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Hepatoprotective Potential of Acaro Calamus against Carbon tetrachloride induced Liver damage in Rats

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Abstract: Objective- The aim of this study was to evaluate the hepatoprotective effect of *Acorus calamus* against carbon tetrachloride induced liver injury in rats.
Method – In the present study, the protective effect of *Acorus calamus* was investigated against carbon tetrachloride induced hepatotoxicity and compared with silymarin, a standard hepatoprotective reference drug. Total 30 animals were taken and divided into five groups .Group I is control group and group II induction group and group III and IV are test groups to which administered the ethanolic extract of powder of Acorus calamus at doses of 30mg/kg and 100mg/kg and group V administered standard drug Silymarin 5mg/kg orally for 07days respectively .On seventh day single dose of 1mg/kg.b.wt.i.p with olive oil 1:1 was administered.
Result-Administration of ccl_4 induced mark increase in serum hepatic enzyme level of SGPT, SGOT, ALP and decrease in normal protein as compared to normal control.
Conclusion-Pretreatment of rats with Acorus calamus 30mg/kg and 100mg/kg prior to ccl_4 administration caused significant reduction in values of SGPT, SGOT and increase in total protein level but ALP and total bilirubin not affected significantly.
Keywords- Hepatotoxicity, Carbontetrachloride, Acorus calamus.

Introduction

Liver is an important organ in the body as it provides protection from potentially injurious exogenous and endogenous compounds and in this process it gets affected[1]. Thus protective mechanism of liver is of special concern. Conventional medicines are used for treatment of liver diseases have adverse side effect and are costlier. So there is need to evaluate natural compound as effective alternative which are safer and cost effective.

Acorus calamus Linn. belongs to the family Acoraceae, commonly known as “sweet flag” or “calamus”, is a semiaquatic, perennial, aromatic herb with creeping rhizomes. The plant is found in the northern temperate and subtropical regions of Asia. Many ethnomedicinal and ethnobotanical uses have been ascribed to the rhizomes of the plant. A. calamus Linn. has been used as traditional Indian prescriptions for its beneficial effects on antiproliferative [2], antidiarrhoeal [3], antioxidant [4] and hypolipidemic activity [5].

Materials and Method

Drugs and chemicals

Commercially available vekhand powder was obatained from Lokhande Ayurvedic Stores Pune Maharashtra. Its ethanolic extract at doses of 30mg/kg b. wt. and 100mg/kg b.wt. was administered orally.

Experimental Animals

The institutional animal ethical committee National Toxicology Centre, Pune, India approved the experimental design (Proposal No.32032,) 30Albino (*Wistar*) male rats of 200-250g, 12 female swiss albino mice were used for the study. Animals were housed in well ventilated room (temperature 22 $\pm$ 2^{o}C, humidity 40-60% and 12h light/dark cycle) at national toxicology centre. Animals were fed with standard pellet diet and water. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Anima.

Table 1: Experimental design and evaluation of hepatoprotective activity

Sr. No.	Group (6 animal in each group)	Description
1	Group -I	Rats were received 1ml of Vehicle orally for 07 days
2	Group –II (CCl_4 control)	Rats were received vehicle orally for 07 days + singal dose of CCl_4 1.0ml/kg. b. wt. , ip with olive oil , 1:1 on the 7th day was administered.
3	Group –III (Test Acorus calamus + CCl_4)	Rats were received ethanolic extract of Acorus calamus 30mg/kg b.wt. P.O. for 07 days + CCl_4 single dose of 1.0ml/kg. b. wt., ip. With olive oil 1:1 on the 7th day was administered.
4	Group -IV (Test Acorus calamus + CCl_4)	Rats were received ethanolic extract of Acorus calamus 100mg/kg b.wt. P.O. for 07 days + CCl_4 single dose of 1.0ml/kg. b. wt., ip. With olive oil 1:1 on the 7th day was administered.
5	Group-V (standard Silymarin+ CCl_4)	Rats were received Silymarin (5mg/kg b.wt. P.O.) for 7 days + CCl_4 single dose of 1.0 ml/kg.b.wt. i.p with olive oil1:1 on the the 7th day was administered.

