Phytochemical And Pharmacological Screening Of *Moringa Oleifera* For Hepatoprotective Activity On Rats

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Abstract
Moringa oleifera (*M.oleifera*) has been used as food and folk medicine to recover several diseases. In the present study is effect of ethanolic extract of *M.oleifera* on . Isoniazid and Rifampicin induced hepatotoxicity on rats. Isoniazid and Rifampicin was administered a 100 and 50 mg/kg bw dose to induced hepatotoxicity. *M. oleifera* (300 and 500mg/kg, p.o) and Silymarin 100mg/kg, p.o. were administered once daily for 30 days. The degree of liver injury was estimated by liver function parameters ALP,AST,ALT,TP and TB. Isoniazid and Rifampicin drugs significantly increases in serum ALP,AST,ALT,TP and TB and reduction in liver weight and changes in histopathology. Treatment with *M.olefiera* extract significantly changing in weight liver and histopathology of liver as well as Biochemical parameters in serum ALP,AST,ALT,TP and TB compare to control group. The biochemical parameters and histopathology provide evidence that the ethanolic extract of *M.oleifera* has shown hepatoprotective activity.

Key words: *M.oleifera.* INH+RF,Liver injury

INTRODUCTION

Liver is the major organ which plays a key roles in metabolisms , biochemical ,physiological functions and Detoxification of endogen and exogenous compounds, such as drugs and xenobiotics, homeostasis, growth, energy and nutrient supply.[1-2] Tuberculosis (TB) is a disease that affects one third of the world's population; nearly 9.6 million cases were reported and close to 2 million deaths [3-4] RIF, INH and PZA are basic for treating sensitive or monoresistant TB, and these mainly cause liver damage [5].

M. oleifera a food and folk medicinal plant (family moringaceae), is a small or middle sized tree, usually grows10 -12 m in height .The plant is indigenous and abundantly seen in India, Pakistan, Bangladesh and Afghanistan. *M. oleifera* produce drumstick-like fruits. The flowers are white and quite small. It has teardrop shaped round and small leaves. Fruits and leaves are edible which are generally eaten as green vegetable [5-6]. *M. oleifera* contain primary and secondary phytochmicals, has been isolated and reported from various parts are carbohydrates,protein amino acid, flavonoids, resin, minerals , fatty acids vitamin and minerals [7-9] A number of pharmacological activity has also been reported are and anti-oxidant and radical scavenging activity[10], anti-convulsant activity[11] , antidiabetic[12] , Anti-asthmatic activity[13], anti cancer[14] , and Anthelmintic activity[15] , However our present study was ethanolic extract of *M. oleifera* on Isoniazid and Rifampicin induced liver damage on rats .

MATERIALS AND METHODS

The leaf of *M. oleifera* were collected in the month of August-September from the local areas of Hyderabad and make herbarium. The plants were identified, confirmed and authenticated by Dr.Vijaya Bhasker Reddy, Assistant Professor, Department of Botany , Osmania university, Hyderabad. A voucher specimen (No.OUAS-165).

PREPARATION OF EXTRACTION

The fresh leaf around 2kg shade dried for 15 days; leaf material was powdered using mixer grinder and passed through sieve no 85. Weight About 150gm of dried fruit powder was subjected to soxhlet's apparatus extraction using ethanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator

Preliminary phytochemical analysis: the extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents [16].

Experimental animals procured

Adult wistar rats of male 9 to 11 week age, weighing 160–180gm were procured from Mahaveera enterprises, Hyderabad. Animals were housed in standard laboratory conditions at 25°c with 12 hr light-dark cycle with free access to chow and water *ad libitum*. The research protocol was approved by (HKES/COP/MTRIPS/IAEC/105/2022)

Evaluation of HepatoProtective Activity:

Hepatic injury: A dose of 50 mg/kg and 100 mg/kgb.w Isoniazid and Rifampicin respectively in Aqueous 1% CMC through oral for 28days.

