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Evaluation of Hepato Protective Activity of Methanolic Stem Bark Extract of Mangifera indica in Rats

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Abstract:
The present study was undertaken to evaluate the effect of methanolic stem bark extract of mangifera indica for its hepatoprotectivity activity. It was evaluated in normal and INH, rifampsin induced hepato toxic rats. Liv.52 was used as a standard drug at a dose of 500mg/kg body wt given in oral route. Albino wistar rats with INH's rifampsin induced hepato toxicity were divided in to 4 groups of 6 each in the study. Hepatoprotective effect as two different doses (200 & 400 mg/ kg body wt PO) of MEMI will be investigated for 28 days to evaluate dose dependant activity. Effect of MEMI on SGPT , SGOT, ALP, total cholesterol, total bilirubin, were investigated for Zero day, 7th, 14th, 21st, 28th day and anti oxidant parameters were checked on 28th day of the scarification.

Keywords:
Hepato Protective Activity in rats, Methanolic Stem Bark of Mangifera indica, Rifampsin.

Introduction

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its function may leads to complications on one's health[1]. Drug-induced liver injury (DILI) is a major health problem that challenges not only health care professionals but also the pharmaceutical industries and drug regulatory agencies. According to the United States, DILI accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (APAP, 39%) and idiosyncratic liver injury triggered by other drugs (13%)[2]. Because of the significant patient morbidity and mortality associated with DILI, the U.S. food and drug administration (FDA) has removed several drugs from the market, including bromfenac, ebrotidine, and troglitazone[3]. Other hepatotoxic drugs, such as risperidone, trovafloxacin, and nefazodone, have been assigned "black box" warnings[4]. DILI is the most common cause for the withdrawal of drugs from the pharmaceutical market[5,6].

The development of new diagnostic and therapeutic methods is regularly improving the management and prognosis of most of the diseases but it is also associated with the occurrence of new iatrogenic diseases. Drug-induced liver injuries were one of the major problems. Liver toxicity is developing and remains the first cause of drug-induced death and withdrawl of drugs from the pharmaceutical market. Despite improvement in toxicological studies and in the safety analysis of clinical trials, the frequency of drug hepatotoxicity has not decreased from the last decade. The spectrum of liver damage caused by drugs is very broad. Indeed, all cells present in the liver can be affected by drugs. Almost the entire spectrum of liver injuries can be reproduced by drugs. This explains the concern of physicians, health authorities and pharmaceutical companies about drug hepatotoxicity[6].

World Health Organization (WHO) estimates that about 80% of populations living in developing countries rely almost exclusively on traditional medicines for their primary health care needs. Since the medicinal plants are the backbone of traditional medicine, that, 3300 million people in the under developed countries utilize medicinal plants on a regular basis. This assumption does not include the developed countries where there has been a great fascination for the herbal medicines and dietary food supplements in the last 10 years.

Due to the toxic and adverse effect of synthetic medicines being observed round the globe, herbal medicine has made a comeback to improve the fulfillment of our present and future health needs[7]. Consumption of medicinal herbs or herbal preparations is tremendously increasing in order to identify alternative approaches to improve the quality of life and maintain a good health9. Developing drugs from natural products may reduce the risk of toxicity and maintain its therapeutic effectiveness, when the drug is used clinically[8].

Tuberculosis (TB) is one of the most common infectious diseases which gradually swallows the life span of human beings. The global prevalence of tuberculosis was 32% (1.86 billion people) and mortality rate was 23%[9]. The number of estimated cases of tuberculosis was 7.96 million in 1997, with 80% of all incident tuberculosis cases being found in 22 countries and more than 40% in five south-east Asian countries. In India, pulmonary tuberculosis is one of the

major causes for adult deaths. Isoniazid (isonicotinic acid hydrazide-INH) was the first effective bactericidal drug used to treat tuberculosis and till far, an important part of most antitubercular drug regimens. Rifampicin (RIF), which is another effective bactericidal drug, was added to the regimen in 1962 and has remained the most effective antitubercular combination along with isoniazid. Although both these drugs are very potent against the tuberculosis bacillus, both are well-known hepatotoxic drugs[10].

