Protective Effects of Zingiber Officianale on Isoniazid Induced Hepatotoxicity in Albino Rats: A Histomorphological Study

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Abstract

Objective: Tuberculosis (TB) continues to exist as a fatal, rampant disease in most developing countries of the world, including Pakistan. Isoniazid (INH) is an effective drug of choice for the disease, but poses a significant risk of hepatotoxicity. Studying the ability of natural supplements like Ginger to counter the hepatotoxicity of INH may lead to a breakthrough in Tuberculosis treatment. Our study hypothesized to explore the potential hepatoprotective properties of Zingiber officinale (Ginger) against INH-induced hepatotoxicity in albino rats, as evidenced by histomorphological alterations.

Methods: This experimental study was conducted over a period of six months on 40 adult albino rats at the Institute of Basic Medical Sciences, DUHS, Karachi. The rats were divided by random sampling into four groups of ten each: Control group, Ginger group, INH group and INH+Ginger group. The livers of the rats were dissected and examined under light microscope for recording histomorphological changes. The data underwent analysis using IBM SPSS Version 23.0.

Result: Present study highlighted that none of the specimens of the Control or Ginger groups (0%) showed any features of alteration in liver architecture. In the INH group, periportal hepatitis was moderate in 90% of cases, whereas in the INH+Ginger group, it was mild in 90% of cases. Both, INH and INH+Ginger groups displayed varying degrees of confluent necrosis, focal and portal inflammation, with fibrosis observed in 100% of the INH group compared to only 10% in the INH+Ginger group. Steatosis was found in 80% of the INH group versus only 10% of INH+Ginger group. All findings showed significant association between groups ($p<0.001$).

Conclusion: Our findings substantially conclude that Ginger exhibits hepatoprotective effects against INH-induced hepatotoxicity in male albino rats. This suggests a promising avenue for augmenting strategies in the treatment of Tuberculosis.

Keywords: Ginger; Zingiber officinale; Tuberculosis; Isoniazid; Hepatotoxicity

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Introduction

Tuberculosis B is an infectious disease that spreads through airborne particles and continues to be a significant health problem in the world today. more than 10 million individuals contract tuberculosis annually, making it a significant contributor to global mortality rates. TB is a great public health concern in Pakistan as well. In 2020, there were approximately 200,000 notified cases of TB in Pakistan[1].

Isoniazid (INH) is widely employed as the primary medication for treating tuberculosis. However, liver toxicity is a prominent adverse effect experienced by 3% to 20% of patients during its administration. The severe form of HPT is exhibited as hepatic injury including hepatic necrosis found in 1-2% of patients which can lead to discontinuation of treatment. If not identified and treated promptly, it may also become irreversible and lead to death[2]. The INH-induced HPT in humans and animals is due to production of free radicals through cytochrome

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2E1 (CYP2E1). These radicals behave as activators of lipid peroxidation and thereby cause death and destruction of cell membrane[3].

The American Diabetics Association defines Nutraceuticals as the materials regarded as part of food or food itself that impart health or medicinal benefits, including suppression and cure of diseases[4]. Ginger (*Zingiberofficinale)* is extensively used for flavoring and seasoning food and as a condiment in every day cooking throughout the world. Ginger (GIN) is registered in ''Generally Recognized as Safe'' (GRAS) certificate of the American Food and Drug Regulatory Authority (FDA) and is regarded safe at dosages of up to 4 grams daily[5]. GIN is being used for centuries in alternative medicine as a remedy to cure a wide range of diseases[6]. GIN is deemed to possess hypolipidemic, antioxidant and hepato-protective properties. It comprises active agents like phenolic compounds (gingerol, paradol & shogoal) which possess anticancer[7], anti-inflammatory and anti-atherosclerotic properties[8]. GIN compounds act by inhibiting xanthine oxidase, the enzyme accountable for generating reactive oxygen species (ROS) such as superoxide anion[9].

Because of the reasons stated above, it is worthwhile to consider the role ginger administration may play in attenuating the damaged and impaired morphological and morphometric parameters induced in the liver by INH. Significant findings in rats would open the doors for similar studies in humans and may lead to an innovative solution for the adverse effects associated with Tuberculosis treatment.

