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Renal Protective effects of vitamin C against toxicity induced by tuberculosis drug "isoniazid" on rats.

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Abstract

It is known that the combination of antibiotics, such as isoniazid, tuberculosis drug, causes oxidative kidney damage. This study focused on biochemically and histopathologically investigating the vitamin C effects on renal tissue damage by the isoniazid drug treatment. six healthy control rats (250–300g) as group 1, INH was orally administrated to 6 rats at dose 50 mg/kg/day as group 2 and Vitamin C (100 mg/kg/day) + Isoniazid (50 mg/kg/day) was orally administered to 6 rats as group 3. This procedure was carried out for 14 days. At the end of this period renal function parameters as urea, creatinine and uric acid levels were measured in blood samples, and renal tissues were also examined histopathologically. The administration of INH increased blood urea, creatinine, and uric acid levels, which are indicators of kidney function. Vitamin C decreased oxidative kidney damage induced by isoniazid administration. These findings indicated that vitamin C may be beneficial in preventing nephrotoxicity caused by isoniazid.

Key Words: Isoniazid, renal toxicity, renal function, vitamin c, antioxidant.

1. **Introduction: Tuberculosis is a disease caused by bacteria infection. It affects the lungs and other organs (Ravi *et al.*, 2010). It is the second highest cause of deaths from communicable diseases worldwide (Ramachandran *et al.*, 2015). Affecting 1/3 of the world's people (Lonnroth *et al.*,2008). In 2011, approximately 60% of new TB cases were found in Asia (WHO,2012).**

Isoniazid (INH) isonicotinic acid hydrazine is an antibiotic that is effective against tuberculosis. It works by stopping the bacteria's lipid and DNA synthesis, and

cell wall formation (Hussain *et al.*, 2003). However, drug induced nephrotoxicity causes acute kidney failure in 66% of cases (Kohli *et al.*, 2000). Mahmoud *et al.* (2015) recorded that the use of isoniazid lead to oxidative damage in renal tissue.

Vitamin C, known as a vital extracellular fluid free radical scavenger, operates through rapid electron transfer to reactive oxygen species. Its protective role from kidneys external injuries and shortening the required kidney repair time (Rehman *et al.*,2012).

Vitamin C, also called L–ascorbic acid, is a water–dissolvable vitamin. It is in certain food sources. Most animals, humans cannot produce vitamin C inside (Li & Schellhorn, 2007).

The aim of our study was to investigate the effect of vitamin c on kidney damage caused by using isoniazid (INH) in rats.

3.Methods of Research and the tools used

Animals :

Adult male rats weighing 250– 300 g were get from Schistosoma Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Cairo, Egypt. Randomly divided into three groups, containing 6 rats each. All rats stay for two weeks, at a temperature 25±2°C, humidity 50±5% and light and dark cycle. The rats were fed on standard rat diet and tap water.

Experimental procedure:

Random placing of six animals each in 3 groups A, B, and C was done. Group A served as control, Group B administered oral dose of isoniazid 50mg/kg/day for 14 days, and Group C was administered isoniazid with Vitamin C 100 mg/kg/day orally for 14 days.

Rats were fasted overnight, anaesthetized the rats by placing them in an anaesthetic box filled with vapour of ether on a cotton wool on the base of the box. Then the animals (both control and treated ones) were sacrificed, and samples of blood were collected for biochemical studies. The animals were dissected, and kidney exposed. The kidney from each rat was taken and processed for histological examination.

Biochemical analyses Blood Samples were collected and placed in a clean dry centrifuge tube for each rat, then centrifuged at 5000 r.p.m. for 15 min. The serum was frosted at –20°C for subsequent analysis.

Statistical Analysis

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. The 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.

4. Results of Research

Biochemical result

The data of this study illustrated that animals those were treated orally with the isoniazid drug for two weeks significant increase in serum urea, creatinine, and uric acid activities. But the treatment with vit.c and drug revealed improvement in the increasing comparing with control rats (table 1).

Histopathological results

Isoniazid is commonly used in the treatment of TB. Its administration to the experimental group of rats allows for the evaluation of any potential histopathological alterations induced by the drug in the kidney.

