Hepatoprotective effect of Carica Papaya and Ficus Bengalensis Latex in Isoniazid as an antituberculosis drug Induced liver injury in Rats

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ABSTRACT: : *Hepatotoxicity is one of the major side effects of anti-tubercular drug like Isoniazid. The aim of Study was designed to evaluate the hepatoprotective activity of Carica Papaya Latex at the dose 400mg/k.g. and Ficus Bengalensis Latex at the dose 300mg/k.g. per oral induced by isoniazid at the dose 50mg/k.g. per oral. Group -I serve as a normal control received Saline 1ml/Kg. Group-II were received Isoniazid (50mg/kg.)- per oral. Group-III were received Carica Papaya Latex 400mg/Kg. b.w./oral 30 min, prior to Isoniazid (50mg/kg .). Group-IV were received Ficus Bengalensis Latex 300mg/Kg./oral 30 min, prior to Isoniazid (50mg/kg .). Group-V were received Silymarin suspended in 1% of CMC 100mg/Kg./oral 30 min, prior to Isoniazid (50mg/kg .) in sterile saline once daily for 30 days. Animals were sacrificed on 30 days. Blood were collected by Cardiac puncture. Hepatoprotective activity was observed by using bio-chemical parameters such as Serum-Glutamate Pyruvate Transaminase and Serum-Glutamate Oxalate Transaminase (SGPT and SGOT), total protein, Bilirubin, Alkaline Phosphate (ALP).*

Carica papaya Latex and Ficus Bengalensis Latex formed major hepatoprotective effect by decreasing the activity of serum enzyme as SGPT, SGOT, Bilirubin, ALP, and prominent total protein level. Both plant latex were observed to prevent the toxic effect of Isoniazid on above serum parameters.

Key Words:

INTRODUCTION

Antitubercular drugs are major cause of drug induced liver injury. The first line antitubercular drug having the highest hepatotoxic potential are pyrazinamede and Isoniazid[1]. The hepatotoxicity of Isoniazid is usually under reported but it has still been reported to be a leading cause of drug induced liver damage[2]. Isoniazid is first line antituberculosis drug[3]. Evidence shows that it produces hepatotoxicity is 12% of patients as serious adverse effect[4].

The hepatotoxicity of Isoniazid is thought to be initiated by cytochrome P450 mediated metabolism of IMH to acetyl hydrozene and hydrazine [5].Tuberculosis is a prevalent cause of morbidity and mortality in developing countries like India[6]. Drug induced liver injury has been identified to be a leading cause of hepatic dysfunction. The mechanism of the hepatic injury in most of the cases of drug induced liver damage remains unknown. Few suggested mechanism are direct injury to the hepatocytes by drug or its metabolite by generation of free radicals as reactive oxygen species[7]. Three mechanism i.e. direct cell stress, specific immune reaction as direct metochoandrial impairment are involved in drug induced hepatotoxicity[8]. Among the antituberculosis drugs, Isoniazid has been reported to be the drug that most frequently induces hepatoxitotoxicity[9]. It remains a widely used efficient first line agent for the treatment of tuberculosis[10]. Tuberculosis is a common problem in India and worldwide. Especially after the recent increase in incidence of AIDS and is a leading cause of adult deaths[11]. Carica papaya linn plant belongs to family Caricaceae is a tropical tree. It is native to the tropics of an Americas but now widely cultivated in other tropical regions of the world. Plant is available throughout the year[12]. Aqueous extracts of unripe Carica papaya have been reported antisickling and reversal of sickling properties [13]. Ficus Bengalensis plant is commonly known as Banyan tree in Ayurveda[14]. The extracts of Ficus Bengalensis have been reported to decrease insulinase activity from liver and kidney [15].

MATERIAL AND METHODS

Plant materials

Carica Papaya and Ficus Bengalensis were collected from Mathura, Uttar Pradesh. The authentication and identification was done by (Prof.) Dr. D.K. Singh, Department of Botany, KR (PG) College , Mathura, Uttar

Pradesh.

Carica Papaya Latex Collection

Latex was collected locally in early morning 7:00 to 8:00 am, as the flow of latex is low during the day. Collection was done by making 1-2 mm deep vertical incisions on the skin of unripe fruit, but mature fruit. The latex was then dry at room temperature till it became brittle and non-sticky. The dried latex was triturated using a mortar and pestle. It was stored at 4-8 ºC until use [16].

