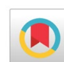

Hepatoprotective effect of Hydro-alcohol extract of *Mimusops elengi* root against antitubercular drug-induced hepatotoxicity in rats

Prakash Dabadi[1], Fouad Saleih Resq AL-Suede[2,3*], Chandrashekhar VM[4*], Mallappa Shalavadi [4], Ashok Gnanasekaran[5]

Abstract

Introduction: Nowadays, different chemicals and drugs are causing liver injuries including first-line antitubercular drugs (ATDs). There is a need to find safe and potent moieties of hepatoprotective drugs against liver diseases from different natural resources including medicinal plants. So, the current study aimed to evaluate the hepatoprotective property of *Mimusops elengi* root.

Methodology: *Mimusops elengi* root extract (200-400mg/kg) was evaluated in an induced hepatotoxicity model of oxidative stress in Wistar rats by ATDs orally for 14 days. Markers indicating oxidative stress and hepatic damage such as serum transaminase (SGOT / SGPT), and alkaline phosphatase (ALP) were measured. Biomarkers of antioxidant status, superoxide dismutase, catalase, glutathione reductase, and marker of lipid peroxidation, were evaluated using standard procedures. The hematological lipid profile, bilirubin, glucose, and histopathological examination were also assessed.

Results: intoxication with ATDs markedly reduced the hematological indices and elevated the biochemical enzyme markers (SGOT, SGPT, ALP $p<0.001$) and lipid profile ($p<0.001$), decreased and glucose was elevated. However, pretreatment with *Mimusops elengi* root extract significantly ($p<0.001$, $p<0.01$) improved this alteration and sustained the antioxidant potential. The Histopathological and biochemical data support hepatoprotective action.

Conclusion: The results of the current study reinforced the extract of *Mimusops elengi* possesses significant hepatoprotective and antioxidant activity against ATDs-induced hepatotoxicity.

Key Words: Hepatoprotective, *Mimusops elengi*, Isoniazid, Rifampicin, Anti-TB.

Significance | Development of therapeutics for the treatment of hepatotoxicity and oxidative stress induced by ATDs

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1. Introduction

According to World Health Organization (WHO), tuberculosis has been declared a global health emergency, with almost one-third of the world's population estimated to be infected. Directly observed treatment, short-course (DOTS) is the mainstay of tuberculosis treatment, according to the RNTCP(Begum, Parveen, Sultana, Fatima, & Fareedullah, 2020). The liver is the largest solid organ in the body as well as the largest gland. The liver plays a vital role in lipid, protein, and carbohydrate metabolism. Every year, over 20,000 people die as a result of liver illnesses (Anoopa, Kannappan, & Manojkumar, 2020).

The cornerstone of TB care is a 6-month course of anti-TB medications that includes isoniazid, rifampicin, pyrazinamide, and ethambutol for two months in the intensive phase. Compliance is life-threatening in the treatment of tuberculosis. Adverse effects naturally harm defiance because they often need a change in treat-

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ment, which might have unfavorable implications for the treatment outcome. Anti-TB drug-induced hepatotoxicity is also one of the side effects that can impact TB therapy outcomes (Molla, Wubetu, & Dessie, 2021).

Anti-TB drug hepatotoxicity mechanism of action, Isoniazid induced hepatotoxicity (INH) is a serious clinical problem, and the current mechanistic explanation involves metabolic idiosyncrasy, which is the bioactivation of acetyl hydrazine, which causes cytotoxicity. Acetyl hydrazine is an INH metabolite. Diacetyl hydrazine and monoacetyl hydrazine are formed when acetyl isoniazid is metabolized. The enzyme cytochrome p-450 converts monoacetyl hydrazine into hazardous compounds. Hepatotoxicity caused by anti-TB medications is mostly caused by CYP2E1 and P-4502E1. The CYP2E1 C1/C1 genotype produces more hepatotoxins, which increases CYP2E1 activity. Isoniazid inhibits the activities of CYP1A2, 2A6, 2C19, and 3A4. INH may cause toxicity by inducing or inhibiting enzymes (Metushi, Cai, Zhu, Nakagawa, & Uetrecht, 2011; Tostmann et al., 2008).

The deacetylation of rifampicin results in the synthesis of diacetyl rifampicin, which is then hydrolyzed to create 3-formyl rifampicin. It causes hepatocellular dysfunction in the early stages of treatment. It increases the formation of hazardous metabolites from acetyl hydrazine (Ohno et al., 2000).Further Rifampicin converts isoniazid to hepatotoxic isonicotinic acid and hydrazine. When INH and rifampicin are used together, the risk of liver necrosis increases.

Pyrazinamide Microsomal amides, a liver enzyme, converted pyrazinamide to pyrazinoic acid (PA), which was then oxidized by xanthine oxidase to 5-Hydroxypyrazinoic acid (5-OH-PA). PZA metabolites have been shown to have hepatotoxic properties. Hepatotoxicity and hyperuricemia are also linked to PA (Ramappa & Aithal, 2013; Yew & Leung, 2006).

