PROTECTIVE EFFECT OF CORIANDRUM SATIVUM L. (CORIANDER) ETHANOLIC LEAF EXTRACT ON ISONIAZID INDUCED HEPATOTOXICITY IN ALBINO MICE

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ABSTRACT

Objective: To study the antioxidant effects of ethanolic extract of coriander leaves on INH induced hepatotoxicity.
Methodology: Total 27 male albino mice were randomly divided through balloting into three groups containing nine animals each. Group A served as control while INH toxicity was produced by oral administration (INH 100mg/kg) to group B. Hepatoprotectivity was investigated by co-administration of single oral dose of ethanolic extract of coriander leaves (200mg/kg) along with INH in groups C for 30 days.
Results: On gross examination, liver of group C animals were found to be normal. Biochemical analysis of liver markers of group B showed that these enzymes were markedly raised while experimental group C showed significant decreased level of liver enzymes. Histological examination of group C revealed normal histology with little evidence of inflammation, vascular congestion, necrosis, pyknosis, apoptosis and vacuolar degeneration as compared to group B.
Conclusion: This study showed the ameliorating effect of ethanolic extract of *Coriandrum sativum* L. against INH induced hepatotoxicity.

Key words: Coriander, Hepatotoxicity, Isoniazid, Hepatoprotective.

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INTRODUCTION

Tuberculosis (TB) is a disease; caused by *Mycobacterium tuberculosis* (MTB), its mortality rate is highest among other microbial diseases[1]. According to the World Health Organization (WHO) one third of World's population is infected by *Mycobacterium tuberculosis* (MTB) among which 9 million people develop active tuberculosis each year[2]. This number increased to 9.6 million in 2014 out of which 1.5 million patients died[3].

Since 1952, isoniazid (INH) is used to treat active tuberculosis, and also as prophylactic therapy against MTB[4]. Isoniazid, rifampicin, pyrazinamide and ethambutol are first line antituberculus drugs but isoniazid is highly selective choice for prophylactic therapy. All antimicrobial agents cause antituberculus drug-induced hepatitis (ATDH) especially INH as liver is the main organ to be affected by drug toxicity[5]. Despite of its effective response, the drug is also associated with deleterious effects of elevation of liver enzymes which is mild in case of 20% patients and severe in 1-2% of patients receiving this treatment[6], whereas 10-20% patients on isoniazid therapy show transient harmless increase in liver enzymes; where the rate of hepatotoxicity increases with increasing age[7] and in also malnourished patients. This condition is 8-20% higher in

developing countries as compared to developed countries[8].

Isoniazid induced hepatotoxicity is clinically characterized by signs and symptoms similar to viral hepatitis[9], evident by elevated serum ALT, AST, ALP and total serum bilirubin[10]. Serum levels of these enzymes rise progressively over 15-90 days of INH administration due to disruption of integrity of plasma membrane of hepatocyte, indicative of hepatotoxicity.

Since ages, variety of herbs has been used to treat different diseases especially the liver dysfunctions because of their cost effectiveness, easy availability and fewer side effects[11].

There are however, certain limitations reducing their popularity which include lack of standardization, clinical trials and deficient knowledge about their active ingredients in addition to lack of toxicological evaluation[12].

Coriandrum sativum L. belongs to Apiaceae family, cultivated as spice, to obtain essential oils and as food preservative. All parts of the *Coriandrum sativum* L. possess antioxidant potential with highest levels detected in leaves[13]. Studies by Melo et al[14]. showed that phenols and carotenoids were its major antioxidant components. Among phenol, caffeic acid was in higher concentration in the aqueous extract whereas beta-carotene was found to be the potent constituent in etheric extract. Hydrophobic regions of phenol decrease its solubility in aqueous extract thereby decreasing its antioxidant potency[15]. Later work by Melo et al[16]. showed that aqueous extract of *Coriandrum sativum* L. has less antioxidant activity in liver homogenate. This was also reported by Altimimi et al[17]. that *Coriandrum sativum* leaves inhibit lipid peroxidation due to its anti-oxidant properties.

