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Hepatoprotective effect of ethanolic extract of *Prosopis cineraria* seed on CCl_4 induced Hepatotoxicity in Albino Rats

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Abstract
The ethanol fractions of *Prosopis cineraria* were subjected to preliminary phytochemical investigation and *in-vitro* antioxidant activity, the results of phytochemical investigation revealed the presence of carbohydrate, glycosides, flavonoids, tannins and proteins The sample VTRF-1 and VTRF-2 have shown potent antioxidant activity further were investigated for *in-vivo* antioxidant and hepatoprotective activity against carbon tetrachloride induced hepatotoxicity using biochemical markers. The test drug *Prosopis cineraria* samples (VTRF-1 & VTRF-2) showed significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity as evident by restoration of serum transaminases, alkaline phosphatase, bilirubin, triglycerides, cholesterol and protein level. Restoration of lipid peroxidation, glutathione and catalase contents suggests the antioxidant and hepatoprotective activity of VTRF-1, was more potent compare to VTRF-2 ethanol fraction.

Keywords: *Prosopis cineraria, in vivo,* hepatotoxicity, CCl_4

Introduction
The Hepatotoxicity suggests chemical by product driven liver damage or harm. Drug-induced liver harm could be a cause of intense and unremitting liver disease caused particularly by solutions and the foremost common reason for a sedate to be pulled back from the advertise after approval [1-3]. The liver plays a central part in changing and clearing chemicals and is helpless to the poisonous quality from these specialists. Certain restorative specialists, when taken in overdoses and now and then indeed when presented inside helpful ranges, may harm the organ. Other chemical operators, such as those utilized in research facilities and businesses, normal chemicals (e.g., microcystins), and home grown cures (Two conspicuous cases being kava, component obscure, and comfrey, through its pyrrolizidine alkaloid substance) can too actuate hepatotoxicity. Chemicals that cause liver harm are called hepatotoxins [4].

Liver is an critical organ that plays an fundamental part in directing different physiological forms within the body. It is included in a few crucial capacities, such as digestion system, discharge & storage. It has awesome capacity to synthesize valuable standards that control inside chemical environment. The most work of liver is digestion system & mien of xenobiotics by uncovering straightforwardly or in a roundabout way. Hepatotoxic operators such as carbon tetrachloride (CCl4), Paracetamol, Nitrosamine, Polycyclic fragrant hydrocarbons and overabundance utilization of liquor harm liver cells or degenerative maladies primarily inducing lipid peroxidation & free radicals.

Paracetamol is utilized around the world for its pain relieving and antipyretic activities. It incorporates a range of activity comparable to that of NSAIDs and takes after especially the COX-2 particular inhibitors. Paracetamol is, on normal, a weaker pain relieving than NSAIDs or COX-2 specific inhibitors but is regularly favored since of its superior resistance. In spite of the likenesses to NSAIDs, the mode of activity of paracetamol has been questionable, but it is presently for the most part acknowledged that it represses COX-1 and COX-2 through digestion system by the peroxidase work of these isoenzymes. This comes about in restraint of phenoxyl radical arrangement from a basic tyrosine buildup fundamental for the cyclooxygenase movement of COX-1 and COX-2 and prostaglandin (PG) blend.

Paracetamol appears selectivity for inhibition of the blend of PGs and related components when moo levels of arachidonic corrosive and peroxides are accessible but on the other hand, it has small action at considerable levels of arachidonic corrosive and peroxides [5]. There are many sedate which cause various side impact conjointly cause poisonous quality so there's a plant based normal sedate.