Antitubercular drug-induced hepatotoxicity is found to be mediated through oxidative damage and free radical damage to hepatocytes. They damage the Protein, DNA and cell membrane by lipid peroxidation (Foaud Saleih R Al-Suede et al., 2014; Basini, Mohanalakshmi, & Anitha, 2013). Furthermore, Hydrazine depletes the liver's reserved glutathione (GSH) level, causing oxidative stress and cell death. Because oxidative stress is a major contributor to antituberculosis drug-induced hepatotoxicity and liver damage, the goal of this investigation is to see how works as a supplemental agent for INH, RIF, and PZA-induced liver dysfunction in rats (Huang et al., 2003).

Despite considerable advances in modern medicine, there are currently no effective drugs that boost liver functions, protect the liver from injury, or aid in the regeneration of hepatic cells (Jain et al., 2008). Modern medicine is still struggling with how to treat liver illness. Herbs play an important role in the management of liver problems, for the treatment of liver problems, many local herbs are used. Oral administration of *M. oleifera* leaf extract can restore normal liver activity in rats after INH, RIF,and PZA-induced hepatic injury (Pari & Kumar, 2002). *Mimusops elengi* Linn (family Sapotaceae), often known as the Spanish cherry or Bullet wood in English, is a tree native to South India's western peninsula. The plant parts can be used to treat a variety of diseases, including astringent, cooling, anthelmintic, tonic, liver, diuretic, cardiotonic, reduce inflammation, aphrodisiac, and febrifuge effects. According to Ayurveda, and is effective in reducing Kapha and pitta doshas (Baliga, Pai, Bhat, Palatty, & Boloor, 2011). Plant parts contain chemical constituents are taraxerol, flavonoids, taraxerone, ursolic acid, and betulinic acid, gallic acid according to chemical analyses (Tabana et al., 2016), α-spinosterol, β-sitosterol, lupeol, triterpenoid (Fouad Saleih R Al-Suede et al., 2014), mimusopsides A and B, mimusopin, saponins, and a new steroidal saponin (Akhtar, Ali, & Alam, 2010). It is aued as antiulcer (Prakash, Koti, Vijay, & Chandrakala, 2011), antioxidant (Dabadi, Chandrashekhar, & Shalavadi, 2021), antibacterial, antifungal, anti-cariogenic, free radical scavenging, antinociceptive, and diuretic activities (Baliga et al., 2011).

There is an insufficiency of research on *Mimusops elengi* root on hepatoprotective activity. As a result, the current study was carried out to determine the hepatoprotective effects of a hydroalcohol extract of *Mimusops elengi* root (HEME) on anti-tubercular drug isoniazid Rifampicin and Pyrazinamide induced liver damage in rats.

2. Materials and Methods

2.1 Drugs and Chemicals

2-Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), and 5, 5'-Dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA), Sodium nitroprusside (Qualigens., Mumbai), and Ascorbic acid (Burgoyne Burbidge's and Co., Mumbai). Serum GOT GPT, ALP, Total Bilirubin, and LDL-cholesterol enzyme test kits were purchased from Erba Diagnostic, Germany (Nice Chemicals, Cochin), Standard Silymarin free sample from Himalaya Drug Co., Bombay, and Isoniazid, Rifampicin, and Pyrazinamide free samples from Himalaya Drug Co., Bombay (HiMedia Lab. Pvt. Ltd, Mumbai). The rest of the reagents and solvents used in the experiment were of analytical quality. Refrigerator Centrifuge (MPW-350R), Spectrophotometer (UV) (UV-1601, Shimadzu Corporation, Kyoto, Japan).

2.2 Plant material

The plant *Mimusops elengi* was found in Davanagere, Karnataka, in a garden area. Professor. L C Kulkarni, Department of Botany, PC Jabin Science College, Hubli-580031, Karnataka. Identified and authenticated it.

2.3 Preparation of extract

Mimusops elengi roots were collected, washed, and air dried before coarse powdering and passing through sieve # 44 to achieve consistent powder size. The powder was first defatted with petroleum ether, and then 1000 g of defatted root powder was macerated for 48 hours in 6 L of hydroalcohol solvent [water: ethanol (7:3)] with intermittent shaking. To get a clear extract, the macerate was decanted and filtered through cloth and then filter paper. With the same volume of hydro-ethanol combination, the operation was repeated. Macerates were pooled and collected in trays, then evaporated to dryness at 30-35°C, lyophilized, and kept in the air-tight container under refrigeration. The obtained extract was used for hepatoprotective activity.

2.4 Animals

Wistar-Albino rats of either sex (200-250g) were procured from the H. S. K. College of Pharmacy and Research Centre, Bagalkot central animal house. The animals were kept at room temperature (22-28°C) with a relative humidity of (65 ± 10 %) and fed normal laboratory feed (Amruth, Sangli, Maharashtra(Al-Suede, 2021)) and water *ad libitum* for a 12-hour dark and light cycle. The study was approved by the Institutional Animal Ethics Committee and carried out according to their guidelines [File no: IAEC/HSKCOP/ January/2017/Ph. D1].

