AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Evaluation of Hepatoprotective Activity of Methanolic Extract of *Justicia Beddomei* (Clarke) Bennett Against INH and Rifampicin Induced Hepatotoxicity

Akash Marathakam[1]*, Kannappan N[2], Santhiagu A[3]

1.Department of Pharmaceutical Chemistry, National College of Pharmacy, Calicut, Kerala
2.Department of Pharmacy,Annamalai University, Annamalai Nagar 608002, Tamil Nadu.
3.Department of Biotechnolgy , National Institute of Technolgy , Calicut, Kerala

ABSTRACT

The hepatoprotective effects of Methanolic Extract of Justicia Beddomei (MEJB)on Isoniazid and Rifampicin (INH and RIF) induced hepatotoxicity and the probable mechanism(s) involved in this protection were investigated in rats. Male wistar rats were divided in to five groups and used for the study. Hepatotoxicity was induced in animal models and was compared with the protective effect of standard drug Silymarin. In the present study it was noted that the administration of INH, Rifampicin decreased the level of total protein, albumin and increased the level of liver enzymes AST, ALT, ALP and TC. These parameters were brought back to the near normal level in control group treated with MEJB. The histopathological studies also revealed that treatment with Methanolic Extract of Justicia Beddomei (MEJB) showed a protection against injuries due to the effect of INH, Rifampicin. These results suggets that MEJB showed significant hepatoprotective activity against Isoniazid and Rifampicin induced hepatotoxicity and this might be due to the presence of flavanoids and phenolics. Further research is sought to explore the mechanism of action and phytoconstituents responsible for the pharmacological action.

Keywords:- INH ,Rifampicin, Hepatoprotective, Justicia Beddomei

*Corresponding Author Email: akashmarathakam@gmail.com
Received 15 January 2014, Accepted 20 January 2014

Please cite this article in press as: Marathakam A *et al.*, Evaluation of Hepatoprotective Activity of Methanolic Extract of *Justicia Beddomei* (Clarke) Bennett Against In and Rifampicin Induced Hepatotoxicity. . American Journal ofPharmTech Research 2014.

INTRODUCTION

The liver's highly specialized tissues regulate a wide variety of high volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions[1]Liver disease is a worldwide health problem among which Jaundice and hepatitis are two major hepatic disorders that account for high death rate[2.] In the present study Isoniazid (INH) and Rifampicin (RIF) are used to induce hepatotoxicity. These drugs are the most important first line anti-tubercular drugs which have been used for the treatment of TB are hepatotoxic[3].The frequency of hepatotoxicity is increased when these drugs are used in combination [4]. A meta-analysis of studies involving several anti-tubercular drugs regimens estimates the incidence of liver toxicity is 2-6% with co-administered isoniazid and rifampicin and 1.1% with rifampicin alone[5]. Anti-tubercular drugs mediated oxidative damage is generally attributed to the formation of the highly reactive oxygen species, which acts as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane. Increased protein catabolism and urea formation that are seen in anti-tubercular drug induced hepatocellular damage may also be responsible for the increased levels of amino transferases in liver [6]. Various experimental animals are used to investigate hepatitis; however INH and RIF induced hepatitis rat models have been used in model of drug induced hepatitis[7].

There is a growing trend to follow systematic research methodology and to evaluate the scientific basis for traditional herbal medicines that are claimed to possess herbal medicines that are claimed to possess hepatoprotective potential [8]. The genus Justicia comprises about 300 species world over and nearly 50 species occur in India [9,10]. About 20 species have been chemically investigated and reported to contain lignans, triterpenoidal glycosides and amide. The plants of *Justicia* genus are rich sources of bioactive lignans particularly arylnaphthalides [11]. The plant has been found to be having anti diabetic, anti-inflammatory and anthelmintic properties [12, 13]. The plant is distributed in the hilly regions of Kerala. Leaves of Justicia beddomei are used in diarrhoreal bleeding and irritative cough. Flowers are used in ophthalmic preparations and the roots along with the leaf juice are used in pthisis, cough, and asthma [14]. In traditional folk medicine the plant is extensively used in single dosage medicament for jaundice .Since no hepatoprotective studies has not been reported for the plant justicia beddomei and the methanolic extract showing excellent anti-oxidant ability[15] has been used for hepatoprotective studies.

