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An Investigation of Hepatoprotective Activity of Methanolic extract of *Ziziphus jujuba* on Experimentally Induced Ethanol Hepatotoxicity in Rats

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Abstract : This study investigated the hepatoprotective activity of aqueous extract of *Ziziphus jujuba* leaves against ethanol-induced liver injury in experimental rats. *Ziziphus jujuba* has been traditionally used in the Ayurvedic system of medicine as a chief ingredient in many polyherbal formulations as an antioxidant. The present study sought to evaluate the protective effect of orally administered methanolic extracts (150 and 300 mg/kg/day b.w, p.o) for 21 days on ethanol induced oxidative stress. Silymarin (100 mg/kg p.o.) was used as a standard reference. The following serum parameters such as Serum Glutamate Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), total and direct bilirubin, Total Protein (TP) and tissue antioxidant levels such as Glutathione (GSH) and Lipid Peroxidation (LPO)were evaluated and on the basis of the study we conclude that the control group did not exhibit increase in serum parameters, but ethanol toxicant group showed significant increase in serum parameters, Silymarin and aqueous extract of *Z. jujuba* (150 mg/kg p.o, 300 mg/kg p.o.) treated groups showed significant decrease in serum parameters.
Keywords : Hepatoprotection, *Ziziphus jujuba*, Total protein, Lipid peroxidation.

Introduction:

Liver is one of the largest organs in the human body and the main site for intense metabolism and excretion. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision, and reproduction[1]. Hepatic damage is associated with distortion of these metabolic functions[2] and sometimes, resulting in serious health problems. Over consumption of alcohol has been increased and is now a serious problem in Indian society and worldwide.Scientific research in herbal medicine with hepatoprotective activity may be a great benefit as an alternative therapy in alcohol-induced liver diseases. Three pathologically life-threatening liver diseases induced alone by alcohol abuse are fatty liver (steatosis), hepatitis and cirrhosis.

The main function of the liver is to expel toxic substances from the blood stream. This process may be interrupted if toxins prepare to enter the blood stream at a rate faster than the liver's ability to break them down

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and this can cause liver toxicity[3]. Increase in the level of liver enzymes ALT and AKP in serum in combination with increased bilirubin level are literally considered to be the most relevant sign of liver toxicity. In the absence of a consistent liver protective drug in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the healing of liver disorders [4].

In allopathic medicinal practices consistent liver protective drugs are not available, but herbs play a major role in the management of liver disorders[5]. In view of severe undesirable side effects of synthetic agents, there is an emergent focus to follow the systematic research methodology and to evaluate the scientific source for the traditional herbal medicines that are claimed to acquire hepatoprotective activity. Management of liver disease is still a challenge to the current medicine. In the absence of consistent liver-protective drugs in the allopathic medical practices, herbs show a vital role in the management of liver disorders.

Herbal medicine is an achievement of popular therapeutic diversity[6]. The world is now touching towards the herbal medicine or system, which can then properly fight foreign invaders and aid to destroy offending pathogens without toxic side-effects[7]. Herbal medicines are cheaper, easily accessible and their method of preparation is also trouble-free and above all it suits the societal and cultural needs of peoples[8].

Silymarin is promoted as one of the standard hepatoprotective herbal formulations. Among the medicinal plants, *Ziziphus jujuba* (family: Rhamnaceae) is a useful Indian medicinal plant which has been qualified with therapeutic properties to treat several diseases. It functions as an antidote, astringent, emollient, expectorant, hypnotic, narcotic, pectoral, tranquillizer and diuretic, it is used to treat cancer, skin and GI tract diseases. An active and safe drug is needed for the treatment of liver disorders. *Z.Jujuba* is an effectual herbal remedy. It supports weight gain, improves muscular strength and increases stamina. In Chinese medicine *Z. jujuba* was prescribed as a tonic to strengthen liver function. The leaves are astringent and febrifuge it is also said to promote hair growth[9].*Z. Jujuba* helps to resourcefully get the energy from food. It also gives energy and has an emotionally calming effect. In-vivo experiments illustrate that *Z.jujuba* can increase the phagocytosis of mice's mononuclear-phagocyte system[10]. The leaves are applied as poultices and are helpful in liver troubles, asthma and fever and it also strengthens liver function and stimulates immune system[11], the leaves are antipyretic and diminish obesity [12]. Extracts of jujuba leaves can appreciably inhibit dimethyl benzene-induced auricular inflammation in mice. They are used to form a plaster in the treatment of strangury[13].Ziziphin, a compound in the leaves of the jujube, stifles the ability to perceive the sweet taste in humans. On the basis of these facts, the present study was undertaken to evaluate the hepatoprotective effect of *Z.Jujuba* in rodent experimental model.