Isoniazid can cause mild to moderate elevation of plasma liver enzyme activity in 10-20% of patients, and severe hepatotoxicity in approximately 0.5-2%. The incidence of hepatotoxicity is approximately 2.6% with isoniazid-rifampicin co-administration but only 1.6% with isoniazid treatment alone and 1.1% with rifampicin alone; suggesting the higher incidence of severe hepatotoxicity in patient's co-treated with these two drugs[11,12]. The base of evidence for isoniazid hepatotoxicity is a level of risk in the range of 5 to 20 cases of hepatotoxicity expected per 1000 persons receiving treatment with a 1% to 10% case[13].

As hepatotoxicity is potentially a serious adverse effect of these currently used anti-tubercular chemotherapeutic regimens, a search for an alternative drug useful for the prophylactic treatment of hepatotoxicity induced by anti-tubercular remains important. Efforts to explore hepatoprotective effect of any natural product thus carry a great clinical significance. Therefore, the present study was undertaken for the hepatoprotective activity of Mangifera indica stem bark extract against antitubercular treatment (ATT) of INH and RIF in rats.

Materials and Methods

Plant Profile

Mangifera indica is one the most important tropical plants marketed in the world .It is a large tree that grows in tropical and subtropical regions, whose fruits are widely appreciated by the population. There are many traditional medicinal uses for the bark, leaves and roots of mangifera indica thorough globe.

Chemical constituents and properties

Mangiferin; mangin; piuri-yellow dye; benzoic acid; citric acid; tannin, 10%.The leaves contain 43-46 percent euxanthin acid and some euxanthon.Seed contains a fixed oil, oleostearin. The bark exudate yields a resin, gun, ash, and tannin. Mangostine, 29-hydroxymangiferonic acid, mangiferin and flavonoids have been isolated from the stem bark. Leaves and flowers yield an essential oil containing humulene, elemene, ocimene, linalool and nerol.

Properties

- Root, diuretic; bark, astringent; seeds, astringent and mifuge; leaves, pectoral.
- Considered antiseptic, antibacterial, anti-inflammatory, diaphoretic, stomachic, vermifuge, cardiotonic and laxative.

Parts used

Leaves, kernel, bark and fruit.

Uses

- Good source of iron (deficient in calcium); excellent source of vitamins A, B, and C. Fruit contains citric, tartaric and mallic acids.
- Decoction of root is considered diuretic.
- Bark and seeds are astringent.
- Resin is used for aphthous stomatitis.
- **Cough:** Drink infusion of young leaves as needed.
- **Diarrhoea:** Take decoction of bark or kernel as tea.
- Hot lotion from bark used for rheumatism.
- Gum resin from bark, mixed with coconut oil, used for scabies and other parasitic skin diseases.
- Juice of leaves used for dysentery.
- Tea of leaves with a little honey used for hoarseness and aphonia, 4 glasses daily.
- Powdered dried leaves, 1 tbsp to a cup of warm water, 4 times daily, used for diabetes.
- Ashes of burned leaves used for scalds and burns.
- Infusion of young leaves used in asthma and cough.
- Tea of powdered dried flowers, 4 times daily for diarrhoea, urethritis.
- Juice of peel of unripe mangoes used for skin diseases.
- Seed is vermifuge and astringent.
- Infusion of powdered dried seeds used for asthma, diarrhoea, dysentery, menorrhagia, bleeding piles, round worms.
- In Indian traditional medicine, seeds used for diarrhoea.

Plant collection

Mangifera indica was collected from surrounding areas of Tirupati, authentified by Dr. K. Madhava chetty, Asst -Prof. of Botany, Sri Venkateswara University, Tirupathi. Bark was separated, washed in water, chopped into pieces and then shade

dried.

Preparation of extract:

50 g of powder of Mangifera indica bark was weighed and extracted with 300ml of various solvents like pet ether, benzene, chloroform, ethyl acetate, methanol and aqueous in a soxhlet apparatus for 72 hrs. Then the solvent is subjected to distillation and concentrated the extracts. The extract is concentrated with rotary evaporator under reduced pressure and the dried extract was weighed and the percentage yield of the extracts was calculated. The percentage yield of extracts was tabulated in the Table no.4.1

Table No. 01: Percentage Yield Extracts of Mangifera indica

S.NO	Extract	Percentage yield
1	Petroleum ether	5.92
2	Benzene	4.13
3	Chloroform	4.05
4	Ethyl acetate	2.65
5	Methanol	6.89
6	Water	3.98

Phyto chemical tests

Test for carbohydrates:

Molisch's test: To the 2 ml of test solution, alcoholic napthol was added and then few drops of Conc. Sulphuric acid was added through sides of the test tube. Purple to violet colour ring appeared at the junction.