The objective of this study was to evaluate and analyze the impacts of ginger root extracts on hepatotoxicity induced by Isoniazid in albino rats. The assessment was carried out by histological analysis of the rats' liver sections.

Methodology

It was an experimental study conducted at Institute of Basic Medical Sciences, Dow University of Health Sciences (DUHS) in collaboration with Animal House and diagnostic research and reference laboratory, Karachi, Pakistan, after taken ethical approval from the scientific committee and Institution Review Board (IRB). The study was completed over a period of 24 weeks from July 2020 to Jan 2021. A total of 40 adult albino rats (approximately 200 grams each) were included and randomly allocated into four groups, each comprising 10 rats: Control, Ginger, INH, and INH+Ginger groups. Sample size was calculated by parametric method of ANOVA for four groups[10]. (Minimum significant sample for animal study is 30; we have taken 1/3rd of the study group for control, so the total sample is 30+10=40.)

Control group was given 4ml of 0.9% Normal Saline daily for 4 weeks via nasogastric (NG) tube. One 500 mg Ginger Root capsule from GNC (General Nutrition Corporation, Pittsburgh, PA 15222) was mixed in 5 ml distilled water to form a 100 mg/ml homogenized solution.

The safe GIN dose for rats is 500mg/kg[11]. The average weight of each rat was 0.2 kg. Therefore, each rat in the Ginger group received 0.5-1 ml of ginger root capsule solution daily for 4 weeks via NG tube. Two 100 mg tablets of INH[12] from Macleods ® Pharmaceutical Industries, Mumbai, India, were grounded and mixed in 5 ml distilled-water to achieve a concentration of 40 mg/ml. The INH dose is 50mg/kg. Therefore, each rat in the INH group received 0.25 ml of INH solution daily for 4 weeks via NG tube. The INH+Ginger group received both ginger solution (1ml) and INH solution (0.25 ml) daily for 4 weeks via NG tube.

After completing the experiment's duration, all rats were reweighed and then sacrificed using anesthesia. Their livers were dissected out, observed for gross morphological features and weighed using a Sartorius balance. The livers were then preserved in 10% formalin for 24 to 48 hours for fixation before being sent to the histopathology laboratory for further processing. In the laboratory, the liver specimens underwent dehydration in increasingly concentrated alcohol solutions from 70% - 100%, followed by washing in xylene and fixed firmly & deeply in paraffin blocks. Sections measuring five-micrometers in thickness were prepared from these blocks, suspended in a hot water bath at 42°C for

24 hours and finally mounted on ready to use glass slides in sequential order. The slides were then examined under a light microscope.

For the purpose of concision, only microscopic features will be reported and discussed in this article. Microscopic features of the liver including general architecture, arrangement of hepatic chords, and structures at portal triad were observed through Hematoxylin and Eosin staining at 10X and 40 X magnifications. The distribution of collagen fibers was demonstrated with Masons Trichrome technique at 10X and 40X magnification[13]. Liver slides were evaluated using the Ishak grading system[14] to assess histological features. Since steatosis wasn't covered in the Ishak grading system, it was evaluated separately based on Brunt's scoring criteria[15].

Periportal or periseptal interface hepatitis where, Grade-0: absence of periportal or periseptal interface hepatitis. Grade-1: Mild involvement, observed in focal areas with few portal regions affected. Grade-2: Mild to moderate involvement, noted in focal areas with most portal regions affected. Grade-3: Moderate involvement, where the hepatitis is continuous around less than 50% of the tracts or septa. Grade-4: Severe involvement, with the hepatitis being continuous around more than 50% of the tracts or septa.

Confluent necrosis where, Grade-0: Absence of confluent necrosis. Grade-1: Confluent necrosis observed in focal areas. Grade-2: Zone 3 necrosis present in some areas. Grade-3: Zone 3 necrosis observed in most areas. Grade-4: Zone 3 necrosis along with occasional portal-central bridging. Grade-5: Zone 3 necrosis along with multiple portal-central bridging. Grade-6: Presence of panacinar or multiacinar necrosis.