Materials and Methods:

Collection of plant material:

The leaves of Ziziphus jujuba were collected from the hill forest of Chittoor District, Andhra Pradesh. The characters were confirmed by Dr. K. Madhava Chetty, Assistant professor, Department of botany, S.V.University, Titupathi. A voucher specimen has been kept in the department for further reference.

Preparation of Extract:

15g of the powdered sample was taken in a thimble and placed in a Soxhlet apparatus and was extracted by the hot percolation method. The extract was carried out until the plant material, become colourless. The extract was collected and evaporated in a boiling water bath at 60°C. The residue was mashed and stored in an airtight container in a refrigerator.

Preparation of dose:

Based on previous studies, two doses (150 and 300 mg/kg/day) were selected.The experiments were conducted 1 h after the oral administration. In multiple dose study, the animals daily received the suitable oral dose of the aqueous extract of for a period of 21 days. The parameters were assessed on the 22nd day.

Animals:

Wistar rats weighing 150-250g procured from central animal facility of Institute. The animals were maintained in controlled temperature (24 ± 20C) as well as humidity (60-70%) in 12 –h light –dark cycles with standard diet and water will provide ad libitium. The care and the use of these animals were in accordance with the guidelines of the CPCSEA. An experimental protocol was approved by IAEC.

Assessment of hepatoprotective activity:

The animals of either sex weighing between 200 to 250 gm were divided into main five groups, six animals in each group. Group I received water (5ml/kg. p.o) and served as normal control. Group II received water (5 ml/kg. p.o) and 40% ethanol (v/v, 2.0ml/l00g body wt, p.o.). Group III received standard drug Silymarin (25 mg/kg. p.o.) and 40% ethanol (v/v, 2.0ml/l00g body wt, p.o.).Group IV received MEZJ (150 mg/kg) and 40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.) and Group V received MEZJ (300 mg/kg) and 40% ethanol (v/v, 2.0ml/l00g body wt, p.o.).The study was carried out for 21 days. On the 22nd day of the study the animals were sacrificed liver and blood samples were collected from each animal to generate the liver and serum biochemical assay.

Assessment of liver functions:

After 21 days of treatment with MEZJ, on 22nd day blood is collected by the puncture of retro-orbital plexus and then rats were sacrificed to collect liver which was immediately perfused with phosphate buffer solution. Serum was separated by centrifugation at 10,000 rpm in a laboratory centrifuge and assayed for Serum Glutamate Oxaloacetic Transaminase (SGOT)[12, 13], serum glutamate Pyruvate Transaminase (SGPT)[14], direct and total bilirubin[12], and Total Protein (TP)[15] using standard method. The tissue homogenate was analysed for antioxidant parameters such as Lipid Peroxidation (LPO) and glutathione (GSH)[16-19].

Histopathology studies:

A portion of liver tissue of all the animal groups was excised and then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48h and then with bovine solution for 6 h and were then processed for paraffin embedding. By using a microtome, sections of 5 micron thickness were taken and stained with hematoxylin and eosin.

Results:

Control group rats treated with ethyl alcohol developed a significant hepatic damage as evidenced by elevated serum levels of hepatospecific enzymes like SGPT, SGOT, total bilirubin, TP, and LPO and significant decrease in GSH when compared to normal control. Pretreatment with MEZJ 150 mg/kg showed better protection. Silymarin (100 mg/kg) and MEZJ 300 mg/kg showed highest protection against ethanol-induced toxicity to liver. Significant reduction was observed in elevated serum enzyme levels and LPO, and elevation in GSH in MEZJ treated animals compared to toxic control animals.

Table 1:Effect of MEZJ on SGOT, SGPT, T. Bilirubin, and T. Protein levels in Ethanol induced Hepatotoxic Rats:

Treatment	SGOT levels (U/L)	SGPT levels (U/L)	Total bilirubin (mg/dl)	Total protein (mg/dl)
Normal control	90.46 ± 5.85	60.28 ± 8.06	0.311 ± 0.030	9.53 ± 0.122
Ethanol intoxication (40%ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	138.0±11.28***	115.9±9.05***	5.82±0.043***	4.91±0.057***
Silymarin+40%ethanol (v/v, 2.0 ml/l00 body wt, p.o.)	80.98±5.88***	62.76±6.43***	0.38±0.014***	8.13±0.036***
MEZJ(150mg/kg)+40%ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	108.2±9.90**	79.3± 12.00**	0.52±0.057**	7.96± 0.259**
MEZJ(300mg/kg)+40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	98.98 ± 10.6**	69.86±8.007***	0.40±0.029***	7.65±0.194***

Values are expressed as mean+SEM (n=6), by one way ANOVA followed by Tukey test. Where,* represents significant at p<0.05, **represents highly significant at p<0.01, *** represents very significant at p<0.001compared to positive test.

