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RESEARCH ARTICLE ON CARRY OUT PHARMACOLOGICAL EVALUATION OF AZADIRACHTA INDICA AGAINST ISONIAZID RIFAMPICIN INDUCED HEPATOTOXICITY IN LABORATORY RAT

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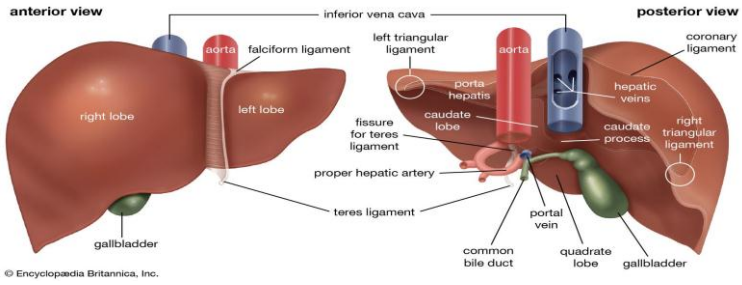
ABSTRACT
Two essential medications for the treatment of tuberculosis, isoniazid (INH) and rifampicin (RIF), are linked to hepatotoxicity, which presents serious clinical difficulties. In this work, laboratory rats' hepatoprotective responses to INH-RIF-induced hepatotoxicity are assessed using Azadirachta indica, or neem. INH-RIF was administered to cause liver damage as part of the experiment, and Azadirachta indica extract was then used as a therapy. In addition to histological analysis of liver tissues, biochemical indicators of liver function such as serum ALT, AST, ALP, and total bilirubin were measured. The findings showed that by restoring histological architecture and normalizing biochemical indicators, Azadirachta indica considerably reduces liver damage. The results imply that Azadirachta indica has hepatoprotective qualities, which may be facilitated by its anti-inflammatory and antioxidant effects. This study highlights the therapeutic potential of *Azadirachta indica* as a complementary strategy in managing drug-induced hepatotoxicity.

KEYWORDS: Azadirachta indica, hepatotoxicity, Isoniazid, Rifampicin, liver protection, antioxidants, anti-inflammatory, tuberculosis therapy, laboratory rats.

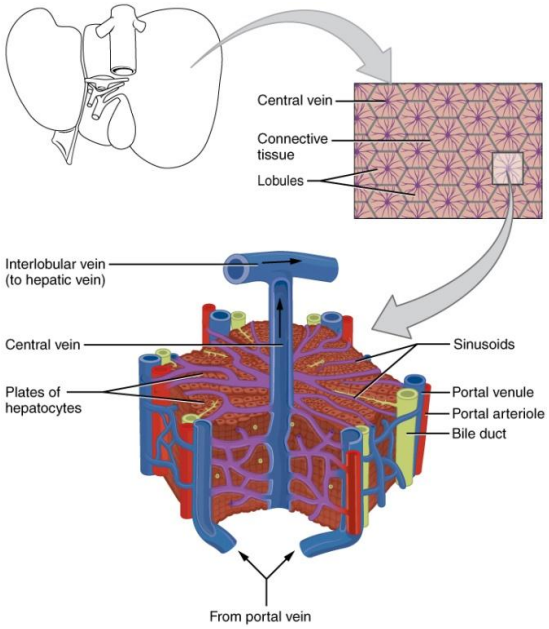
INTRODUCTION
The liver plays a vital role in maintaining homeostasis, regulating metabolism, detoxification, and nutrient storage (Sharma et al, 1991). Hepatotoxicity refers to liver injury caused by drugs or xenobiotics (Navarro & Senior, 2006). Since 1998, drug-induced hepatotoxicity has been identified as an endemic problem (Njoku, 2014). Specific drugs associated with hepatotoxicity were identified by Schiodt (1999), Temple & Himmel (2002), and others. Certain medicinal agents, chemicals, and herbal remedies can cause liver damage, even within therapeutic ranges (Pandit et al, 2012). Over 900 drugs are linked to liver injury, often resulting in abnormal liver enzyme tests.

Liver physiology
The liver, weighing approximately 1500 g, is the largest organ located in the upper right abdomen. It performs over 500 metabolic functions, including synthesis of glucose, plasma proteins, and bile, while storing glycogen, fats, and fat-soluble vitamins. Blood the liver through the portal vein (from the gastrointestinal tract, spleen, pancreas, and gallbladder) and the hepatic artery (from the aorta), with both merging in the liver's capillary bed. The liver then drains blood via the central veins to the inferior caval vein.



Anatomical view of liver

The portal vein, hepatic artery, lymphatics, nerves, and hepatic bile duct all enter the liver at the hilus. They branch within the liver through the portal canal, where the portal vein drains into the sinusoids, the liver's capillary system. In the sinusoids, blood from the portal vein mixes with blood from the hepatic artery. After passing through the sinusoids, blood collects in the central vein and exits the liver via the hepatic vein. The liver's hexagonal lobule structure typically has three portal canals at its corners, all draining into a central vein.



Blood vessel network in liver and liver lobules

The liver lobule consists of hepatocytes arranged in plates surrounding the sinusoids. The acinus, a more functional unit, is centered around the portal canal with central veins at its corners. It is divided into three zones: periportal (around the portal canal), central (near the central vein), and midzonal (between periportal and central zones).

Liver diseases can be classified into several types:

Viral Hepatitis: Caused by hepatitis A, B, C, D, and E viruses, leading to liver inflammation. Hepatitis A and E cause acute infections, while B, C, and D can cause both acute and chronic infections, potentially progressing to cirrhosis or liver cancer.

Cirrhosis: A chronic condition where liver tissue is replaced by scar tissue, leading to portal hypertension and end-stage liver disease. Liver transplantation is the only curative option.

Hepatocellular Carcinoma (HCC): A primary liver malignancy often resulting from chronic liver disease or cirrhosis, particularly in regions with high hepatitis B and C prevalence.

NAFLD: A fatty liver disease linked to metabolic syndrome, insulin resistance, and abnormal lipid metabolism.

ALD: Caused by excessive alcohol consumption, progressing from steatosis to cirrhosis and HCC.