Ficus Bengalensis Latex Collection

Latex was collected locally in early morning 7:00 to 8:00 am, as the flow of latex is low during the day. Collection was done by making 1-2 mm deep vertical incisions on the skin [16]. At room temperature the Latex was extracted by maceration process (48h) in methyl alcohol after defatted with petroleum ether at (72h). The extracted was dried by rotatory evaporator under reduced pressure [17]. The dried latex was triturated using a mortar and pestle. It was stored at 4-8^{0}C until use.

Phytochemical studies

Phytochemical analyses was carried out on Carica Papaya Latex and Ficus Bengalensis Latex for the detection of various phytochemicals by following standard methods described in practical pharmacognosy by Trease and Evans.

Phytochemical Screening

Phytochemicals are chemical compounds that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not established as essential nutrients. The presence of, alkaloids, general test for glycosides (reducing sugars), tannins, anthraquinones, sterols and saponins ,flavonoids,were tested by simple qualitative methods (Trease and Evans, 1989)[18].

Experimental Animals

Albino rats of either sex (Wister strain) weighing 150-200g and female albino mice weighing 20-25g were used in this study. The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at twenty seven ºC ± 2 ºC , relative humidity sixty five ± 10% under 12 hours light/ dark cycle. The animals were fed with rodent pellet diet (Gold Mohur Lipton India Ltd.) and water *ad libitum.*

Ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethics Committee (IAEC) and registration number 1334/a/10/CPCSEA.

Determination of acute toxicity LD_{50}

The acute toxicityty for Carica Papaya Latex and Ficus bengalensis latex were determined in female albino mice. The animals were fasted overnight prior to the experiment, fixed dose method of OECD guideline No. 420; (Annexure 2d) of CPCSEA was adopted for this purpose. Group of three mice were taken for each test dose and $1/10^{th}$ of LD_{50} cut off value of test latex selected as screening dose for Hepatoprotective activity [19].

ISONIAZID INDUCED HEPATOTOXICITY

Group I- Serve as control (Saline 1ml/kg./p.o.)
Group II- Received Isoniazid (50mg/kg .)- P.O
Group III- Received Isoniazid (50mg/kg .) and carica papaya latex 400mg./kg./p.o.
Group IV- Received Isoniazid (50mg/kg .) and Ficus Bengalensis Latex 300mg./kg./p.o.
Group V- Received Isoniazid (50mg/kg .) and Silymarin(100mg/kg)/p.o.

Animals were divided in 5 groups in six animals in each. Group -I serve as a normal control received Saline 1ml/Kg for 30 days. Group-II were received Isoniazid (50mg/kg.)- P.O in sterile saline.Group-III were received Carica Papaya Latex 400mg/Kg. b.w./oral 30 min, prior to Isoniazid (50mg/kg .)in sterile saline once daily for 30 days. Group-IV were received Ficus Bengalensis Latex 300mg/Kg. b.w./oral 30 min, prior to Isoniazid (50mg/kg .) in sterile saline once daily for 30 days. Group-V were received Silymarin suspended in 1% of CMC 100mg/Kg. b.w./oral 30 min, prior to Isoniazid (50mg/kg .) in sterile saline once daily for 30 days.

Animals were sacrificed on 30 days. Blood were collected by Cardiac puncture under ether anesthesia and allowed to clot for 30 min. at rt. Serum were separated by centrifugation at 2500 rpm at 30º C for 15 min. Serum sample used for estimation of SGOT, SGPT Bilirubin, ALP and total protein through the auto analyzer for the study of the Toxic effect of Isoniazid and also therapeutic effect of plants Latex.

Biochemical Analysis

Estimation of SGPT on blood serum was carried out using AGD Clinipak from AGD Biomedicals Pvt. Ltd. Mumbai.

Estimation of Bilirubin on blood serum was carried out using diagnostic kit from SIEMENS Ltd., Vadodara,

Gujrat.

Estimation of Alkaline Phosphate, Total Protein, SGOT on blood serum was carried out using Diagnostic test kit from Beacon Diagnostic Pvt. Ltd., NAVSARI.

Histological studies

The Liver were isolated from the animals and washed with normal Saline. The liver was fixed in Formalin diluted to 10% with normal Saline then processed further for histological studies. The results were analyzed by student t-test.