After 36 hrs of CCl_4 administration under the chloroform anesthesia, blood samples were collected in centrifuge tube via cardiac puncture. The abdomen was then cut open and collected all organs. Liver and kidney samples were used for histological evaluation.The blood samples were kept in centrifuge tube at room temperature for 1Hr. for clotting time serum was separated by centrifuge at 2500 rpm at 37^{oc} for 10 min and following biochemical parameters were estimated.

SGPT – Serum Glutamate Pyruvate Transaminase.

SGOT- Serum Glutamate Oxaloactate Transaminase

ALP- Serum alkaline phosphate.

TP- Total Protein

SB- Serum bilirubin

Were assayed according to Standard method.

Table 2: Effect of Acorus calamus on liver marker enzymes and protein content in serum of control and carbon tetrachloride intoxicated rats

Parameters	Group-I (Normal control)	Group-II (CCl_4 control)	Group-III Test. HP01+ CCl_4	Group-IV Test: HP01+CCl_4	Group-V Std – silymarin + CCl_4
SGPT/ALT (U/L)	100± 5.514	209±6.186	182±6.512**	158.3±6.563**	134.2±5.707**
SGOT/AST(U/L)	106.2±5.193	211.2 ±6.047	177±4.733***	149.5±4.506***	128.3±3.502***
ALP (U/L)	334.83±129.8	505±182.02	510.83±56.55^{NS}	356.66±98.83 ^{NS}	342±138.52 ^{NS}
Total Bilirubin (mg/ 100ml)	0.135±0.056	0.495±0.237	0.39±0.270 ^{NS}	0.355±0.285 ^{NS}	0.315±0.159 ^{NS}
Total protein gm/100ml	8.017±0.8704	5.283±1.303	6.8±0.6542***	7.733±0.9812***	8.5±1.014***

Statistical analysis—

Values are mean ± SD of 6 rats from each group. Group 2 is compared to group-I , Group3, 4, 5 are compared to group 2. Values are not sharing a common superscript differ significantly at p values: ***<0.001, **,0.01,*,0.05(DMRT).Values are means ± SD of 6 rats from each group. Group 2 is compared to group 1, Groups 3, 4, 5 are compared to group 2. Values not sharing a common superscript differ significantly at p values : ***<0.001, ** < 0.01, * < 0.05 (DMRT).

