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Caesalpinia crista seeds-*in vivo* screening of hepatoprotective efficacy against CCL_4 induced hepatotoxicity

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***Abstract*---**Ethanolic extract of *Caesalpinia crista*(commonly known as Latakaranja) seeds, belonging to family Caesalpiniaceae, was evaluated for hepatoprotective efficacy in Wistar rats against hepatic injury carbon induced tetrachloride. The toxicant CCl_4(1:1 mixture in olive oil)was administered to rats (3 mL/kg)*i.p.*, twice a week for 4 weeks to induce liver toxicity. The ethanolic extract of seeds of *Caesalpinia crista*was administered at doses of 150 and 300 mg/kg *p.o.,* daily for 4weeks and Silymarin (100 mg/kg *p.o.*) was used as a standard drug. Biochemical parameters such as serum alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin were assessed and histopathological studies of the liver were performed to evaluate the hepatoprotective efficacy of the extract. Treatment of rats administered with *C. crista* seed extract showed a significant decrease in CCl_4-induced elevated serum enzyme levels as well as a significant

increase in total protein levels that was lowered by the hepatotoxic compound used and the results were comparable to Silymarin.

Keywords---*Caesalpiniacrista,* Wistar rats, carbon tetrachloride, hepatoprotective efficacy.

Introduction

The liver is the body's largest internal organ as well as the largest gland. At the same time it serves as a metabolic as well as a biochemical transformation factory. It receives blood carrying substances via the portal vein and oxygen-rich blood via the hepatic artery (Muriel). As a result, the liver is referred to as a "bidirectional biofilter" (Arias) that is capable of performing a wide range of tasks (mohan and Muriel).

As the liver assists nearly every organ in the body; is imperative for endurance and due to its essential positioning and multifaceted functionalities, it is additionally inclined to numerous illnesses (Eswar).Also as the liver's role is to eliminate substances through the portal circulation, it is vulnerable to constant attack by exogenous agents, resulting in hepatic diseases (Bodakhe). Hepatic problems thus continue to be one of the most serious threats to overall health, posing serious health concerns around the world (Asha).

Extended drug treatment, unrestrained utilization of a number of regularly utilized medicines like paracetamol or acetaminophen, diclofenac and so forth; liquor addiction, vulnerability to particular xenobiotics, toxins and diseases influence liver working to a certain extent and sooner or later result in several liver disorders like hepatitis, cirrhosis, cancer and alcoholic hepatic disorder (Adewusi and Afolayan, 2010).

Due to the absence of any compelling medication that can restore hepatic functions, provide safety from harm or help in recovering hepatic cells (Chattopadhyay, 2003); inaccessibility of well-advised treatment in present day medication and no or extremely more negative impact of synthetic medications in liver-damage; have encouraged specialists in this field to anticipate natural medications with better hepatoprotective activity (Ravishankar and Bhavsar, 1993).Powerful and intense herbal medications require assessment by standard scientific techniques in order to be approved for the treatment of an illness (Jannu *et al.,* 2012).

Utilizing natural medications for treating hepatic disorders has a long background and medicinal plants alongwith their derivatives are as yet utilized everywhere in the world in one form or the other for this reason. Hepatoprotective plants contain an assortment of chemical components like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes (Bhawna and Kumar, 2009). The retardation of free radical generation also can provide an effortless model for assessing the action of hepatoprotective agents (Mohamed Saleem *et al.,* 2008).
Caesalpinia crista, commonly known as Fever nut or Latakaranja[Khare] has been traditionally used in Ayurveda to treat a vast range of diseases like fever, malaria,

skin diseases, and inflammation. It is found to possesses anthelmintic[Jabbar], antidiabetic[Gupta], analgesic and antipyretic[Ishan; Gill], wound healing[Patil], antiulcer [Chauhan], antibacterial and antiviral[Afrin; Patil], and cardioprotective[Kumar] activities. Different parts of the plant like leaves, flowers, fruit, root, bark, seeds and seed oil were used medicinally[Suryawanshi]. Taking into account the physiologically active nature of *Caesalpinia crista,* the current study seeks to assess the hepatoprotective potential of *C. crista*seeds against carbon tetrachloride-induced hepatotoxicity.

Materials and Methods

Chemicals

All chemicals and solvents utilized in the study were of analytical grade; DPPH (Sigma Chemicals Co.,); and Silymarin (Micro Labs Ltd) were procured from local supplier of Ghaziabad. The assay kits employed for the estimation of various biochemical parameters were acquired from ERBA (Himachal Pradesh, India).