2.5 Experimental groups

Hepatotoxicity wasinduced with isoniazid (INH), rifampicin (R), and pyrazinamide (Z). The daily doses of drugs for rats were INH (50mg/kg), rifampicin (100mg/kg), and pyrazinamide (350mg/kg) (Eminzade, Uras, & Izzettin, 2008; Saram, Imanuel, Kailasam, Narayana, & Venkatesan, 1986). INH and rifampicin were administered intraperitoneally (i.p.) whereas pyrazinamide was given orally. Hydro-alcohol extract of *Mimusops elengi* (HEME) root.The extract was administered orally in doses of higher and lower doses in rats, and silymarin (Std) was administered at a dose of 50 mg/kg orally(Anbarasu, Rajkapoor, & Kalpana, 2011). All the drugs were administered daily for 14 days. The anti-tubercular drugs, HEME, and silymarin were administeredusing 2% gum acacia.

Experimental design

Wistar albino rats, five groups were divided each group having six rats in it (n=6) animals in each group. Group 1 received a vehicle 10mg/kg body weight as a normal group. Group 2 received anti-TB drugs (INH-50mg/kg +R-100mg/kg + Z-350mg/kg) induced oxidative stress as negative control. Group 3 treated by Silymarin (50mg/kg) on anti-TB drugs (INH-50mg/kg + R-100mg/kg + Z-350mg/kg) induced oxidative stress. Whereas group 4 & 5 treated with HEME (200mg/kg) with anti-TB drugs (INH-50mg/kg + R-100mg/kg + Z 350mg/kg)and HEME (400mg/kg) with anti-TB drugs (INH-50mg/kg + R-100mg/kg + Z- 350mg/kg) induced oxidative stress respectively. Upon completion of the experimental period the blood was collected, the animals were sacrificed and liver samples are collected.

2.6 Evaluation of biochemical parameters

2.6.1 Hepatoprotective activity

Serum biochemical estimation: After 24 h of the last dose of treatment animals were under light ether anesthesia blood samples were collected by puncturing the retro-orbital plexus. The blood samples were allowed to clot for 30 min and centrifuged (3000 rpm for 10 min) to separate serum and analyzed for various biochemical parameters.

2.6.2 Biochemical estimations

Serum levels of Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) ,Total Bilirubin, Alkaline Phosphatase, Total Cholesterol, Glucose (Varley, 1980), Total protein, in serum concentrations were estimated for assessing Hepatoprotective activity against anti-TB drugs (INH+R+Z) induce Hepatotoxicity on Wistar albino rats (Remirez, Commandeur, Groot, & Vermeulen, 1995).

2.7 Antioxidant estimations in tissues

2.7.1 Preparation of Post Mitochondrial Supernatant

The rats were euthanized after the blood samples were taken, and their livers were perfused with ice-cold normal saline to eradicate any remaining blood contents. The liver was then removed and placed on ice, then blotted on filter paper, weighed, and homogenized in cold phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 rpm for 10 minutes at 4˚C (MPW-350R, Korea), and the post-mitochondrial supernatant (PMS) was used to calculate total protein and lipid peroxidation. The supernatant was centrifuged for 1 hour at 4˚C at 15000 rpm. The resulting supernatant was utilized to calculate SOD, CAT, GSH, and total thiols.

2.7.2 Lipid peroxidation (LPO)

The method was used to calculate the amount of thiobarbituric acid reactive compounds (TBARS) in the homogenate (Prabhakar et al., 2006). Briefly, 0.5 ml of 10% homogenate was incubated at 95 ºC for 15 minutes with 15% TCA, 0.375 % TBA, and 5N HCl, then cooled, centrifuged, and absorbance of the supernatant measured at 512 nm against a blank. TBARS nmoles /mg of protein were calculated using = 1.56 x 10^5 $M^{-1}cm^{-1}$ and represented as TBARS nmoles /mg of protein (Braughler, Chase, & Pregenzer, 1987).

2.7.3 Superoxide dismutase (SOD)

The ability of SOD to block the auto-oxidation of adrenaline to adrenochrome at alkaline pH was used to measure superoxide dismutase activity (Misra & Greenwald, 1985). The production of adrenochrome was detected at 295 nm using 25µl of the supernatant obtained from centrifuged liver homogenate and 0.1 mM epinephrine in carbonate buffer (pH 10.2) in a total volume

of 1ml. Using the usual plot, the SOD activity (U/mg of protein) was estimated.

2.7.4 Catalase (CAT)

Claiborne'stechnique was used to measure catalase activity .In a total amount of 3.0ml, the assay mixture contained 1.95ml phosphate buffer (0.05M, pH7.0), 1.0ml hydrogen peroxide (0.019M), and 0.05ml homogenate (10 % w/v). At 240 nm, changes in absorbance were measured. Catalase activity was measured in nM H_2O_2 consumed/min/mg protein.