Jaundice: A yellowing of the skin and eyes caused by elevated bilirubin levels in the blood.

Epidemiology of hepatotoxicity

The true incidence of drug-related liver disease is unclear because many cases go unreported. A study in France showed the vulnerability of hepatotoxicity, with an annual incidence of hepatic reactions to drugs being 139 cases per 1 million people, 16 times higher than reported cases. A lack of accurate diagnosis is a significant limitation, with about 50% of reactions reported to regulatory authorities found unrelated to the drug (Aithal et al., 1999). The incidence of drug-induced hepatotoxicity has been increasing due to greater drug exposure, better knowledge of liver toxicity, and improved diagnostic tests for viral hepatitis (Dossing & Andreasen, 1982; Friis & Andreasen, 1992). Hepatotoxicity may result from direct toxicity of the drug or its metabolite, or from an immune-mediated response affecting hepatocytes or liver vasculature (Saukkonen et al., 2006; Deng et al., 2009; Singh et al., 2011).

Causes of drug-induced hepatotoxicity fall into two categories:

1. Direct hepatic injury: The drug or its metabolite directly causes toxicity, like acetaminophen, which induces dose-related hepatotoxicity through GSH depletion and oxidative stress, leading to liver cell death (Reid et al., 2005; Lee, 2003).

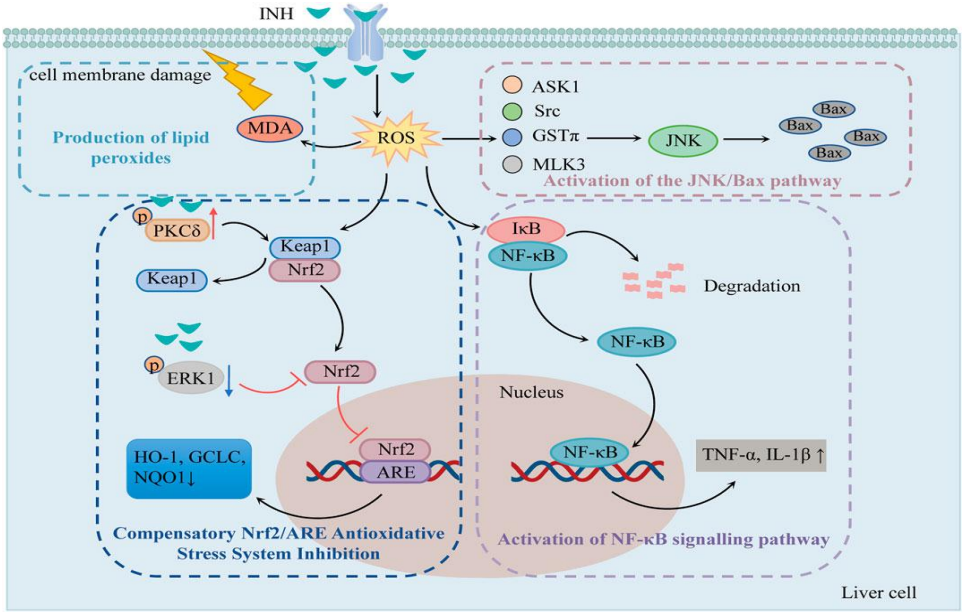
2. Idiosyncratic reactions: These vary and can be host-dependent (genetic factors or metabolic causes) or host-independent (immunologic causes, which may also have genetic or metabolic underpinnings) (Njoku et al., 2014).

Various drugs, such as bromfenac, cyclophosphamide, methotrexate, and others, can induce hepatocyte necrosis. Carbon tetrachloride (CCl4), a widely used hepatotoxin in laboratories, causes liver damage through the formation of reactive metabolites like the trichloromethyl radical (CCl3), which initiates lipid peroxidation and impairs cellular functions, leading to fatty degeneration, steatohepatitis, or even hepatic cancer (Lee, 2003). CCl4-induced toxicity involves complex molecular processes, including the activation of TNF-α and transforming growth factors, which push the liver towards apoptosis or fibrosis, while IL-6 and IL-10 may aid recovery by counteracting these effects (Weber et al., 2003).

Genetic involvement in drug-induced hepatotoxicity is associated with Phase I and Phase II drug metabolism enzymes (Njoku et al., 2014). Immunologically, drug hepatotoxicity is often categorized as immuno-allergic or autoimmune, with immune cells like neutrophils, Kupffer cells, and macrophages playing key roles. Neutrophils, in particular, are early responders to hepatocyte damage in acetaminophen hepatotoxicity, though their role remains debated (Jaeschke, 2005; Ramaiah & Jaeschke, 2007). Kupffer cells may also offer direct protection during hepatotoxic events (Ju et al., 2002).

Isoniazid and Rifampicin-induced hepatotoxicity

Isoniazid (INH) and rifampicin (RFP) are first-line antitubercular drugs, with their use linked to liver injury, contributing to 5%–22% of acute liver failure cases (Devarbhavi et al., 2013). Natural medicinal ingredients offer multi-target regulation and show promise in reducing liver injury risk, enhancing repair, and improving symptoms (Hong et al., 2015). The molecular mechanisms of INH-induced liver injury (INH-ILI) involve oxidative stress, mitochondrial dysfunction, drug metabolism enzymes, protoporphyrin IX accumulation, endoplasmic reticulum stress, bile transport imbalance, and immune response. Oxidative stress from INH is linked to dysregulated Nrf2/ARE pathways and ROS accumulation.



The mechanisms of oxidative stress injury caused by INH (Compensatory Nrf2/ARE antioxidative stress system inhibition, production of lipid peroxides, activation of NF-κB signaling pathway and the JNK/Bax pathway play important roles in INH-induced oxidative stress injury)(Zhuang X, et al. 2022)

Notes: INH, isoniazid; ROS, reactive oxygen species; MDA, malonic dialdehyde; Nrf2, nuclear erythroid 2-related factor 2; Keap1, kelch-like ECH-associated protein 1; PKCδ, protein kinase Cδ; ERK1, extracellular signal-regulated protein kinase 1; ARE, antioxidant response element; HO-1, heme oxygenase 1; GCLC, glutamine-l-cysteine ligase; NQO1, quinone oxidoreductase 1; NF-κB, nuclear factor-κB; IκB, NF-κB inhibitory protein; JNK, c-jun N-terminal kinase; ASK1, apoptosis signal-regulating kinase 1; Src, Src kinase; GSTπ, glutathione S-transferase π; MLK3, mixed-lineage protein kinase 3.