Table2:Effect of MEZJ on Glutathione and LPO in Ethanol induced Hepatotoxic rats:

Treatment	Glutathione	LPO
Normal control	0.029 ± 0.002	0.078 ± 0.001
Ethanol intoxication (40%ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	0.007±0.003***	0.150±0.003***
Silymarin+40%ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	0.026±0.001***	0.076±0.011***
MEZJ(150mg/kg)+40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	0.024±0.003**	0.120 ± 0.009**
MEZJ(300mg/kg)+40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	0.022±0.005***	0.100±0.004***

Values are expressed as mean+SEM (n=6), by one way ANOVA followed by Tukey test. Where, * represents significant at p<0.05, **represents highly significant at p<0.01 ,*** represents very significant at p<0.001compared to positive test.

Table 3:Determination of liver weight of Rats:

Treatment	Liver weight in gm/100g
Normal control	6.54±0.06
Ethanol intoxication (40%ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	8.54±0.28*
Silymarin+40%ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	7.66±0.48***
MEZJ(150mg/kg)+40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	8.40±2.48**
MEZJ(300mg/kg)+40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.)	7.20±0.76***

Values are expressed as mean+SEM (n=6), by one way ANOVA followed by Tukey test. Where, * represents significant at p<0.05, **represents highly significant at p<0.01, *** represents very significant at p<0.001compared to positive test.

Histopathology:

Histopathological studies of liver also provided a supportive evidence for biochemical analysis. Histological changes such as steatosis (fatty changes in hepatocyte) and perivenular fibrosis wereobserved in ethanol treated (toxic) control group (**B**), no changes was observed in control(**A**),treatment with the standard (**C**) and plant extracts (**D,E**) has prevented these histological changes.

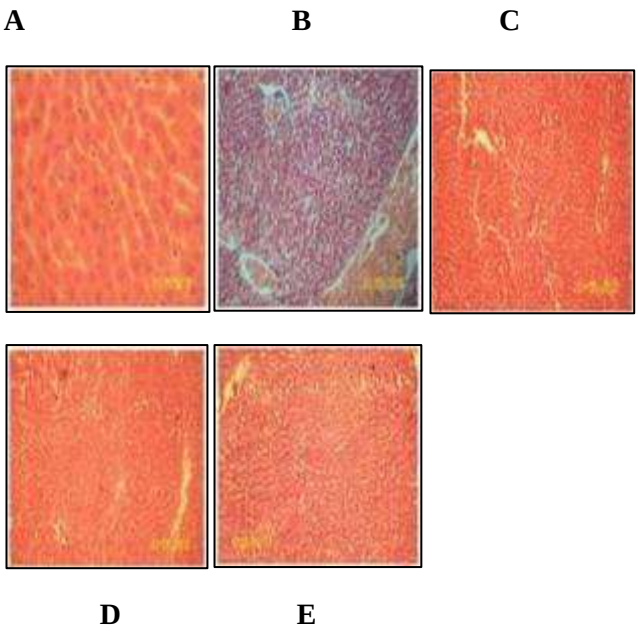

Figure 1:

Discussion:

It is well known that hepatocytes participate in various metabolic activities. Hepatocytes follow three main pathways for ethanol metabolism to produce acetaldehyde; the microsomal ethanol oxidizing system which is located in the endoplasmic reticulum, the alcohol dehydrogenase pathway of cytosol, and catalase located in the peroxisomes.The feeding of ethanol in this is chronic which results in appearance of a form of cytochrome P-450 which differs from other cytochrome P-450 species[20]. Furthermore, administration of alcohol leads to slight depletion of GSH due to conjugation of GSH with acetaldehyde. Alcohol produced marked liver damage as expected at given doses. The serum levels of SGOT, SGPT,total bilirubin increased and TP significantly decreased. Antioxidant parameters such as LPO increased and GSH decreased on administration of ethanol due to increased formation of lipoperoxides, conjugated dienes and malondialdehyde. Pre-treatment with MEZJ showed a dose dependent protection against injurious effects of alcohol which markedly decreased the levels of SGOT, SGPT, total bilirubin, and increase in TP resulting in the hindarance of the formation of hepatic free radicals. Pre-treatment with MEZJ also showed restoration of depleted GSH levels to the normal and also brought down the elevated levels of LPO. No side effects were also seen in the rats during the experiment. Therefore it has been suggest that hepatoprotective activity shown by methanolic extract of *Z.Jujuba* was confirmed by the biochemical and histopathological parameters.

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