MATERIALS AND METHOD

Plant material

The plant material was collected from hilly regions of Kerala, India. The plant was botanically identified by Dr.V. Chelladurai, Research Officer Botany, (Rtd) CCRAS, Government of India. A voucher Specimen has been kept in the department of chemistry (NCP/CH/PS/JB01), National College of Pharmacy, Calicut.

Extraction

The aerial parts of Justicia beddomei were dried, grounded into fine powder and sieved through No. 20 mesh sieve. About 100 g of powdered aerial part was subjected to soxhlet extraction with 500 ml methanol. After extraction, the extract was concentrated and dried at room temperature. The percentage yield of methanolic extract was 8.8%w/w.

Chemicals and instruments:-

All the materials used for this experiment were of analytical grade. The chemicals used were manufactured by Sigma Chemical Co. Diagnostic kits for the estimations were manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Standard or gastric cannula was used for oral drug administration.

Acute Toxicity studies:-

The acute toxicity for 70% MEJB was determined on male wistar rats ,maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose method of OECD guideline No.420 given by CPCSEA was adopted for toxicity studies

Animals:-

Male wistar rats (180-220 gm) were supplied by central animal house, KMCP. Throughout the study, rats were housed in temperature controlled rooms with 12h light dark cycle, and were free access to food and water. For hepatotoxicity model, 100 mg/kg per day of INH and RIF each was used in the study [16]. INH and RIF solution were prepared separately in sterile distilled water. Rats were treated with INH, co-administered with RIF for 21 days. For the hepatoprotective model, 200 mg/kg and 400mg/kg per day of Methanolic extract of Justicia Beddomei (MEJB) along with INH&RIF solution was administered.

Hepatoprotective activity:-

Animals were divided in to five groups Group I animals were given normal saline only (10ml/kg).Group II were the hepatotoxicity control animals which were given INH & RIF orally for 21 days. Group III were the standard group the animals on INH+RIF+SILYMARIN orally for 21 days.Group IV were given INH+RIF+ Methanolic extracts of Justicia Beddomei (MEJB) (200 mg/kg orally for 21 days).Group V animals were given INH+RIF+ Methanolic extracts Justicia Beddomei(MEJB) (400 mg/kg) for 21 days). Both Group IV & Group V animals were

the treatment group of animals. The hepatoprotective activity was evaluated biochemically and histopathologically.

Liver Function test:-

On day 21 the rats were anaesthetized and sacrificed 1 h after administration drug. The blood was collected by retro-orbital plexus method; the serum was separated by centrifugation and used for the estimation of Serum alkaline phosphatase (ALP),Serum AST,Serum ALT,Total protein and Albumin, Serum cholesterol.

Histopathological Studies:-

The liver was then subjected to histopathological study. Rats were sacrificed, livers excised, cleaned with saline and they were transferred into 10% neutral formalin solution, after one week liver tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5section, stained with haematoxylin-eosin dye and then observed under photomicroscope.

Statistical analysis:-

All the values are expressed asMean ±SEM and data analyzed by one-way ANOVA.. The level of significancewas found out by Newman Keul's multiple range tests wherein all thegroups are compared against control.

RESULTS AND DISCUSSION

During metabolism of INH, hydrazine can be produced both directly (from INH) and indirectly from acetyl hydrazine.Isoniazide metabolite, acetyl hydrazine and hydrazine have been implicated as the causative hepatotoxins.Oxidative activation of these metabolites in liver by cytochrome P450 (Cyto P450) monooxygenase and free radicals which are capable of causing liver injury in animals [17,18]. Thus cyto P450 enzymes are critical in hepatotoxicity that leads to reactive, toxic metabolites.