Studies reported above cleverly showed that hepatoprotectivity is quite common in preparations from herbs however no work is available on antioxidant properties of coriander, therefore, current study was planned to investigate the protective effect of coriander against INH induced hepatotoxicity.

METHODS

This was a randomly controlled experimental study carried out in experimental research laboratory of Anatomy department, University of Health Sciences Lahore. Twenty-seven adult male albino mice used in this study were divided in three groups of nine animals each. Group A served as control and received 0.5 ml distilled water while group B received INH 100mg/kg [10] [10] dissolved in distilled water for 30 days orally. Groups C received 200mg/kg of ethanolic extract of *Coriandrum sativum* L. leaf extract (EEC) [18] along with 100mg/kg INH for 30 days dissolved in distilled water orally. Herbal extract was prepared at PCSIR laboratories Lahore. Animals were anesthetized 24 hours after the last dose of drugs. Blood samples were drawn for biochemical analysis through cardiac puncture and ALT, AST, ALP and total bilirubin were determined through automated analysis. Animals were dissected and livers were removed and preserved for histological examination. Three to five microns thick sections were cut on a rotary microtome (Leica) and slides were stained with H&E, and were studied under light microscope (Leica DM 1000).

Results were analyzed using SPSS version 20. Mean ± S.E were calculated for quantitative variables while percentages were calculated for qualitative variables. One way ANOVA was applied to compare mean and Fisher's Exact test was applied to observe associations between qualitative variables. p- Value ≤ 0.05 was considered statistically significant.

RESULTS

Biochemical Analysis: Animals of group A (control) and C (INH 100mg/kg and ethanolic coriander extract 200 mg/kg togather) were healthy and active while group B animals which were given INH (100mg/kg) were docile and quite sluggish at the end of experiment. Animals of group C also showed decrease in liver enzymes as compared to group B although this difference was not statistically significant for ALT and AST; however, ALP and total bilirubin decreased significantly (Table 1).

Histological Analysis: Histological study of liver from group A showed normal radiating cords and sheaths of hepatocytes constituting hepatic lobule (Fig.1). In the centre of hepatic lobule was central vein and portal triad located at periphery. Hepatic sinusoids, central vein, hepatic artery and portal vein were normal in appearance. Slides from group B showed loss of general architecture of hepatic lobule with congested vessels and moderate periportal inflammatory infiltrate. There was significant increase in number of necrotic cells and apoptotic bodies. Signet ring cells were also seen around central vein alongwith inflammatory infiltrate. Inflammatory cells were mainly around portal triade and few focal areas of inflammation were also seen. (Fig. 2).

Histological preparations of group C stained with H & E showed preservation of general architecture of hepatic lobules having central vein lined with flattened epithelium at the centre and radiating cords of hepatocytes of one or two cell thickness (Fig.3). Hepatocyte cords enclose sinusoids that were lined with discontinuous flattened epithelium and contain specialized stellate cells called Kupffer cells towards their lumen. Routine H & E staining showed mild periportal inflammation and few dilated vessels in portal triad.

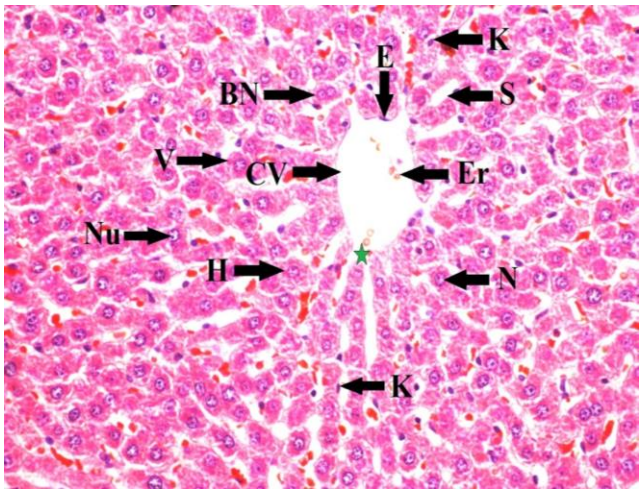

Fig. 1: Photomicrograph of liver from group A showing central vein (CV) lined with endothelial cell (E). Lumen of central vein contain erythrocytes (Er) and sinusoid opening ( ) into the lumen. Sinusoid (S) lined by polyhedral hepatocytes (H) with round central nucleus (N) and prominent nucleoli (Nu), some of them are binucleated (BN). Cytoplasmic vacuoles (V) are also visible. Kupffer cells (K) with rounded nuclei project into the lumen of sinusoid. H & E. X 400.