The Nrf2/ARE pathway regulates antioxidant defenses. During oxidative stress, Nrf2 detaches from Keap1, translocates to the nucleus, and activates antioxidant enzymes like HO-1 and NQO1 (Iranshahy et al., 2018). INH disrupts Nrf2 activation through PKCδ phosphorylation, ERK1 inhibition, and KPNB1 reduction (Verma et al., 2015, 2018). Excessive ROS, generated

via CYP2E1, causes oxidative damage, amplifies through MDA, and induces apoptosis and liver necrosis (Guo et al., 2015a). ROS also triggers NF-κB and JNK activation, leading to inflammatory responses and apoptosis (Shi, 2015; Chen et al., 2018).

Review of literature

Test Drug

1) Name of test drug & Diagram

Test Drug Name

Neem- Azadirachta indica

Neem

Taxonomic Details

- Domain: Eukaryotae
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order : Rutales
- Suborder: Rutinae
- Family: Meliaceae(Alzohairy *et al*. 2016, Ahmad *et al* 2019)

Recommended scientific name

Azadirachta indica

Preferred Common Name-The neem tree

Parts of Used

Several elements of the Neem tree, including as the leaves, bark, fruit, flowers, oil, and gum, are said to be used in the previously stated medical folklore to treat specific ailments. Over 80% of people worldwide trust phytomedicines, according to the World Health Organisation (WHO, 2002). Teeth paste, cosmetics, soaps, and insect repellents have all employed seed oil.

The tree's parts are used to treat a variety of conditions, including rheumatism, constipation, respiratory issues, gastrointestinal (GIT) illnesses, leprosy, fever, headaches, and chicken pox.(Saleem S *et al*.2018)

Chemical Constituents

From various parts of the Neem tree, about 135 distinct structural compounds have been isolated and identified.

Two categories have been established for these compounds:

a. Isoprenoids That include C-secomeliacins such as azadirachtin, nimbin, and salanin, as well as limonoids, protomeliacins, gedumin, azadirone, and vilasinin.(Tiwari *et al*. Sarkar *et al* 2007)

b. Non-isoprenoids

That contains aliphatic chemicals, amino acids, polysaccharides, polyphenolics such as flavonoids, sulphurous compounds, glycosides, dihydrochalcone, tannins, coumarin, and polyphenols.(Bhat *et al* 2008, Atawodi *et al*., 2009)

Medicinal uses

BIOLOGICAL ACTIVITY

The **hepatoprotective role** of neem (Kapgate *et al* 2017) studied hepatoprotective of neem. Wistar rats were treated with ATT drugs in combination in various doses up to 4-8 weeks. Total nine experiments were conducted to achieve successful hepatotoxicity. The aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were the biochemical parameters of assessment. The successful attempt to induce hepatotoxicity can be achieved with the doses of INH - 100, RMP - 300, PZA - 700 mg/kg. The findings were confirmed by the raised ALT, AST, and ALP levels compared with baseline.

Antioxidant and hepatoprotective activity of fresh Juice of young (tender) stem bark of s*Azadirachta indica* A. (Gomase *et al* 2011) were evaluated against carbon tetrachloride (CCl4)-induced hepatic damage in albino rats. The hepatoprotective activity of *A. indica* was evaluated by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) The serum levels of total proteins and bilirubin were also estimated. Silymarin was used as standard drug. Administration of fresh juice *A. indica* young stem bark extract of *A. indica* was good hepatotoxic agent at a dose level of 500mg/kg. the plant extract has decreased the enzyme level of SGOT, SGPT, ALP, Bilirubin by the dose of 500mg/kg and these result are statisticallysignificant $P < 0.01$ when compared with CCl4 group, while juice extract increases the proteins serum level too.

Hepatoprotective effect

Kale *et al.* 2003 evaluated the Hepatoprotective effect of aqueous leaf extract from Azadirachta indica (AI) on hepatotoxicity induced by antitubercular medicines in albino rats.

Rats were subjected to a 30-day oral suspension of isoniazid, rifampicin, and pyrazinamide in order to induce hepatotoxicity.

Anti-Bacterial Activity

Ghonmode *et al.* 2013 evaluated the antibacterial activity of the bark, leaf, seed and fruit extracts of Azadirachta indica (neem) on bacteria which was isolated from adult mouth and results showed that bark and leaf extracts of Neem showed antibacterial activity against test bacteria

used. Also, seed and fruit extracts exhibited antibacterial activity only at higher concentrations. Results revealed that Neem bark extract (NBE) significantly blocked HSV-1 entry into cells at concentrations ranging from 50 to100 μg/mL.

Anti-Malarial Activity

Osanaiya, *et al.,* conducted an experiment in which the anti-malarial activity of extracts using Plasmodium berghei infected albino mice was used and reports led that Neem leaf and stem bark extracts reduced the level of parasitaemia in infected mice by about 51–80% and 56–87%, respectively.

Hepatoprotective Activity

Althaiban *et al.* 2019 done the study to determine whether neem leaf extract (NMLE) could prevent liver damage brought on by RIF. Group I consisted of forty male rats that were not given any treatment; Group II received RIF at a dose of 54 mg/kg/day for thirty days; Group III and IV were intoxicated rats that were given NMLE orally at a dose of 250 and 500 mg/kg/day, respectively, for thirty days. On day 30, the liver was histopathologically inspected and blood was drawn for biochemical examination. The findings demonstrated that the NMLE at the two dose levels dramatically reduced serum levels of MDA and liver enzymes while concurrently increasing GSH and SOD activities and exhibiting ant-inflammatory effects as demonstrated by considerablythe administration of NMLE has been shown to have hepatoprotective effects in hepatotoxic rats via an antioxidant and ant-inflammatory pathway, as evidenced by decreased levels of TNF-α and IL-1α compared to RIF group II.