The study design is divided into 5groups, six rats in each. After 28 days of treatment study, animals were sacrificed and the following are weight of liver and liver profile for biochemical enzymes parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and liver histopathology.[16-17]

Group No	No. of Rats	Treatment	Dose
1	6	Control-Aqueous 1% CMC	10ml/kg b.w
2	6	Positive control – INH + RIF	50 mg/kg +100 mg/kg btw,
3	6	INH + RIF+ *M. oleifera*	50 mg/kg +100 mg/kg btw+400mg/kg
4	6	INH + RIF+ *M. oleifera*	50 mg/kg +100 mg/kg btw+600mg/kg
5	6	INH + RIF + Silymarin 100	50 mg/kg +100 mg/kg btw +5mlg/kg

Table1: Treatment of *M. oleifera* on INH+RF induced liver injury

After 28 days of treatment study, animals were sacrificed and the following morphological and biochemical parameters such as

1. Weight of liver,
2. Histopathology of liver,
3. Biochemical enzymes parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)[16-18]

Histopathological Investigation:

The liver tissues were excised out, washed with the cold saline, fixed in 10% buffered formalin for 12 hours and processed and stained with hematoxylin and eosin dye for photomicroscopic observations.

Statistical Analysis

The results were expressed as mean ± SEM, The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test , *p* values <0.05, <0.01 and <0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

Results

Preliminary Phytochemical Screening:

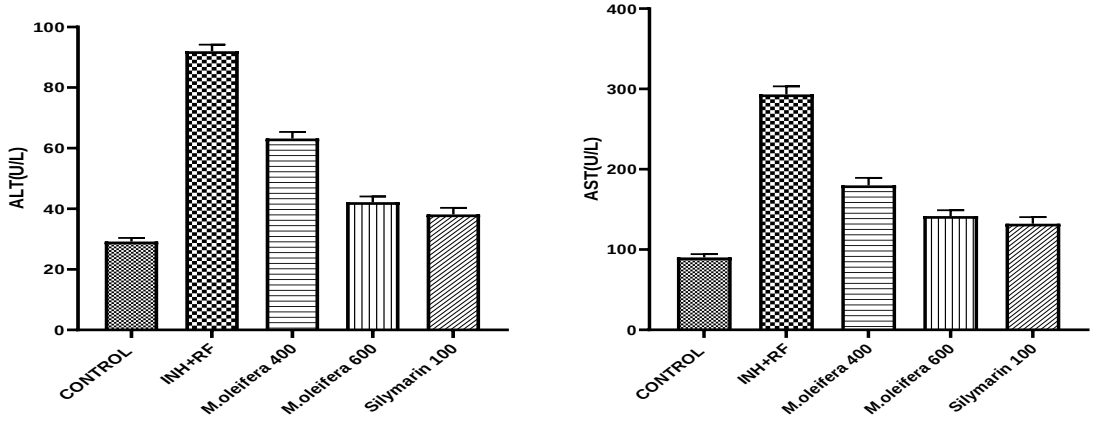
The preliminary phytochemical studies were performed for testing different phytochemical constituents present in *M. oleifera* . The observations showed the presence of alkaloids, flavonoids, steroids, Carbohydrate, aminoacids, tannins and poly phenolics, which were found in extract.

Effect of ethanolic extract of *M. oleifera* on liver weight in rats

Anti-TB drug INH+RF treated group significantly decrease the liver weight compared to control group. *M. oleifera* treatment markedly ameliorated the effect of anti-TB drug on liver weight. Impact of *M. oleifera* was comparable to the effect of Silymarin on hepatic weight.

Effect of *M. oleifera* extract on liver function tests

The administration of INH+RF significantly increased the level of AST, ALT, ALP, TP, and TB in serum compared to the control group. The hepatotoxicity induced with INH+RF was ameliorated by the co-administration of *M. oleifera* to INH+RF administered in rats. The protective effects of *M. oleifera* on AST, ALT, ALP, TP, and TB were significantly decreases the liver serum in a dependent manner. The level of AST, ALT, ALP, TP, and TB in the liver serum Silymarin administered groups remained impervious compared to the control group as shown in (figure1)