Barfoed's test: To the 1 ml of test solution, 1ml of Barfoed's reagent was added and heated on a water bath, the formation of cupric acid confirmed the presence of the monosaccharide.

Fehling's test: Fehling's A&B of equal volumes was taken and heated on a water bath. To this 2ml of test solution was added and again heated. Brick red precipitate was formed.

Benedict's test: To the 2ml of sample solution, 2 ml of Benedict's reagent was added and heated on a water bath. Reddish brown precipitate was formed.

Test for proteins and amino acids:

Biuret test: To the 2 ml test solution, 2 ml of Biuret reagent was added. The formation of violet colour confirmed the presence of proteins.

Millon's test: To the 2 ml of test solution, 2 ml of Millon's reagent was added. The formation of white precipitate confirmed the presence of amino acids.

Ninhydrin test: To the 2ml test solution, 2ml of Ninhydrine solution was added and boiled on a water bath. The formation of violet colour confirmed the presence of amino acids.

Test for steroids:

Salkowski test: The extract was treated with few drops of concentrated sulphuric acid, the formation of red colour at lower layer indicated the presence of steroids or the formation of yellow coloured lower layer indicated presence of triterpenoids.

Libermann-Burchard test: The extract was treated with few drops of acetic anhydride, boiled and cooled. After adding the conc. Sulphuric acid from sides of test tube, a brown colour ring was formed at the junction between two layers and upper layer turned into green which confirmed the presence of steroids and formation of deep red colour indicated presence of triterpenoids.

Test for alkaloids:

Dragendroff's reagent: To the 2 ml of sample solution, Dragendroff's reagent (potassium bismuth iodide solution) was added. Reddish brown precipitate was formed.

Mayer's reagent: To the 2 ml of sample solution, Mayer's reagent (potassium mercuric iodide solution) was added. Cream colour precipitate was formed.

Hager's reagent: To the 2 ml of sample solution, Hager's reagent (saturated solution of picric acid) was added. Yellow colour precipitate was formed.

Wagner's reagent: To the 2 ml of sample solution, Wagner's reagent (Iodine-potassium iodide solution) was added. Reddish brown precipitate was produced.

Test for glycosides:

Cardiac glycosides:

Baljet's test: 2 ml of test solution was treated with picric acid or sodium picrate. Orange color was formed Keller-killani test (test for deoxy sugars): The drug was extracted with chloroform and evaporated to dryness. 0.4 ml of glacial acetic acid was added containing trace amount of FeCl3. Transfer the solution to a small test tube-acetic acid layer shows blue colour.

Saponin glycosides:

Foam test: Place 2 ml solution of drug in water in test tube, shake well, stable froath was formed.

Anthraquinone glycosides:

Results

The results obtained for the effect of methanolic stem bark extract of Mangifera indica on SGPT, SGOT, ALP, effect on total cholesterol, HDL, bilirubin(serum analytical methods) and the effect on SOD,Catalase, reduced GSH ,lipid peroxidation (tissue bio chemical methods) and histology are as follows

Table No. 03: Effect of Methonolic bark extract of Mangifera indica on SGPT

Group	Treatment	SGPT (IU/L)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	34.5±3.5	34.7±3.5	34.9±4.1	35.4±5.7	35.1±2.1
II	Control INH+RIF (200 mg/kg)	30.2±5.5	112.8±17.2	143.4±2.3	165.5±6.1	188.7±4.1
III	Standard INH+RIF (200 mg/kg) + liv52 (100 mg.kg)	37.3±2.3	68.3±5.7	92.3±3.9	101.2±7.2	113.1±5.4
IV	INH+RIF (200 mg/kg) + Mangifer indica(200 mg/kg)	32.4±3.9	88.2±4.6	102.5±4.8	122.4±8.9	133.3±6.2
V	INH+RIF (200 mg/kg) +	38.9±6.2	74.7±9.2	97.9±5.1	113.8±3.3	135.7±3

a = $p < 0.001$, when compared to normal animals
b = $p < 0.001$, when compared to control animals