The control group provides a reference point to compare the histological changes observed in the experimental groups. The microscopic examination of renal tissue in the control group showed normal renal structures including glomerular tuft and Bowman space, renal tubules, and interstitial without any inflammatory reaction (fig. 1). But The INH treated rat's kidney showed mild histopathological changes compared to the normal control rats in group 1, there were few glomeruli with shrinkage of Malpighian corpuscles, degenerative changes, congestion in glomeruli and dilatation in Bowman's space (fig. 2).

Finaly microscopic examination of the kidney of rats of the 3-rd. group showed almost no degenerative changes in renal glomeruli and convoluted tubules and mild atrophy in some glomeruli when compared to the drug treated rats (fig. 3).

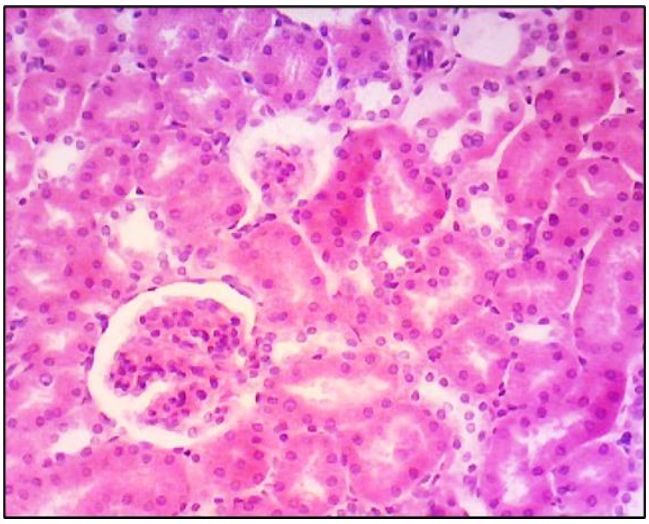
Table (1): Effect of daily oral administration of isoniazid drug (50 mg/kg b.w), vitamin c (100 mg/kg b.w) , and both Vitamin C and isoniazid drug for 14 days on level of creatinine (mg/dl), urea (mg/dl) and uric acid (mg/dl) level in rat model.

Parameter	Control	drug	% D	Drug+vit c	% D	p-value
Urea	$3.4^{a} \pm 0.3$	$19.4^{b} \pm 0.58$	+470.5%	$3.9^{a} \pm 0.29$	+14.7%	0.000
Creatinine	$47.5^{a} \pm 1.9$	$85.3^{b} \pm 1.43$	+79.5%	$46.8^{a} \pm 2.58$	-1.5%	0.000
Uric acid	$0.65^{a} \pm 0.2$	$1.6^{b} \pm 0.03$	+146.1%	$0.62^{a} \pm 0.03$	-4.6%	0.000

Significance between groups at p value < 0.0

Statistically significant means (P value < 0.05) are given different letters and statistically non-significant means are given the same letter.

%D: Percentage difference [(Treated value – Control Value) / Control Value] x 100



