Research Article

HEPATOPROTECTION BY *ACACIA CATECHU* IN CCL_4 INDUCED LIVER DYSFUNCTION

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ABSTRACT

The present study investigates the hepatoprotective action of heartwood powder of *Acacia catechu* in the treatment of liver damage in rats exposed to carbon tetrachloride. The evaluation has been carried out using liver function marker enzymes in blood plasma, Liver tissue biochemistry supported by histopathology due to CCl_4 induced hepatopathy the marker enzymes leak into the blood. The extent of recovery has been compared with the natural liver regeneration after CCl_4 damage and normal liver. The heartwood powder of *Acacia catechu* has been treated in the form of aqueous slurry. From the findings of blood biochemical parameters it could be inferred that the treatment with this plant material is effective against CCl_4 induced dysfunction of the liver, justifying the use this plant by folk healers. The decreased levels of serum bilirubin after treatment with heartwood powder of *Acacia catechu* restores the normal functional status of the liver. This hepatoprotective effect was supported by light microscope studies.

Keywords: *Acacia catechu,* enzyme markers, liver dysfunction.

INTRODUCTION

Acacia catechu is a commonly occurring tree in INDIA upto height of 1500m. This plant material is used as anodyne, astringent, bactericide, refrigerant, stimulant, styptic, masticatory expectorant and antiphlogistic. It is also used in asthma, cough, bronchitis, colic, diarrhoea, dysentery, boils, in skin affections and sores and for somatitis. The bark is used as an anthelmentic, antipyretic, antiinflamatory, in bronchitis, ulcers, anaemia and gum troubles.[1-4]

In Ayurvedic medicine, Acacia leaves, flowers, and pods have long been used to expel worms, to staunch bleeding, heal wounds, and suppress the coughing up of blood. Its strong astringent action is used to contract and toughen mucous membranes throughout the body in much the same way as witch hazel or oak bark.[5-7]

Liver disorders are a serious health problem. In allopathic medicinal practices reliable liver protective drugs are not available but herbs play important role in management of liver disorders. Numerical medicinal plants are used for the same in ethnomedical practices and in traditional system of medicines in India. Some hepatoprotection is achieved by herbal medicines based on *phyllanthus* species but these plants are declared as endangered plants by Indian Systems of Medicines. *Acacia catechu* is the plant which has ability to set right the disorder created in liver function.[8-10]

The present work was carried out to investigate the hepatoprotective action of this plant material on CCl_4 (Carbon tetra Chloride) induced liver damage in rats. Blood and tissue biochemical assays have been studied for evaluation of hepatoprotection. From the results of these parameters it is clear that *Acacia catechu* gave best recovery for hepatoprotection.[11-13]

MATERIALS AND METHODS

Plant Material

The plant material was collected around Rajgurunagar, Pune District, Maharashtra, India. After collection of the required quantity, it was carefully segregated, and dried in shade to constant weight. The plant material was kept in preset oven for a week at 40°C and powdered in high speed electronic mixer and sieved through a BSS Mesh No. 85 sieve and stored in an airtight container. This plant material was used for animal trials.

Acute toxicity Study

The acute toxicity study of heartwood powder of *Acacia catechu* was carried out on Swiss mice with a dose of 2, 4 and 6 g/Kg body weight orally. The single administration exposure of the heartwood powder of *Acacia catechu* in the form of aqueous slurry was carried out and the exposure route was oral with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There is no sign of toxicity. There was no mortality recorded even at the highest dose level of 6gm/kg body weight.[14-16]

Animals for Hepatosuppression Study

Albuno Wistar rats of either sex, weighing 130–150g, were used. Animals were housed under controlled conditions of temperature (25 ± 2^0C) and with 12-h light/dark cycles and fed with Amrut food pallets and water.[15,16]

Induction of hepatic injury

Hepatic injury was induced in rats by a single dose of 0.7 ml/kg $CC1_4$ in 0.5ml liq. Paraffin as a vehicle.

Experimental protocol

Animals were grouped into five groups and administered following dose mentioned in Table I.

Table I: DAILY DOSE REGIME

DAYS	Group I Vehicle Control	Group II CCl4 control	Group III CCl4 treated Natural recovery	Group IV Silymarin treated	GroupV CCl4 + plant slurry treated
1	0.5cc liq. Paraffin & 2 cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. And 2cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. And 2cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. and 0.4gm/kg plant material in 2cc d/w orally
2	2cc d/w orally	2cc d/w orally	2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w orally	0.4gm/kg plant material in 2cc d/w orally
3	2cc d/w orally	2cc d/w orally	2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w orally	0.4gm/kg plant material in 2cc d/w orally
4	Sacrifice	Sacrifice	2cc d/w orally	Sacrifice	Sacrifice
5	-	-	2cc d/w orally	-	-
6	-	-	2cc d/w orally	-	-
7	-	-	Sacrifice	-	-

Note: 1. The above dosage is for an individual animal of the group; 2.The number of animals in each group = 6;
3. i.p. = intra peritoneal; 4. d/w = distilled water; 5. liqd. paraffin = liquid paraffin.