Statistical Analysis

All the values are expressed as mean ±S.D. result were analyzed statistically by analysis of variance (ANOVA) followed by student T-Test was used for determining significance.

RESULT AND DISCUSSION

Result were observed that serum parameters like SGPT ,SGPT,Bilirubin,ALP,values were elevated and decreased the total protein level in the Isoniazid induced rats.This indicated that damage of liver in the Isoniazid induced rats .

Animals treated with Carica Papaya Latex at dose 400mg/kg/p.o and Isoniazid at dose 50mg/kg/p.o were exhibited to decrease in level of Serum parameters SGPT (116.8±0.25), SGOT(152.80±10.8), Bilirubin(1.58±0.02), ALP(138.16±8.6) and increased total protein level (6.44±0.06) compare to Isoniazid treated group at dose 500mg/kg b.w., SGPT(228.5±0.48), SGOT(274.16±8.6), Bilirubin(2.2±0.06) , ALP(228.23±12.4) and total protein level (5.64±0.043).

Whereas animals treated with Ficus Bengalensis Latex at dose 300mg./kg./p.o. and Isoniazid at dose 50mg/kg/p.o were showed to decrease in level of Serum parameters SGPT (132.4±0.18), SGOT(162.6±12.4), Bilirubin(1.64±0.08), ALP(148.24±4.4) and enhanced total protein level (6.17±0.03) compare to Isoniazid treated group.,

Result of Isonizid and Silymarin treated group were showed significant decrease in above said parameters results are shown in table No.1.

The result of present study found that administration of Carica Papaya Latex and Ficus Bengalensis latex were showed significant protection against Isonizid induced liver injury.

Histological observations

Histological study of Control groups animal were show normal architecture compare to Isonizid treated group.

Isoniazid treated group were exhibited macro vesicular fatty change around a central wane and large area of necrosis. Group treated with Carica Papaya Latex(400mg/kg .b.w.), Ficus Bengalensis Latex (300mg/kg .b.w.) and Silymarin (100mg/kg .b.w.) were observed the protection against Isoniazid drug induced hepatotoxicity and it shows the recovery of Macro vascular fatty change. Silymarin treated animals was shown the recovery of Hepatic architecture which was nearly similar to Control group. It shown in figure no. 1 to 5.

TABLE NO .1: Effect of Carica papaya Latex & Ficus Bengalensis Latex in Isoniazid induced Hepatotoxicity in Albino rats

S. No.	Groups	SGPT (1u/L)	SGOT (1u/L)	Bilirubin (myp/L)	ALP	T. Protein
1.	Control	56.32±0.16**	84.34±0.42**	0.92±0.04**	114.8±1.8**	7.36±0.012
2.	Isoniazid treated(50mg/Kg) p.o.	228.5±0.48	274.16± 8.6	2.2±0.06	228.23±12.4	5.64±0.043
3.	Isoniazid+ Carica Papaya Latex(400mg/kg/b.w/p.o)	116.8±0.25*	152.8±10.8*	1.58±0.02**	138.16±8.6	6.44±0.06
4.	Isoniazid+ Ficus Bengalensis Latex(300mg/kg/b.w/p.o)	132.4±0.18*	162.6±12.4	1.64±0.08*	148.24±4.4	6.17±0.03
5.	Isoniazid suspended in 1%of CMC+ silymarin suspended in 1%of CMC	64.22±0.24**	96.48±0.26**	0.86±0.06**	112.8±2.8	7.12±0.04

All values are expressed in mean ±SD; n=6 Control vs *P<0.05, **P<0.001

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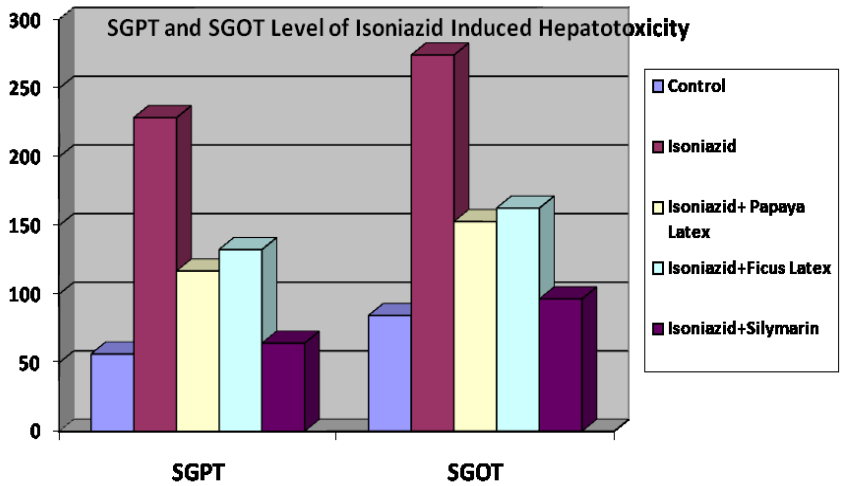