Liver Function test:-

The present study has been attempted to demonstrate the role of hepatoprotective activity of Methanolic Extract of Justicia beddomei in INH, Rifampicin induced hepatotoxicity at different doses. The results of hepatoprotective activity of MEJB at the dose of 200mg/kg b.w. and 400 mg/kg on rats intoxicated with INH, Rifampicin were illustrated in Table.1.

In Control group(G1) the basal level of liver enzymes [ALP, AST, ALT] in control were 120.39 ± 4.02, 145.07 ± 4.40, 75.38 ± 3.50 respectively. Total protein and albumin levels were 8.14 ± 0.47, 4.91 ± 0.35 respectively. The total cholesterol in the normal control were 80.61 ± 4.39.In Toxic control (G3)INH and Rifampicin administration for 21 drugs resulted in increased level of

cholesterol (158.11 ±8.17), accompanied by significant decrease in level of total protein (4.24 ± 0.20), total albumin (2.12 ± 0.22) and also significant increase in ALP (307.59 ± 6.49), ALT(176.14±8.88),AST(254.23±8.69) as compared to the control. In Treatment control there was significant decrease in cholesterol (96.43 ± 6.09), accompanied by significant increase in level of total protein (7.84 ± 0.75) total albumin level (4.54 ± 0.34) and also significant decrease in ALP (203.96 ± 5.42), AST (175.08 ± 6.53), ALT (83.03 ± 5.82) as compared to the toxic c4ntrol.

Table 1:Effect of Methanolic Extract of Justicia Beddomei on Serum Enzymes

Group	Total Protein(g%)	Albumin (g/dl)	Total Cholesterol(mg/dl)	ALT (u/l)	AST (u/l)	ALP (mg%)
G1	8.14±0.47	4.91±0.35	80.61±4.39	75.38±3.50	145.07±4.40	120.39±4.02
G2	4.24±0.20**a	2.12±0.22**a	158.11±8.17**a	176.14±8.88**a	254.23±8.69**a	307.59±6.49**a
G3	7.84±0.75**b	4.54±0.34**b	96.43±6.09**b	83.03±5.82**b	175.08±6.53**b	203.96±5.42**b
G4	6.79±0.35*b	3.95±0.33**b	114.12±7.12**b	123.03±6.01**b	210.09±7.39**b	264.77±6.03**b
G5	7.32±0.45**b	4.28±0.37**b	107.04±5.01**b	103.61±5.70**b	196.16±9.41**b	258.89±6.75*b

All values are expressed as Mean ± SEM for n=6.

G1-Normal Control, G2-Toxic Control, G3-Standard Control, G4-Treatment Control, MEJB (200mg/kg), G5-Treatment Control, MEJB (400mg/kg)

**a-values are significantly different from control (G1) (P<0.001),

**b-values are significantly different from toxic control (G2) P<0.001

All values are found out by using one way ANOVA followed by Newman Keul's multiple range tests.

In G4 group of animals there was significant decrease in cholesterol (114.12 ± 7.12), accompanied by significant increase in level of total protein (6.79 ± 0.35), total albumin (3.95 ± 0.33) and also significant decrease in ALP (264.77 ± 6.33), AST (210.09 ± 7.39), ALT (123.03 ± 6.01) as compared to the toxic control. In G5 group of animals there was significant decrease in cholesterol (107.04 ± 5.01), accompanied by significant increase in level of total protein (7.32 ± 0.45), total albumin (4.28 ± 0.37) and also significant decrease in ALP (258.89 ± 6.75), AST (196.16 ± 9.41), ALT (103.61 ± 5.70) as compared to the toxic control.