Focal (spotty) lytic necrosis, apoptosis & focal inflammation where, Grade-0: Absence of focal lytic necrosis, apoptosis, and focal inflammation. Grade-1: One focus or less per 10x objective. Grade-2: Two to four foci per 10x objective. Grade-3: Five to ten foci per 10x objective. Grade-4: More than 10 foci per 10x objective.

Portal inflammation where, Grade-0: Absence of portal inflammation. Grade-1: Mild inflammation, observed in some or all portal areas. Grade-2: Moderate inflammation, noted in some or all portal areas. Grade-3: Moderate to marked inflammation, affecting all portal areas. Grade-4: Marked inflammation, affecting all portal areas.

Fibrosis (Ishak Stage) where, Stage-0: No fibrosis.Stage-1: Fibrous expansion of some portal areas, possibly with short fibrous septa. Stage-2: Fibrous expansion of most portal areas, possibly with short fibrous septa. Stage-3: Fibrous expansion of most portal areas with occasional bridging. Stage-4: Marked bridging of portal areas, including portal to portal and/or portal to central. Stage-5: Marked bridging with occasional nodules (incomplete cirrhosis). Stage-6: Cirrhosis, probable or definite.

Steatosis Score (Modified Brunt Criteria and Grading NAFLD) Score-0: No macrovesicular steatosis. Score-1: Minimal steatosis, involving less than 10%. Score-2: Mild steatosis, affecting 10% to 33%. Score-3: Moderate steatosis, involving 33% to 66%. Score-4: Severe steatosis, affecting more than 66%.

The data were stored and analyzed utilizing latest version of SPSS (23.0). The histomorphological features were analyzed using a two-tailed Student's T-test.

Results

The following six microscopic variables were studied:

Periportal hepatitis was not observed in any specimens of Control (0%) (Fig. 1) and Ginger groups (0%) (Fig. 2). However, in the INH group, it was mild/moderate in 1 rat (10%) and moderate in 9 rats (90%) (Fig. 3). In the INH+Ginger group, it was absent in 1 rat (10%) and mild in 9 rats (90%) (Fig. 4). Our findings demonstrated a highly significant association among the groups. Our findings revealed highly significant differences among the groups ($p<0.001$), as depicted in Table-1.

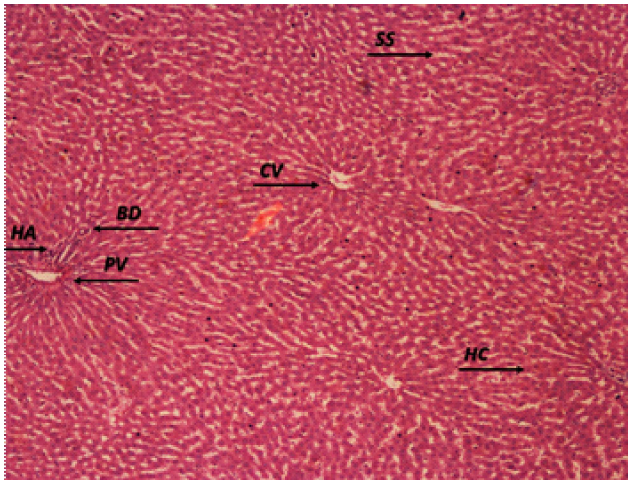

Fig 1. Photomicrograph of H & E stained section of liver of albino rat of Control Group at 10X magnification. The CV surrounded by HC and SS. PV is shown alongside HA and BD. The field appears clear with no sign of inflammatory cell infiltration.