Plant collection and authentication

The seeds of *Caesalpiniacrista* were procured from the local market of Old Delhi, India. The seeds of the plant were authenticated by an emeritus scientist, Dr. Sunita Garg of Raw Material Herbarium and Museum (RHMD), CSIR- National Institute of Science Communication and Information Resources, Delhi (taxonomic reference number: NISCAIR/RHMD-3632-33).

Preparation of the extract

The procured seeds of *Caesalpinia crista* were washed properly and dried. They were then ground to powder, first with the help of pestle-mortar and then an electric grinder. The powdered seeds were subjected to continuous extraction with hot ethanol using Soxhlet apparatus (Harborne, Evans). The extract obtained, denoted as EECC, was filtered and the filtrate was made solvent free using a vacuum evaporator, yield was calculated and then preliminary phytochemical analysis was performed (Bulugahapitiya).

Total phenolic and flavonoid content

Total phenolic content of ethanolic extract of *Caesalpinia crista*was determined using Folin-Ciocalteu reagent method with some modifications, (Sen *et al* (2013) taking gallic acid as standard. All tests were done in triplicate and the result of total phenolic content for all plant extracts was expressed as milligrams of gallic acid equivalent (GAE) to per gram of extract (mgGAE/ g).Similarly total flavonoid content of EECC was determined spectrophotometrically by the method using aluminium chloride (Sandip *et al* (2014) withs light modifications. The samples were prepared in triplicate and average absorbance was noted. Concentration of total flavonoid contents in the extracts was expressed as milligram Quercetin Equivalents (QE) per gram of the plant extract (mg of QE/ g of plant extract).

In vitro antioxidant activity

To study the antioxidant efficacy, the DPPH scavenging potential of *Caesalpiniacrista* seed extract was determined (sodhi). Ascorbic acid was used as a positive control, due to its well-known antioxidant properties. The bleaching of DPPH indicates the free radical scavenging capacity of ethanolic seed extract of *Caesalpiniacrista*, the activity being highest at 71.6±0.42% as compared to the standard ascorbic acid, 84.6±0.45% by DPPH free radical scavenging method. As a result, the findings demonstrated that EECC could work as a natural antioxidant as well as a free radical inhibitor or scavenger.

Animal studies

Wistar rats of either sex weighing between 300-350 g, were acquired from the animal house of MIET, Meerut, Uttar Pradesh, India. They were housed in polypropylene cages with dust free rice husk as a bedding material and maintained under standard laboratory conditions (controlled temperature of 23 ± 2°C, relative humidity of 40 ± 10% and natural, 12 hour each, light-dark cycle) for acclimatization for seven days prior to and during experimentation as per guidelines of Institutional Animal Ethics Committee. They were fed with standard rodent pellet diet and water *ad libitum.* The research protocol of this study was approved by IAEC of Meerut Institute of Engineering And Technology (registration no. IAEC/MIET/2021/27).

Acute toxicological studies

Wistar rats (n=3) were selected by random sampling technique and OECD-423 guidelines (OECD) were followed to study the acute oral toxicity. The ethanolic extract was administered orally starting with 5 mg/kg body weight up to 2000 mg/kg body weight. Initially for the first an hour after administering the dose, individual animals were monitored, at least once and then regularly throughout the first 24 hours, with specific care being given during the first 4 hours, every-day after that for a total of 14 days.

Hepatoprotective activity against carbon tetrachloride induced hepatotoxicity

Carbon tetrachloride, a hepatotoxin, was used to induce liver injury and Silymarin was used as reference standard. The animals were randomly divided into 5 different groups (n=6) as follows:
Group I: Served as normal control, normal saline (10 ml/kg of body wt.) p.o. was administered to rats daily for 4 weeks.
Group II: Served as disease control, CCl_4 dissolved in olive oil (1:1) was administered to rats (3 mL/kg of body wt.)intraperitoneally, twice a week for 4 weeks.
Group III: Carbon tetrachloride (CCl_4) was pre-administered as in group 2to rats followed by Silymarin(100 mg/kg *p.o.*) daily for 4weeks.
Group IV: CCl_4 was pre-administered as in group 2 to rats followed by ethanolic extract of stem bark of *Caesalpinia crista*(150 mg/kg *p.o.*) daily for 4weeks.
Group V:$CCl_4$was pre-administered as in group 2to rats followed by ethanolic extract of seeds of *Caesalpinia crista*(300 mg/kg *p.o.*) daily for 4weeks.

After 24 hours of the last dose, the animals were euthanized and liver and blood samples were collected for the biochemical assessment and histopathological analysis.