In the present study it was noted that the administration of INH, Rifampicin decreased the level of total protein, albumin and increased the level of liver enzymes AST, ALT, ALP and TC. These parameters were brought back to the near normal level in Group 4 and Group 5 treated with Methanolic Extract of Justicia Beddomei (MEJB). Treatment with Methanolic Extract of Justicia Beddomei (MEJB) showed a protection against injuries due to the effect of INH, Rifampicin that may result from the interference with CYP450, resulting in the hindrance of formulation of hepatotoxic free radical. Attainment of near normal values of AST, ALT, ALP levels in INH and Rifampicin intoxicated animal groups with MEJB treated animals confirms the

hepatoprotective activity. The results of biochemical parameters and histopathological parameters supports the hepatoprotective activity of Justicia Beddomei.

Histopathological Studies

Rifampicin is a power inducer of mixed function oxidase contributes to hepatotoxicity of INH by enhancing the production of toxic metabolites. It is becoming increasingly apparent that many reactive intermediate formed during isoniazid metabolites are free radicals [19,20].The combination of INH and RIF was reported to result in a higher rate of inhibition of biliary secretion and a increase in liver cell lipid peroxidation and cytochrome P450 was thought to be involved in the synergistic effect of RIF on INH [21].

Histopathological observation of liver section from normal control group (G_1) shows normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein.(Figure:-1) InToxic control (G_2) INH, Rifampicin intoxicated group animal (G_2) showed total loss of hepatic architecture with centrilobular hepatic necrosis and fatty changes(Figure:-2),whereas Silymarin treated group (G_3) had normal liver architecture and occasional inflammatory cells with no traditis or necrosis.(Figure:-3). Histopathological pattern of the liver of rats ofTreatments (G_4)treated with INH, Rifampicin & lower dose of Methanolic Extract of Justicia Beddomei (MEJB) showed minimal necrosis, mild inflammation & less steatosis.(Figure:-4).

In Treatment group (G_5) Pretreatment with higher dose of Methanolic Extract of Justicia Beddomei (MEJB) shows mild inflammation and complete regeneration of hepatocytes & lobular architecture.(Figure:-5)

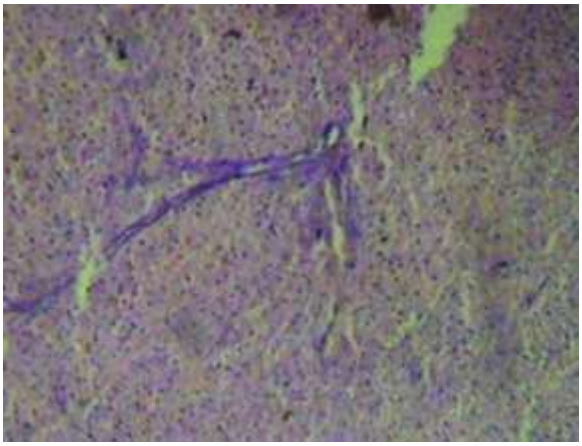

Figure 1 Normal Control Rat: Section of liver parenchyma with hepatocyte which appear normal, and central vein & portal tract are normal.

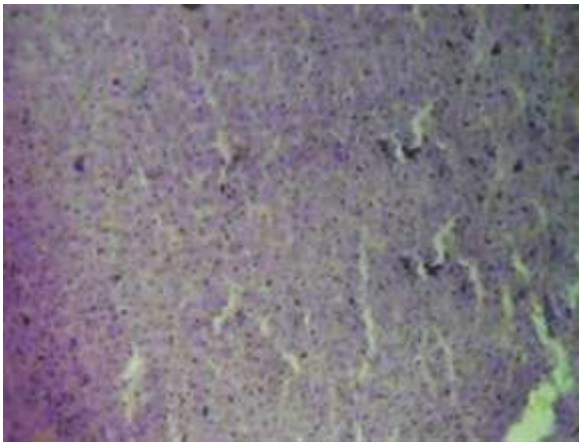

Figure 2 Toxic Control :-Section of liver parenchyma with scattered focal area of necrosis of hepatocyte.