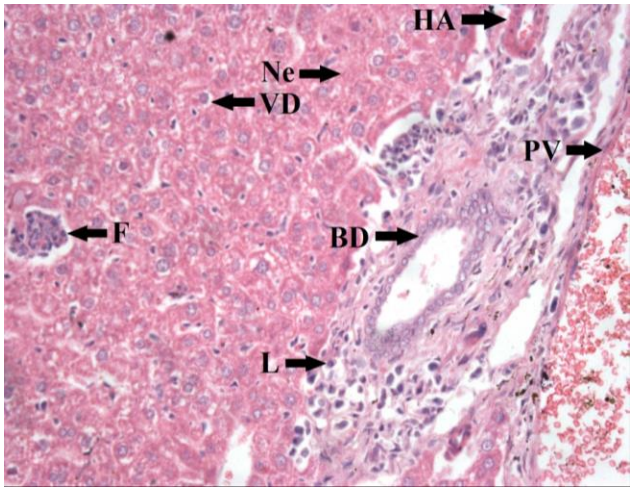

Fig. 2: Photomicrograph of liver from group B showing dilated portal vein (PV), hepatic artery (HA) and bile duct (BD) in portal triad infiltrated with lymphocytes (L) and focal inflammatory infiltrate (F). Hepatocytes show vacuolar degeneration (VD). Areas of necrosis (Ne) are also visible. H & E. X 400.

Hepatocytes appear to be of normal size comparable to group A with increased eosinophilia. Many binucleated hepatocytes were also present. Vacuolar degeneration, pyknosis, apoptosis and fatty change was absent. Binucleated hepatocytes with rounded nuclei containing prominent nucleoli and increased eosinophilia represent the signs of regeneration. On staining with PAS, slides showed magenta red granules indicative of glycogen stores within cell with few lymphocytes (Fig. 4).

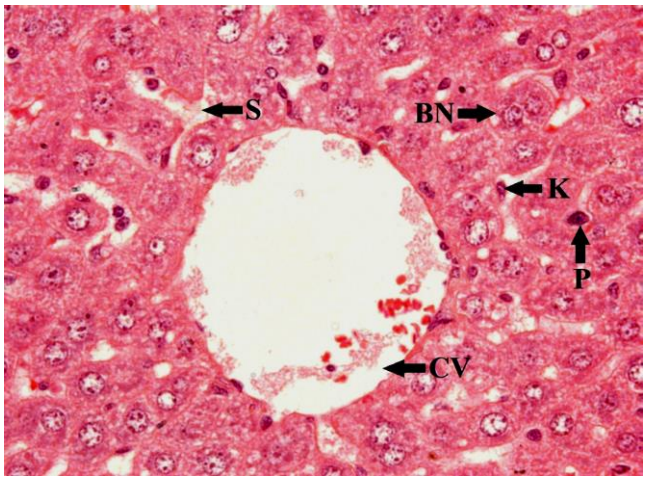

Fig. 3: Photomicrograph of liver from group C showing general hepatic architecture having central vein (CV), enclosing sinusoids (S) which contain kupffer cells (K). Binucleated (BN) cell are indicative of regeneration. Some of pyknotic nuclei (P) are also present. H & E. X 400.