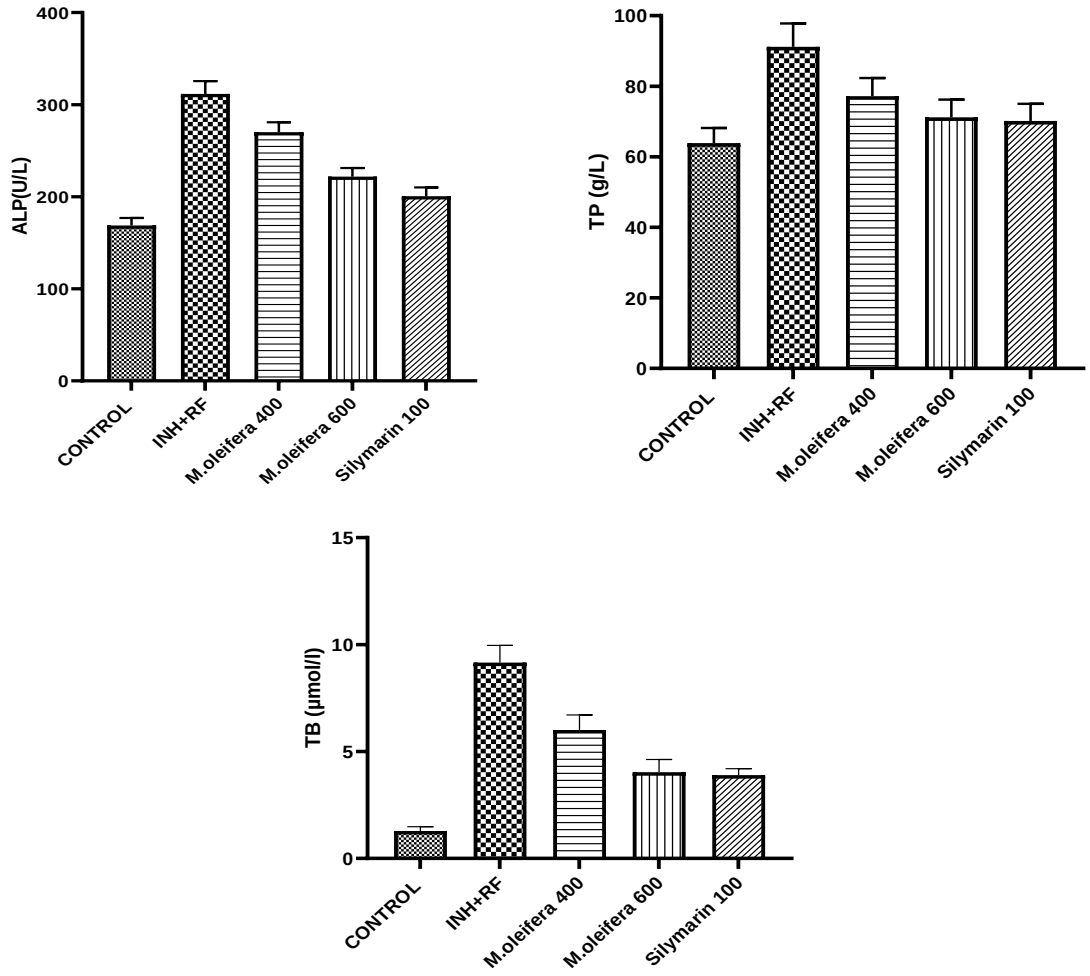
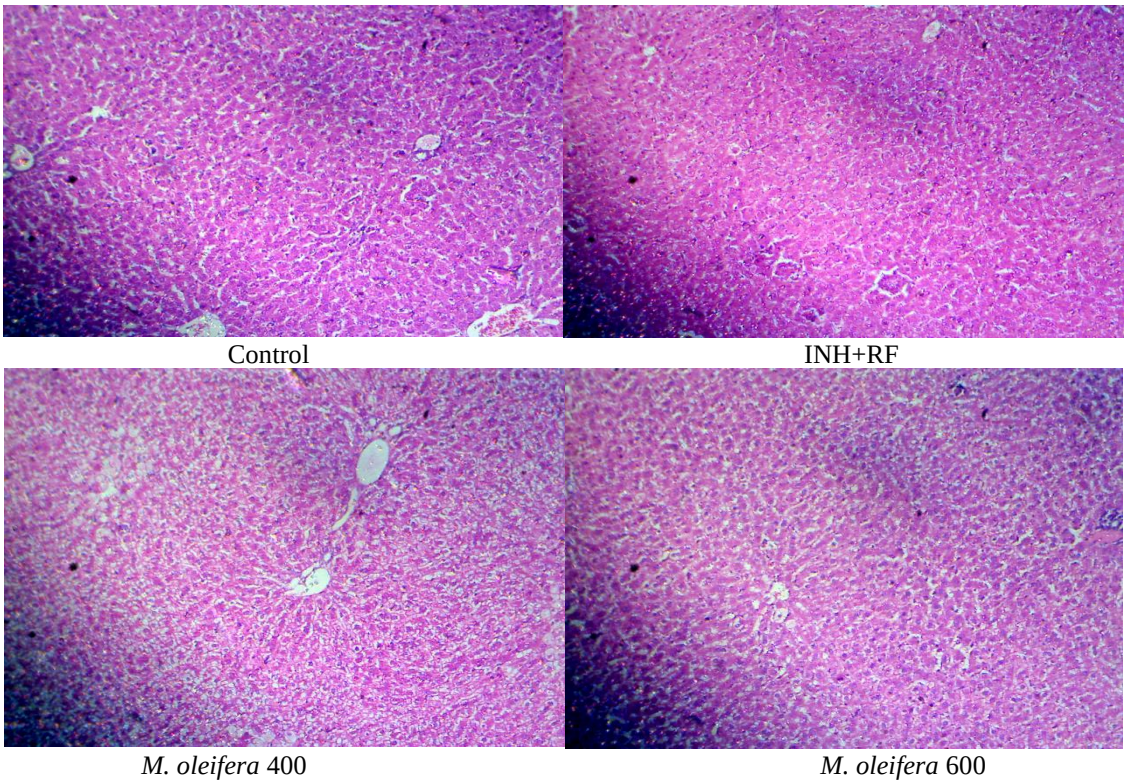


