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Research Article

PROTECTIVE EFFECT OF *MIRABILIS JALAPA* LEAVES ON ANTI-TUBERCULAR DRUGS INDUCED HEPATOTOXICITY

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ABSTRACT

Objectives: The present study was undertaken for investigating the protective effect of ethanolic extract of *Mirabilis Jalapa* Linn leaves on anti-tubercular drugs induced hepatotoxicity.

Methods: Anti-tubercular drugs were used to induce hepatotoxicity in rats. Silymarin was used as standard drug (100 mg/kg p.o.). Ethanolic extract of leaves of *Mirabilis Jalapa* Linn (250 & 500 mg/kg p.o.) was administered along with one hour prior administration of anti-tubercular drugs once daily for 35 days.

Results: Liver biomarkers such as SGOT, SGPT, ALP, TB, total cholesterol were elevated and total HDL were reduced on anti-tubercular drugs administration. The treatment of ethanolic extract of *Mirabilis Jalapa* Linn leaves 250 mg/kg and 500 mg/kg with anti-tubercular drugs were significantly reduced liver biomarker enzymes. Antioxidant parameters such as SOD, CAT, GSH, GPx and GRx were suppressed and increased TBARs levels in anti-tubercular drugs administration but restored these antioxidant levels in the treatment of ethanolic extract of *Mirabilis Jalapa* Linn leaves at a dose of 250 mg/kg and 500 mg/kg.

Conclusion: The result of the present study was indicated that *Mirabilis Jalapa* Linn leaves showed protective effect on hepatotoxicity induced by anti-tubercular drugs.

Keywords: Hepatoprotective activity, Anti-tubercular drugs, Liver biomarkers, Mirabilis Jalapa, Antioxidant parameters.

INTRODUCTION

Drug induced hepatotoxicity is a serious adverse drug reaction of anti-tubercular drugs [1]. Isoniazid, Rifampicin, Pyrazinamide and Ethambutol are anti-tubercular drugs used in the treatment of tuberculosis (TB) by DOTS (Directly Observed Treatment Shortcourse) regimen. Hepatotoxicity due to anti-tubercular drugs is found to be mediated through oxidative stress and free radical damage to hepatocytes [2].

Hepatotoxicity as injury to the liver that is allied with diminished liver function. Numerous medical plants and their formulations are being used for liver disorders in ethnomedical practices and in traditional system of medicine in India [3]. Conventional drugs used in the treatment of liver disease are often inadequate [4]. It is therefore search for supplementation/ alternative drugs for the treatment of hepatic damage caused by anti-tubercular drugs.

Mirabilis Jalapa (family: Nyctaginaceae), known as four O' clock plant or marvel of peru, is a perennial herb. It can also be grown as an annual, tall herbaceous climbing plant with opposite leaves, large showy flowers, coriaceous obovoid fruits and prominent tuberous roots, planted as an ornamental plant throughout the world. It is used in traditional medicine by the people from different countries for the treatment of diarrhea, dysentery, conjunctivitis, edema, inflammation, swellings, muscular pain and abdominal colics [5,6] and its extract has antibacterial, antiviral, and antifungal activities [7]. It is also found to posses antispasmodic and antinociceptive properties. It is rich in many active compounds including triterpenes, flavanoids, alkaloids, steroids and amino acid-based proteins called antiviral proteins. Phytochemical investigations revealed the constituents of this plant alanine, alpha-amyrins, arabinose, beta-amyrins, campesterol, C-methy labronisoflavone, stigmasterol, tartaric acid, trigonelline. A number of active compounds were extracted from different parts of *Mirabilis Jalapa*, including ribosome-inactivating protein (RIP) associated with antiviral activity, antifungal phenolic compounds [8], antimicrobial peptides [9] and rotenoids showing inhibition of HIV-1 reverse transcriptase [10], further isolation of active components is under progress. It is well known antioxidant plant [11]. Hence, the present study is focused on protective effect of ethanolic extract of *Mirabilis Jalapa* Linn (EMJ) leaves against anti-tubercular drugs induced hepatotoxicity.