Prosopis cineraria (L.) Druce. (Family Leguminosae, subfamily Mimosoideae), is locally known as Ghaf. The plant impacts socially, ethnologically, customarily and remedially in the life of the individuals. The propositionis to summarize the current thinks about on up-to-date and comprehensive data, highlighting bioactive potential of the plant. The survey pointed to revise the data accessible with respect to the helpful exercises distributed in logical diaries and to supply a comprehensive arranged information in such a way that it is valuable for scholarly analyst. This paper incorporates a amalgamation of data, the utilize of innate, conventional and scientific information gathered within the past a long time driving to helpful potential of the plant. A through logical look of *P. cineraria* (L.) was carried out on phytochemical constituents *viz*. leaf, stem, case, bark, and their pharmacological data and security. The plant impacts socially, ethnologically, customarily and remedially within the life of the individuals. The *Prosopis cineraria* (L.) Druce happens in most of the world's hot bone-dry and semi-arid locales as local or presented species. The Incredible Indian Leave, prevalently known as the Thar, the bone-dry locales is characterized by the amazingly parched climate with moo and sporadic precipitation, dry air and tall wind speeds. *P. cineraria* (L.). Its populace is centered on the of India and Pakistan, but littler populaces happen in Iran, Afghanistan, and the Arabian Peninsula [6-10].

Material and Methods

Animals

The Albino Wister rats weighing 150-200g were used for the study. The inbred colonies of rats were collected from IIRT, GLP certified Laboratory, Ghaziyabad UP. They were maintained in the animal house of SET's IIRT, GLP certified Laboratory, Ghaziyabad UP for experimental purpose. The animals were maintained under controlled conditions of temperature (23±2 °C), humidity (50±5%) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad *libitum*. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of IIRT Labs, Ghaziyabad, UP, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India [11, 12].

Plant material

The whole plant of *Prosopis cineraria* Linn was collected from in and around Jaipur district, Rajasthan, India and was authenticated by Dr. Choubey Lecturer in Botany, Janta P.G. College, Rewa, M P (India). A voucher specimen has been kept in the herbarium of pharmacognosy.

Preparation of Extract

Preparation of crude powder

The collected seed material will be manually cleaned to remove course impurities and then air dried in shade at a well-ventilated place in the laboratory. Further drying will be done in the incubator to remove moisture at a temperature of 40 °C. The dried bark will be crushed and grounded in electric mixer-grinder to form crude powder and stored in air tight container.

Preparation of alcoholic extracts of seed of *Prosopis cineraria*

The authenticated *Prosopis cineraria* seed will be extracted successively with Ethanol 90%.

Qualitative chemical investigation

Endophytic fractions from *Prosopis cineraria* were subjected to qualitative chemical investigation. A saturated solution of the fractions were dissolved in methanol and filtered. Then the solutions were used for chemical tests. The following chemical tests were performed, *viz* [12-14].

Test for carbohydrates

Molish's test was performed as the general test, followed by test for reducing sugars (Fehling's test and Benedicts test), monosaccharides (Barfoed's test),hexose sugars (Cobalt chloride test, and Selwinkoff's test), non-reducing sugars and non-reducing polysaccharides (Iodine test, Tannic acid test for starch).

Test for proteins

Biuret test was performed as general test, followed by Millon's test, Xanthoprotein test, test for proteins containing sulphur, and precipitation test.

Test for Amino acids

Ninhydrin test as general test, followed by test for tyrosine, tryptophan and cystein.

Test for Steroids

Salkowski test, Libermann-Butchard reaction, Libermann reaction.

Test for Triterpenoids

Salkowski reaction, and Libermann-Butchard reaction.

Test for Glycosides

Test for Alkaloids

Dragendroff's test, Mayer's test, Hager's test and Wagner's test.

Test for Flavonoids

Shinoda test and ferric chloride test.

Pharmacological evaluation

Acute Toxicity Study

The acute toxicity tests for endophytic fractions from *Prosopis cineraria* Linn were performed on albino mice of either sex weighing between 20-30 gm as per OECD Guidelines. The animals were fasted overnight prior to the experiment. The Up and Down method was adapted for toxicity studies. The maximum non-lethal dose was found to be 2000 mg/kg body weight. Hence $1/10^{th}$ of the dose was taken as effective dose (200 mg/kg body weight) for the

fractions to evaluate hepatoprotective and antioxidant activities.

