CHAPTER 8

HEPATOPROTECTIVE ACTIVITY OF ROOT BARK OF *CROTON CAUDATUS* GEISELER

8.1: Introduction

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. According to the United States, Acute Liver Failure Study Group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%) ***(Michael and Cynthia, 2005).***

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles. It is the second largest organ after skin and largest internal organ of the human body **(*Guyton and Hall, 1996*).**

Drugs are an important cause on liver injury. Approximately 75% of the idiosyncratic drug reaction results in liver transplantation (or) death (***Zimmerman, 1978***). Drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately -half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease **(*Kaplowitz, 2001*).** Different types of drugs such as acetaminophen, chloroquine and isoniazid are inducing hepatotoxicity in world. Isoniazid and rifampicin, the first line drugs used for tuberculosis therapy are associated with hepatotoxicity

(*Tasduq et al., 2005*). The rate of hepatotoxicity has been reported to be much higher in developing countries like India (8% - 30%) as compared to that in advanced countries (2% - 3%) with a similar dose schedule **(*Sharma, 2004).***

Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated **(*Mascolo et al., 1998).***

But there is not much drug available for the treatment of liver disorders **(*Karan et al., 1999, Chaterrjee, 2000*).** There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown **(*Gupta et al., 2005*).** Therefore, many folk remedies from plant origin are tested for its hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts ***(Rubinstein, 1962, Suja et al., 2002).***

From the above studies (Chapter 5, 6, 7) we confirmed that the methanol extracts of root bark of Croton *caudatus* Geiseler have higher activity than that of *Phyllanthus acidus* (L)Skeels. So, we selected the methanol extract of root bark of *Croton caudatus* Geiseler for further study of hepatoprotective activity of this particular plant.

8.2: Material and Methods

8.2.1 Extract preparation:

The collected root bark of *Croton caudatus* Geiseler were washed, air dried, powdered and exhaustively extracted with methanol and filtered. The filterate was concentrated and completely dry of the solvent to give a dry methanol extract.

8.2.2 Animals:

Swiss albino mice of weighing between 25-30g were collected from Pasteur Institute (Shillong) was used for the study. The mice were divided into six groups (I, II, III, IV, V and VI) of six animals each. The animals were maintained in closely inbred colony under conventional laboratory conditions at room temperature and in 12hr.light/dark cycle. Mice were provided with standard food pallet and water *ad libitium*. All experimental procedures followed animal research guidelines.

8.2.3 Drugs and chemicals:

Carbon tetrachloride (CCl_4) was obtained from Merck Ltd., Mumbai, India. SGPT, SGOT, ALP from SYNERGY-BIO, Quantum Bilogical Pvt.Ltd. Cholesterol from Medsource Ozone Biomedical Pvt.Ltd, Silymarin from MICROLABS LIMITED. All chemicals used in the study were of analytical grades.

8.2.4 Acute Toxicity Studies:

Acute toxicity studies were performed according to Organization for Economic Co-Operation and Development **(OECD)-423** guidelines ***(Ecobicon, 1997).*** Swiss albino mice were selected by random

sampling technique were employed in this study. The animals were fasted for 4 hours with free access to water only. *Croton caudatus* Geiseler was administered orally at a dose of 5 mg/kg initially. Mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. Since no mortality was observed in our study. The higherdoses (50, 300, 2000 mg/kg) of were *Croton caudatus* Geiseler employed for further toxicity studies.

8.2.5 Experimental procedure:

The animals were divided in to 6 groups of 6 animals each. Group-I, which served as normal control received distilled water (1ml/kg., p.o.), Group–II received equal mixture of CCl_4 and olive oil (50 % v/v, 0.5 ml/kg i.p.) once daily for 7 days **(*Rao et al., 1993*).** Group–III received equal mixture of CCl_4 and olive oil along with *Croton caudatus* Geiseler (500 mg/Kg, p.o.) simultaneously once daily for 7 days. Group–IV received equal mixture of CCl_4. and olive oil along with *Croton caudatus* Geiseler (1000 mg/Kg, p.o.) simultaneously once daily for 7 days. Group–V received equal mixture of CCl_4 and olive oil along with plant extracts(1500 mg/Kg, p.o.) simultaneously once daily for 7days ***(Sheweita et al., 2002).*** Group–VI received equal mixture of CCl_4 and Silymarin standard. Animals were sacrificed under light chloroform anesthesia on 8^{th} day, 48hrs after CCl_4 administration. The blood was collected by direct cardiac puncture and serum was separated for various biochemical estimations. The body weight of each animal before starting the experiment and just

before sacrifice was recorded. After sacrifice the liver weight of each animal was also recorded. The livers were excised quickly and fixed in 10% formalin and stained with haemotoxylin and eosin for further histopathological studies.