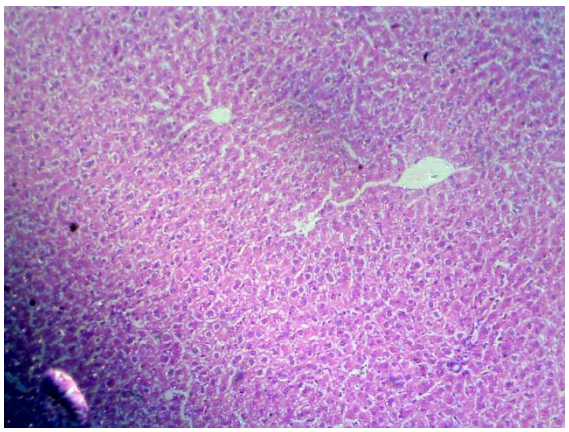
Figure: 1 Effect of *M. oleifera* in serum liver function on INH+RF induced Liver injury in rats

Histopathology of Liver:



Control INH+RF

M. oleifera 400 *M. oleifera* 600



Silymarin

Figure2: Histopathology of liver of *M. oleifera* on INH+RF induced Liver injury in rats.

The histopathology of liver stain with Hematoxylin and eosin are shown in (figure 2) the control group showed the normal lobular structure of liver. INH+RF drugs liver was altered and fatty changes were prominent. The lobular structure was disrupted and there was congestion of blood vessels, a severe degree of hemorrhage, necrosis with fatty vacuolations. There were degenerative changes and the chromatin material showed clumped morphology. The cell membrane hepatocytes in some of the areas were not distinguished. Treatment with *M. oleifera* 400 , 600 and Silymarin protected the liver from the toxicity of anti-tuberculosis drug , the changes induced with an anti-tuberculosis drug were observed in the histopathology of liver are no congestion of blood vessels, a low degree of hemorrhage, no necrosis with fatty vacuolations , were compare to liver was near to the control group.

DISCUSSION:

Liver is an important organ in human body it's actively involved in metabolic functions. Liver diseases are known to be associated with INH+RF damage hepatic metabolizing capacity and impaired activity of various hepatic enzymes[20]

Isoniazid is used to cause liver injury by forming acetyl radical which combines with cellular lipids and protein the presence of oxygen to induce lipid peroxidation by hydrogen abstraction. This result in the changes in the structure of endoplasmic reticulum and other membrane, it induces loss of metabolic enzyme that leads to liver damage.

AST and ALT are found in both cytosol and mitochondria of hepatocytes. A significant rise in AST and ALT level could be taken as index of liver damage. This elevation could be due to toxic injury to the liver by toxic agent[21], of these two enzymes, such as AST and ALT, ALT is better indicates of liver injury by toxins. ALT level is the chief application diagnosis of hepatocellular destruction [12]. In the present study the increased level of AST and ALT were observed in isoniazid intoxicated rats. Administration of *M. oleifera* to INH+RF intoxicated rats to restore the level of AST and ALT. This is to confirm the hepatoprotective effect of *M. oleifera*.

CONCLUSION:

In conclusion, the result of the present study indicated that under the present experimental conditions. Etanolic extract of *M. oleifera* possesses potent antioxidant activity, which may be due to presence of antioxidant component in the *M. oleifera*.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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