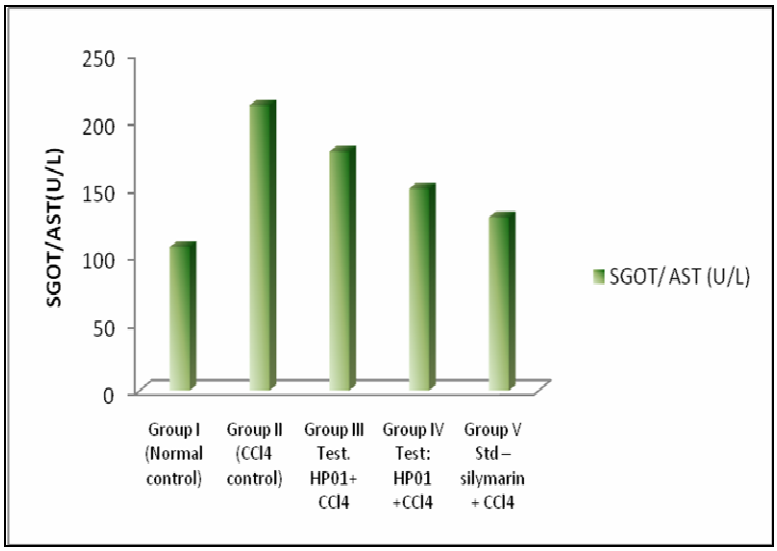


Fig. 1: Effect of standard drug and test drug on SGOT enzyme level

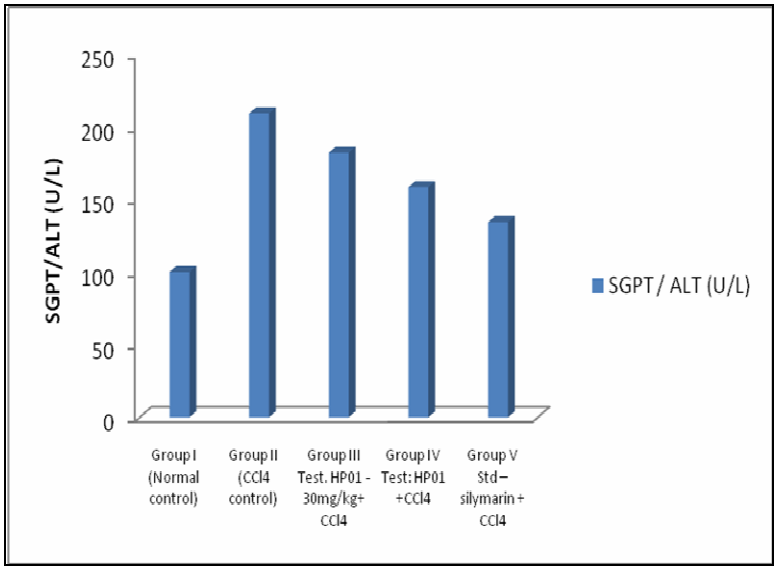


Fig. 2: Effect of standard drug and test drug on SGPT enzyme level

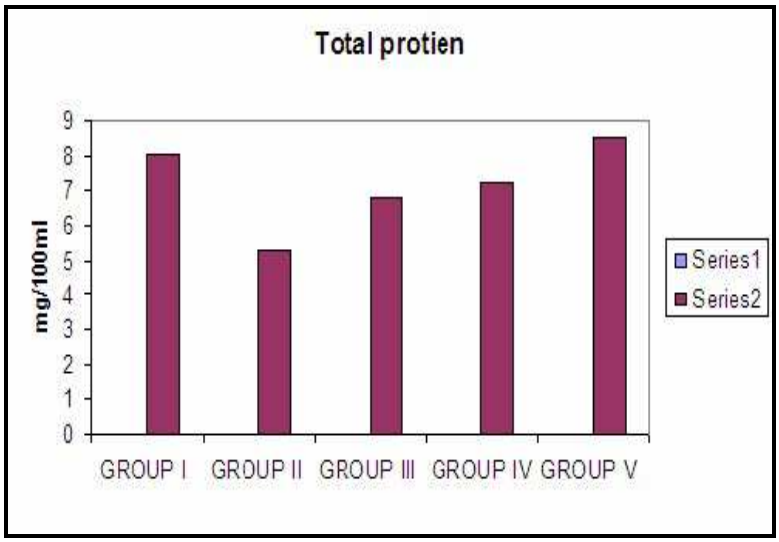


Fig. 3: Effect of standard drug and test drug on total Proteins level

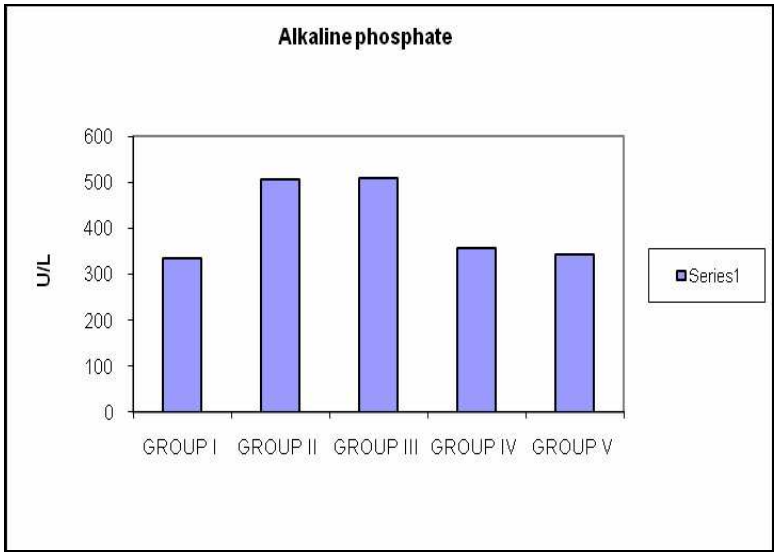


Fig. 4: Effect of standard drug and test drug on Serum Alkaline Phosphate enzyme level

