PROTECTIVE EFFECT OF *EMBELIA TSJERIAM-COTTAM* FRUIT EXTRACTS ON ISONIAZID INDUCED HEPATOTOXICITY IN WISTAR RATS.

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ABSTRACT

The objective of the present investigation was to study hepatoprotective activity of various fruit extract of *Embelia tsjeriam-cottam* in isoniazid induced liver damage in Wistar rats. Liver damage was produced by isoniazid (50gm/kg, p.o. For 30days). The Plant extracts (200mg/kg, p.o.) were administered every 24 hrs for thirty days, while standard group received Liv52. At the end of the study the marker enzymes in serum and histopathological analysis were carried out. The aqueous as well as alcoholic extract showed significant hepatoprotective activity.

Keywords: *Embelia tsjeriam-cottam; Hepatoprotective*; Isoniazid.

1. INTRODUCTION

Drug induced hepatotoxicity account for 9.5% of all suspected adverse drug reaction, and are the most common reason for withdrawal of drug from the market.[1]The liver is the central metabolizing organ, so it more susceptible to metabolism-dependent injury. Thus injury may be a direct toxic effect or immunological reaction to either of the drug or an active metabolite formed by bioactivation.[2] It is reported that 62% of withdrawn drugs have active metabolite[3]. Dose dependent hepatotoxicity is due to prolong administration or single toxic dose. The predominant clinical presentation is acute hepatitis, although almost any clinical pathological pattern of acute or chronic liver disease can occur. Several plants have been investigated and reported to possess antioxidant property and hepatoprotective activity e.g. *Baliospermum montanum*[4], *Ocimum sanctum*[5], *Tamarindus indica*[6] etc. Similarly *Embelia tsjeriam-cottam* is a widely distributed plant throughout the greater part of India, The fruit is given as anthelmentic and for piles. It is sometimes used as an antispasmodic and carminative.[7]

The survey revealed that *Embelia tsjeriam-cottam* leaf along with milk is used by the traditional healers for the treatment of jaundice and patient feedback is quite encouraging. However hepatoprotective activity of *Embelia tsjeriam-cottam* has not been scientifically investigated. Therefore, the present study is planned to investigate the effect of aqueous as well as other extracts of *Embelia tsjeriam-cottam* fruit in isoniazid induced liver damage in Wistar rats.

2. MATERIALS AND METHODS

2.1 Preparation of *Embelia tsjeriam-cottam* Extract:

Embelia tsjeriam-cottam fruits were collected from open field around the Belgaum city in the month of September were identified and authenticated by the taxonomist Dr. Harsha Hegde and the herbarium (voucher No. RMRC 487) has been preserved at Regional Medical Research Centre (Belgaum). Shade dried leaves were powdered to moderately coarse grade. Alcohol & aqueous extracts of fruits were obtained by using soxhlet extractor. The extraction was continued for 12 cycles or until the solvent in the thimble was clear. After evaporating the solvent, the dark brown semisolid extract was kept in an air tight container at 4^0c for future use. Suspensions of each extract were freshly prepared using 0.1% Tween 80, for experimental use.

2.2 Animals:

The complete course of the experiment was carried out using healthy adult male Wistar rats obtained from registered breeders (Venkateshwara Enterprises) Bangalore and were maintained at the Animal House of the Institution. They were fed on commercial laboratory animal feed (Amrut brand, Sangli) and tap water *ad lib*. The rats weighing between 120-150 g were housed for about a week for acclimatization with natural 12:12hr light – dark cycle. The animals were starved overnight with tap water *ad lib* prior to the day of experimentation. Ethical clearance was obtained from Institutional Animal Ethics Committee constituted as per CPCSEA guidelines.

2.3 Acute Toxicity Study

Acute toxicity studies were carried out for all the extracts as per OECD guideline 425[8] in Swiss mice weighing 25 to30gms by administering a dose 2000 mg/kg orally. The groups were almost continuously observed for mortality and behavioral changes during first 24hr and then daily for a fortnight. The oral LD_{50} was found to be more than 2000mg/kg.