MATERIAL AND METHODS

Collection and Autentication of Plant Material

Leaves of *Mirabilis Jalapa* Linn were collected from Tirumala hills, Andhra Pradesh. The plant was identified, authenticated and certified by Dr. K. Madhavachetty, Assistant Professor, Department of Botany, S.V.University, Tirupathi, and A.P.

Preparation of plant extract

The air dried powder was extracted in Soxhlet apparatus using ethanol as solvent. Appearance of colorless solvent in the siphon tube was taken as the end-point of extraction. The extract was concentrated to ¾ of its original volume by distillation.

Acute toxicity studies

Acute toxicity studies were performed for EMJ according to OECD guidelines 423 [12]. 10 mice were selected for the study and oral administration of EMJ at a dose of 5, 50, 300, 2000 mg/kg given at 48 hrs interval simultaneously. In this acute toxic study, animals were observed for any changes in consumption of food and water, body weight, behavioural changes and mortality rates.

Animals

Healthy adult albino rats (150–250 gm) were used and they were purchased from Invivo biosciences, Bangalore. The animals were housed in clean metabolic cages, maintained in controlled temperature (22±3°c) and light cycle (12 hour light and 12 hour dark). They were fed with standard pellet diet and water *ad libitum*. The protocol was approved by the Institutional animal ethical committee (IAEC) of Krishna Teja Pharmacy College **(1521/PO/a/11/CPCSEA)**.

Study protocol

Hepatotoxicity was induced by using H-Isoniazid (27 mg/kg, p.o), R-Rifampicin (40 mg/kg, p.o), Z-Pyrazinamide (66 mg/kg, p.o) and E-Ethambutol (53 mg/kg,p.o) for 35 days and Silymarin (100 mg/kg, p.o) was used as the standard. The oral doses of anti–tubercular drugs were extrapolated from daily human dose using the conversion table based on body surface area [13].

Experimental procedure

Experimental animals were randomly divided in to 5 groups, each group containing 6 animals and the treatment schedule for 35 days as follows. **Group I**: Control (Normal saline 1ml/kg, p.o), **Group II**: Toxic control (anti–tubercular drugs – HRZE, p.o.), **Group III**: Silymarin (100 mg/kg, p.o) + one hour prior administration of anti–tubercular drugs, **Group IV**: EMJ (250 gm/kg, p.o)+one hour prior administration of anti- tubercular drugs and **Group V:** EMJ (500 gm/kg, p.o) + one hour prior administration of anti- tubercular drugs. On 36th day, blood is collected for estimation of liver biomarker enzymes. On the same day, liver is removed and stored in 10% formalin solution for the estimation of antioxidant parameters; and processing for histopathological studies.

Estimation of Biochemical and Antioxidant Parameters

SGOT and SGPT were estimated by Reitman and Frankel method, ALP was estimated by kind king's method. Total Bilirubin, total cholesterol were estimated by Jendrassik and Grofs method and CHOD/POD method respectively. Antioxidant parameters were estimated by according to reported methods SOD [14], CAT [15], GSH [16], GPx [17], GRx [18] and lipid peroxidation [19].

Histopathological studies

Livers from rats were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin Eosin (H&E) for light microscopic analyses.

Statistical analysis

The results are presented as Mean ± S.E.M (n=6 in each group). Analyses were performed using One-way ANOVA followed by Tukey posthoc for the difference between the control and treatment groups.

RESULTS

On acute toxicity studies

The ethanolic extract of *Mirabilis Jalapa* Linn leaves was found to be safe since no animal died even at the dose of 2000 mg/kg when administered orally and the animals did not show any gross behavioral changes.