8.2.6 Statistical analysis:

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA one way analysis, ORIGIN v 3.0) followed by student's t-test. P values <0.01 were considered significant.

8.2.7 Bio-chemical analysis:

Serum samples were used for estimation of the activities of SGPT, SGOT ***(Bergmeyer et al., 1978)*****,** alkaline phosphate (ALP) were measured by the method of ***Kind &King (1954)***. The results were expressed as units/litre (U/L).

8.2.8 Calculation of hepatoprotection:

Percentage of hepatoprotection for each biological parameter is calculated by assuming that there was no protection (100%) damage in CCl_4 control group (***Subramoniam et al., 1998).***

$$\text{Percentage of hepatoprotection} = 100 - \frac{100}{(\text{Toxin control-Normal control})} \times (\text{CCGE and toxin - Normal control})$$

8.3: Results and Discussions

8.3.1 Acute Toxicity Studies:

All the doses (5, 50, 300, 2000mg/kg, p. o.) of *Croton caudatus* Geiseler employed for acute oral toxicity studies were found to be non-toxic. *Croton caudatus* Geiseler did not produce any mortality even at the highest dose (2000mg/kg p.o.) employed. Three sub-maximal doses

(500mg/kg, 1000mg/kg, and 1500mg/kg) which were found to be safe were employed for further pharmacological investigation.

8.3.2 Bio-chemical analysis:

The results of biochemical estimation of blood serum of bark of *Croton caudatus* Geiseler on CCl_4-treated albino mice are shown in Table 8.3.2.

Table 8.3.2: Effect of methanol extract of root bark of *Croton caudatus* Geiseler on serum enzymes (SGOT, SGPT, and ALP) and cholesterol in albino mice treated with CCl_4.

(Acute, 7 day treatment, Values are MEAN±SEM, n=6)

Group	Dose(mg/kg)	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	Cholesterol (Mg/dl)
Normal Control	1ml dis.water	23.42 ± 1.34	34.5 ± 3.68	47.9 ± 2.91	58.83 ± 1.36
CCl_4 Control	2ml/kg	112.28 ± 5.07**	117.27 ± 4.91**	126.2 ± 8.4**	154.22 ± 3.43**
CCGE	500mg/kg	87.1 ± 1.05**	87.9 ± 1.69**	95.65 ± 1.8**	88.15 ± 1.84**
	1000mg/kg	51.68 ± 1.9**	57.3 ± 2.58**	82.43 ± 1.12**	76.88 ± 3.75**
	1500mg/kg	38.2 ± 2.08**	39.7 ± 1.2**	66 ± 2.37**	71.39 ± 1.31**
Silymarin (Reference)	100	37.45 ± 1.23**	37.2 ± 1.66 **	65.53 ± 2.23**	61.18 ± 4.46**
One way ANOVA	F	175.67	131.06	48.9	143.5
	df	5,30	5,30	5,30	5,30
	p	0.05	0.05	0.05	0.05

Note: CCGE-*Croton caudatus* Geiseler extract

** $P<0.01$ as CCl_4 Control group was compared with the Normal Control group and the rest of the other group compared with the CCl_4 treated group.