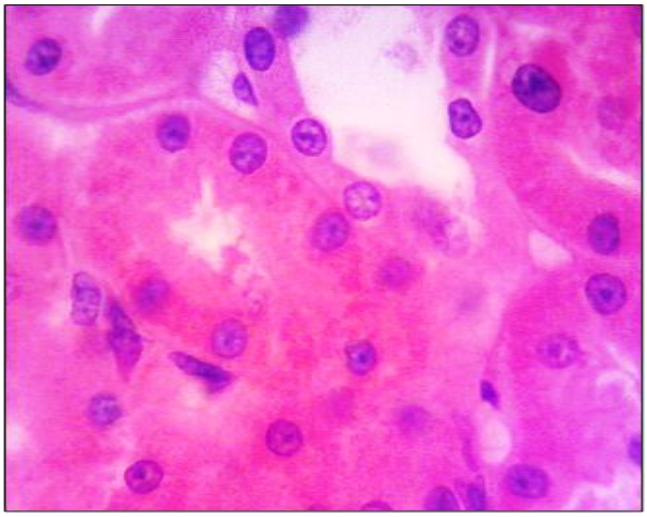
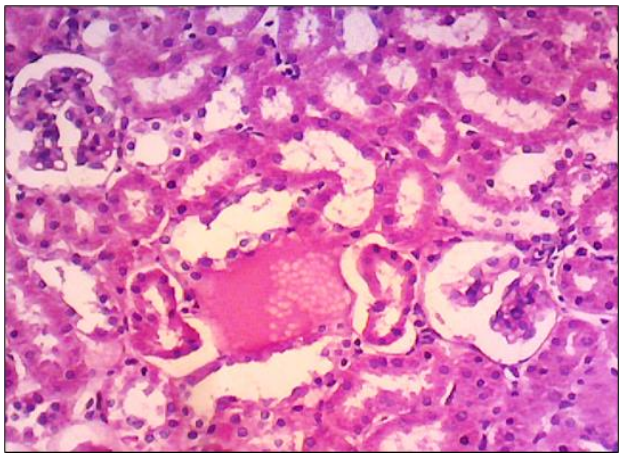
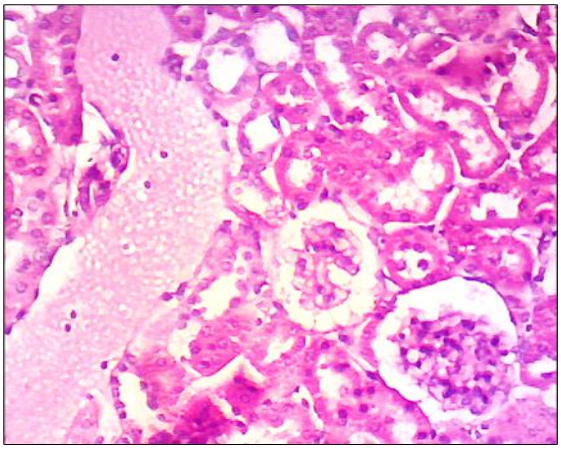


Fig.1a **Fig.1b**

Figures (1a &b): Photomicrographs of transverse sections of normal rat kidney revealed normocellular glomerular tuft with Bowman space , no inflammatory cellular infiltrate, normal interstitial tissue and renal tubules.

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





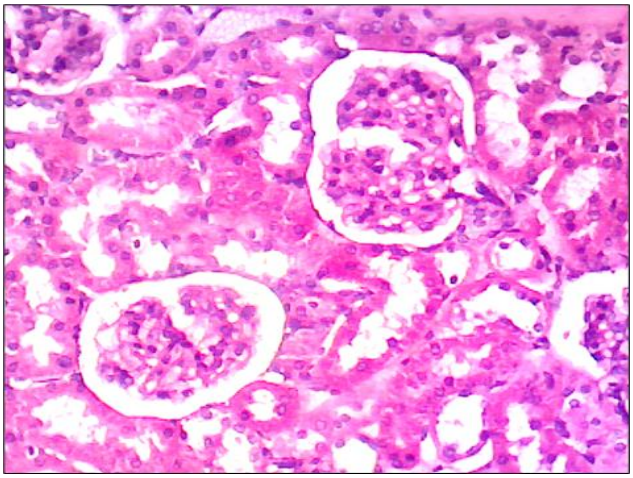


Fig.2c

Figures (2a,b,c): Sections of isoniazid drug treated rat kidney showing shrinkage of the glomerular tuft and dilated bowman space, tubular hyaline casts, interstitial tissue inflammatory cellular infiltrate (congestion)