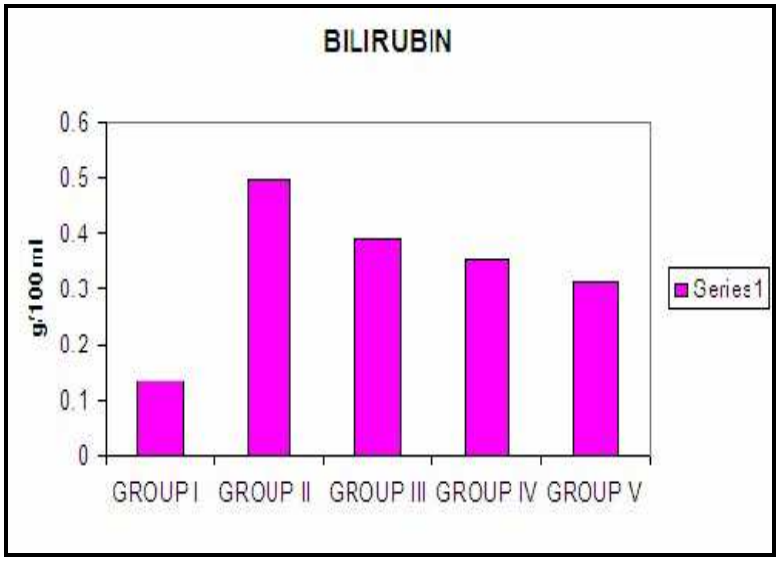
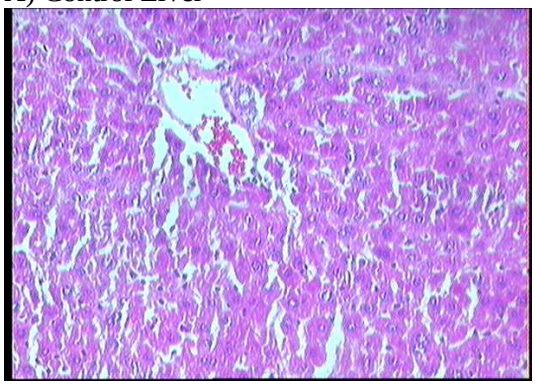


Fig. 5: Effect of standard drug and test drug on Bilirubin enzyme level

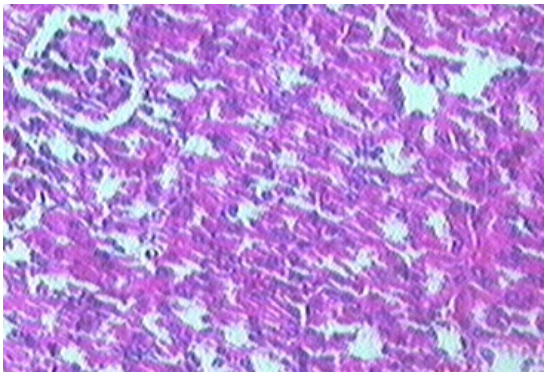
Histopathological Studies

Immediately after the sacrifice, a portion of the liver was fixed in 10% formalin, then washed, dehydrated in descending grades of alcohol and finally rinsed with xylene. The tissue were then embedded in molten paraffin wax, Section were cut at 5mm thickness, stain with haematoxylin and eosin was observed microscopically for histopathological changes.