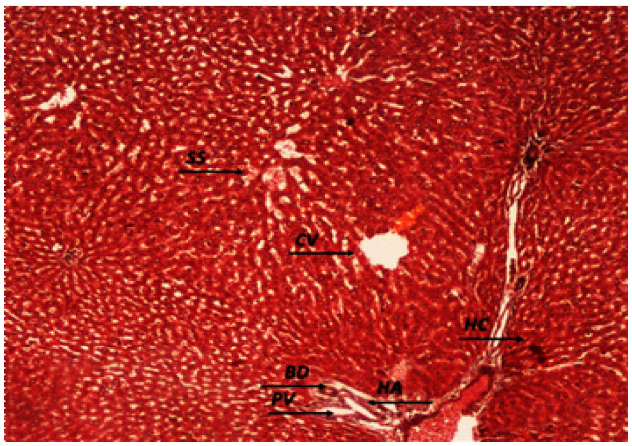

Fig 2. Photomicrograph of Masson's Trichrome Stained Section of liver of albino rat of Ginger Group at 10X magnification. The CV surrounded by HC and SS. PV shown alongside HA and BD. The field appears clear with no sign of inflammatory cell infiltration.

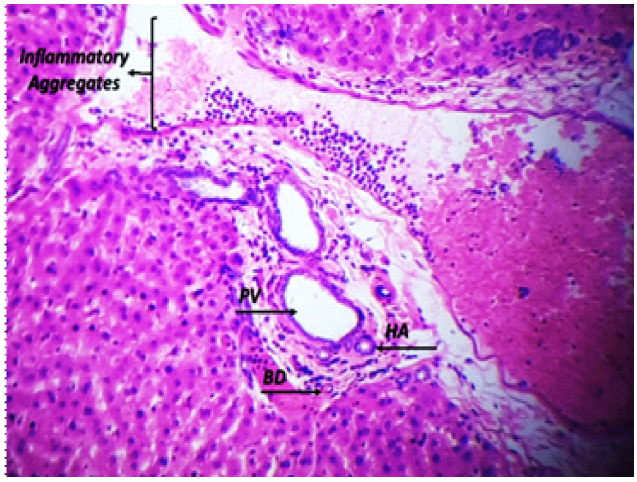

Fig 3. Photomicrograph of H & E Stained Section of liver of a rat of INH Group at 40X magnification. Moderate periportal lymphoid and macrophage infiltrate observed near portal area containing PV, HA and BD.

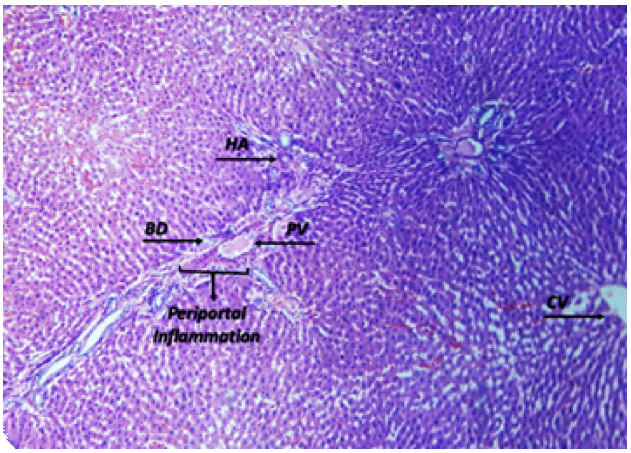

Fig 4. Photomicrograph of H & E Stained Liver Tissue from INH+Ginger Groupat 10X magnification. Mild periportal inflammations with no fibrosis observed around PV, CV, BD and HA are also seen.

Where, CV: Central vein, HC: Hepatic cords, SS: Sinusoids, PV: Portal veins, HA: Hepatic artery, BD: Bile duct.

Confluent necrosis was not observed in any specimens of the Control (0%) (Fig. 1) and Ginger groups (0%) (Fig. 2). However, in the INH group, it was present in some areas of zone 3 in 9 rats (90%) and in most areas of zone 3 in 1 rat (10%) (Fig. 5). In the INH+Ginger group, focal necrosis

was observed in 8 rats (80%), and necrosis in some areas of zone 3 was found in 2 rats (20%) (Fig. 6). Our findings revealed highly significant differences among the groups (p<0.001), as depicted in Table-1.