On Biochemical Parameters

Animals treated with anti-tubercular drugs (toxic control) showed a significantly elevated levels (P<0.05) of SGOT, SGPT, ALP, total bilirubin and total cholesterol levels; and significantly decreased (P<0.05) in HDL levels when compared to control group. EMJ 250 mg/kg and 500 mg/kg given with one hour prior administration of anti-tubercular drugs showed a significant decreased serum diagnostic liver enzymes and increased HDL levels in a dose dependent manner when compared to toxic control. The results are presented in the **table 1**.

Table 1: **Effect of ethanolic extract of *Mirabilis Jalapa* Linn leaves on serum SGOT, SGPT, ALP, TB, Total cholestrol and HDL in rats**

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (IU/L)	Total cholesterol (mg/dl)	HDL (mg/dl)
Group-I	Control	122.67± 6.38	63 ±3.02	182 ±6.27	0.06 ±0.01	83.5±6.3	33.8 ±2.1
Gropu-II	Anti-TB Drugs (Toxic control)	386.50 ± 14.60#	639.33 ±17.96 #	315.83 ±7.46 #	0.39 ±0.01#	131.3±16.2#	17.4 ±1.9
Group-III	Silymarin (100mg/kg)	166.67 ±2.90***	136.33 ±5.76*	138 ±9.65***	0.08 ±0.02**	88.7±5.9***	33.6± 2.4***
Group-IV	EMJ (250mg/kg)	306.67 ±9.89*	282 ±13.43*	208.66 ±6.93**	0.15 ±0.01*	129.3 ±14.2**	20.9 ±3.6*
Group-V	EMJ (500mg/kg)	201.67 ±12.90**	192.33 ±16.64**	160.66 ±4.84*	0.08 ±0.04**	118.1 ±13.1**	27.7 ±3.1*

Data are expressed as Mean ± S.E.M (n=6), One-way ANOVA Tukey posthoc; #p≤0.05 vs. Control (Group I); *p≤0.05 vs. Toxic control (Group II); **p≤0.01 vs. Toxic control (Group II); ***p≤0.001 vs. Toxic control (Group II).

Invivo Antioxidant parameters

In the present study, antioxidant parameters were assessed in the liver homogenate. Oral administration of anti-tubercular drugs (toxic control) significantly (P<0.05) decreased SOD, CAT, GPx, GRx, GSH and significantly (P<0.05) increased TBARS when compared to control group. EMJ 250mg/kg and 500mg/kg with one hour prior administration of anti-tubercular drugs showed significantly increased the enzymatic and non-enzymatic levels and significantly decreased TBARS levels in a dose dependent manner when compared to toxic control. The results are presented in **table 2**.

Table 2: Effect of ethanolic extract of *Mirabilis Jalapa* Linn leaves on antioxidant parameters in rats

Group	Treatment	SOD (µmol/min /mg)	CAT (µmol/mg/ min)	GPx (µmol/mg/ min)	GRx (µmol/mg/ min)	TABRS (nM/min/ mg)	GSH (nM/min/ mg)
Group I	Control	3.55±0.26	34.99 ±0.98	28.99 ±0.64	30.81 ±0.94	34.95 ±1.98	2.47 ±0.12

Group II	Anti-TB Drugs (Toxic Control)	1.62 ±0.66#	15.49 ±0.57#	12.64 ±0.66#	18.34 ±0.31#	88.29 ±5.88#	0.74 ±0.06#
Group III	Silymarin (100mg/kg)	3.71 ±0.19***	33.89 ±0.08**	29.91 ±0.82**	30.86 ±0.44***	33.89 ±3.23***	2.27 ±0.09**
Group IV	EMJ (250mg/kg)	2.08 ±0.78*	23.44 ±0.89*	20.32 ±0.18*	22.89 ±0.26*	47.28 ±4.10*	1.14±0.04*
Group V	EMJ (500mg/kg)	2.53 ±0.38*	29.33 ±0.53**	25.51 ±0.24*	27.55 ±0.62**	35.84 ±2.43**	1.77 ±0.06**

Data are expressed as Mean ± S.E.M (n=6), One-way ANOVA Tukey posthoc; #p≤0.05 vs. Control (Group I); *p≤0.05 vs. Toxic control (Group II); **p≤0.01 vs. Toxic control (Group II); ***p≤0.001 vs. Toxic control (Group II).