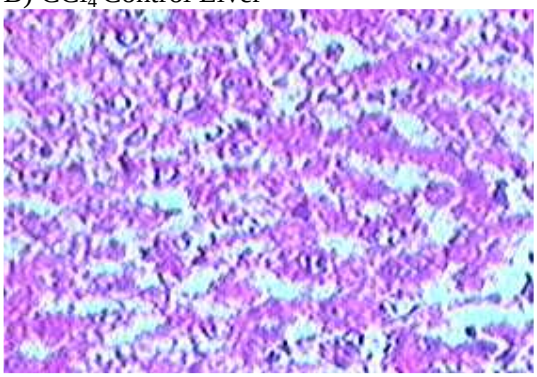
A) Control Liver



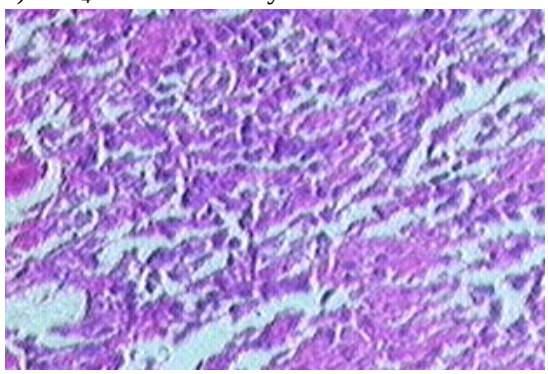
A) Control Kidney



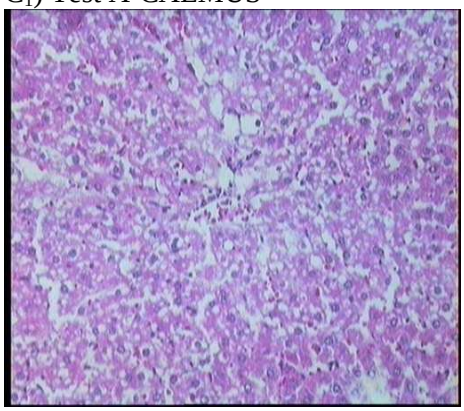
B) CCl_4 Control Liver



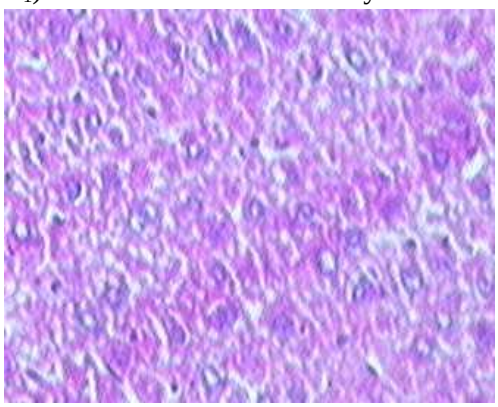
B) CCl_4 Control Kidney



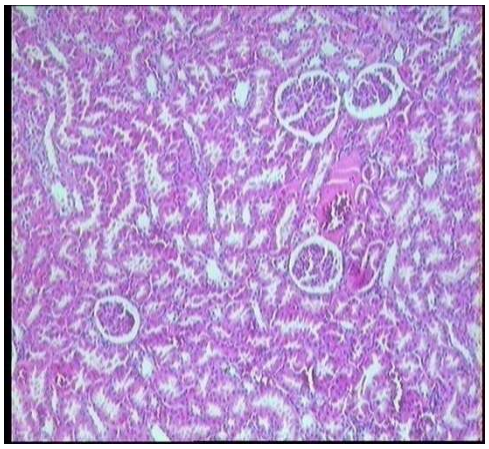
C_1) Test A-CALMUS



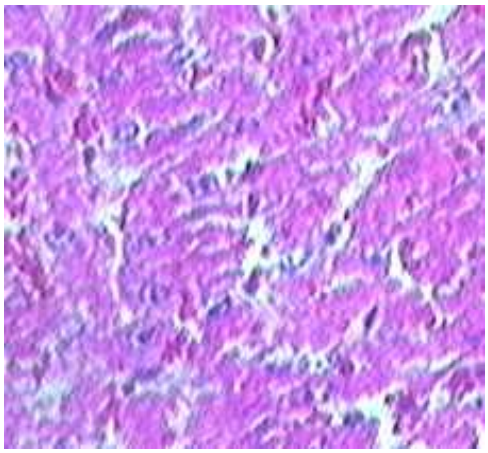
C_1) Test-A-CALAMUS Kidney



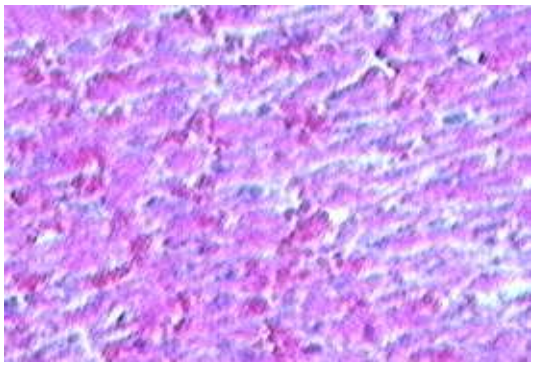
C_2) Test-A-CALAMUS



CTest-A-CALAMUS Kidney



D) Standard Drug (Silymarin) Liver



D) Standard Drug (Silymarin)Kidney

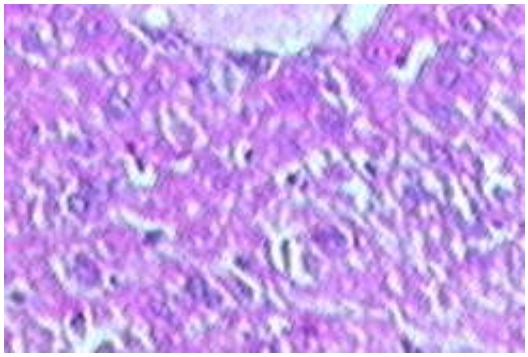


Fig- 6: Histopathological monograph of extract and standard. a. control b. Carbon tetrachloride (1ml/kg)alone c_1. Carbon tetrachloride+ Acorus calamus(1ml/kg +30mg/kg) c_2. Carbon tetrachloride+ Acorus calamus(1ml/kg +100mg/kg) d. Carbon tetrachloride+ Standard Silymarin(1ml/kg+ 5mg/kg)

Result

Hepatoprotective activity

The increses in the level of liver marker enzymes clearly indicated the damage of the hepatic cells. However treatment with Acorus calamus prevented alteration in the above levels similar to those in control rats. ccl4 (1 ml/kg ip body weight) induced mark increased in the serum hepatic enzyme levels of SGPT,SGOT,ALP, and decreased in total protein as compared to the normal control . Pre treatments of the rats HP01 30mg/kg and 100 mg/kg prior to ccl_4 administration caused significant reduction in values of SGPT,SGOT and increase in total protein levels but ALP and total bilirubin not affected significantly. Values are mean +- SD of 6 rats from each group. Group 2 is compared to group-I, Group3, 4, 5 are compared to group2. Values are not sharing a common superscript differ significantly at p values: ***<0.001, **,0.01,*,0.05(DMRT) Values are means ± SD of 6 rats from each group. Group 2 is compared to group 1, Groups 3, 4, 5 are compared to group 2. Values not sharing a common superscript differ significantly at p values: ***<0.001, ** < 0.01, * < 0.05 (DMRT).

Histopathological results-

