were randomly assigned. Group B received an oral dose of isoniazid 50 mg/kg/day for 14 days, whereas Group C received an oral dose of isoniazid plus vitamin C 100 mg/kg/day for 14 days. Group A acted as the control group throughout this time.

After an overnight fast, the rats were put to sleep by putting them in an anesthetic box filled with ether vapor, which was achieved by putting liquid on cotton wool at the box's base. Following the animals' sacrifice, blood samples were taken for biochemical research. The animals' livers were uncovered after being dissected. Every rat was given its liver, which was then prepared for a histological analysis.

biochemical evaluations for each rat, blood samples were taken, allowed to clot in a clean, dry centrifuge tube, then centrifuged for 15 minutes at 5000 r.p.m. For further examination, a portion of the serum from the clear supernatant was frozen at –20°C.

Statistical Analysis: Our data were analyzed using SPSS 18.0. An analysis of variance, or ANOVA, was used in one way to determine group differences. The Post Hoc Tukey test was utilized to observe any variations in means between the groups. Fisher exact test and Chi–square test were used to observe the relationship between groups' qualitative factors. A statistically significant p–value was defined as < 0.05.

3. Results

Biochemical results

The study's data illustrated that animals that were treated orally with the (INH) drug for two weeks showed a substantial rise in ALP, AST, and ALT activity in the serum. But the treatment with vit.c and (INH) drug revealed improvement in the increasing with comparing of control group (table 1).

Histopathological results

All the rats' liver tissue was examined under a microscope. Every animal in the control group had normal morphology, showing hepatic lobules formed of plates of hepatocytes with well-preserved cytoplasm, and nucleus or binucleate cells. Additionally, the portal spaces were normal; no distribution of fibroblasts or an infiltration of inflammation were seen. (fig 1).

But liver tissue in the treated with isoniazid drug rats showing hepatocytes with fatty changes, hepatocytes showing vacuolar changes, dilated sinusoids, mild infiltration of lymphocytes and kupffer cell hyperplasia (fig 2). The histological results were associated with significantly elevated plasma ALT, AST, and ALP activity, treated with only significantly increases activities. When compared to the drug group that received the same treatment, the outcomes of Group C, who received vitamin C, demonstrated a notable improvement. Histopathological study of this group showed normal architecture of hepatic plates, polygonal hepatocytes with regular nucleus and cytoplasm, clear broad central vein, and absence of centrilobular necrosis (fig 3).

Table (1): Effect of daily oral administration of isoniazid drug (50mg/kg b.w.), vitamin C (100 mg/kg b.w.) , and both Vitamin C and of isoniazid drug for 14 days on Levels of AST, ALT, and ALP in IU/L in rat model.

parameter	Control	drug	% D	Drug+vit c	% D	p-value
ALP	80.7^{a} ±3.2	290.9^{b}±0.8	+260.4%	102.5^{a}±1.3	+27%	0.000
AST	33.8^{a} ±1.2	95.1^{b}±1.3	+181.3%	45.7^{a}±1.3	+35.2%	0.000
ALT	36.2^{a} ±1.1	90.6^{b}±1.2	+150.2%	39.9^{a} ±0.5	+10.2%	0.000

Group significance with a p-value of less than 0.0

Different letters are assigned to statistically significant means (P value < 0.05) while the same letter is assigned to statistically non-significant means.