2.7.5 Glutathione (GSH)

The method of Sedlak and Lindsay was used to estimate GSH in various tissues (Moron, Depierre, & Mannervik, 1979) . To give a brief, 5% tissue homogenate was prepared in 20 mM EDTA, pH 4.7, and 100µl of the homogenate or pure GSH was added to 0.2 M Tris-EDTA buffer (1.0 ml, pH 8.2) and 20 mM EDTA, pH 4.7 (0.9ml), followed by 20µl of Ellman's reagent (10 mmol /l DTNB in methanol). After 30 minutes of room temperature incubation, samples were centrifuged and measured at 412 nm.

2.7.6 Total thiols

This assay is based on the principle of sulfhydryl groups forming a rather stable yellow color in the presence of DTNB (D. Singh, Cho, & Upadhyay, 2016). 0.2 ml of liver homogenate, 40µl of 10 mM DTNB, and 3.16ml of methanol were combined with phosphate buffer (pH 8). The absorbance of this mixture was measured at 412 nm against suitable blanks after a 10 min incubation period. Using = 13.6 x 10^{31} cm^{-1} M^{-1}, the total thiol content was calculated.

2.7.7 Protein

The protein concentration was quantified in samples as described previously (Al-Suede, 2021).

2.8 Histopathological studies

The livers of the control and experimental groups were fixed in 10% formalin, embedded in paraffin wax, and sliced into 5µm thick longitudinal sections. For histopathological examination, the sections were stained with haematoxylin and eosin dye (10X and 40X).

2.9 Statistical analysis

All the data are expressed in mean ± SEM. The significance of differences in means between control and treated animals for different parameters was determined by One-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test.

3. RESULTS

3.1 *In-vivo* Hepatoprotective against Isoniazid, Rifampicin, and Pyrazinamide induced hepatotoxicity in rats.

The *In-vivo* hepatoprotective activity of Hydro-alcohol extract of *Mimusops elengi*rootwith the dose of 200mg/kg and 400mg/kg against Isoniazid, Rifampicin and Pyrazinamide induced hepatic damage.

3.2 Serum biochemical levels of isoniazid, rifampicin, and pyrazinamide induced hepatotoxicity.

3.2.1 Effect of Hydro-alcohol extract of *Mimusops elengi* root on Serum GOT:

In the isoniazid, rifampicin, and pyrazinamide treatment group, SGOT levels were significantly higher ($p<0.05$) than in the control group. The groups given a Hydro-alcohol extract of *Mimusops elengi* root showed a substantial reduction in SGOT level at doses of 200mg/kg and 400mg/kg ($p<0.001$). Similarly, when compared to the control group, Standard Silymarin-treated rats showed significant ($p<0.05$) protection against isoniazid, rifampicin, and Pyrazinamide-induced hepatotoxicity (Figure 1).

3.2.2 Effect of Hydro-alcohol extract of *Mimusops elengi* root on Serum GPT:

In the isoniazid, rifampicin, and pyrazinamide treatment group, SGPT levels were significantly higher ($p<0.01$) than in the control group. At a dose of 200mg/kg and 400mg/kg, the groups treated with *Mimusops elengi* root hydro-alcohol extract demonstrated a significant ($p<0.001$) reduction in SGPT level. When compared to the control group, standard Silymarin-treated rats had significantly lower levels of SGPT against isoniazid, rifampicin, and pyrazinamide-induced hepatotoxicity ($p<0.01$) (Figure1).

3.2.3 Effect of Hydro-alcohol extract of *Mimusops elengi* root on Serum ALP:

When compared to the normal group, the isoniazid, rifampicin, and pyrazinamide treated group had a substantial ($p<0.001$) elevated level of serum ALP. The blood ALP level was reduced significantly in the groups treated with *Mimusops elengi* hydro-alcohol extract ($p<0.05$) at doses of 200mg/kg and 400mg/kg. Similarly, when compared to the control group, standard Silymarin-treated animals showed a significant ($p<0.05$) lower level of serum ALP against isoniazid, rifampicin, and pyrazinamide-induced hepatotoxicity, as well as a decreased level of Serum ALP activity (Figure 2).

3.2.4 Effect of Hydro-alcohol extract of *Mimusops elengi* root on Total Bilirubin:

When comparing the isoniazid, rifampicin, and pyrazinamide in the control group, there was a significant ($p<0.001$) rise in Total Bilirubin. The total bilirubin level was significantly reduced in the groups treated with *Mimusops elengi* root hydro-alcohol extract at doses of 200mg/kg and 400mg/kg ($p<0.001$). When compared to the control group, standard Silymarin-treated rats had a significantly lower level of total bilirubin against isoniazid, rifampicin, and pyrazinamide-induced hepatotoxicity ($p<0.001$) (Figure 2).