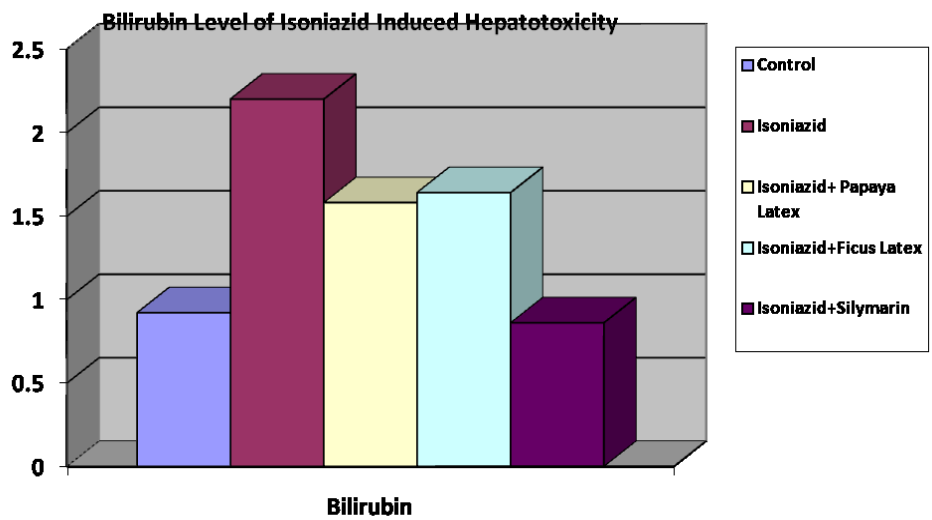

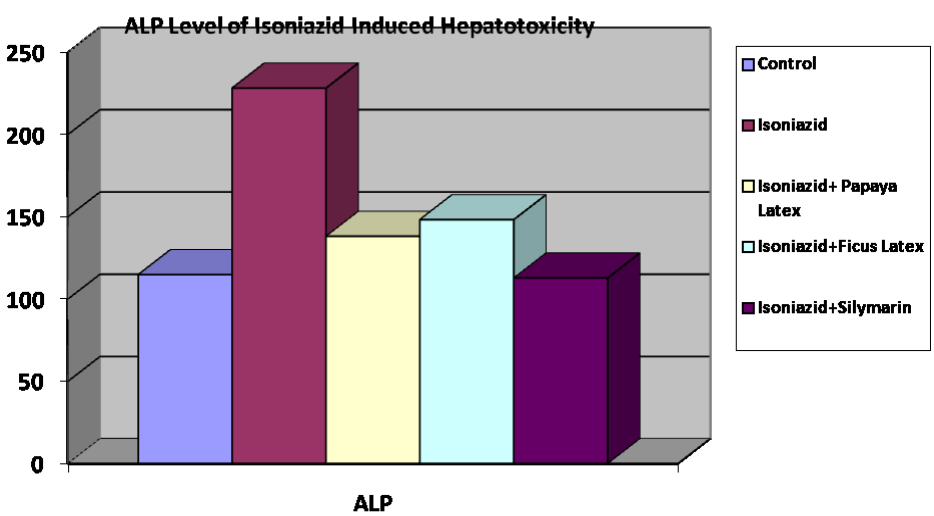

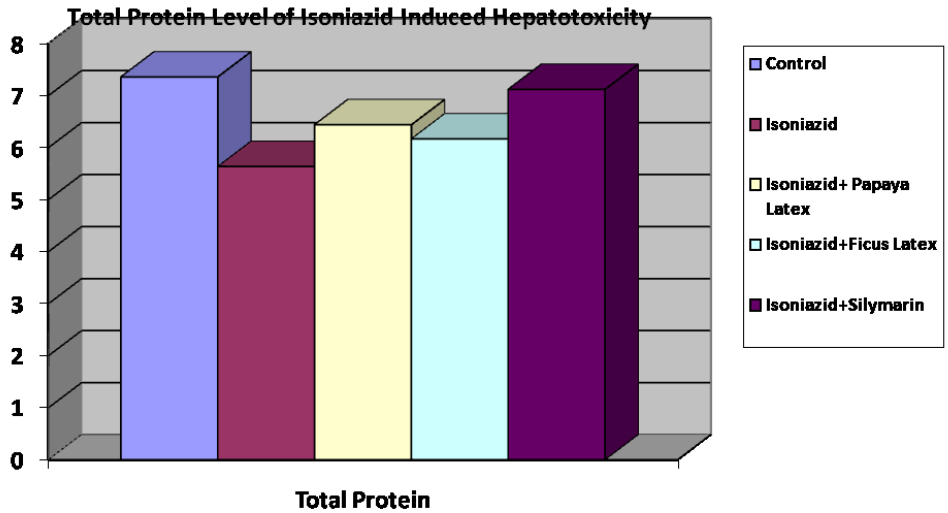

HISTOLOGY

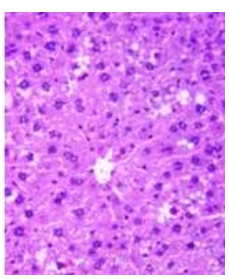

Figure 1: Control group

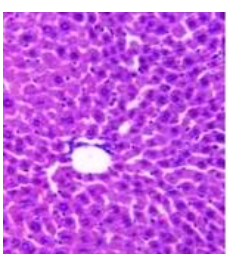

Figure 2: Isoniazid Treated Group

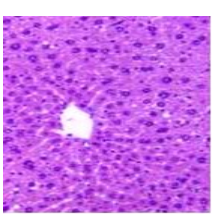

Fig. 3: : Isoniazid + C. Papaya Latex

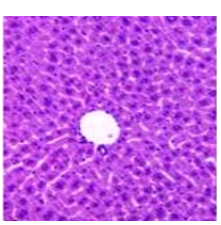

Fig. 4: Isoniazid + Ficus Bengalensis

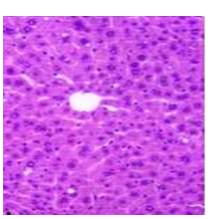

Figure 5: Silymarin treated group

Isonizid is a widely used drug for treatment of Tuberculosis but significant Hepatotoxicity has often been reported with the use of this drug[20].

Administration of Isonizid reduce many metabolic and detoxifying sites for anti tubercular drug. Isonizid get converted to acetylisoniazid by enzyme NAT2 which is eliminated by kidney, acetylisoniazid is further transformed into acetylhydrazine and then to potential hepatotoxic metabolite acetyldiazine by the CYP enzymes which generated reactive acetyl onium ion, acetyl radical and ketene, which cause irreversible damage to the liver tissue[21].

Groups treated with Carica Papaya (400mg/kg b.w.), Ficus-Begalensis (300mg/kg b.w.) and Silymarin (100mg/kg b.w.) were significant reduction in serum SGPT, SGOT, Bilirubin, , ALP and increased total protein level .It is shown in Table No.1.

Rats treated with Carica Papaya Latex were found that it is more significant reduction in enzymes compare to Ficus Bengalensis Latex. It shown in Table no. 1.

Phytochemical studies was found that Both plant carica papaya latex and ficus bengalenesis latex contains flavonoid ,carbohydrate, tannins ,saponins alkaloid, glycoside, protein.

CONCLUSION

This study concluded that both plant Carica Papaya Latex and Ficus Bengalensis Latex posses a hepatoprotective activity .

We found that the treatment with both latex prevented elevation of serum parameter like SGPT , SGOT, Bilirubin , ALP and increase the decreased level of total proteins due to Isoniazid , challenge models.

Treatment with both latex found that it improved the liver anatomy / hepatic anatomy with normalization of histological observation

A finding from the hepatoprotective study indicates that both latex were showed significantly reduced the elevated serum parameter induce by different hepatotoxin in rats .Where as Carica Papaya Latex was found more effective compare to Ficus Bengalensis Latex .

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