%D: The percentage difference between the treated and control values x 100

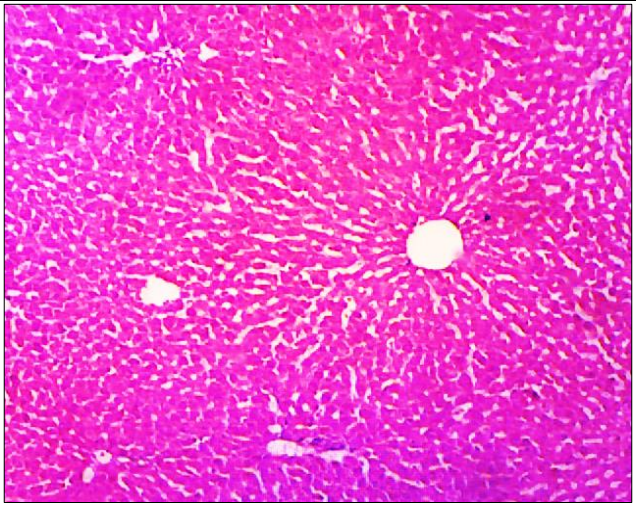


Fig. 1a

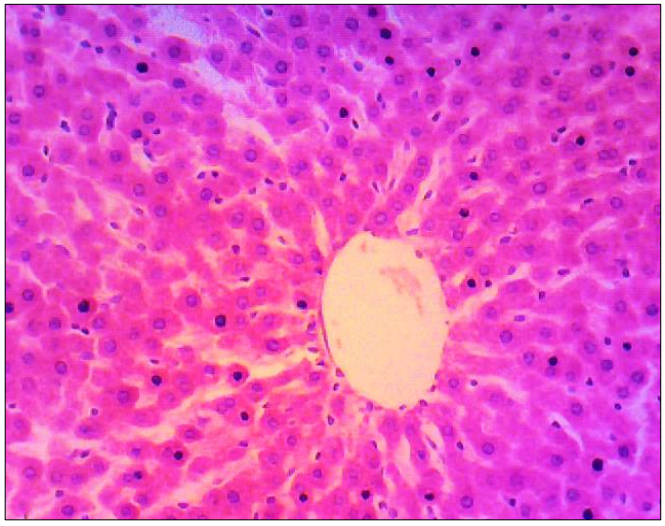


Fig. 1b

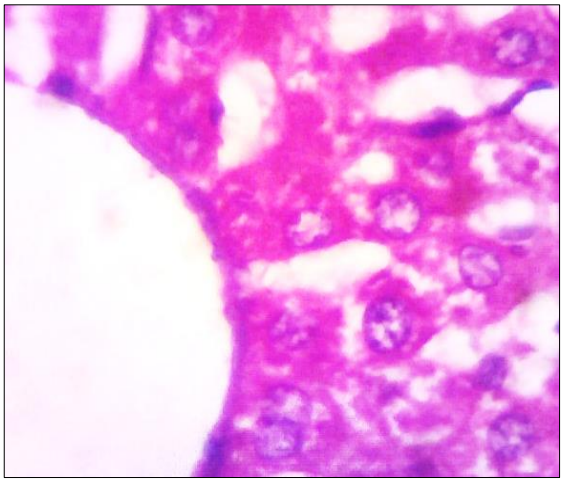


Fig. 1c

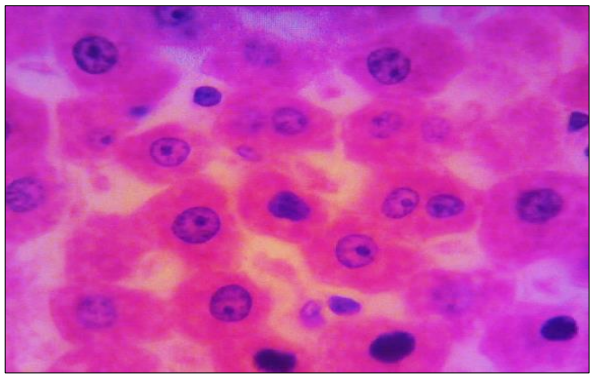
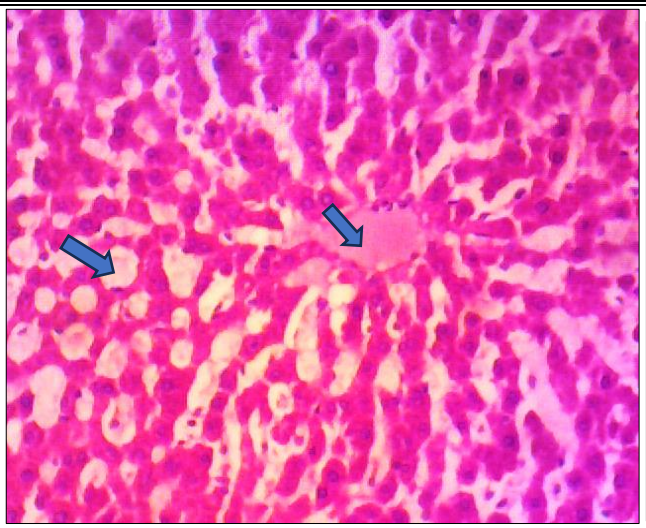


Fig. 1d

Figure (1a, b, c, d): A Control rat liver showing hepatic lobules formed of plates of hepatocytes (with well-preserved cytoplasm, and nucleus, which is surrounded by sinusoids and radially distributed toward the centrilobular veins, along with a few binucleate cells. Additionally, the portal spaces showed no aberrant distribution of collagen or fibroblasts, nor was there any infiltration of inflammation.