Table II: Effect of heartwood powder of *Acacia catechu* on body weight

Groups	Body weights of rats in grams						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th day	7th Day
Group I Normal Control	141.4 ± 2.2	142.33 ± 3.2	144.2± 2.4	SACRIFICE	-	-	-
Group II CCl_4 Control	145.3 ± 3.2	144.2± 3.4	142.2± 4.0	SACRIFICE	-	-	-
Group III CCl_4 Recovery	134.4 ± 4.2	135.2± 3.5	137.3± 3.7	137.8± 4.0	139.1± 3.2	140.4±2.04	SACRIFICE
Group IV Silymarin Control	143.1 ± 3.2	144.7± 6.20	146.7± 6.2	SACRIFICE	-	-	-
Group V Plant control	128.2±3.10	129.6± 3.4	130.9± 3.3	SACRIFICE	-	-	-

Table III: Effect of heartwood powder of *Acacia catechu* on Biochemical Parameters

Parameter	Gr. I Normal control	Gr. II CCl_4 control	Gr. III CCl_4 Recovery	Gr. IV Silymarin control	Gr. V Plant extract control
GPT(B)	55	63	51	70	57
GOT(B)	53	59	61	57	52
Cholesterol(B)	62	70	72	74	63
Bilirubin(B)	0.45	0.78	0.69	0.62	0.53
Triglycerides(B)	121	106	112	128	125
Gamma GT(B)	11	38	31	16	22
Alk. PO4(B)	140	163	151	148	146
Glycogen(T)	24	20	22	23	26
Total Protein(T)	05	20	10	08	08
Cholesterol(T)	02	2.2	01.8	02.1	01.9
DNA(T)	0.18	0.23	0.70	0.20	0.40
RNA(T)	2.1	4.2	3.2	4.0	3.0
Liver to Body wt Ratio	0.04	0.04	0.05	0.038	0.04

(B): Blood Biochemical Parameter; (T): Tissue Biochemical Parameter

RESULTS

In present study it is observed that there was significant decrease in body weight of CCl_4 treated group as compared to normal control group given in Table II. Treatment of Silymarin and heartwood powder of *Acacia catechu* showed an increase in body weight as compared to CCl_4 treated group indicating the sign of recovery. The plant material shows significant inhibition of the acute elevation of serum markers induced by CCl_4 than Silymarin.

Blood and Tissue Biochemical Marker enzymes

All the blood biochemical marker enzymes, *viz.*, ALT, AST, Cholesterol, Bilirubin, Triglycerides, Alkaline Phosphate and GGT as well as tissue biochemical markers like glycogen, Total protein, Cholesterol, DNA and RNA reported increased activity in CCl_4 treated rats as compared to normal control group as the marker enzymes leaks into the blood due to hepatopathy. In plant material administered group, the levels of these enzymes were found close towards normalcy. The mean values of blood and tissue biochemical parameters are given in table III.

The level of blood and tissue biochemical parameters reported shows significant increase in CCl_4controlled group as compared to those of normal control group. All these biochemical changes showed signs of returning more towards the normalcy in group plant material control group as compared to the natural recovery and Silymarin control group.

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxin and is well documented[13-15]. Carbon tetrachloride is biotransformed under the action of cytochrome. The microsomal compartment of the liver to trichlomethyl radical which readily reacts with molecular oxygen to form trichloromethyloethoxy radical. This free radical in the presence of oxygen may cause peroxidation of lipid on target cell resulting in extensive damage of liver. The administration of $CC1_4$ intraperitonially to wistar rats produced hepatotoxicity showed by significant increase in the serum levels of GOT, GPT and alkaline phosphate in comparison to the control group. Also the total protein levels were significantly decreased in CCl_4 control groups from normal control group. The dose of heartwood powder of *Acacia catechu* not only prevented the rise in serum level of GOT, GPT, alkaline phosphates but also improved serum lipid profile. The results were found to be well comparable with plant material treated group, hence the plant material reports better recovery.

Liver histology

The light microscopy of normal rat liver reveals almost regular structures. The hepatocytes in thin sections appear to radiate from the central vein. The hepatocytes are polygonal with well-defined borders, with single nucleus in each. The thin sections show a portal tract with distinct endothelial lining surrounded by terminal portal venules, hepatic artery and small bile duct. (Fig. 1).