Experimental design

1. **Group 1:** Normal Control rats treated with 0.9% NaCl-2 ml/kg day.
2. **Group 2:** Rats treated with CCl4 (2ml/kg i.p. in olive oil).
3. **Group 3:** Rats treated with fraction VTRF-1 (200mg/kg p.o) + CCl4.
4. **Group 4:** Rats treated with fraction VTRF-2 (200mg/kg p.o) + CCl4.
5. **Group 5:** Rats treated with Silymarin (100 mg/ kg p.o) + CCl4.

Results

The extract fractions of VTRF-1 and VTRF-2 were obtained by flash rotary evaporation process with solvent ethanol. The % yield were found to be 0.78 -1.4% for VTRF-1 and 0.89-1.65% for VTRF-2.

Preliminary phytochemical screening

The qualitative chemical investigation of ethanol extracts of *Prosopis cineraria* Linn. was carried out to find the presence of various chemical constituents and the results are given in Table 1. It is observed that flavonoids, glycosides, protein, triterpinoid and tannins were present in the fractions.

Table 1: Results of Qualitative Chemical Tests

Phytochemical Constituents	Ethanol fractions			
	VTRF-1	VTRF-2	VTRF-3	VTRF-6
Alkaloids	- -	- -	- -	- -
Carbohydrates	+	+	+	+
Flavonoids	++	++	+	++
Glycosides	++	+	+	+
Tannins	+	+	+	- -
Steroids	- -	- -	- -	- -
Saponins	- -	- -	- -	- -
Triterpinoid	+	+	+	+
Proteins	+	+	+	+

Note: - absent ++ more clarity + Present +++ better response

Effect of VTRF-1 & VTRF-2 on biochemical markers in CCl4 induced hepatotoxicity

There was a marked increase in AST (IU/L) levels observed in CCl4 treated group (264.34±17.83 as shown in Table 2). However, the AST levels were reversed to near normal levels with the treatment of 200 mg/kg VTRF-1 to (185.98±28.03^{b}) and 200 mg/kg VTRF-2 to (182.15±18.24^{b}) with significance of (p<0.01) in both fractions. Serum ALT levels have been also elevated in the CCl4 treated group (247.50±64.3).

(13) Treatment with standard Silymarin 100 mg/kg has brought back the ALT levels to the near normal levels (115.78±13^{b}) However treatment with VTRF-1 and VTRF-2 have restored the ALT levels towards normal in dose of 200 mg/kg up to (116.78±13^{b}) and (136.19±6.6^{a}) respectively, which is statistically significant (p< 0.01 and p< 0.05 respectively).Rise in ALP (IU/L) serum levels were remarkable in CCl4 treated group (346.00±43.86) and with the 200 mg/kg dose of VTRF-1 and VTRF-2 had reduced to (239.16±13.25^{b}) and (257.83±13.30^{a}) with significant (p< 0.01 and p< 0.05) respectively, whereas standard Silymarin (100 mg/kg) responded well and restored the ALP levels to (227.5±30.15^{b}) with significance (p<0.01) when compared to positive control group (p< 0.001).

Table 2: Effect of VTRF-1, VTRF-2 fractions on liver weight and enzymes AST (SGOT) ALT (SGPT), and ALP levels in blood serum of CCl4 induced hepatotoxicity.