3.2.5 Effect of Hydro-alcohol extract of *Mimusops elengi* root on LDL-Cholesterol:

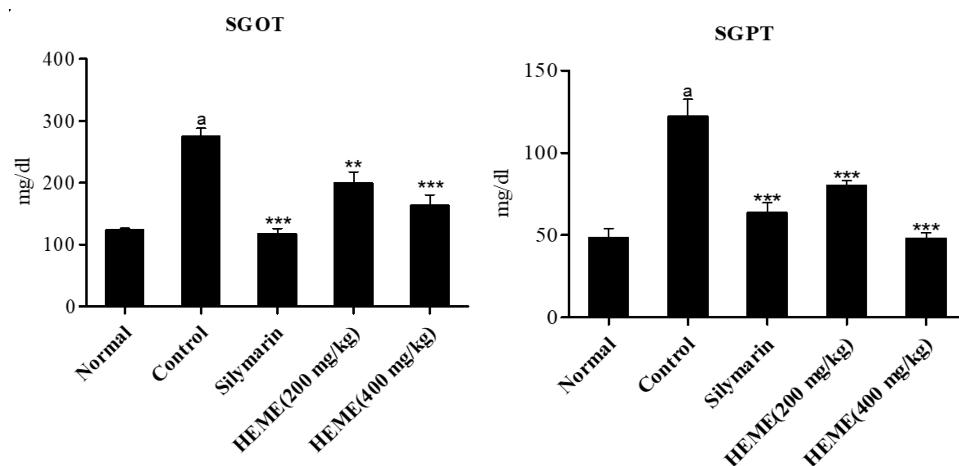

Figure 1: Effect of Hydro-alcohol ext *Mimusops elengi* root on SGOT and levels. All values were presented as a M SEM, One Way Analysis of Variance (AN followed by multiple comparisonsof T test. [a]p<0.001 as compared with the group and ***p<0.001, **p<0.01, and *p< compared with the control group. H Hydro-alcohol extract of *Mimusops eleng*

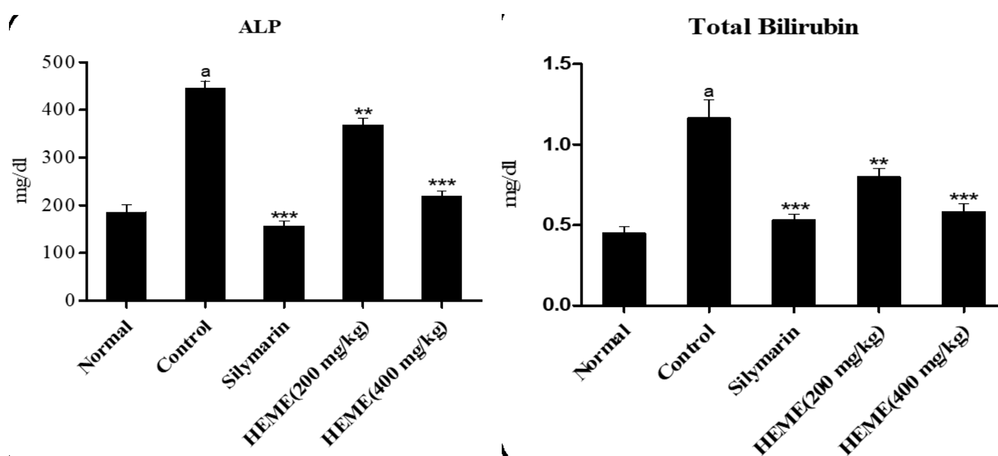

Figure 2: Effect of Hydro-alcohol extract of *Mimusops elengi* root on ALP and Total bilirubin level. All values were presented as a Mean ± SEM, One Way Analysis of Variance (ANOVA) followed by multiple comparisonsof Tukey's test. [a]p<0.001 as compared with the normal group and ***p<0.001, **p<0.01, and *p<0.05 as compared with the control group. HEME= Hydro-alcohol extract of *Mimusops elengi* root.