Table No. 04: Effect of Methonolic bark extract of Mangifera indica on SGOT

Group	Treatment	SGOT (IU/L)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	68.3±9.4	68.8±7.2	69.4±7.2	71.3±5.2	73.4±2.9
II	Control INH+RIF (200 mg/kg)	133.3±11.1	133.3± 11.1a	151.7± 8.5a	169± 3.7a	189± 9.2a
III	Standard INH+RIF (200 mg/kg) + liv52 (100 mg.kg)	71.3±8.9	71.3± 8.9b	89.2± 3.3b	94± 6.4b	103± 7.1b
IV	INH+RIF (200 mg/kg) + Mangifera indica (200 mg/kg)	95.4±6.2	95.4± 6.2b	103.4± 2.4b	113± 5.2b	129± 6.1b
V	INH+RIF (200 mg/kg) + Mangifera indica (400 mg/kg)	87.0±9.8	87.0± 9.8b	95.3± 1.9b	97± 3.2b	109± 4.8b

a = $p < 0.001$, when compared to normal animals
b = $p < 0.001$, when compared to control animals

Table No. 05: Effect of Methonolic bark extract of Mangifera indica on ALP

Group	Treatment	ALP (IU/L)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	135.2±4.1	138.2±3.1	147.2±4.1	153.1±5.8	158.4±6.9
II	Control INH+RIF (200 mg/kg)	134.1±5.3	274.3±44.2	298.4±3.2a	321.4±4.1a	351.3±5.3a
III	Standard INH+RIF (200 mg/kg) + liv52 (100 mg.kg)	132.2±6.2	167.0±9.9	191.3±5.3c	213.7±6.9c	223.5±7.2c
IV	INH+RIF (200 mg/kg) + Mangifera indica (200 mg/kg)	137.3±1.9	192.3±5.4	214.7±3.3	237.5±3.9c	248.7±5.4

V	INH+RIF (200 mg/kg) + Mangifera indica (400 mg/kg)	135.7±2.3	179.2±3.5	193.9±4.1c	218.3±4.8c	235.2±5.1c

a = $p < 0.001$, when compared to normal animals
b = $p < 0.05$, when compared to control animals
c = $p < 0.001$, when compared to control animals

Table No. 06 Effect of Methonolic bark extract of Mangifera indica on total cholesterol

Group	Treatment	Total cholesterol (mg/dl)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	158.39	159.42	157.89	160.6	161.05
II	Control INH+RIF (200 mg/kg)	154.31	206.57±2.1	248.38±6.2a	272.59±6.2a	298.39±5.3a
III	Standard INH+RIF (200 mg/kg) + liv52 (100 mg.kg)	161.42	173.41±9.8	193.21±5.4c	219.14±7.1c	211.18±7.1c
IV	INH+RIF (200 mg/kg) + Mangifera indica (200 mg/kg)	158.72	197.61±8.1b	213.41±3.9c	229.31±8.7b	231.17±1.9c
V	INH+RIF (200 mg/kg) + Mangifera indica (400 mg/kg)	168.34	183.2±7.2	205.4±8.1c	217.8±4.9c	209.2±3.1c

a = $p < 0.001$, when compared to normal animals
b = $p < 0.01$, when compared to control animals
c = $p < 0.001$, when compared to control animals

Table No. 07: Effect of Methonolic bark extract of Mangifera indica on HDL-cholesterol

Group	Treatment	HDL- cholesterol (mg/dl)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	42.40 ± 5.606	45.06 ± 7.679	49.40 ± 7.352	52.12 ± 6.612	51.07 ± 6.725
II	Control INH+RIF (200 mg/kg)	58.79 ± 6.149	21.34 ± 5.859	26.21 ± 6.496	27.43 ± 5.852	20.89 ± 4.335a
III	Standard INH+RIF (200 mg/kg) +liv52 (100 mg.kg)	56.46 ± 5.251	36.02 ± 7.229	31.53 ± 8.148	35.47 ± 6.728	51.40 ± 6.029b
IV	INH+RIF (200 mg/kg) + Mangifera indica (200 mg/kg)	59.18 ± 5.224	32.03 ± 7.936	31.89 ± 7.322	33.02 ± 6.538	46.98 ± 5.610b
V	INH+RIF (200 mg/kg) +Mangifera indica (400 mg/kg)	51.68 ± 5.338	34.19 ± 7.984	35.19 ± 8.221	35.62 ± 5.993	50.44 ± 6.871b

a = $p < 0.05$, when compared to normal animals
b = $p < 0.05$, when compared to control animals

Table No. 08: Effect of Methonolic bark extract of Mangifera indica on total bilirubin