Standard Drug

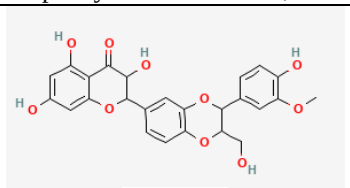
Silymarin

The complex and well-known polyphenolic antioxidant combination known as silymarin chemicals is derived from the seeds of the milk thistle plant (Silybum marianum). This plant is a member of the Asteraceae family, which has been investigated for its potential as a natural remedy for cancers, liver conditions, cardiovascular conditions, and neurological disorders(Taleb *et al*., 2018). Of the several biological effects that silymarin compounds may have, hepatoprotective activity is the primary focus. The primary biological activity associated with silymarin compounds is hepatoprotective activity, out of various possible biological activities (Asgarshirazi *et al*., 2017, Elyasi *et al.,* 2016 Elyasi *et al*., 2017, Marjani *et al*., 2016). Clinical trials substantiate the biological activity against β-thalassemia major by, for example, lowering serum iron and ferritin levels by 2–5, improving liver and heart health (Darvishi-Khezri *et al*. 2016), and improving liver functions (specifically, lowering liver enzymes and raising albumin and bilirubin levels) in individuals with hepatitis C virus(Fathalah *et.al.,* 2017). Furthermore, a number of research characterized the hepatoprotective (Saleem et al., 2018, Simon et al., 2018) and anticancer activities of natural extracts using silymarin compounds as a positive control. This circumstance is crucial for this family of compounds, enhancing its potential as a natural remedy to pharmaceuticals used to treat a variety of illnesses.

Mechanisms of action

The hepatoprotective properties of silymarin are achieved through a number of methods, including as antioxidation, prevention of lipid peroxidation, improved glucuronidation, protection against glutathione depletion, and accelerated liver detoxification by inhibition of Phase I detoxification. According to readings, silymarin has a variety of anti-inflammatory properties, including as blocking the production of prostaglandin and leukotriene, inhibiting Kupffer cells, stabilizing mast cells, and preventing neutrophil migration. Furthermore, it has been demonstrated that silymarin enhances the synthesis of hepatocyte proteins, thereby facilitating the regeneration of liver tissue. Additionally, silybin has been shown in animal tests to decrease the hepatic stellate cells' ability to differentiate into myofibroblasts, which can delay or even reverse fibrosis. Silymarin has been shown in clinical trials carried out in Hungary to have immunomodulatory effects on liver disease.

Table: Physicochemical Properties of Silymarin.

Iupac Name	5,5-diphenylimidazolidine-2,4-dione
Chemical Name Structure	 Silymarin
Molecular Formula	C25H22O10
Drug class	hepatoprotective
Melting point	167^0C
Form	Solid
Colour	white
Solubility	Insoluble in water
Uses	Protective effects on the liver and improve its function

Saller R, Melzer J et al., 2007, Morishima C, Shuhart MC et al., 2010

As previously stated, silymarin's hepatoprotective qualities include inhibiting the conversion of stellate hepatocytes into myofibroblasts, increasing membrane stability when exposed to xenobiotics, scavenging free radicals, raising the cellular content of glutathione, which inhibits lipid peroxidation, and reducing the deposition of collagen fibers. Furthermore, silymarin/silybinin stimulates RNA polymerase I, which in turn promotes ribosomal protein synthesis.

Silybinin effectively reduces high intra-hepatic messenger RNA (mRNA) levels of TNF-α, IFN-γ, IL-2, and IL-4.

Gillessen A, Schmidt HH et al., 2020
Numerous studies have looked into how well silymarin works to stop DILI caused by ATDs. Participants (N = 565) were randomized to receive ATDs with silibin capsules (70 mg, three times a day) or ATDs alone in a prospective, multicenter trial [90]. Following eight weeks of treatment, there were no statistically significant differences between the silibinin-treated patients and the control group in terms of the number of patients with liver injury (2.2% vs. 2.4%), DILI diagnosis (7.2% vs. 9.3%), or ATD treatment stopped due to liver injury and symptoms (3.25% vs. 6.19%).

Kren & Walterova, *et.al*, 2005
Antioxidant Activity Silymarin is a potent antioxidant, neutralizing free radicals and enhancing the body's antioxidant defense system. It increases the levels of endogenous antioxidants such as glutathione (GSH) and superoxide dismutase (SOD). By scavenging reactive oxygen species (ROS), silymarin prevents oxidative damage to hepatocytes, which is a common pathway in liver injury.

Saller, R., Meier, R., & Brignoli, R. et al., 2007
Liver Regeneration Silymarin has been shown to promote liver regeneration by enhancing protein synthesis and stimulating the production of new hepatocytes. This regenerative effect is particularly beneficial after liver injury or partial hepatectomy, where the liver needs to regenerate lost tissue.

AIM AND OBJECTIVES

The aim of present work was to carry out pharmacological investigation of *Azadirachta indica* against isoniazid-rifampin induced hepatotoxicity in laboratory rat.

To determine the effect of standardized extract of Azadirachta indica (100,200, and 400 mg/kg p.o) & Silymarin (100mg/kg p.o) on hepatotoxicity and isoniazid-rifampin induced hepatotoxicity in laboratory rat by following methods.

Pharmacological evaluation of *Azadirachta indica* against isoniazid-rifampin induced hepatotoxicity in laboratory rat.