The hepatoprotective effect of Acorus calamus was confirmed by histopathological examination of the liver tissue of control and treated animals (Fig.1). The histological architecture of control rats was found to be normal with distinct hepatic cells and sinusoidal space. In the group II i.e. the carbon tetrachloride liver sect ion showed congestion, mild centrilobular degeneration of hepatocytes, mild bile duct hyperplasia and multifocal cell infiltration.The histopathological profile of the group III rats showed mild degeneration of hepatocytes and in group IV rats no visible changes were observed confirming the safety of the extract at selected dose. In group V rats treated with Silymarin, intoxicated with Carbon tetrachloride showed less disarrangement and degeneration of hepatocytes .

Discussion

Carbon tetrachloride induced hepatic damage and account for considerable levels of morbidity and mortality. The advantage of this model is that being a dose- dependent toxicant, the experiments are technically easy to perform and most importantly, it is a clinically relevant. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. Administration of ccl_4 induced mark increase in serum hepatic enzyme level of SGPT, SGOT, ALP and decrease in normal protein as compared to normal control. Pretreatment of rats with Acorus calamus 30mg/kg and 100mg/kg prior to ccl_4 administration caused significant reduction in values of SGPT, SGOT and increase in total protein level but ALP and total bilirubin not affected significantly. Bilirubin I a yellow pigment produced when heme is catabolisized .Hepatocytes render the bilirubin water soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyperbilirubinemia may result from the production of more bilirubin than the normal process, damage to liver impairs its ability to excrete normal amounts of bilirubin or obstruction of excretory ducts of liver . Serum bilirubin is considered as one of the true test of liver function since it reflects ability of the liver to take and process bilirubin into bile. Acarocalmus helped not much decreasing significantly alter leves of bilirubin but it reduced significantly level of SGPT, SGOT and it has increased in total protein level.Thus study clearly demonstrate that acarocalmus exhibit hepatoprotective effect when compared with standard drug silymarin.However, further pharmacological evidence at the molecular level is required to estamblish the actual mechanism of action of the drug .

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