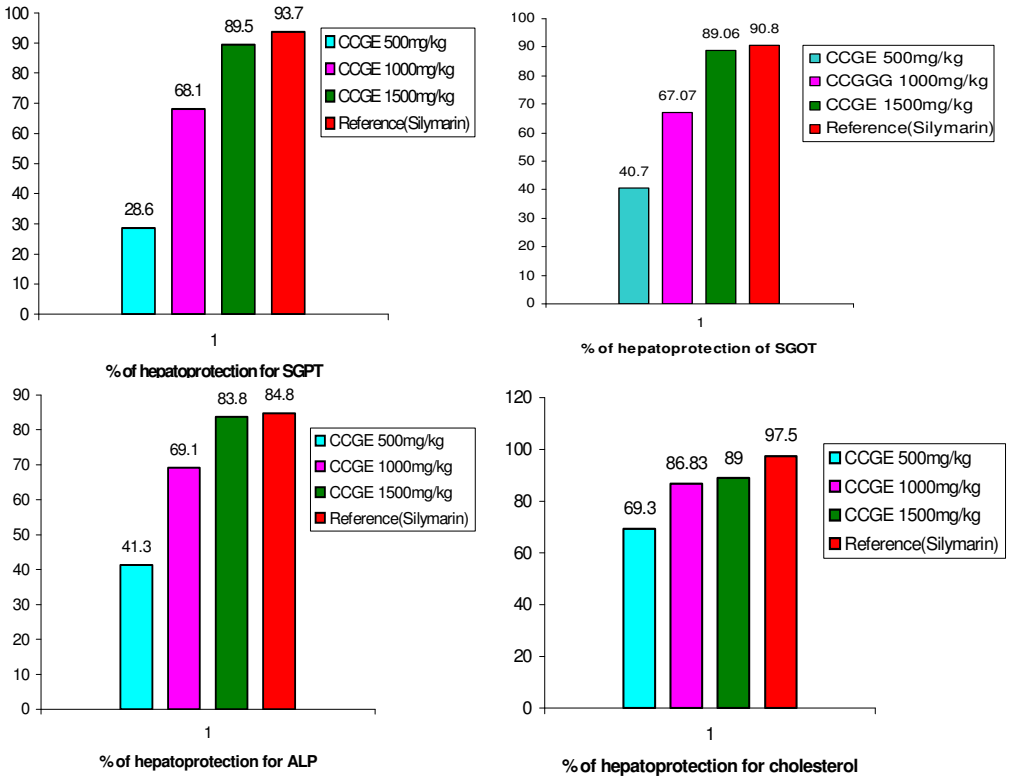

Fig.8.3.2(a): Bar graph showing the percentage of hepatoprotection of various enzymes such as SGOT, SGPT, ALP and Cholesterol.

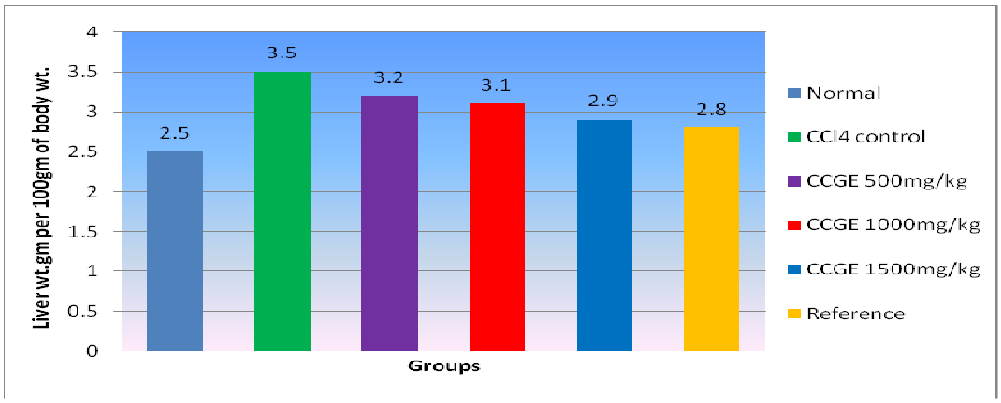

Fig.8.3.2(b): Effect of concurrent administration of root bark of *Croton caudatus* Geiseler, CCl_4 (acute treatment) on body weight (gm) and liver weight in albino mice.

8.3.3 Histopathological studies:

General histopathological analysis of the liver in the control group showed the sinusoids and the portal areas were found to be essentially normal.

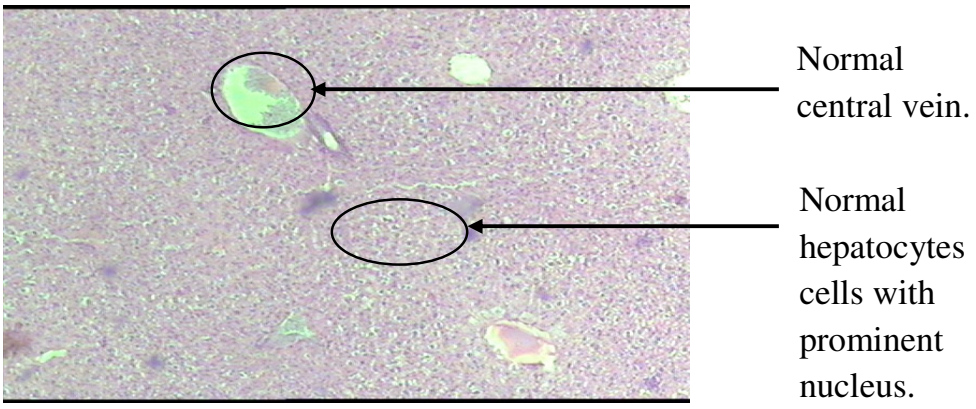

Fig.8.3.3(a): Transverse sections of the liver of control rats receive 1ml/kg, distill water.