A. Induction of hepatotoxicity in rat
B. Parameters to be evaluated

✓ ***In-vivo parameters***

- Body weight
- Liver Weight and Liver weight /Body Weight
- AST, ALT, ALP
- Albumin
- Total bilirubin and Direct bilirubin

✓ ***Ex-vivo parameters***

- Liver oxidative stress (SOD, GSH, MDA, Nitric oxide and Total Protein)
- Histopathology of hepatic tissue

MATERIALS

Animals

Sprague Dawley rats weighing 180-220 g were purchased from Global Bioresearch Solutions Private Limited, H No 251 Nhavi, Tal - Bhor, Dist- Pune, Pune. The animals were housed in polypropylene cages and maintained under environmental condition of temperature 25±1 ºC and relative humidity of 45-55 % under 12h light: 12 dark cycle. The animals had free access to food pellet (Nav Maharashtra Chakan oil mills Ltd., Pune) and water ad libitum. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Loknete shri dada patil Pharate College of pharmacy, Mandavganpharata constituted under the guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is 2168/PO/Re/S/22/CPCSEA

Chemicals

Stand. extract of *Azadirachta indica* (Natural Remedies Pvt. Ltd., Bangalore) and Silymarin tablets (Silybon®) (Micro Labs Ltd, Sikkim-737132, India) shall be purchased from respective vendors. All chemicals are analytical grade.

For test drug

Substances:
Azadirachta indica
Dose: 100, 200 and 400mg/kg
Sites: Oral
Volumes: Not more than 1-2ml
Blood withdrawal: Yes
Volume: 0.3 ml

For std drug

Substances: Silymarin
Dose: 100 mg/kg
Sites: Oral
Volumes: Not more than 1-2 ml
Blood withdrawal: Yes
Volume: 0.3 ml

Isoniazid-rifampicin-inducedhepatotoxicityin laboratory animals

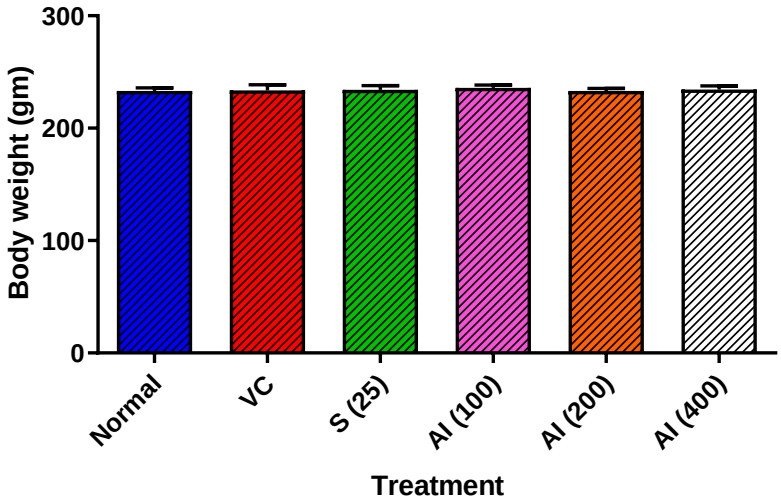
6.1.6.1. Experimental designs

The animals were divided randomly into groups with six rats per group as follows:

- Group I: Normal group
  The rats treated with vehicle (distilled water, 10 mg/kg, p.o.) for 21 days and received saline (250 mg/kg, p.o.) for 21 days.
- Group II: Vehicle control
  The rats were administered a vehicle (distilled water, 10 mg/kg, p.o.) 2h prior to oral administration of Isoniazid + Rifampicin (100 mg/kg, p.o.) for 21 days.
- Group III: Silymarin (25) treated group
  The rats treated with Silymarin(25 mg/kg, p.o.) 2h prior to oral administration of Isoniazid + Rifampicin (100 mg/kg, p.o.) for 21 days.
- Group IV: Standardized extract of *Azadirachta indica* (100)treated group
  The rats treated with Standardized extract of *Azadirachtaindica*at a dose of 100 mg/kg, p.o 2h prior to oral administration of Isoniazid + Rifampicin (100 mg/kg, p.o.) for 21 days.
- Group V: Standardized extract of *Azadirachta indica* (200)treated group
  The rats treated with Standardized extract of *Azadirachtaindica*at a dose of 200 mg/kg, p.o 2h prior to oral administration of Isoniazid + Rifampicin (100 mg/kg, p.o.) for 21 days.
- Group VI: Standardized extract of *Azadirachta indica* (400)treated group
  The rats treated with Standardized extract of *Azadirachtaindica*at a dose of 400 mg/kg, p.o 2h prior to oral administration of Isoniazid + Rifampicin (100 mg/kg, p.o.) for 21 days.

Effect of *Azadirachta indica* on INH+RIF-induced alteration in body weight

Body weight (gm) - Mean ± SEM					
Normal	**Vehicle Control**	**Silymarin (25 mg/kg)**	**AI (100 mg/kg)**	**AI (200 mg/kg)**	**AI (400 mg/kg)**
233.20 ± 1.08	233.70 ± 1.99	234.20 ± 1.49	235.80 ± 1.01	233.20 ± 0.95	234.30 ± 1.31

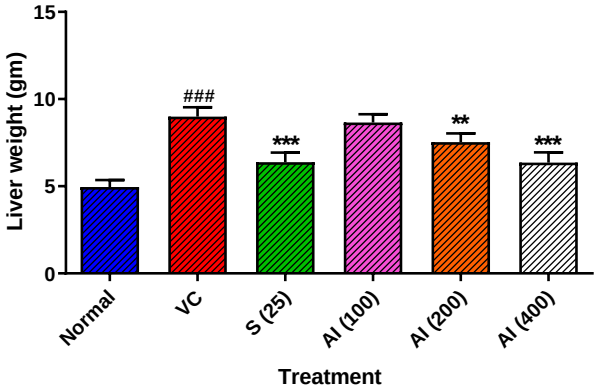


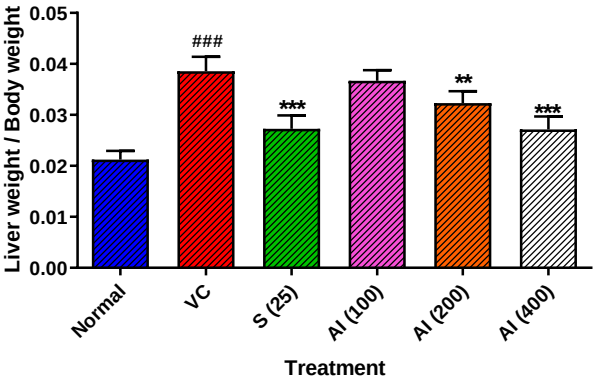
Effect of *Azadirachta indica* on INH+RIF-induced alteration in body weight

Data were analyzed by One-Way ANOVA followed by Dunnett's. When compared to normal group, administration of INH+RIF did not cause any significant changing body weight in vehicle control group. Treatment of silymarin (25 mg/kg) for 21 days and *Azadirachta indica* (100, 200 and 400 mg/kg) also did not show any significant change in the body weight.