Assessment of biochemical parameters

Various biochemical parameters were evaluated for assessing liver functions where estimation of serum enzymes like SGOT, SGPT (Reitman) and serum alkaline phosphatase (Burtis); total bilirubin, total proteins (Doumas, Tietz), serum albumin (Doumas)and TBARS (Wills, Ohkawa)was performed to study the effect of EECC on liver toxicity induced by CCl_4.

Histopathological analysis

The liver tissues fixed in 10% formalin, were dried in graduated amounts of ethanol before being submerged in xylene and then embedded in paraffin. 4 µm thick sections were prepared and stained with haematoxylin and eosin dye (Clayden) to observe gross histopathological changes.

Statistical analysis

The results were given as mean ± standard error mean (SEM). The significance of difference among the control group and various treated groups was analysed using analysis of variance (ANOVA) and $P < 0.05$ was considered statistically significant. The data was statistically analysed using GraphPad Prism 5 software (GraphPad Inc., La Jolla CA).

Results and Discussion

In the Indian system of medicine, a variety of plants are believed to provide relief from liver problems butthe claimed medicinal reputation of such herbs' must be scientifically validated. Thus in the current research work, the ethanolic extract of *Caesalpinia crista*seeds was evaluated for its hepatoprotective potential. The percentage yield obtained from the ethanolic extract was found to be 8.1% and the preliminary phytochemical evaluation revealed the presence of plant secondary metabolites such as alkaloids, flavonoids, tannins, carbohydrates, terpenoids and amino acids in the ethanolic seed extract of *Caesalpinia crista*(table 1). The total phenolic and flavonoid content found to be present in ethanolic extract of *C. crista* seeds was 60.45 ± 8.76 mgGAE/gand 11.78 ± 1.02 mgQE/g, respectively.

Table 1
Preliminary phytochemical screening of ethanolic extract of *Caesalpinia crista*seeds

Phytochemical Group	EECC
Flavonoids	+
Phenols	+
Alkaloids	+

Tannins	+
Steroids and sterols	-
Terpenoids	+
Glycosides	+
Saponins	-
Carbohydrates	+
Amino acids and proteins	+

+ = *Presence of active constituents*
- = *Absence of active constituents*

Considering the presence of greatest amount of phytoconstituents, the ethanolic seed extract of *C. crista* was tested for its hepatoprotective efficacy against carbon tetrachloride.It was found to be nontoxic up to a dose of 2000mg/kg with no signs of mortality in test animals. CCl_4, a best known example of a hepatotoxin, on metabolising forms a hazardous trichloromethyl radical, which causes necrosis and liver failure.

It was perceived that the rats treated with carbon tetrachloride developed a significant hepatic damage as seen from augmented serum levels of hepato-specific enzymes like ALT, AST, ALP, and total bilirubin; and lowered serum total protein levels when compared to normal control. Pre-treatment with Silymarin and EECC showed good protection against carbon tetrachloride induced toxicity to liver. Tests indicate a significant reduction in augmented serum enzyme levels and a considerable increase in total protein levelswith extract treated animals as evident from table 2.

Lipid peroxidation study showed that CCl_4 treated group exhibited significant increase in malondialdehyde level when compared to normal control group whereas EECC was significantly able to lower this rise in MDA level as evident from table 2.

Table 2
Effect of ethanolic extracts of stem bark of *Caesalpinia crista* (EECC) on ALT, AST, ALP, total bilirubin, total proteins, serum albumin and LPO levels in carbon tetrachloride induced hepatotoxicity in rats

Group	Serum ALT Levels (IU/L)	Serum AST Levels (IU/L)	Serum ALP Levels (IU/L)	Total bilirubin Levels (mg/dl)	Total protein levels (g/dl)	Serum albumin (g/dl)	LPO (nMole of MDA/mg of protein)
1	39.88±5.52	41.77±5.42	101.66±11.88	0.72±0.09	7.59±0.86	4.61±0.53	0.78±0.08
2	82.67±10.67	91.35±10.25	226.91±24.34	1.81±0.19	3.06±0.41	1.51±0.18	2.63±0.27
3	51.88±4.96***	61.62±6.40***	127.54±13.45***	1.01±0.14**	5.58±0.3***	3.68±0.40***	1.55±0.18**
4	62.86±	74.65±	171.77±	1.16±	3.99±	2.91±	1.98±

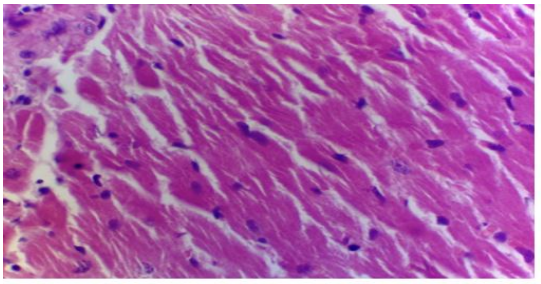
	6.29*	9.32*	17.58*	0.11*	0.4**	0.21**	0.2*
5	39.7± 4.34***	55.87± 6.1**	125.23± 12.06***	0.96± 0.083***	5.97± 0.45*	3.76± 0.31***	1.09± 0.09***

Values are mean ± SEM (n=6) one way ANOVA. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$. All values are compared with toxicant.