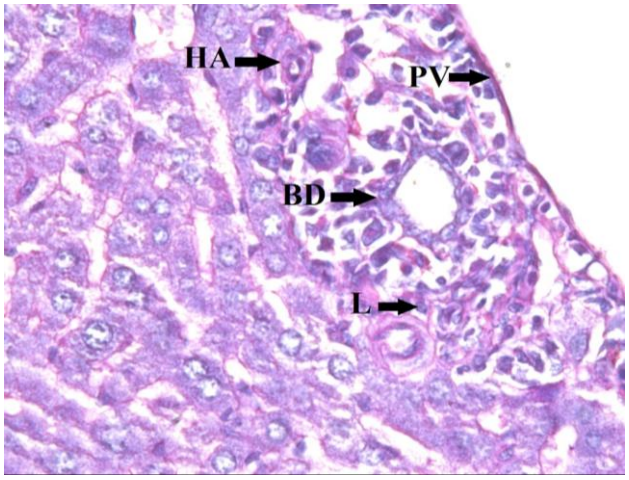

Fig. 4: Photomicrograph from preparation of liver from group C showing magenta red colour with PAS stain. Portal triad having portal vein (PV), hepatic artery (HA) and bile duct (BD) infiltrated with lymphocytes (L). PAS stain X 400

DISCUSSION

Isoniazid causes hepatotoxicity by its reactive metabolite hydrazine that results in lipid peroxidation (LPO) of plasma membrane including mitochondria. This oxidative stress is evident as increase in liver function markers and various histological changes caused by loss of functional integrity and hepatocellular damage to liver[4].

Table 1: Mean values of serum biochemical parameters of mice given INH and/or ethanolic extract of coriander. Each contained nine animals and values given are mean ± SEM.

Serum marker	Group A	Group B	Group C	p value
Serum ALT(U/L)	19.35±1.27	99.12±3.59	79.90±13.22	0.229
Serum AST (U/L)	116.23±18.06	175.31±15.27	131.24±14.53	0.191
Serum ALP (U/L)	88.52±4.02	177.41±15.45	117.55±6.42	0.002*
Serum T. Bilirubin (mg/dl)	0.34±0.01	1.97±0.14	0.85±0.12	0.000*

*p ≤0.05 is statistically significant according to the single factor ANOVA followed by Tukey post hoc test.

The present study showed that the histological preparations from Isoniazid only group (Fig. 2) showed distorted architecture with congested and dilated vessels and moderate focal and general inflammation. Some animals showed fatty infiltration evident as signet ring cell with peripheral nuclei. Humayun et al[19]. reported the distorted architecture with subchronic Isoniazid intoxication. Later Jehangir et al[10]. Also concluded the same results of architectural loss, vascular congestion, dilatation, periportal inflammation with areas of necrosis and apoptosis due to INH toxicity. Our results confirm earlier studies on the hepatic toxicity of Isoniazid. Sarich et al[7]. reported that areas of necrosis were small less than 10% and were not uniformly distributed. Isoniazid treatment also elevated, significantly (p<.001) the serum enzymes i.e., serum ALT, AST ALP and total bilirubin when compared with control animals. Our results are comparable to Humayun et al[20]. WHO studied the effect of propolis on INH induced hepatotoxicity.

The results of present study are comparable to many earlier studies which provide scientific basis that antioxidant compounds are helpful in hepatoprotection against INH induced hepatotoxicity. Many other studies[21-22] also showed the presence of phenolic and caffeic acid compounds which are main constituents of plant extract involved in antioxidant activity. Presence of these compounds in *Coriandrum sativum* could be considered as hepatoprotective against INH induced hepatotoxicity in current study.

It is evident from aforesaid literature that ROS are mainly involved in INH induced hepatotoxicity that can be prevented by antioxidants. EEC contains high levels of antioxidants in form of phenolic and caffeic acids and this corroborated the findings of current study i.e., restoration of liver enzymes near to normal level and preservation of normal histology.

Further studies are recommended to determine the effect of higher dose of coriander extract against INH toxicity.

CONCLUSION

It appears that ROS production by INH is the main cause of its hepatotoxicity that can be prevented by antioxidants. EEC contains high levels of phenolic and caffeic acid contents therefore it counteracted the hepatotoxicity of INH and helped in ameliorating the liver enzymes, total bilirubin and restoring the normal histology.

ETHICAL APPROVAL

The study was approved by the University of Health Science, Lahore. Vide No. UHS/Education/126-13/2641 Dated: September 18th, 2013.

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AUTHOR'S CONTRIBUTIONS

MH: Concept, Principal Investigator, Manuscript writing
AR: Manuscript writing
MT: Proof reading
NN: Microscopic Facilitation.