Effect of *Azadirachta indica* on INH+RIF-induced alteration in absolute and relative liver weights

Time (in days)	Absolute liver weight (gm)and Relative liver weight - Mean ± SEM					
	Normal	**Vehicle Control**	**Silymarin (25 mg/kg)**	**AI (100 mg/kg)**	**AI (200 mg/kg)**	**AI (400 mg/kg)**
Liver weight (gm)	4.95 ± 0.16	9.00 ± 0.22$^{###}$	6.38 ± 0.23***	8.65 ± 0.19	7.53 ± 0.20**	6.36 ± 0.24***
Liver weight / Body weight	0.021 ± 0.001	0.039 ± 0.001$^{###}$	0.027 ± 0.001***	0.037 ± 0.001	0.032 ± 0.001**	0.027 ± 0.001***



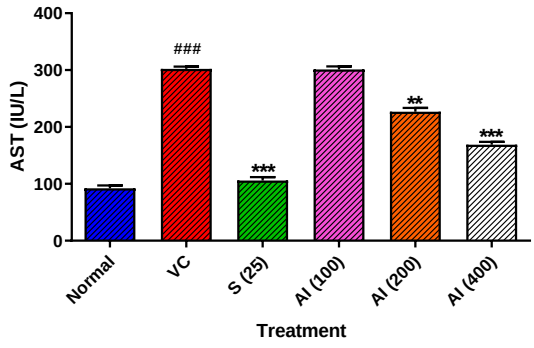


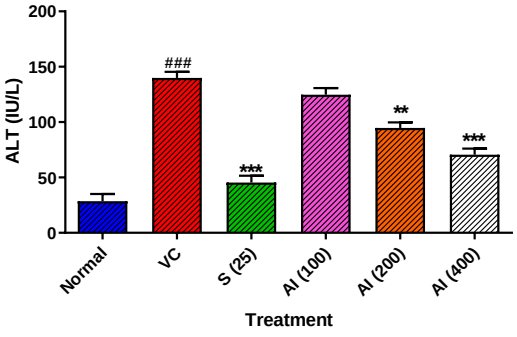
Effect of *Azadirachta indica*onINH+RIF-induced alteration in absolute and relative liver weights

Data were analyzed by One-Way ANOVA followed by Dunnett's. $^{###}P$ <0.001 as compared with normal group and $^{**}P$ <0.01, $^{***}P$ <0.001 as compared with Vehicle Control group on respective days. When compared to normal group, administration of INH+RIF cased a significant increase (P <0.001) in liver weight (absolute) and liver weight to body weight ratio (relative liver weight) in vehicle control group. On the other hand, treatment of silymarin (25 mg/kg) for 21 days resulted in the significant attenuation (P <0.001) of ratio of liver weight to body weight and liver weight as compared with vehicle control group. When compared with vehicle control rats, *Azadirachta indica* (200 and 400 mg/kg) treated rats also showed the significant and dose dependant decreased (P < 0.01 and P < 0.001) in the absolute and relative liver weights. Administration of *Azadirachta indica*(100 mg/kg) did not show any significant protection against INH+RIF-induced increased hepatic weights.

Effect of *Azadirachta indica* on INH+RIF-induced alteration in AST and ALT levels

Parameter	AST (IU/L) and ALT (IU/L)- Mean ± SEM					
	Normal	Vehicle Control	Silymarin (25 mg/kg)	AI (100 mg/kg)	AI (200 mg/kg)	AI (400 mg/kg)
AST (IU/L)	92.18 ± 2.00	302.00 ± 1.70$^{###}$	106.00 ± 2.37***	301.10 ± 2.17	226.70 ± 2.70**	168.90 ± 2.07***
ALT (IU/L)	28.57 ± 2.69	139.90 ± 2.23$^{###}$	45.35 ± 2.54***	124.80 ± 2.43	94.80 ± 2.04**	70.64 ± 2.22***





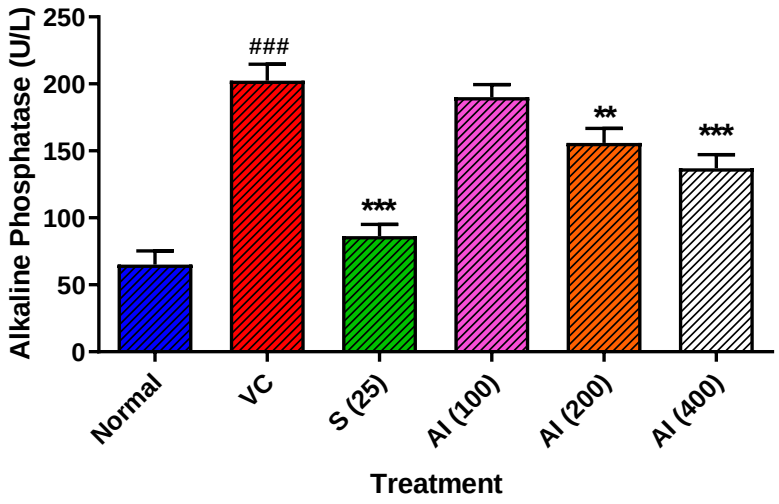
Effect of *Azadirachta indica* on INH+RIF-induced alteration in AST and ALT levels

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P$ <0.001 as compared with normal group and $^{**}P$ <0.01, $^{***}P$ <0.001 as compared with Vehicle Control group.

On 22nd day, the AST and ALT levels in vehicle control group showed a significant (P <0.001) increase administration when compared to normal group. On the other hand, treatment with silymarin (25 mg/kg) showed a significant (P <0.001) decreased in AST and ALT levels compared to vehicle control group. Treatment with *Azadirachta indica* (200 and 400 mg/kg)showed significant and dose dependant decrease (P < 0.01 and P < 0.001) in AST and ALT levels compared to vehicle control group. *Azadirachta indica*(100 mg/kg)did not show any significant decrease in AST and ALT levels compared to vehicle control group.