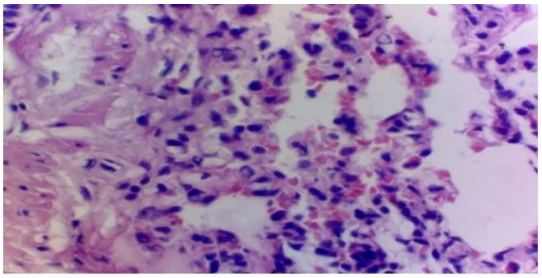
Histopathological studies of the liver in carbon tetrachloride induced hepatotoxicity

The histopathological evaluation of carbon tetrachloride toxicity in all the groups was examined and shown in figure 1.The rat liver section of normal control group showed liver parenchyma with intact architecture (the usual morphology) whereas the architecture of the liver in the disease control group was partially effaced, with certain hepatocytes showing apoptotic changes, perivenular mononuclear inflammatory in filtration, and scattered inflammatory in filtration within the parenchyma as signs of toxicity. On the other hand, liver section of Silymarin treated group showed liver parenchyma with intact architecture, though some of the central veins showed congestion with diffuse congestion of sinusoids. Also, the liver section in EECC treated groups(150 and 300mg/kg)showed intact architecture, few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells that is similar to Silymar in treated group.

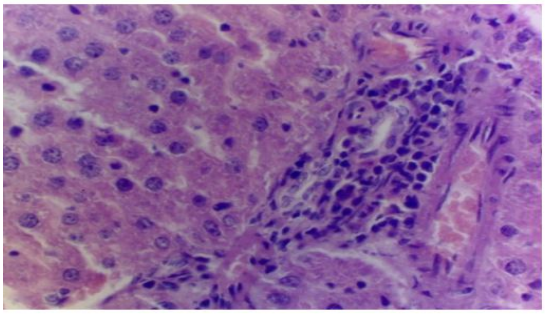
Based on these findings, tannins and flavonoids found to be present in EECC may be responsible for the hepatoprotective efficacy. The outcomes of this study, supported by histological findings, showed that *C. crista*promises to be a pharmacologically effective treatment for a variety of liver illnesses, demonstrating an improvement in liver function, similar to Silymarin.



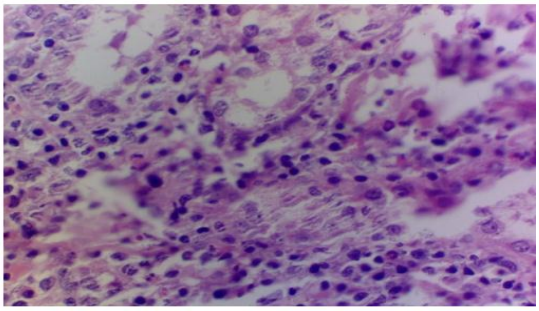
Normal Control



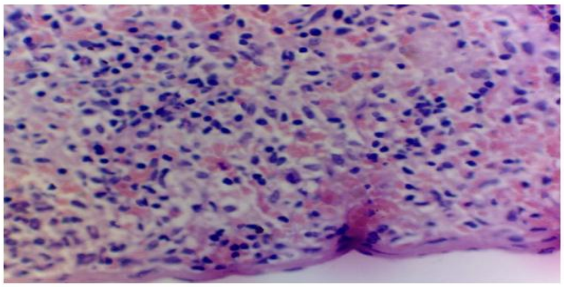
CCl_4 treated



Standard Silymarin (100mg/kg)



EECC treated (150 mg/kg)



EECC treated (300 mg/kg)

Figure 1: Histopathology of the rat liver in carbon tetrachloride induced hepatotoxicity

Conclusion

Pursuant to the results, treatment with an ethanolic extract of *Caesalpiniacrista* seeds afforded considerable protection against CCl_4-induced hepatotoxicity. In contrast to 150 mg/kg, the hepatoprotective potential of 300 mg/kg was somewhat higher. The extract's therapeutic effects were endorsed by histological findings.

Conflicts of interest: the authors declare no conflicts of interest.

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