Group	Treatment	Total bilirubin (mg/dl)				
		0 day	7th day	14th day	21st day	28th day
I	Normal	0.83 ± 0.05783	0.94 ± 0.06560	1.01 ± 0.03490	0.76 ± 0.02472	0.89 ± 0.03337
II	Control INH+RIF (200 mg/kg)	1.06 ± 0.1468	3.24 ± 0.1049a	3.44 ± 0.03480a	2.98 ± 0.03347a	3.14 ± 0.02915a
III	Standard INH+RIF (200 mg/kg) +liv52 (100 mg.kg)	0.98 ± 0.1640	2.74 ± 0.05023c	1.97 ± 0.04248c	1.47 ± 0.02708c	0.98 ± 0.04781c
IV	INH+RIF (200 mg/kg) + Mangifera indica (200 mg/kg)	0.78 ± 0.1028	2.91 ± 0.06546b	2.11 ± 0.04757c	1.85 ± 0.04370c	1.12 ± 0.03851c

V	INH+RIF (400 mg/kg) + Mangifera indica (200 mg/kg)	0.97 ± 0.1031	2.37 ± 0.03838c	2.03 ± 0.03748c	1.34 ± 0.02717c	0.87 ± 0.03055c

a = $p < 0.001$, when compared to normal animals
b = $p < 0.05$, when compared to control animals
b = $p < 0.001$, when compared to control animals

Table No. 09: Effect of mangifera indica on various tissue antioxidant parameters

Sl. No	Group	SOD(U/mg protein)	CAT(µM H2O2consumed/mg protein)	Reduced GSH(µg of GSH/mg protein)	MDA (nM of MDA/ mg protein) protein)
1	Normal	8±0.80	10.68±0.54	9.854±1.002	0.448±0.07
2	Control INH+RIF (200 mg/kg)	4.394±0.51b	6.136±0.45b	5.35±0.64b	1.288±0.14f
3	Liv 52 (50 mg/kg b.w)	9.808±0.83f	10.99±0.50	10.52±0.64	0.4384±0.05
4	INH+RIF (200 mg/kg) + Mangifera indica (200 mg/kg)	7.658±0.73	10.73±0.87d	9.516±0.41e	0.718±0.059e
5	INH+RIF (200 mg/kg) + Mangifera indica (400 mg/kg)	8.556±0.72d	10.79±0.63f	10.58±0.69d	0.4924±0.11e

All values are shown as mean ± SEM and n=6.
a indicate $p< 0.05$, b indicate $p<0.01$, c indicate $p<0.001$ when compared to normal group.
d indicate $p<0.05$, e indicate $p<0.01$, f indicate $p<0.001$ when compared to INH+RIF group.

Discussion on Results

Pharmacological studies

Acute toxicity studies

The Methanolic bark extract of Mangifera indica was found to be safe since no animal died even at the maximum single dose of 4000 mg/kg when administered orally, and the animals did not show any gross behavioral changes. Hence, 1/20 and 1/10 of maximum therapeutic dose (4000 mg/kg) was selected for the present study.

Hepatoprotective activity

Effect on SGPT

Rats treated with INH+RIF (G-II) showed a significant increase in SGPT levels on 7th, 14th, 21st and 28th day, when compared to normal group (G-I). The group (III) rats treated with standard drug liv.52 (500 mg/kg) showed a significant decrease in SGPT levels on 7th, 14th, 21st and 28th day, when compared to control (G-II).

The groups (IV and V) receiving methanolic bark extract of mangifera indica (200 mg/kg and 400 mg/kg) shows a dose dependent decrease on SGPT levels on 7th, 14th, 21st and 28th day when compared to control group (G-II) (Table-6.1).

Effect on SGOT

A significant increase in SGOT levels was observed in rats treated with INH+RIF (G-II) on 7th, 14th, 21st and 28th day, when compared to normal group (G-I). The group (G-III) rats treated with standard drug liv.52 (500 mg/kg) showed a significant decrease in SGOT levels on 7th, 14th, 21st and 28th day, when compared to control (G-II).

The groups (IV and V) receiving methanolic bark extract of Mangifera indica (200 mg/kg and 400 mg/kg) showed a dose dependent decrease in SGOT levels on 7th, 14th, 21st and 28th day when compared to control group (G-II) (Table-6.2).

Effect on ALP

Administration of INH + RIF induced a significant increase in ALP levels on 7th, 14th, 21st and 28th day in control group (G-II) when compared to normal group (G-I) .

On treatment with liv 52 induced a significant decreases in ALP levels on 7th, 14th, 21st and 28th day in standard group (G-III) when compared to control group (G-II).