HISTOPATHOLOGICAL SLIDES

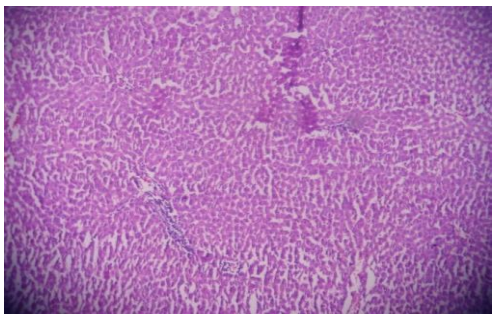

Figure 1: Control (Normal saline 1 ml/kg) – Hepatocytes showed a normal lobular architecture of the liver

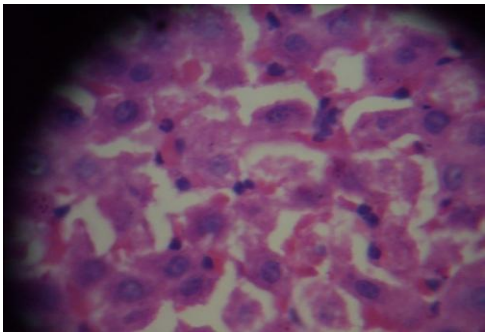

Figure 2: Toxic control (anti-tubercular drugs)– Hepatocytes showed liver cell necrosis & inflammation observed in the centrilobular region with portal triaditis

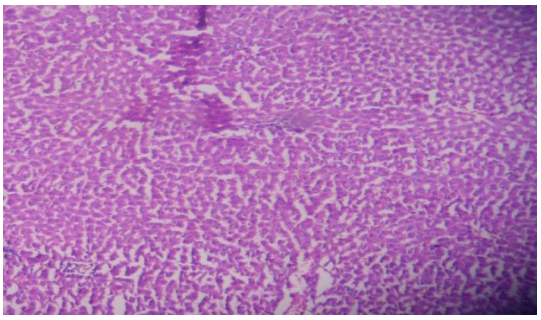

Figure 3: Standard (silymarin-100 mg/kg) +One hour prior administration of anti-tubercular drugs- Hepatocytes showed normal lobular architecture of the liver

Histopathological study of the liver

Hepatic control group animals showed significant liver cell necrosis and inflammation in the centrilobular region with portal triaditis as compared to normal control group. EMJ 500mg/kg showed protective effect on the hepatocellular necrosis and their lobular structure was normal **(figure 1 to 5)**.

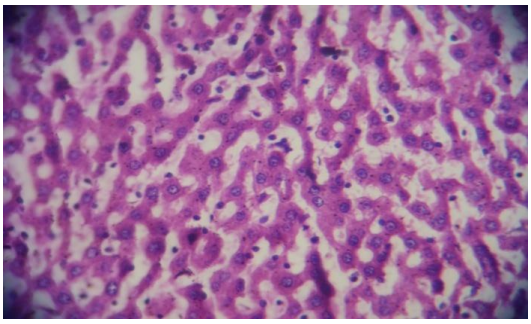

Figure 4: Group VI- (EMJ 250mg/kg) + One hour prior administration of anti-tubercular drugs-Hepatocytes showed minimal inflammation with moderate portal triaditis and their lobular architecture is normal.

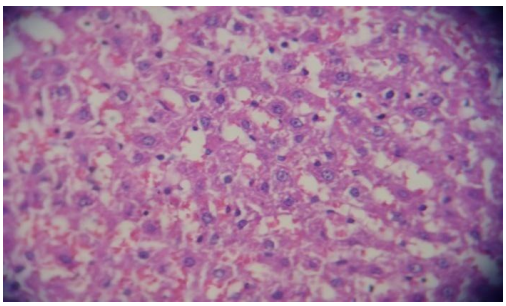

Figure 5: Group V- (EMJ 500mg/kg) + One hour prior administration of anti-tubercular drugs-Hepatocytes showed group showed normal hepatocytes and their lobular architecture is normal.