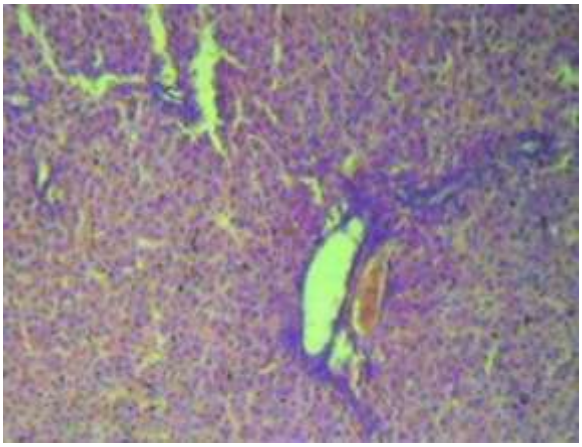

Figure 3 Positive Control Section of liver parenchyma shows normal hepatic architecture

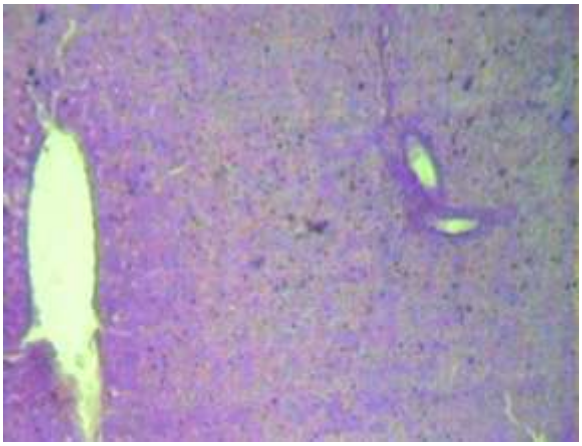

Figure 4 Treatment group (4) Rat Section of liver parenchyma with minimal necrosis, and minimal inflammation

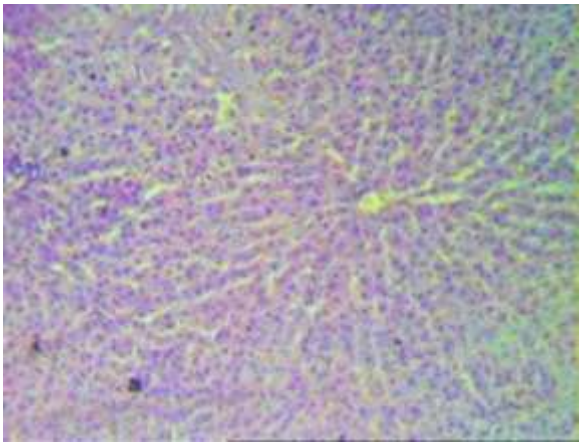

Figure 5 Treatment group (5) Rat Section of liver parenchyma with hepatocyte which appears normal and central vein & portal tract are normal

CONCLUSION:

In the present study, Methanolic Extract of Justicia Beddomei (MEJB) exhibit strong hepato protective activity, afforded protection from INH, Rifampicin induced liver damage. Hepato protective activity of Methanolic Extract of Justicia Beddomei (MEJB) may be due to free radical scavenging activity due to presence of Flavonoids and antioxidants. Additional studies are needed to better understand the mechanism of action of Methanolic Extract of Justicia Beddomei(MEJB) that is responsible for hepatoprotective activity.

ACKNOWLEDGEMENT

The authors are grateful to Dr.Chidambaranathan, KM College of Pharmacy,Madurai for providing valuable support and encouragement throughout the study.