Groups (IV and V) treated with methanolic bark extract of Mangifera indica induces a significant decreases in ALP levels on 7th, 14th, 21st and 28th day both the doses has shown dose dependent when compared to control group (G-II) (Table-6.3).

Effect on total cholesterol

A significant increases in total cholesterol levels in INH + RIF treated group (G-II) on 7th, 14th,

21st and 28th day when compared to normal group (G-I).

Liv.52 treated group (G-III) shows significant decreases in total cholesterol levels on 7th, 14th, 21st and 28th day when compared to control group (G-II). Methanolic bark extract of Mangifera indica treated groups (IV and V) shows a significant decreases in total cholesterol levels on 7th, 14th, 21st and 28th day in both doses has shown dose dependent when compared to control group (G-II) (Table-6.4).

Effect on serum HDL-cholesterol

INH + RIF receiving groups (G-II) shows a significant increases in HDL- cholesterol levels on 7th, 14th, 21st and 28th day when compared to normal group (G-I).

Liv.52eceiving group (G-III) shows significant decreases in HDL- cholesterol levels on 7th, 14th, 21st and 28th day when compared to control group (G-II). methanolic bark extract of Msangifera indica treated groups (IV and V) shows a significant decreases in HDL- cholesterol levels on 7th, 14th, 21st and 28th day in both doses has shown dose dependent when compared to control group (G-II) (Table-6.5).

Effect on total bilirubin

Administration of INH + RIF induced a significant increase in total bilirubin levels on 7th, 14th, 21st and 28th day in control group (G-II) when compared to normal group (G-I) .

On treatment with liv 52 induced a significant decrease in total bilirubin levels on 7th, 14th, 21st and 28th day in standard group (G-III) when compared to control group (G-II).

Groups (IV and V) treated with methanolic bark extract of Mangifera indica induces a significant decreases in total bilirubin levels on 7th, 14th, 21st and 28th day both the doses has shown dose dependent when compared to control group (G-II) (Table-6.6).

In vivo antioxidant parameters

In the present study, various antioxidant parameters were assessed in the pancreas at the end of the study on 29th day.

Administration of INH + RIF diminished the antioxidant status in liver by decreases the catalase, GSH, SOD levels and increases the LPO levels in control group (G-II) when compared to normal group (G-I).

Standard group (G-II) treated with liv.52 increases the antioxidant status in liver by increasing the SOD, catalase, GSH levels and decreases the LPO levels when compared to control group (G-II).

Both doses of methanolic bark extract of Mangifera indica shows the significant increases in antioxidant status in liver by increasing the SOD, catalase, GSH levels and decreasing the levels of LPO has shown dose dependent when compared to control group (G-II) (table6.7) (Graphs-6.7,6.8,6.9,6.10).

Histological examination of liver slices

Histological examination of the normal liver slices showed normal hepatic parenchyma. There was no sign of inflammation or necrosis in these animals (Fig- 6.11). In INH–RIF treated group of animals showed moderate to heavy lobular inflammation and moderate portal triaditis with piecemeal necrosis or focal lobular inflammation (Fig- 6.12).

Pre-treatment with liv.52, 500 mg/kg dose showed almost normal liver lobule with no sign of necrosis and portal triads. Only a few inflammatory cell are observed in the centrizonal area (Figure- 6.13)

In the liver cells of rats treated with hydro-alcoholic root extract of Mangifera indica at 200 mg/kg dose showed reduction of necrosed area and inflammatory infiltrates in centrizonal area with disappearance of inflammatory infiltrate around portal triad (Figure- 6.14). Mangifera indica at 400 mg/kg dose showed greater reduction of the necrosed area and sparse inflammatory cell infiltration (Figure-6.15) as compared to 200 mg/kg dose. Hence, Mangifera indica showed dose dependent reduction in the necrosis and inflammatory infiltration.

Hepatotoxicity of anti-TB drugs is a serious problem because it causes significant morbidity and mortality that requires modification of the drug regimen 98. The incidence of anti-TB drug induced hepatotoxicity has been reported to be higher in developing countries and some factors such as liver disease, incorrect use of drugs, malnutrition and more advanced tuberculosis have been implicated for the increase in hepatotoxicity 98. It is estimated that the incidence of clinically relevant hepatotoxicity is 3% in the US, 4% in UK and 11.5% in India.