Focal inflammation was not detected in any specimens from the Control (0%) or Ginger groups (0%). However, in the INH group, two to four foci per high-power field (HPF) were observed in all 10 rats (100%) (Fig. 7). In the INH+Ginger group, only 2 rats (20%) exhibited two to four foci per HPF, while the remaining rats showed inflammation in one focus or less per HPF (80%). These results reveled highly significant differences among the groups (p<0.001), as indicated in Table-1.

Table 1. Histomorphological findings in control and experimental groups

Histo-morphological features		Groups: Control n	Control %	Ginger N	Ginger %	INH N	INH %	INH+ Ginger N	INH+ Ginger %	p-value
Periportal	None	10	100	10	100	0	0	1	10	<0.01*
hepatitis	Mild	0	0	0	0	0	0	9	90	
	Mild /Moderate	0	0	0	0	1	10	0	0	
	Moderate	0	0	0	0	9	90	0	0	
Confluent-	None	10	100	10	100	0	0	0	0	<0.01*
-necrosis	Focal-necrosis	0	0	0	0	0	0	8	80	
	Zone 3 in some area	0	0	0	0	9	90	2	20	
	Zone 3 in most area	0	0	0	0	1	10	0	0	
Focal-inflammation	None	10	100	10	100	0	0	0	0	<0.01*
	Focus count: d"1/HPF (10x)	0	0	0	0	0	0	8	80	
	Focus count: 2-4/HPF (10x)	0	0	0	0	10	100	2	20	
Portal-inflammation	None	10	100	10	100	0	0	0	0	<0.01*
	Portal Area Involvement: Mild to some or all	0	0	0	0	0	09	90		
	Portal Area Involvement: Moderate to some or all	0	0	0	0	10	100	1	10	
Histo-morphological features		Groups: Control n	Control %	Ginger N	Ginger %	INH N	INH %	INH+ Ginger N	INH+ Ginger %	p-value
Fibrosis	No fibrosis	10	100	10	100	0	0	9	90	
	Portal Area Fibrous expansion: Some areas with or without short septa	0	0	0	0	8	80	1	10	<0.01*
	Portal Area Fibrous expansion: Most areas with or without short septa	0	0	0	0	1	10	0	0	
	Portal Area Fibrous expansion: Most areas with marked bridging	0	0	0	0	1	10	0	0	
Steatosis	None	10	100	10	100	2	20	9	90	<0.001*
	Minimal (<10%-33%)	0	0	0	0	**1**	10	1	10	
	Mild (< 33%-66%) 0	0	0	0	7	70	0	0		

*A significance level of p<0.05 was deemed noteworthy according to the Tukey test.

Portal inflammation was absent in the Control and Ginger groups (Table-1). However, in the INH group, moderate inflammation was observed in some or all portal areas in all 10 rats (100%) (Fig. 8). In the INH+Ginger group, only 1 rat (10%) showed moderate portal area inflammation to some or all, while the remaining 9 rats (90%) exhibited mild inflammation. Our findings demonstrated highly significant differences among the groups ($p<0.001$), as depicted in Table-1.

Fibrosis was absent in the Control and Ginger groups. In the INH group, 8 rats (80%) showed portal area fibrous expansion in some areas with or without short septa, 1 rat (10%) had this in most areas with or without short septa, and 1 rat (10%) exhibited it most areas with marked bridging (Fig. 9, 10).

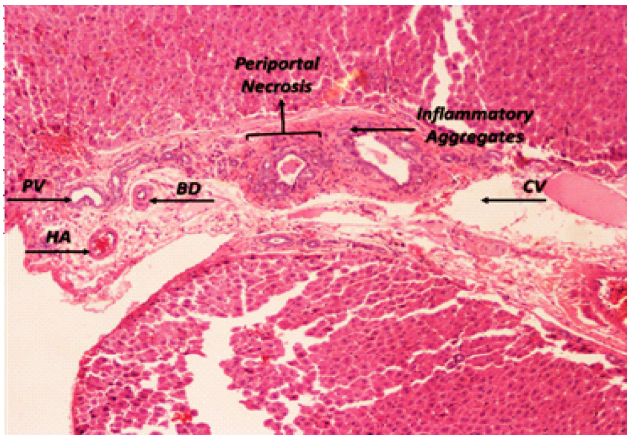

Fig 5. Photomicrograph of H & E-stained section of a rat of INH group at 40X. Periportal necrosis and inflammatory aggregates are observed around PV, HA and BD.