REFERENCES

1. Anthea M. Hopkins J, McLaughlin CM, Johnson S, Warner Q V, David LaHart, Jill D. Wright 1993.Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall.
2. Ahsan R, Monirul Islam KM, Musaddik A, Hague E. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride induced hepatotoxicity in albino rats. Glob J Pharmacol.2009; 3(3): 116-122.
3. Yew WW, Leung CC. Antituberculosis drugs and hepatotoxicity. Respirology. 2006; 11: 699–707.
4. Tahagolu K, Atae G, Sevim T, et al. The management of anti-tuberculosis drug–induced hepatotoxicity. Int Journal of Tuberculosis and Lung Diseases. 2001; 5: 65–69
5. Steele M, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampicin.A meta-

analysis. Chest.1991;99:465–71

6. Sethumadhavan S, Theruvathil K, Sini et al. Hepatoprotective activity of chitosan against isoniazid and rifampicin-induced toxicity in experimental rats. European Journal of Pharmacology. 2007; 572 : 69–73 .
7. Pal R. Valphei K.Singh K. Rana S.V. Garlic confers hepatoprotection in isoniazid rifampicin induced hepatic injury. Ind. J. Gastro.2003; Suppl.1:A100.
8. Dhiman A, Nanda A, Ahmad S. A recent update in research on the anti hepatotoxic potential of medicinaal plants.2012; 10(2):117-126.
9. Rajasekhar D, Vanisree M, Subbaraju GV. Justicia Lignans: Two new aryl naphthalide lignans from Justicia neesii Ramamoorthy. Indian journal of Chemistry.1999; 38:713–17.
10. Subbaraju GV, Rajasekhar D, Kavitha J, Hsu FL, Kur-Ta-Chang. *Justicia* lignans: Part 10-Synthesis of tiruneesiin,the first neolignan from *Justicia* species. Indian Journal of Chemistry. 2007; 46B:357–359.
11. Subburaju GV, Kumar KKK, Raju BL, Pillai KR. Justiciresinol, A New Furanoid Lignan from Justicia glauca. J Nat product.1991; 54:1639–41.
12. Srinivasa U, Venketeswara Rao J, Krupanidhi AM, Divakar K. Antidiabetic activity of Justicia beddomei leaves in alloxan induced diabetic rats. J Res Educ Indian Med.2008; 45–8.
13. Srinivasa U, Krupanidhi AM, Venkateshwara Rao J, Shanmukhappa.Anthelmintic activity of leaves of Justicia beddomei. Ancient Science of Life.2007; 26(3): 1–3.
14. Warrier PK, Nambiar VPK, Ramankutty C. Indian medicinal Plants: A Compendium of 500 Species. New Delhi:Orient Longman 1996; pp.268
15. Marathakam A, Kannapan N etal Studies on phytochemical and In-Vitro antioxidant potential of *Justicia beddomei (Clarke) Bennett* ,Free Radicals and antioxidants.2012; 2(4) :26-29
16. Jiang Y, Ren-xiu P etal. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. Acta Pharmacol Sin.2004;25(5): 699 – 704.
17. Nelson SD, Mitchell JR, Timbrell JA, Snodgrass WR, Corcoran GB.Isoniazid and Iproniazid: activation of metabolites to toxic intermediates in man and rat.Science.1976;193(4256):901-903
18. Timbrell JA, Mitchell JR, Snodgrass WR, Nelson SD.IsoniazidHepatotoxicity,The Relationship. between Covalent Binding and Metabolism In Vivo.J Pharmacol Exp Ther.1980;213(2):364-369.

19. Noda A, Noda H, Ohno K, Sendo T, Misaka A, Kanazawa Y, Isobe R, Hirata M. Spin Trapping of a Free Radical Intermediate formed During microsomal Metabolism Of Hydrazine. Biochem Biophys Res Commun.1985;133(3):1086-91.
20. Trush MA, Mimnaugh EG, Gram TE. Activation of Pharmacologic agents to radical intermediates. Implications for the role of free radicals in drug action and toxicity. Biochem Pharmacol. 1982 ;31(21):3335–3346.
21. Skakun NP, Shman'ko VV.Synergistic Effect Of Rifampicin On Hepatotoxicity Of Isoniazid.Antibiot Med Biotekhnol.1985;30(3):185-189.