It shows normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein, cell cords are normal.

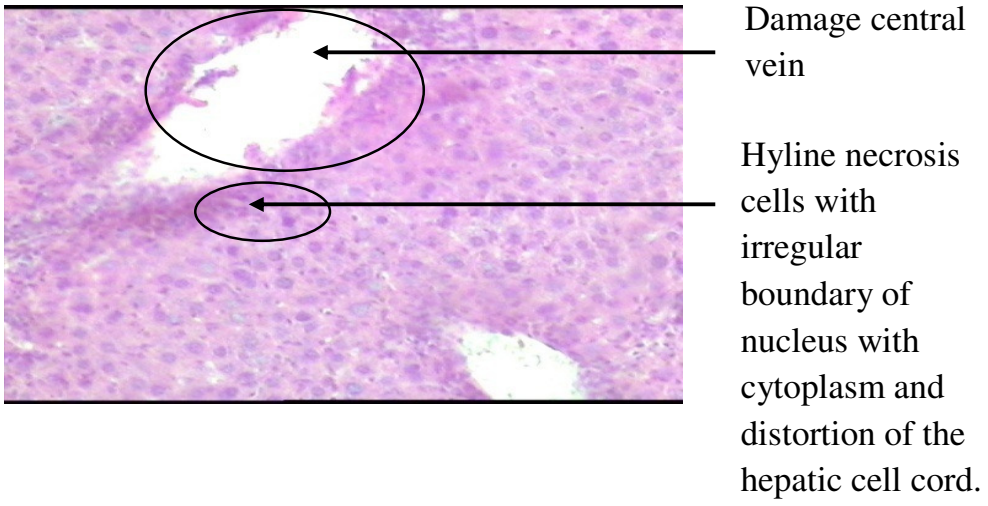

Fig.8.3.3(b): Transverse section of liver from mice which receive CCl_4+olive oil (1:1).

The liver of CCl_4 treated animals showing hydropic changes in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils, congestion of central vein and sinusoids were seen with acute inflammatory cells infiltrating sinusoids mainly in central zone. Cell morphology is not distinct including cytoplasmic outline is not well defined and nucleus are enlarge with irregular margins.

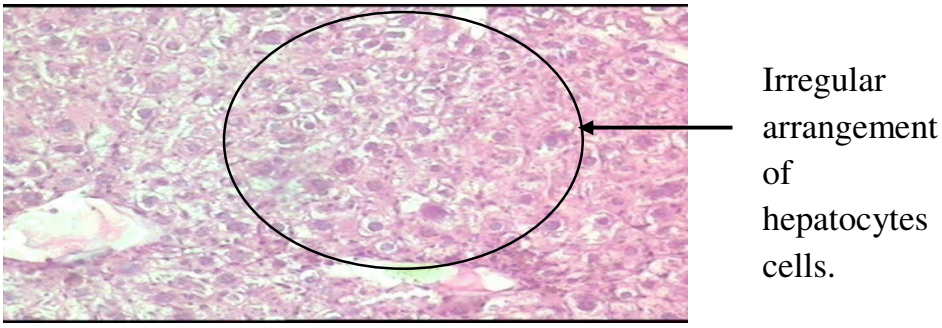

Fig.8.3.3(c): Transverse section of the liver from treatment of root barks of *Croton caudatus* Geiseler (500mg/kg) and CCl_4.

The liver of treated animals showing mild fatty change and mild sinusoidal congestion.