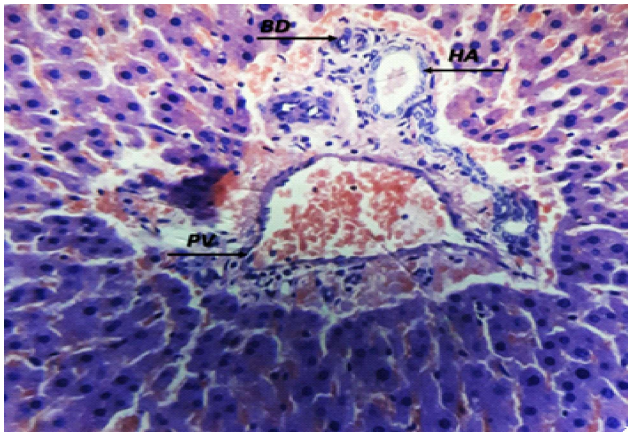

Fig 6. Photomicrograph of H & E-Stained Section of liver of albino rat of INH+Ginger Group at 40X. Mild necrosis around HA, PV and BD.

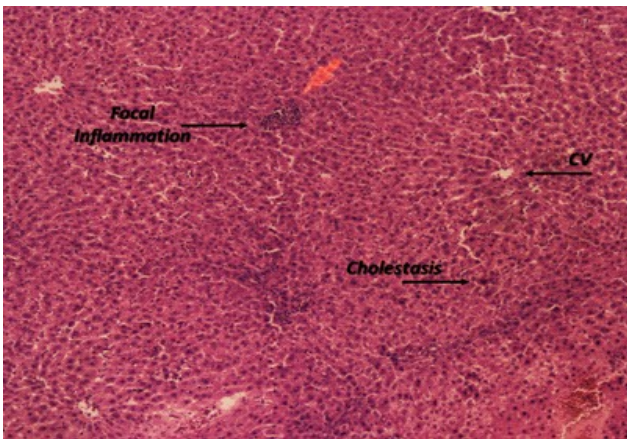

Fig 7. Photomicrograph of H & E-Stained Section of liver of albino rat of INH Groupat 10X. Focal inflammation and cholestasis of bile is observed.

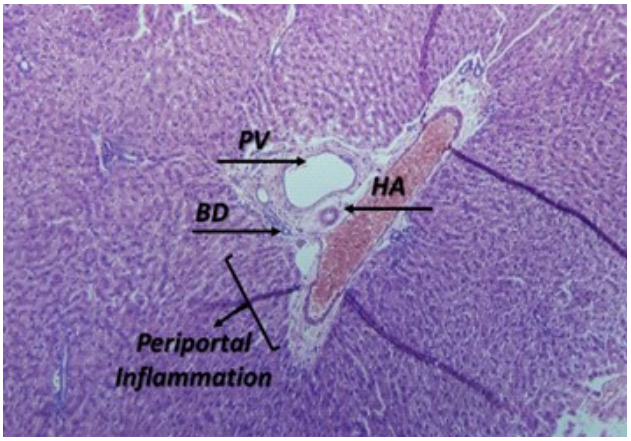

Fig 8. Photomicrograph of H & E-Stained Section of liver of albino rat of INH Group at 10X. Moderate portal inflammations and necrosis observed near PV, HA and BD.

Where, CV: Central vein, HC: Hepatic cords, SS: Sinusoids, PV: Portal veins, HA: Hepatic artery, BD: Bile duct.

In the INH+ Ginger group, there was no fibrosis in 9 rats (90%) and portal area fibrous expansion in some areas with or without short septa in 1 rat (10%). Our findings showed highly significant differences among the groups ($p<0.001$), as depicted in Table-1.