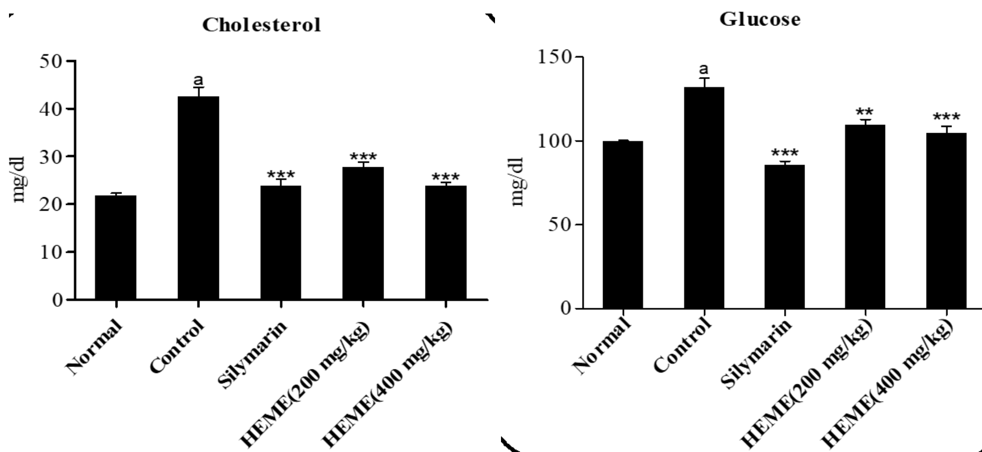

Figure 3: Effect of Hydro-alcohol extract of *Mimusops elengi* root on Cholesterol and Glucose levels. All values were presented as a Mean ± SEM, One Way Analysis of Variance (ANOVA) followed by multiple comparisonsof Tukey's test. [a]p<0.001 as compared with the normal group and ***p<0.001, **p<0.01, and *p<0.05 as compared with the control group. HEME= Hydro-alcohol extract of *Mimusops elengi* root.

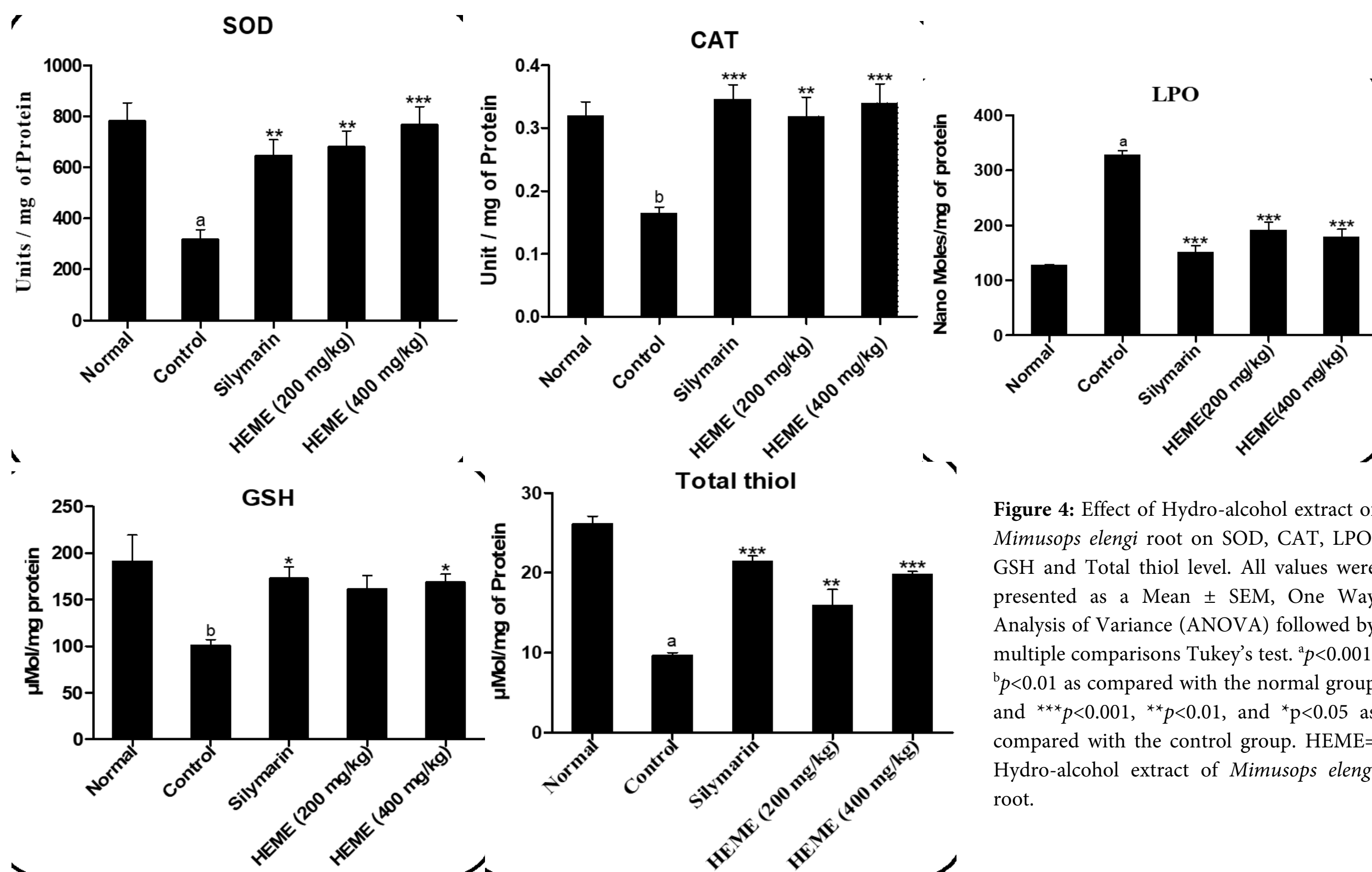

Figure 4: Effect of Hydro-alcohol extract of *Mimusops elengi* root on SOD, CAT, LPO, GSH and Total thiol level. All values were presented as a Mean ± SEM, One Way Analysis of Variance (ANOVA) followed by multiple comparisons Tukey's test. $^{a}p<0.001$, $^{b}p<0.01$ as compared with the normal group and $^{***}p<0.001$, $^{**}p<0.01$, and $^{*}p<0.05$ as compared with the control group. HEME= Hydro-alcohol extract of *Mimusops elengi* root.