Effect of *Azadirachta indica*onINH+RIF-induced alteration in ALP level

Alkaline Phosphatase (U/L) - Mean ± SEM					
Normal	**Vehicle Control**	**Silymarin (25 mg/kg)**	**AI (100 mg/kg)**	**AI (200 mg/kg)**	**AI (400 mg/kg)**
65.15 ± 4.14	202.40 ± 4.98$^{###}$	86.35 ± 3.61***	190.20 ± 3.77	155.90 ± 4.41**	137.00 ± 4.14***



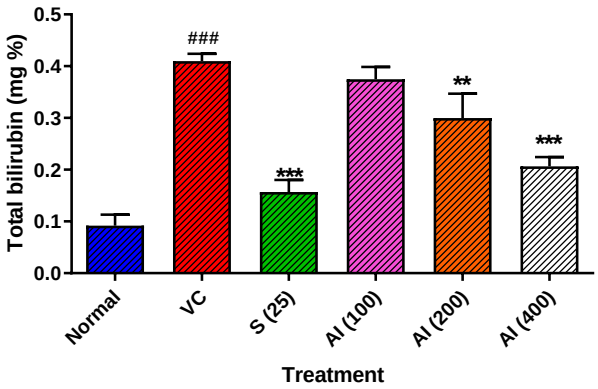
Effect of *Azadirachta indica* on INH+RIF-induced alteration in ALP level

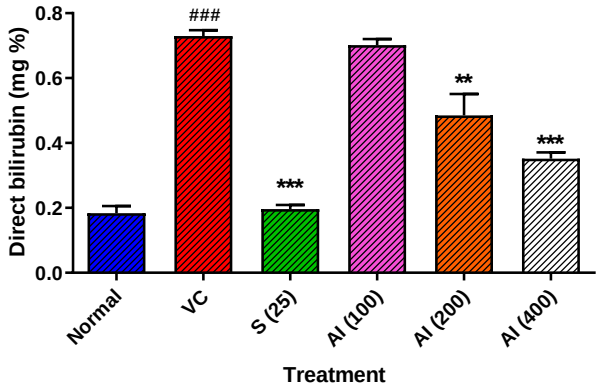
Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P$ <0.001 as compared with normal group and $^{**}P$ <0.01, $^{***}P$ <0.001 as compared with Vehicle Control group.

The significant increased (P <0.001) in ALP level was found after chronic administration of INH+RIF in vehicle control rats as compared to normal rats. This decreased level of ALP was significantly attenuated (P <0.001) by silymarin (25 mg/kg) as compared to vehicle control rats. Treatment with *Azadirachta indica* (200 and 400 mg/kg) also significantlyand dose dependently (P < 0.01 and P < 0.001) decreased the ALP level compared to vehicle control rats. Rats treated with *Azadirachta indica* (100 mg/kg) failed to produce significant decrease in the level of ALP as compared to vehicle control rats.

Effect of *Azadirachta indica*onINH+RIF-induced alteration in totaland direct bilirubin levels

Parameter	**Total bilirubin (mg %) and Direct bilirubin (mg %) - Mean ± SEM**					
	Normal	**Vehicle Control**	**Silymarin (25 mg/kg)**	**AI (100 mg/kg)**	**AI (200 mg/kg)**	**AI (400 mg/kg)**
Total bilirubin (mg %)	0.09 ± 0.01	0.41 ± 0.01$^{###}$	0.16 ± 0.01***	0.38 ± 0.01	0.30 ± 0.02**	0.21 ± 0.01***
Direct bilirubin (mg %)	0.18 ± 0.01	0.73 ± 0.01$^{###}$	0.20 ± 0.01**	0.70 ± 0.01	0.49 ± 0.03**	0.35 ± 0.01***



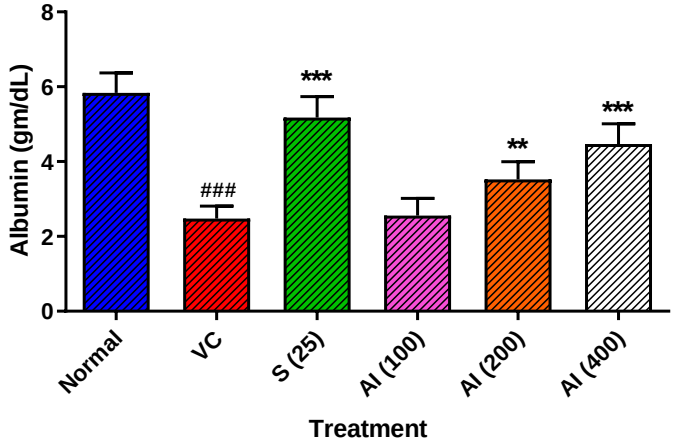


Effect of *Azadirachta indica*on INH+RIF-induced alteration in total and direct bilirubin levels

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P$ <0.001 as compared with normal group and $^{**}P$ <0.01, $^{***}P$ <0.001 as compared with Vehicle Control group.

Effect of *Azadirachta indica*onINH+RIF-induced alteration in albumin level

Albumin (gm/dL) - Mean ± SEM					
Normal	**Vehicle Control**	**Silymarin (25 mg/kg)**	**AI (100 mg/kg)**	**AI (200 mg/kg)**	**AI (400 mg/kg)**
5.84 ± 0.22	2.48 ± 0.14$^{###}$	5.18 ± 0.23***	2.56 ± 0.19	3.52 ± 0.20**	4.47 ± 0.22***