Steatosis was absent in the Control and Ginger groups. In the INH group, it was mild in 7 rats (70%), minimal in 1 rat (10%), and absent in 2 rats (20%) (Fig. 11, 12). In the INH+Ginger group, it was minimal in 1 rat (10%) and absent in 9 rats (90%). Our findings showed highly significant differences among the groups ($p<0.001$), as depicted in Table1.

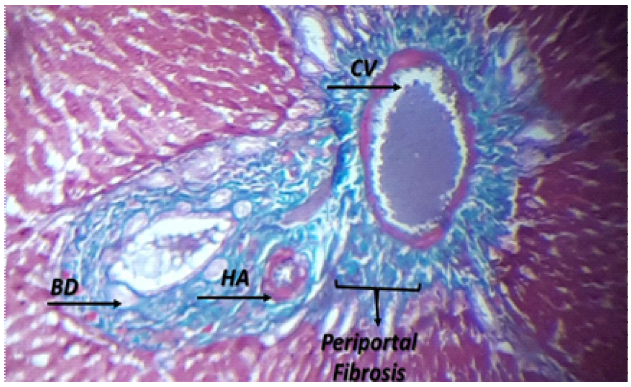

Fig 9. Photomicrograph of H & E Stained Section of liver of albino rat of INH Group at 40X. Periportal fibrosis (blue color) seen around HA, PV and BD.

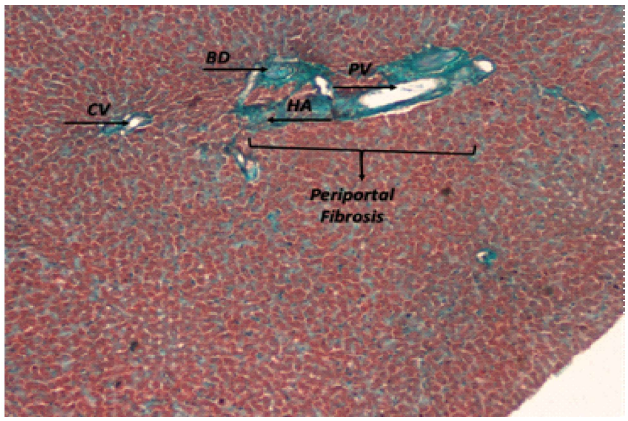

Fig 10. Photomicrograph of Masson's Trichrome Section of albino rat Liver of INH Group at 10X. Periportal fibrosis (blue color) seen around PV, HA and BD.

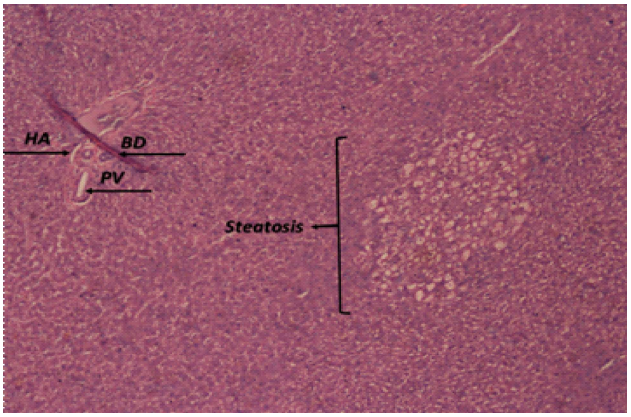

Fig 11. Photomicrograph of H & E Stained Section of rat Liver of INH Group at 10X. Steatosis visible near portal area containing HA and BD.

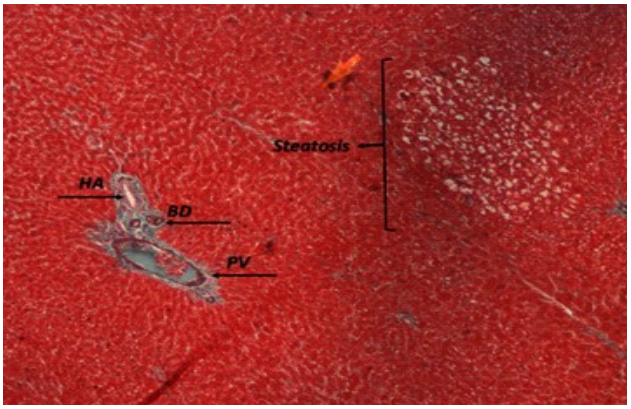

Fig 12. Photomicrograph of Masson's Trichrome Stained section of liver of albino rat of INH group at 10X. Steatosis visible near portal area containing HA and BD.