DIH due to isoniazid prophylaxis has been more commonly observed with advanced age. In published reports, the relative risk of DIH due to isoniazid (INH) prophylaxis ranged from 0/1000 in persons under the age of 20 years; 2.8/1000 in persons aged <35 years, compared to 7.7–19.2/1000 observed in those aged above 50 years. When isoniazid was used to treat TB in combination with other drugs, DIH was found to occur more often in older patients.

The increased risk of hepatotoxicity with INH and rifampicin (RIF) combination has been attributed

to the interaction between the metabolism of isoniazid and rifampicin. INH is metabolized in the liver primarily by acetylation and hydrolysis, and it is these acetylated metabolites that are thought to be hepatotoxins. Acetyl-isoniazid, the principal metabolite is converted to monoacetyl hydrazine. The microsomal p450 enzymes convert monoacetyl hydrazine to other compounds resulting in hepatotoxicity. RIF is thought to enhance this effect by enzyme induction 98. RIF induces cytochrome P450 enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). RIF can also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by RIF and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and RMP in combination.

Serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of liver injury. Serum transaminases are important class of enzymes linking carbohydrate and amino acid metabolism. Aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT) are present majority in the liver and these are also found in the cardiac muscles, skeletal muscles, pancreas, lungs, kidney, brain, etc., SGPT concentration is highest in the liver and therefore, it appears to a more sensitive test to hepatocellular damage than SGOT

There is no suitable drug for treating hepatotoxicity, in regard of these herbs are implicated as potential hepatoprotective agents. Therefore, the present study attempts to study the hepatoprotective and antioxidant property of Mangifera indica against anti-TB drug induced hepatotoxicity, in rats of wistar strain. Biochemical parameters of hepatotoxicity and oxidative stress were analyzed from serum and liver homogenates to assess the hepatoprotective activity. Further histopathological study was also carried out to confirm the pathological changes.

The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due INH and RIF induced peroxidative damage or altered permeability of membrane 35. Increased protein catabolism and urea formation that are seen in antitubercular drugs-induced hepatocellular damage and necrotic lesions in the hepatocytes may also be responsible for the increase of these amino tranferases activities in liver. Similar increase in levels of SGPT and SGOT in the hepatic cells were also observed in the present study, when treated with combination of INH+RIF.

In the present study, co-administration of methanolic bark extract of Mangifera indica with INH+RIF significantly decreased the levels of these diagnostic marker enzymes (SGPT and SGOT) and the effect was observed to be dose dependent.

Alakaline phosphatase was found to increase in the group- II animals treated with INH+RIF. ALP activity on endothelial cell surfaces is responsible for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that results from injury. So, following injury, accumulation of interleukin-6 can lead to production of adenosine by alkaline phosphatase and subsequent protection from ischemic injury. This may be the reason for the increment in ALP in intoxicated rats due to liver cell necrosis 63.

In the present study, co-administration of methanolic bark extract of Mangifera indica with INH+RIF decreased the levels of these ALP marker enzymes in the serum.

Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures 104. The toxic metabolite hydrazine is produced, which further binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In view of this, the reduction in levels of SGOT, SGPT and ALP caused by the Mangifera indica is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by INH+RIF. Similar mechanism for hepatoprotective action were proposed for Ginkgo biloba, Strychno spotatoru and Momordica dioica Roxb 105,104

The major disorder encountered in antitubercular drugs-induced hepatitis is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism 106. In the present study, the levels of total cholesterol were higher in INH and RIF administered rats, indicating that the antitubercular drugs-induces hypercholesterolemic condition. The increase in cholesterol levels in the liver might be due to increased uptake of LDL from the blood by the tissues 107. The abnormal cholesterol deposition is favoured by the dangerous tendency of cholesterol to undergo passive exchange between the plasma lipoproteins and the cell membranes 108. Hence, protective HDL-cholesterol levels were reduced in the animals treated with INH+RIF.
In the present study, co-administration with metha-

nolic bark extract of Mangifera indica reduced the elevation in the levels of total cholesterol induced by anti-TB drugs. The levels of protective HDL-cholesterol were also prominently increased when animals treated with Mangifera indica,

The probable mechanism responsible may be due to the decrease in the biosynthesis of cholesterol in the liver or by inhibiting enzymes responsible for the synthesis of cholesterol, by the chemical constituents of Mangifera indica. This effect may also be responsible for an improvement in the serum HDL-levels. Tridax procumbent is also reported to have beneficial effect on lipid profile due to similar mechanism 109

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function 109. The disaggregation of polyribosomal profiles induced by toxins is also associated with the inhibition of protein synthesis, which may be partially responsible for the fatty liver, probably not necrosis although it contributes to disabling of the cell 63. Hence, decrease in protein levels and increase in total bilirubin was observed in animals when treated with INH+RIF.