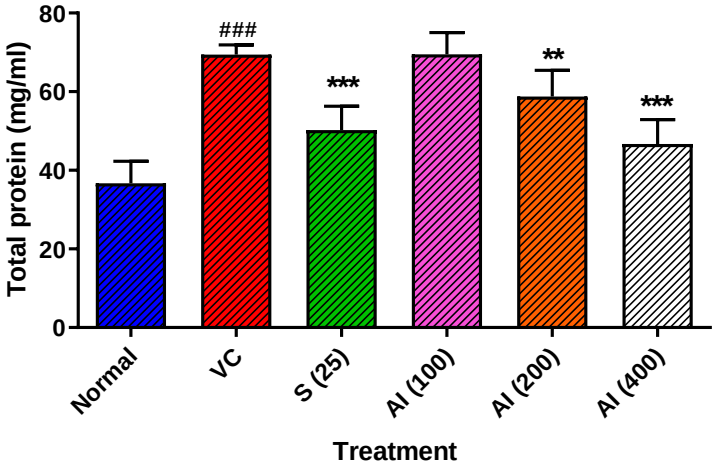


Effect of *Azadirachta indica* on INH+RIF-induced alteration in albumin level

Data were analyzed by One-way ANOVA followed by Dunnett'stest.$^{###}P$ <0.001 as compared with normal group and $^{**}P$ <0.01, $^{***}P$ <0.001 as compared with Vehicle Control group.

Effect of *Azadirachta indica*onINH+RIF-induced alteration in hepatic total protein level

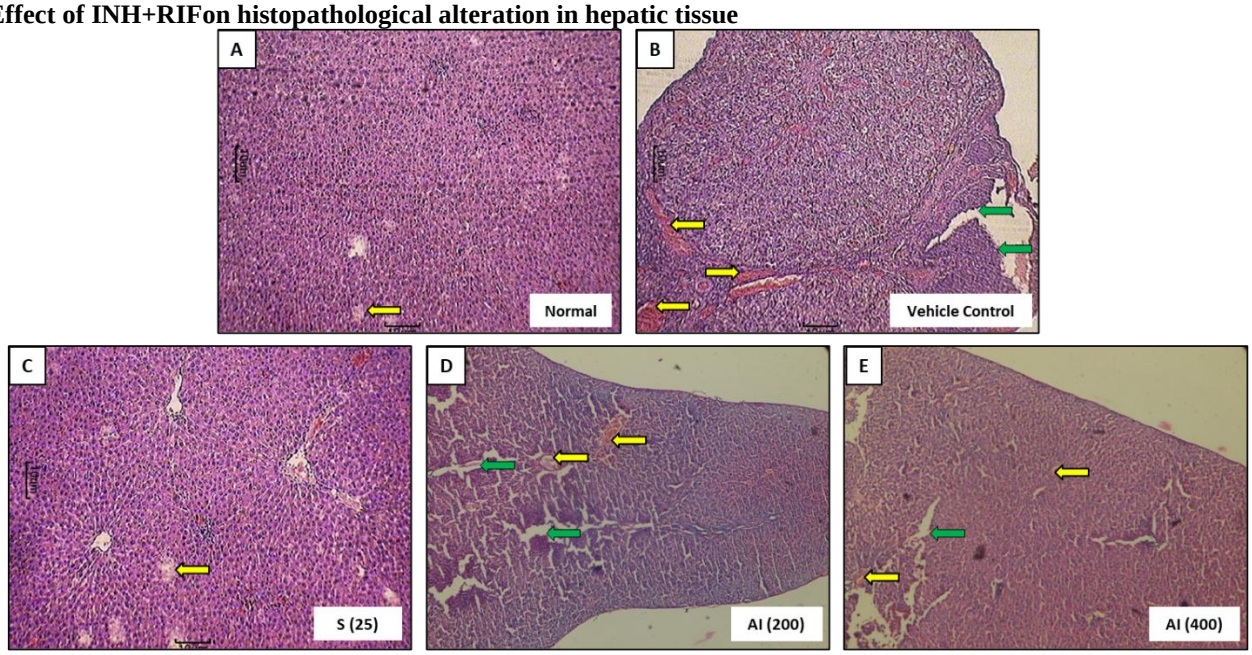
Hepatic total protein (mg/gm) - Mean ± SEM					
Normal	**Vehicle Control**	**Silymarin (25 mg/kg)**	**AI (100 mg/kg)**	**AI (200 mg/kg)**	**AI (400 mg/kg)**
36.73 ± 2.27	69.42 ± 1.02$^{###}$	50.27 ± 2.46***	69.47 ± 2.28	58.74 ± 2.74**	46.71 ± 2.54***



Effect of *Azadirachta indica* on INH+RIF-induced alteration in hepatic total protein level

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P$ <0.001 as compared with normal group and $^{**}P$ <0.01, $^{***}P$ <0.001 as compared with Vehicle Control group.

Effect of INH+RIFon histopathological alteration in hepatic tissue



Histopathological representation of hepatictissuefrom normal rats (A), Vehicle Control rats (B), Silymarin (25 mg/kg) treated rats (C), AI (200 mg/kg) treated rats (D) and AI (400 mg/kg)treated rats (E). Stained with H&E (at 100 X). Infiltration of neutrophils (yellow arrow) and necrotic changes congestion (green arrow) in hepatic tissue.

SUMMARY AND CONCLUSION

Animal studies on AI's hepatoprotective effects have been described. When comparing the serum of rats treated with isoniazid and rifampicin separately and in combination, there was a substantial increase in the levels of AST, ALT, ALP, GSH & SOD, bilirubin, total protein, and total globulin. The current investigation found that pretreatment of rats with AI extract significantly decreased the levels of bilirubin, total protein, total albumin, total globulin, and the enzymes AST, ALT, ALP, and GST And SOD Azadirachta indica demonstrates significant hepatoprotective effects against INH + RIF-induced liver damage in a dose-dependent manner, particularly at 200 and 400 mg/kg. This protective effect is likely mediated through reductions in liver enzyme elevations, improvements in liver histopathology, and enhancement of antioxidant defenses. The treatment resulted in favorable outcomes across biochemical parameters and histopathological findings, suggesting its potential as a therapeutic agent for mitigating hepatotoxicity associated with anti-tuberculosis treatment. Further studies are warranted to elucidate the mechanisms underlying these protective effects.

The study indicated that **Azadirachta indica** (neem) showed significant hepatoprotective effects at doses of **200 mg/kg** and **400 mg/kg**. These doses effectively reduced liver damage markers and improved overall liver health compared to the vehicle control group.

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