Where, CV: Central vein, HC: Hepatic cords, SS: Sinusoids, PV: Portal veins, HA: Hepatic artery, BD: Bile duct.

Discussion

Isoniazid (INH) is a commonly used first-line anti-tuberculous drug, but it is known to cause hepatotoxicity. One key mechanism of this hepatotoxicity is the oxidative breakdown of the lipid bilayer membrane.[3] In this study; we observed how ginger protects against liver damage induced by INH in albino rats. The protective and antioxidant properties of ginger are attributed to its ability to deplete or inhibit the generation of free radicals.[9]

Histological analysis of liver sections stained with H&E from albino rats treated with INH showed significant histomorphological deterioration compared to both control and Ginger-administered groups. Moreover, the concurrent administration of Ginger with INH in the fourth group (INH+Ginger) significantly mitigated the adverse effects of INH.

The hepatotoxic histological alterations induced by INH in our study closely resembled the findings made by Kleiner et al.,. They investigated the prevalence of histological characteristics in drug-induced liver injury and identified the predominant patterns as follows: acute hepatitis (21%) and chronic hepatitis (14%), acute cholestasis (9%) and chronic cholestasis (10%), and cholestatic hepatitis (29%).[16]

Moreover, microscopic examination of the stained slides belonging to INH and INH+Ginger groups showed neutrophilic and lymphocytic cellular infiltration around portal and periportal areas in the liver lobules. As necrosis progressed, only pigmented macrophages were seen that had taken the place of the hepatocytes. This finding is in line with the study by Jahan *et al*., that put forward that the existence of neutrophils generally and lymphocytes especially was an important response of hepatic tissue coming in contact with any harmful substances[17].

It is interesting to note that Ginger has been shown to be protective for hepatotoxic effects of other drugs as well. An animal-based study showed that prior treatment with ginger revealed protective effects towards liver derangement in enzymes markers and histomorphological damage caused by Gabapentin.[18] In another similar animal-based study, ginger treatment adequately accomplished the prevention of diclofenac-generated hepatic failure in rats.[19] Yet another investigative study demonstrated that polyphenol-rich plants like ginger may protect against heavy metal liver toxicity.[20] Hassan *et al*., demonstrated the improvement of hepatic fibrosis induced by CCl4, similar to INH, through simultaneous oral intake of crude ethanolic extract of ginger at a dose of 200 mg/kg[21].

This study is the first of its kind to study the role of Ginger on INH-induced hepatotoxicity. Prior to this, other nutraceuticals investigated in this context include Solanum nigrum (black nightshade), which works by scavenging hydroxyl radicals and 2,2-Diphenyl-1-picrylhydrazyl radicals (DPPH)[22], Turmeric which inhibits extracellular matrix formation by enhancing matrix metalloproteinase expression[23], Garlic which increases anti-inflammatory monocyte IL-10 production[24] and Silymarin (milk thistle) which is also an antioxidant and free radical scavenger.[25]

CONCLUSION

In conclusion, our discussions strongly support Zingiber officinale (ginger) as a promising nutraceutical for mitigating INH-induced hepatotoxicity, indicating potential for human trials. The study's limitation to ginger's impact on INH-induced toxicity prompts future exploration, comparing different nutraceuticals and assessing potential synergistic effects. Our findings suggest ginger's potential in preventing and delaying liver toxicity from single-drug anti-tuberculosis therapy. In resource-constrained Pakistan, efficient, cost-effective anti-TB therapy with minimal failure is crucial. Incorporating inexpensive ginger supplementation may reduce treatment failure, especially in a single-drug regimen. Further research and clinical trials are imperative to validate and translate these promising findings into practical therapeutic strategies.

Conflict Of Interest: None

Disclaimer: None

Source of Fundinf: None

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