In the present study, co-administration of methanolic bark extract of Mangifera indica with INH+RIF increased the levels of these total proteins and decreased the total bilirubin levels in the serum.

Cytochrome P450 is one of the liver enzymes, considered responsible for damage of hepatic cells 32. Mangifera indica may inhibit these enzymes, thus enhance in the level of total proteins and decrease in the levels of total bilirubin. Thus was observed the hepatoprotective action may be mediated through the inhibition of UDP-sugar derivatives, enhancement of glycoprotein biosynthesis and stabilisation of cell membrane and inhibition of lipid accumulation by its hypolipidemic property, which are the few common mechanisms attributed for hepatoprotective activity for natural drugs 110.

The results of histopathological parameters also support the results of biochemical parameters and explain the hepatoprotective activity of Mangifera indica .

Free radicals alter the structural and functional integrity of cells by a variety of mechanisms, including lipid peroxidation, sulfhydryl oxidation and proteolysis and shearing of the nuclear material. Healthy cells can scavenge free radicals effectively by their defensive system (antioxidant effects). In short, there is a dynamic relationship between reactive oxygen species and antioxidants in the human body. In some pathological conditions, such as cells suffering ischaemic insult, the sudden generation of reactive oxygen species can dramatically upset this balance with an increased demand on the antioxidant defence system. Natural antioxidants are depleted accompanied by accumulation of reactive oxygen species. In such a situation, natural products can play an important role in two aspects: enhance the activity of original natural antioxidants and neutralize reactive oxygen species by nonenzymatic mechanisms [111]. It is reported that oxidative stress is also involved in liver damage [112]. Hence, effect of Mangifera indica on oxidative stress induced by INH+RIF was studied.

Isoniazid and rifampicin induced hepatitis is due to their biotransformation to reactive metabolites that are capable of binding to cellular macromolecules [71]. As an alternative to inducing cellular damage by covalent binding, there is evidence that these antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system. The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al., (2000).

Lipid peroxidation is a common event in toxic phenomenon, is regulated by the availability of substrate in the form of polyunsaturated fatty acids (PUFA). Although it occurs to a limited extent under normal physiological conditions, but external factors can augment this process so that it escapes cell control leading to degradation of lipids in the cell membrane and eventually causing membrane damage and death of cell.

In the present study, free radicals formed either by the reaction of metabolites of INH+RIF with oxygen or by the interaction of superoxide radicals with H2O2, seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like MDA that cause loss of integrity of cell membrane and damage to hepatic tissue 114.

Reduced glutathione is one of the most abundant non-enzymatic biological antioxidant present in the liver 114; it efficiently scavenges reactive toxic metabolites of antitubercular drugs. As a substrate for antioxidant enzymes glutathione peroxidase (GPX), glutathione reductase (GR), it protects cellular constituents from the damaging effects of peroxides formed during metabolism and other ROS. Liver injury has been observed when GSH stores are markedly depleted. In our study, similar decrease in GSH was observed on administration of INH+RIF.

It is known that SOD, CAT constitutes a mutually supportive team of antioxidant enzymes which provides a defense system against ROS. Catalase is an enzymatic antioxidant, a heamoprotein which catalyses the reduction of hydrogen peroxide to water and oxygen and protects the tissue from highly reactive hydroxyl free radicals.

In the present study, SOD and catalase decreased in INH+RIF treated animals may be due to an excessive formation of superoxide anions.

Concomitant administration of methanolic bark extract of Mangifera indica and INH+RIF effectively increased the GSH, SOD and CAT activities and also decreased the MDA levels which may be attributed to the scavenging of radicals by methanolic bark extract of Mangifera indica resulting in protection of these enzymes.

Flavonoids, present in the Mangifera indica is known to quench the free radical by maintaing antioxidant levels 115. Thus, it can be suggested that significant antioxidant activity shown by Mangifera indica may be due to presence of mangostin, 29-hydroxymangiferoic acid.

The present study indicates that the methanolic bark extract of Mangifera indica may be used as an effective hepatoprotective agent. Further studies on isolation and structural determination of active principles might be worthy.

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