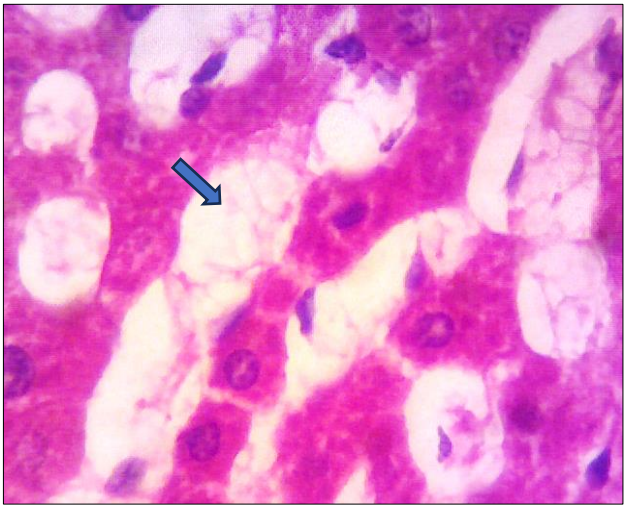
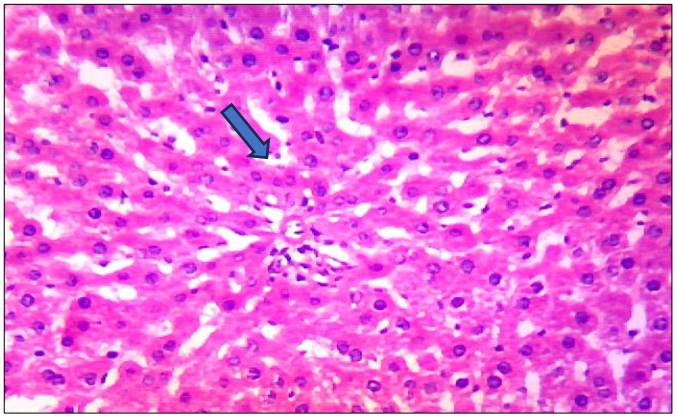


Fig. 2a **Fig. 2b**

Figure (2a, b): A Liver tissue in the treated with isoniazid drug rats showing hepatocytes with fatty changes, dilated sinusoids, hepatocytes showing vacuolar changes, mild infiltration of lymphocytes and kupffer cell hyperplasia.



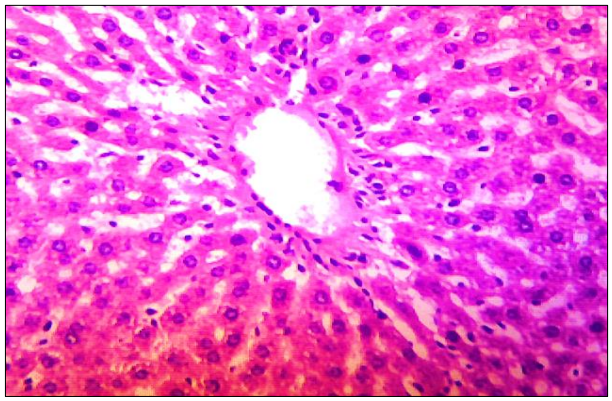


Fig. 3a **Fig. 3b**

Figure (3 a, b): T.S. Photomicrograph of rat liver treated with mixture of vit c and drug showing normal architecture of hepatic plates, polygonal hepatocytes with regular nucleus and cytoplasm, few vaculations of hepatocytes and dilated sinusoids. The presence of kupfer cells, clear broad central vein, and absence of centrilobular necrosis.

4– Discussion

The most prevalent class of medications known to generally cause severe hepatotoxicity is anti–TB medicines. In 5–28% of patients receiving INH treatment, hepatotoxicity linked to anti–TB medications has been documented (Adhvaryu *et al.*, 2007). Approximately 20% of the patients receiving anti–TB drugs have a symptomatic elevation in liver enzymes and caused liver injury (Ramappa, 2013).

Isoniazid (INH) is still used for the treatment of tuberculosis, even though it can cause liver disfunction. Our finding agrees with research and reveals the increase in ALT, AST, and ALP activities by treatment with INH is consistent with Yue *et al.*'s findings (2004 and 2009) and Bais and Saiju (2014).

The main finding was that Administration of vitamin C in rats was associated with a decrease in liver damage caused by isoniazid. These antioxidant properties may be the cause of vitamin C protective actions against INH–induced hepatotoxicity (Williams, 1974& Smith, 1985). Vitamin C has evaluated to have effect of hepatic damage evident by normalize of transaminases and inhibition of lipid peroxidation (Oyinbo, 2006)

In the current investigation, the histological alterations in the drug group showed periportal inflammation, vacuolar degeneration, necrosis, and congestion in the central vein. Similar forms of histological changes brought on by INH treatment in animals have also been documented by some writers (Jaeschke *et al.*, 2002; Tasduq, 2005& Tasduq, 2006).

5– Conclusion

Through the restoration of the physiological and histological structural alterations in the liver tissue of treated rats, vitamin C exhibits a protective effect against INH–induced hepatotoxicity. Consequently, the recovery effects against INH drug–induced hepatotoxicity in our investigation pointed to the importance of vitamin C's antioxidants.

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