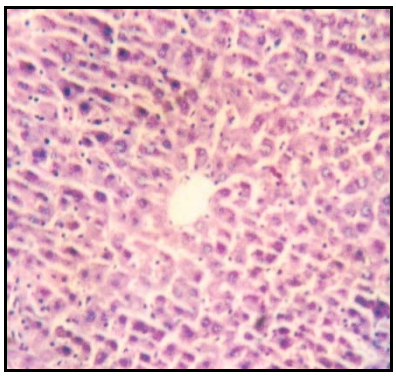

Figure 1: Light micrograph of normal rat liver

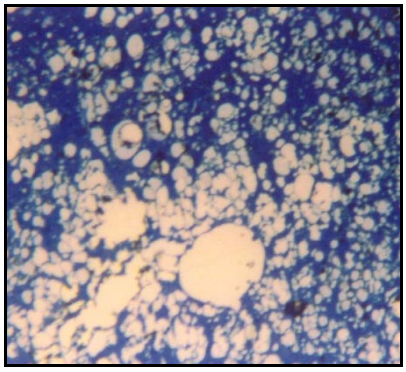

Figure 2: Light micrograph of rat liver after CCl_4 treatment

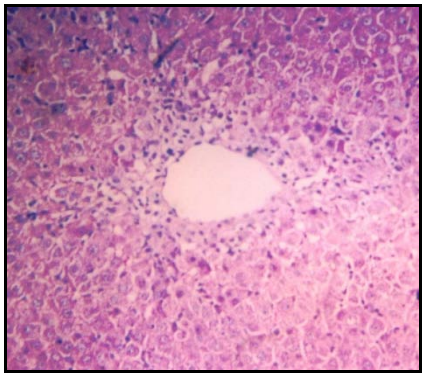

Figure 3: Light micrograph of rat liver after Natural Recovery

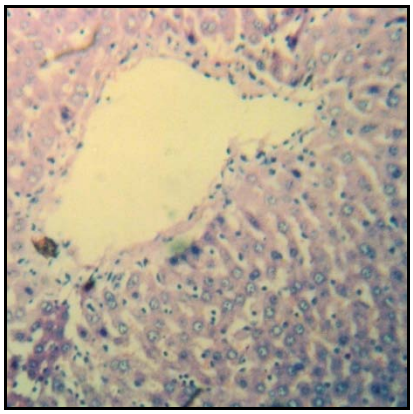

Figure 4: Light micrograph of rat liver treated with CCl_4 and Silymarin

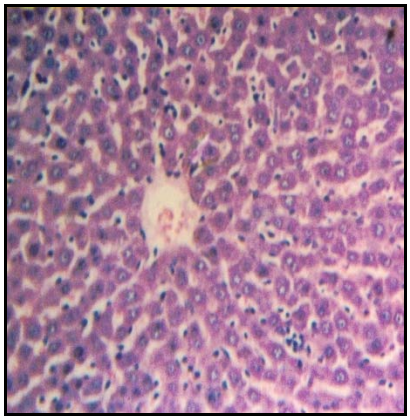

Figure 5: Light micrograph of rat liver treated with CCl_4 and plant material

The rat liver after CCl_4 treatment shows distinct centrilobular necrosis with hepatocytes of these areas showing distinct vacuolation. The nucleus appears pynotic in these cells. The periportal region appears normal. There is distention of sinusoidal lumen in the centrilobular area. There is also distinct enlargement of hepatocytes and few areas show infiltration of mononuclear cells especially near the portal veins. (Fig. 2).

In natural recovery group the histological pictures under the light microscope revealed almost normal liver with very mild swelling of sinusoids and no congestion. The nuclei were normal indicating the recovery of the liver after the toxicant treatment. The rat liver after CCl_4 treatment in electron microscopy shows there is distinct absence of lipid accumulation and reduced mitochondrial activity as compared to CCl_4 treated cells. The microvilli appear normal. There is however, abundance of rER in hepatocytes. (Fig. 3).

The liver of the rats after combined treatment of CCl_4 and heartwood powder of *Acacia catechu* in the form of aqueous slurry shows mild congestion in some of the sinusoids. The dilation of sinusoids is evident in the centrilobular areas. The vacuolation seen after CCl_4 treatment is significantly absent. The liver showed distinct signs of overall recovery. Bile capillaries are dialated. Focal necrotic areas were not visible with vacuolated hepatocytes. Mild congestion was seen in few areas with mononuclear cell proliferation (Fig. 4).

The liver of the rats after combined treatment of CCl_4 and Sylimarin shows more focused regions of recovery. The dilation of sinusoids is evident in the centrilobular areas were distinctly visible. Vacuolated hepatocells and ballooned hepatocells were also seen. Congestion was significant. (Fig. 5).

CONCLUSION

On the basis of above findings, this may be concluded that the probable mechanism by which the heartwood powder of *Acacia catechu* exerts its protective action against CCl_4-induced hepatocellular alterations through synthesis of proteins, or due to bioactivation of CCl_4 and accelerated detoxification. The potential to minimise the effects of free radicals including the peroxy radicals and its antioxidant activity in association with the inhibition of lipid peroxidation, thereby the *Acacia catechu* plant material can be considered as hepatoprotective agent by the combined synergistic effect of its constituents and micronutrients rather than any single factor through free radicals activity.

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