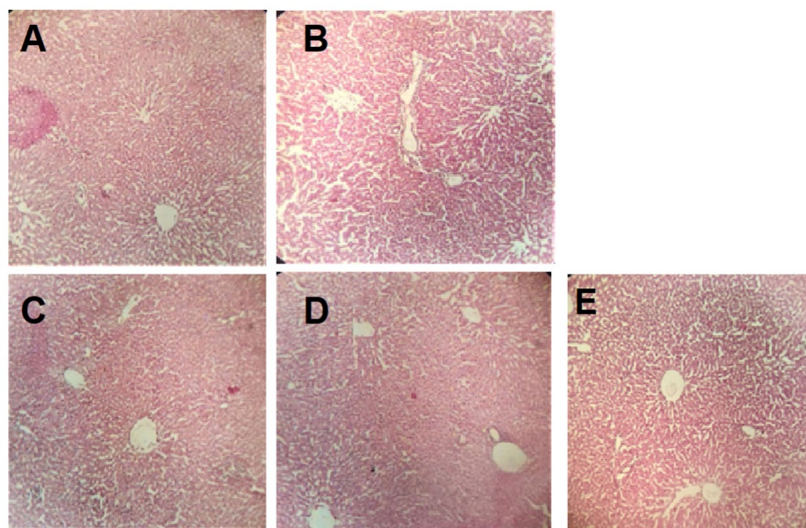

Figure 5: Effect of different doses of Hydro-alcohol extract of *Mimusops elengi* root on Isoniazid, Rifampicin, and Pyrazinamide-induced liver damage in Wister rats. **10 X** magnified Photographs of liver from different treatment groups stained with Haemotoxoline and Eosin. Plates; **A**: Normal group, **B**: Control group, **C**: Standard silymarin treated group, D: HEME 200mg/kg treated group, **E**: HEME 400mg/kg treated group In plate **A** shows portal vein constriction and plate **B** shows portal vein dilation and also there was completely alerted hepatic cells architecture, centrilobular necrosis, hepatic steatosis, and macrovascular fatty changes noticed. But Plate **C**, **D,** and **E** showed almost normal architecture and an absence of centrilobular necrosis, and hepatic steatosis.

When comparing the isoniazid, rifampicin, and pyrazinamide in the control group, there was a significant (p<0.001) rise in LDL-Cholesterol. At doses of 200mg/kg and 400mg/kg, the groups treated with *Mimusops elengi* root hydro-alcohol extract proveda significant (p<0.001) reduction in serum LDL-Cholesterol level. When compared to the control group, Standard Silymarin-treated rats demonstrated a substantial (p<0.001) drop in LDL-Cholesterol level in response to isoniazid, rifampicin, and pyrazinamide-induced hepatotoxicity (figure 3).

3.2.5 Effect of Hydro-alcohol extract of *Mimusops elengi* root on Glucose:

A significant (*p*<0.001) increased level of glucose was observed in the isoniazid, rifampicin and pyrazinamide treatedgroup as compared to the normal group. The groups treated with Hydro-alcohol extract of *Mimusops elengi* root showed a significant reduction in serum glucose level at doses of 200mg/kg and 400mg/kg (*p*<0.001) Similarly, Standard Silymarin treated animals showed a significant (*p*<0.001) decrease level of glucose against isoniazid, rifampicin, and pyrazinamide -induced hepatotoxicity as compared to control group (Figure 3).

3.3 Biochemical estimation

Table 2 and Graphs 4, 5, and 6 describe the enzymatic and non-enzymatic estimations, which demonstrated possible hepatoprotective activity of *Mimusops elengi* root hydro-alcohol extract. When compared to the normal group, the liver homogenate of control group rats exhibited significantly lower enzymatic activities of SOD (p<0.001), CAT (p<0.001), GSH (p<0.001), the non-enzymatic activity of total thiols (p<0.001), and enhanced lipid peroxidation (p<0.05 and p<0.001). When compared the HEME root 200mg/kg and 400 mg/kg administered groups, exhibited significant protection by lowering LPO (p<0.001) and boosting SOD (p<0.05 and p<0.001), CAT (p<0.001), GSH (p<0.01 and p<0.001), and total thiols (p<0.001) levels.

3.4 Histopathological study:

Normal group animals exhibited normal hepatic architecture, no centrilobular necrosis or macrovesicular fatty alterations, no dilatation of the portal vein, and no inflammation or infiltration of tissue on histological examination. The animals in the control group had a lot of centrilobular necrosis, vacuolization, macrovesicular fatty alterations, and a deformed central vein architecture with a lot of intracellular space. The hepatic architecture of the Silymarin-treated animals was almost identical to that of normal animals. However, the different doses of *Mimusops elengi* root hydro-alcohol extract treated group rats showed considerable liver protection against Isoniazid, Rifampicin, and Pyrazinamide causes liver injury, as evidenced by normal hepatic cords, the absence of necrosis and fatty infiltration, and the absence of portal vein dilatation (Figures 5).