2.4 Drugs used and their Doses:

In two groups (n=6, in each) of animals Alcoholic and aqueous, extracts of fruits were administered with the dose of 200mg/kg b.w. third group received Liv52 5ml/kg

b.w[9]. While fourth group received isoniazid 50mg/kg b.w., fifth group received equivalent volume of vehicle. All the treatments were administered orally for 30days.

2.5 Methodology

All the treatments were given for a total period of 30days, on the 31st day all the rats were anaesthetized by halothane to withdraw cardiac blood and the animals were sacrificed by over anesthesia to dissect out liver for histopathological studies and oxidative stress markers. Blood was allowed to coagulate for 30 min and serum was separated by centrifugation at 2500rpm, to estimate alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and billirubin content[12]. Liver is kept in cold conditions. It was cross chopped with surgical scalpel into fine slices in chilled 0.25M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in 10mM Tris-HCl buffer, pH7.4(10%w/v) with 25strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm.The clear supernatant was used for oxidative stress markers assays like lipid peroxidation[13], Reduced Glutathione[14], Superoxide dismutase[15] and Catalase[16].

2.6 Histopathological Studies

Five mm thick piece of the liver was fixed in Bouin's solution (mixture of 75ml of saturated picric acid, 25ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hr and then embedded in paraffin by conventional method and cut into 5nm thick sections. The sections stained with haematoxylin and eosins were observed under microscope (20 X) for histopathological changes.

2.7 Statistical analysis

The results were analysed by ANOVA followed by Tukey's multiple comparison test and $p \leq 0.05$ was considered as significant.

3. RESULTS

The groups treated with isoniazid alone (positive control) showed significantly elevated level of ALT, AST, billirubin and significantly decreased total protein content as compared to negative control (not challenged with isoniazid) animals. The animals treated with aqueous, alcoholic extract and Liv52 showed significant reduction in all the biochemical parameters. (Table 1) In vivo lipid peroxidation study reveals that rats of paracetamol treated group showed significant group showed significant increase in malondialdehyde (MDA) when compared with rats of normal control group. The alcoholic and aqueous extracts were able to significantly blunt this rise in MDA level. There was a marked decrease in the level of GSH and the activities of SOD and CAT in isoniazid treated group when compared with normal control group. The GSH level and activities of SOD and CAT were significantly increased in alcoholic and aqueous extract treated group. (Table 2)

Histopathological examination of liver from control group showed normal hepatic hexagonal lobules and normal morphology (Fig.a). The liver of the rats intoxicated with isoniazid there was vacuolation, sinus congestion, mild inflammation and centrilobular degeneration of hepatic cells with centrilobular necrosis (fig.b). Alcoholic extract, aqueous extract and Liv52 prevented the isoniazid induced hepatotoxicity. (fig.-c, fig-d, e).

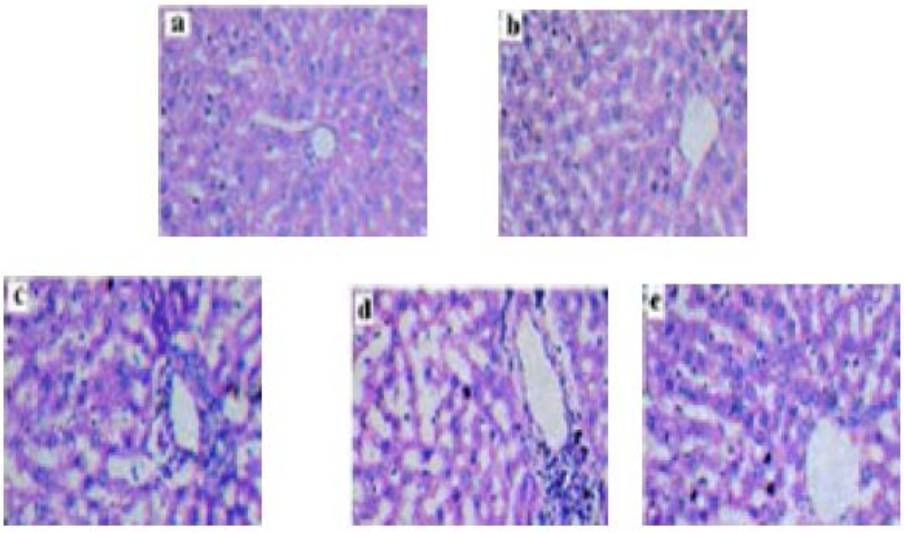

Figure a: Normal rat liver; **Figure b:** isoniazid treated rat liver, **Figure c:** liver treated with alcoholic extract, **Figure d:** liver treated with aqueous extract, **Figure e:** liver treated with Liv52