Treatment	Liver weight in Gms	SGOT (IU/L)	SGPT (IU/L)	SALP s(IU/L)
Normal	5.32±0.42	156.04±6.54	102.09±19.8	207.00±10.04
CCl_4 (2ml/kg)	7.44±0.43	264.35±17.83	247.50±64.3	347.00±43.86
VTRF-1(200mg)+ CCl_4 (2ml/kg)	6.09±0.20	185.98±28.03^{b}	115.78±13^{b}	239.16±13.25^{b}
VTRF-1(200mg)+ CCl_4 (2ml/kg)	5.92±0.16	182.15±18.24^{b}	136.19±6.6^{a}	257.83±13.30^{a}
Sylimarin(100mg) + CCl_4 (2ml/kg)	5.95±0.43	165.18±14.22^{c}	115.39±8.5^{b}	227.5±30.15^{b}

Each value represents Mean ± SEM, n=6, ^{a}p<0.05; ^{b}p<0.01; ^{c}p<0.001 compared to CCl4 group {+control (Ccl_4)}. One way ANOVA followed by Turkey's t-test. Acute toxicity studies were carried out according to OECD guidelines (Up and Down method). No mortality was observed at 2000 mg/kg body weight for VTRF-1 & VTRF-2. Therefore 1/10th doses were taken as effective dose for all further *in-vivo* studies [16]

Discussion

In this study, rats were treated with CCl4 developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the activities of serum (SGOT) AST, (SGPT) ALT, ALP, Total Bilirubin, Direct Bilirubin, Total Triglyceride, Total Cholesterol and Total protein. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver.

Liver damage was assessed by biochemical studies and by histopathological examinations. CCl4 produced an experimental damage that histologically resembles viral hepatitis. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite CCl3 radical is produced which further reacts with oxygen to give trichloromethyl peroxy radical. Cytochrome P450-2E1 is the enzyme responsible for this conversion. This radical binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In this view, the reduction in levels of AST, ALT,

ALP and Bilirubin treated with endophytic fractions exhibited stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl4. This effect was in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. Thus, administration of endophytic fractions has decreased the elevated levels of biochemical markers like AST, ALT, ALP, Bilirubin, Triglyceride and Cholesterol levels. Similarly, a histopathological observation showed that hepatic globular architecture was normalized, fewer lymphatic infiltrations were seen and Kuffer cells proliferation appear to be normal. This observation suggests that the fractions from *Prosopis cineraria* Linn possessed hepatoprotective activity against CCl4 induced hepatotoxicity [12-15].

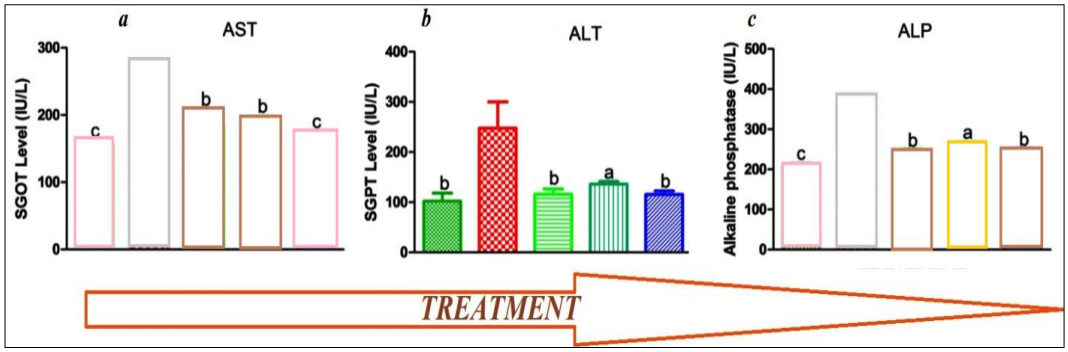

Fig 1: AST, ALT and ALP levels in various groups

Conclusion

We conclude that the ethanol fractions TRF-1 and TRF-2 of endophytes from *Prosopis cineraria* Linn have potent antioxidant and hepatoprotective activity in CCL_4 induced hepatic damage in rats. Collectively, these findings indicate that the antioxidant effects of endophytic fractions may be an important contributor to their hepatoprotective activity. The present investigation has also opened avenues for further research in Pharmacological studies especially with reference to the endophytes from medicinal plants in development of potent biomedicine for treatment of various chronic diseases.

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