4. Discussion

Hepatic cells participate in a wide range of metabolic processes. After a liver injury, the transport function of hepatocytes is particularly disturbed due to the degradation of the plasma membrane. The liver's cell membranes lose their functional integrity as a result of this. Most hepatotoxic substances injure the liver by generating lipid peroxidation either directly or indirectly in higher animals. Peroxyl radicals cause lipid peroxidation, damaging cell membrane integrity and causing liver injury, atherosclerosis, and kidney damage (Tandon, Khajuria, Kapoor, Kour, & Gupta, 2008). Hepatotoxicity caused by anti-tubercular chemotherapy regimens comprising isoniazid, rifampicin, and pyrazinamide is a potentially severe side effect. All of these medications have the potential to be hepatotoxic on their own, but when taken together, their toxicity is amplified synergistically (Tostmann et al., 2008).

INH is processed by the hepatic enzyme N-acetyl transferase, which causes acetylation. INH is acetylated to produce acetyl isoniazid, which is then hydrolyzed to produce acetyl hydrazine and isonicotinic acid. INH is pushed into a secondary metabolic route that produces hydrazine via slow acetylators. Hydrazine and acetyl hydrazine are both hazardous by-products that produce free radicals(C. Singh, Jodave, Bhatt, Gill, & Suresh, 2014).

Rifampicin increases the synthesis of hazardous metabolites from acetyl hydrazine via inducing the cytochrome p450 enzyme (AcHz). Rifampicin can also cause INH to be converted to isonicotinic acid and hydrazine, which are both hepatotoxic. Rifampicin shortens the plasma half-life of AcHz (an INH metabolite), and AcHz is rapidly converted to its active metabolites by raising the oxidative elimination rate of AcHz, which is linked to the increased incidence of liver necrosis induced by INH and rifampicin in combination. In addition, pyrazinamide in conjunction with INH and rifampicin has been linked to an increased risk of hepatotoxicity. In addition to these pathways, oxidative stress-induced liver damage is an essential mechanism in anti-tubercular drug-induced hepatotoxicity.[38]This results in reduced levels of glutathione, catalase, and superoxide dismutase. Glutathione represents the non-enzymatic scavengers while glutathione-S transferase, catalase, and superoxide dismutase, the enzymatic systems are involved in the detoxification of free radicals (Malviya, Jain, Jain, Jain, & Gurjar, 2010).

The results of the present study suggested that different doses of Hydro-alcohol extract of *Mimusops elengi* root treated groups increased the antioxidant enzymes such as GSH, SOD, CAT, and Total thiols and decreased the LPO and decreased the AST, ALT, Total Bilirubin, Alkaline Phosphatase, Total Cholesterol, and Glucose levels in INH, rifampicin, and pyrazinamide induced hepatotoxicity. The effect was comparable to the standard drug

Silymarin a proven hepatoprotective agent. The *Mimusops elengi* containsphenolic components, such as phenolic acids, flavonoids, Gallic acid, tannins, and phenolic diterpenes which have redox properties, and can play an important role in fascinating and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides for the measurements of the reductive ability.

Histopathological examinations further proved the overall hepatoprotective effect of the hydro-alcohol extract of *Mimusops elengi*. On phytochemical screening, HEME indicated the occurrence of flavonoids, steroidal alkaloids, gallic acid, tannin, triterpenes, and glycosides are the major chemical constituents. These phytochemical moieties are acknowledged for their inherent antioxidant property; hence, the hydro-alcohol extract of *Mimusops elengi* may reduce the induced oxidative stress by INH + R+ PZA in addition to the other properties such as anti-inflammatory and thereby preventing liver damage. Hence, the mechanism of hepatoprotection of *Mimusops elengi*might be since of its inherent antioxidant property present in these phytochemicals by reducing the induced oxidative stress that occurred due to the administration of antitubercular drugs in addition to others similar to anti-inflammatory and curative properties which possibly will prevent hepatic damage. Additional investigations are required for the identification of specific active constituents accountable for the hepatoprotection.

5. Conclusion

In conclusion, the antitubercular drugs isoniazid, rifampicin, and pyrazinamide caused changes in protein metabolism and the hepatic antioxidant defense system, which were normalized by the Hydro-alcohol extract of *Mimusops elengi* root treated groups showing a possible cytoprotective role of *Mimusops elengi* against drug-induced hepatotoxicity. Thus, *Mimusops elengi* can be categorized as a hepatoprotective plant.

Author Contributions

PD, CV, MS and AG have designed the experiments. PD, CV, MS , FSRA and AG have conducted the experiments and analysis the data. AG, FSRA, PD and CV have drafted the manuscript. All the authors have read and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest

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