Table 1: Effect of *Embelia tsjeriam-cottam* in Isoniazid induced Hepatotoxicity

Biochemical Parameters					
Treatment/groups	AST (IU/L)	ALT (IU/L)	Total protein (g/dl)	Bilirubin (mg/dl)	
				Total	Direct
	Mean ± SEM				
Normal	45.67 ± 2.89	35.76 ± 1.40	7.60 ± 0.11	0.77 ± 0.03	0.30 ± 0.07
Isoniazid Control	83.33 ±1.49 #	188.3 ± 1.81 #	5.22 ± 0.16 #	1.90 ± 0.02 #	0.12 ± 0.01 #
Alcoholic Extract	56.00 ± 1.15 ***	85.35 ± 1.23 ***	7.21 ± 0.05 ***	0.86 ± 0.01 ***	0.23± 0.01 ***
Aqueous Extract	73.50 ± 1.62**	106.1 ±1.21***	6.44± 0.08***	0.53 ± 0.18***	0.24± 0.01***
Liv 52	56.50 ± 1.25 ***	80.21 ± 1.13 ***	6.76 ± 0.12 ***	0.92 ± 0.02 ***	0.24 ± 0.01 ***

ANOVA: *** p<0.001 considered significant as compared to isoniazid control group.
# p<0.001 considered significant as compared to Normal control group.

Table 2: Effect of *Embelia tsjeriam-cottam* on Biochemical parameters in Isoniazid induced Hepatotoxicity

Treatment/ groups	MDA	GSH	SOD	CAT
Normal	5.93 ±0.28	101.4± 1.31	45.36 ±1.22	280.9± 1.50
Isoniazid Control	25.96± 0.51#	50.35 ±1.26#	19.16±0.28#	92.75 ±1.43#
Alcoholic Extract	8.16 ±0.26***	81.56± 0.60***	36.30 ±0.56***	246.4 ±0.55***
Aqueous Extract	15.70± 0.23***	98.44± 0.44**	30.64 ±0.48**	259.5 ±0.89**
Liv 52	10.47± 0.12***	84.79± 0.60***	32.92± 0.62***	254.5 ±1.86***

One way ANOVA followed by Tukey's multiple comparison test. #P<0.001 when compared with Normal group.
***P<0.001when compared with INH control group

4. DISCUSSION

Findings of the present study clearly indicate that both water and alcoholic extracts of *Embelia tsjeriam-cottam* showed significant Hepatoprotective activity against isoniazid induced hepatic injury. Alcoholic extract appears to be better than aqueous extract since it significantly elevated total serum protein in contrast to aqueous extract. No similar reports could be traced in available literature.

Liv-52 which contains the various herbal plants mainly *Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis and Achillea millefolium* shows the hepatoprotective activity by the virtue of their antioxidant property and this is due to the presence of flavanoids, cynogenic glycosides and triterpines. *Embelia tsjeriam-cottam h*ave been reported to contain quercetin, rutin, hyperin, ferulic acid and beta sitosterol[17] in addition to alkaloids, tannins, saponins etc. beta-sitosterol, hyperin, quercetin and rutin have been reported to posses antioxidant activity.[18] Hepatoprotection offered by *Embelia tsjeriam-cottam* extracts could be attributed to these constituents. Since antioxidants have been reported to posses Hepatoprotective activity[19]. Phytochemical analysis of alcoholic extract had flavanoids, sterols, caratenoids, aqueous extract showed the presence of caretenoids probably extracts in providing hepatoprotection.

The present study was not aimed to elucidate hepatoprotective mechanisms of *Embelia tsjeriam-cottam* extracts. In order to confirm their antioxidant potential and to identify various enzymes involved in generating oxygen free radicals further studies are essential.

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