DISCUSSION

Hepatotoxicity of anti-tubercular drugs is a serious adverse drug reaction because it causes significant morbidity and mortality. Isoniazid, rifampicin, and pyrazinamide each initself are potentially hepatotoxic, when given in combination their toxic effects are enhanced [20]. In the present study, the combination of anti-tubercular drugs was used as a tool to induce the hepatotoxicity in experimental animals [21].

As shown in table 1, daily administration of anti-tubercular drugs (HRZE) for 35 days result in hepatic injury as confirmed by elevated levels of serum diagnostic enzymes such as SGOT, SGPT and ALP levels. At the time of hepatic injury, these enzymes leak out from liver into the blood circulation due to liver tissue damage. The treatment of EMJ, the levels of these liver marker enzymes in serum were near to normal, this may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by anti-tubercular drugs. Hepatotxicity is characterized by cirrhotic liver condition which in turn increases the bilirubin release [22]. The treatment of EMJ restored the level of bilirubin to near normal may be due to the inhibitory effect on mitochondrial enzymes responsible for the metabolism of anti-tubercular drugs.

The cholesterol levels are increased which might be due to uptake of LDL from the blood by the tissues [23]. Thus, EMJ may be effective on reduced cholesterol synthesis, and there by causes increased HDL levels.

SOD, CAT and GPx constitute a mutally supportive team of antioxidant enzymes which provide a defense system against reactive oxygen species (ROS) [24]. In the present study, SOD activity decreased significantly in toxic control animals due to an excessive formation of superoxide anions. The activities of H_2O_2 scavenging enzymes CAT and GPx decreased significantly in hepatic control animals. Reduction in these enzyme activities can be explained by excessive superoxide anions may inactivate SOD, thus, resulting in an activation of the H_2O_2 scavenging enzymes. The treatment of EMJ effectively prevented the decrease in SOD, CAT and GPx activities.

Attri *et al* 2000 reported that antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system. The depletion of antioxidant defenses and/or rise in free radical production deteriorates the prooxidant-antioxidant balance, leading to oxidative stress induced cell death. A marked increase in the concentration of TBARS in toxic control animals indicated that enhanced lipid peroxidation. The treatment of EMJ showed ability to prevent the anti-tubercular drugs induced elevation of TBARS level, suggesting that *Mirabilis jalapa* inhibited the hepatic lipid peroxidation. It implies that reduction in free radicals production and subsequent decrease in damage to the hepatocellular membrane.

In oxidative stress, GSH is converted into glutathione disulfide and depleted leading to lipid peroxidation. Hence, the role of GSH is a marker for the evaluation of oxidative stress. In toxic control animals observed depletion of may be due to increased utilization. The treatment of EMJ restored hepatic GSH content. The effect of EMJ may be due to an initial reduction in hepatic peroxidative activities, thereby leading to restoration of the GSH content

A histopathological observation shows EMJ has reduced cloudy swelling, fatty degeneration, heavy haemorrhage and hepatocellular necrosis. The treatment with EMJ normalized the anti-tubercular drugs induced histopathological changes, therefore it is suggested that hepatoprotective activity of EMJ against anti-tubercular drugs induced hepatotoxicity might be due to its property of reducing oxidative stress.

CONCLUSION

From the results, it is clear that EMJ has hepatoprotective activity at the dose of 500 mg/kg as compared to toxic control. On phytochemical investigation of EMJ revealed the presence of alkaloids, carbohydrates, reducing sugars, phenolic compounds, tannins, flavonoids, and glycosides, which contributes antioxidant potential and probably this, may be responsible for hepatoprotective activity.

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