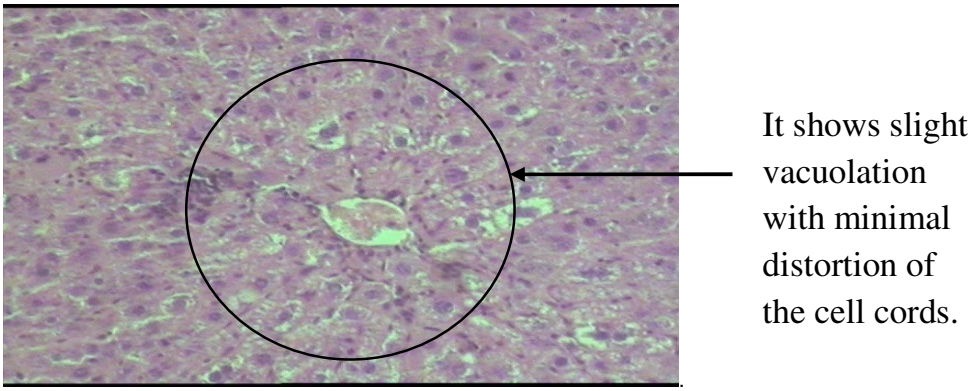

Fig.8.3.3(d): Transverse section of the liver, after simultaneous treatment of root bark of *Croton caudatus* Geiseler (1000mg/kg) and CCl_4

The liver of treated animal showing residual hepatocellular necrosis with cords of regeneration hepatocytes and maintain more architecture.

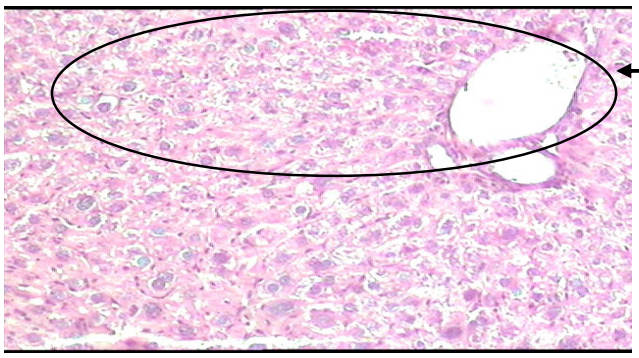

Fig.8.3.3(e): Transverse sections of the liver, after simultaneous treatment of root bark of *Croton caudatus* Geiseler (1500mg/kg) and CCl_4.

Regeneration/Recovery of hepatocytes after simultaneous treatment of root extract of *Croton caudatus* Geiseler .Cells are more prominent and well defined as normal one.

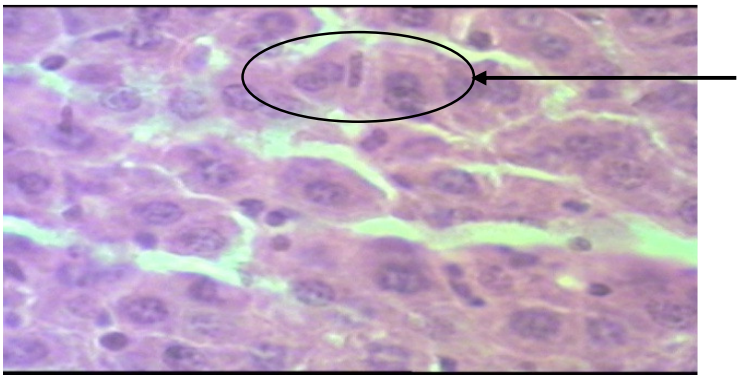

Fig.8.3.3(f): Transverse section of the liver, after simultaneous treatment of silymarin 140mg/kg and CCl_4

The treated animals showing mild central venous congestion and mild fatty vacuolation.

CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases (***Johnson and Kroening, 1998).***

The serum SGOT, SGPT, ALP and Cholesterol are liable markers of the liver function. They are significantly increased in the CCl_4 treated group. On the other hand, in group III treated with 500mg/kg, the serum levels are decreased significantly ($p<0.01$). Simultaneous treatment of root bark extract of *Croton caudatus* Geiseler and CCl_4 caused significant recovery from the damage induced by CCl_4 treatment. The fall in serum enzymes suggests a protective effect of *Croton caudatus* Geiseler on the liver against CCl_4-induced toxicity.

8.4: Conclusion

Histopatholoical studies showed that CCl_4 caused centrilobular necrosis, congestion of central vein and sinusoids effect on liver of mice.*Croton caudatus* Geiseler administration exhibited protection against CCl_4-induced hepatotoxicity, which is also confirmed by the results of biochemical studies. From the above studies, it can be concluded that the root bark extract of *Croton caudatus* Geiseler is more hepatoprotective than silymarin, the standard reference drug.

8.5: Bibliography

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