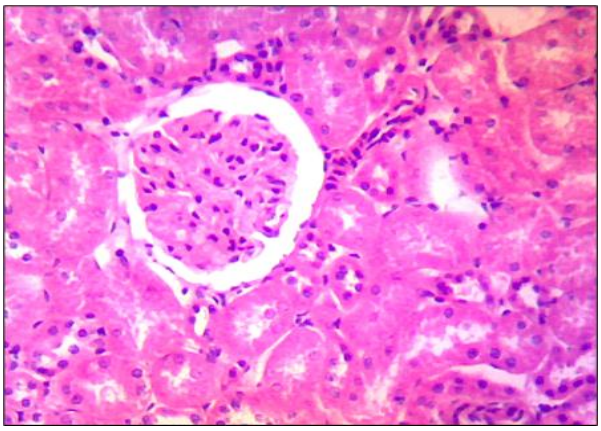


Fig.3a

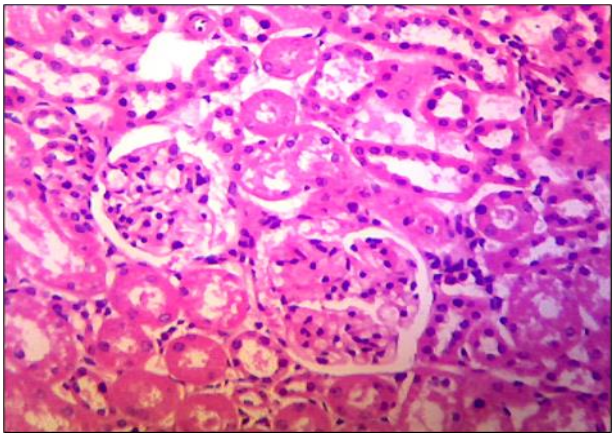


fig.3b

Figures (3a, b): Photomicrograph of transverse sections of rat kidney treated with both vitamin – c and drug showed nearly normal glomeruli, normocellular convoluted tubules and mild atrophy in some glomeruli. the glomeruli, proximal and distal convoluted tubules partly restored their normal configuration. The epithelial cells of the renal convoluted tubules have nearly normal nuclei

Discussion

Treating TB is essential to prevent its spread, but the main drugs against it can have side effects. Sometimes, it causes acute kidney injury. This can force stopping treatment and leave kidney damage (De– Vriese *et al.*, 1998).

Nephrotoxicity is an adverse reaction to many drugs, including INH (Isoniazid) treatment (Singh *et al.,* 2003 and Tostmann *et al.,* 2008). Isoniazid causes nephrotoxicity and kidney damage, in some patients. The mechanism of isoniazid–induced nephrotoxicity is not understood, but it may involve oxidative stress, inflammation, and apoptosis of renal tubular cells (Kisaoglu *et al.*, 2013).

Das *et al.* also used measuring creatinine levels to study the effects of oxidative stress on renal function in animals and found that these levels increase with oxidative stress, (2010). An increase in level of serum creatinine means renal tissue injury (VanBiesen *et al.*, 2006). Our biochemical results matched the histopathological evidence. In the isoniazid group urea, creatinine, and uric acid levels were high and, histological alteration such as tubular necrosis, vacuolization, glomerular dilatation, and haemorrhage occurred in kidney tubular epithelial cells. Isoniazid caused congestion in glomeruli. But vitamin C reduced the pathological damage from isoniazid into normal like renal tissue.

Sharma *et al.* showed that INH drug caused histopathological damage in renal tubules and glomeruli (2019). Sahu *et al.* The treatment by INH alone causes oxidative stress in renal tissue based on biochemical and histopathological evidence.

Vitamin C is an antioxidant agent that can eliminate reactive oxygen species (ROS) and reduce oxidative damage in various tissues. It may also have anti–inflammatory and anti–apoptotic effects by altering the expression of cytokines and chemokines. Therefore, vitamin C have a protective effect against isoniazid–induced nephrotoxicity. Some studies investigated the effect of vit. C on isoniazid–induced nephrotoxicity in animal models. For example, study by Mirjalili *et al.* In 2017 found that vit. C (100 mg/kg) reduced kidney problems, tissue changes, oxidative stress in rats given isoniazid. In another study by Mirzaei *et al.,* vit. C pre–treatment prevented the increase in serum creatinine, blood urea nitrogen, and kidney weight in mice that were exposed isoniazid and improved their antioxidant level.

These and our studies suggest that vit. C can block or reduce the severity of isoniazid–induced nephrotoxicity by supplementing the antioxidant defines system and protect the renal structure and function.

Conclusion

The biochemical and pathological evaluation of serum and kidney samples from the control, isoniazid-treated, and isoniazid plus vitamin C-treated rats can help determine if drug induces any histopathological alterations in the kidney and whether co-administration of vit. C has a protective effect. This research can provide a better understanding of the potential renal effects of isoniazid and the role of vit. C in reducing any adverse effects.

Our biochemical and pathological examinations demonstrated that isoniazid, a medication, caused oxidative stress or imbalance in the kidney tissue of animals. vitamin C may have the potential to protect the kidney from damage caused by isoniazid.

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