Hepatoprotective Activity of *Cassia fistula* root against Carbon tetrachloride-Induced Hepatic Injury in rats *(Wistar)*

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ABSTRACT:

The protective effects of the alcoholic extract of *Cassia fistula root*; against CCl_4 induced hepatic failure in male albino rats (wistar strain) was investigated. For acute and massive invasion of hepatopathy, CCl_4 (s.*c)* injection of CCl_4+Olive Oil in 1:1 ratio; 2ml/kg) was used and the insidious intoxication was evidenced by significant turmoil of various biochemical parameters followed by significant ($p<0.001$) weight loss in toxic control group.

The administration of alcoholic root extract (200mg/kg and 100mg/kg of body weight) for 7 days, elicited protective action since the elevated levels of marker enzymes (SGOT, SGPT, ALP) of liver functions were found to be decreasing progressively in a dose dependent manner. The final body weight was also significantly ($p<0.001$) increased when compared with the toxic control group. The serum total protein and the serum albumin were also approaching normal values. The results found in alcoholic extract 200mg/kg treated rat were quite promising and were comparable with a standard drug Silymarin. In the alcoholic extract 200mg/kg treated rat group all the marker enzymes were analyzed to be decreasing significantly.

The statistically processed results support the conclusion, that the alcoholic root extract of *Cassia fistula root* (200mg/kg and 100mg/kg) possesses dose dependent, significant protective activity against CCl_4 induced hepatotoxicity.

KEY WORDS: *Cassia fistula*, hepatopathy, biochemical parameters, SGOT, SGPT, ALP, Silymarin.

INTRODUCTION:

The entire world population is turning towards natural drugs because of the widespread belief that "green medicines" are healthier and safer than synthetic ones[1]. It is also gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality of life[2]. Natural products have also been the starting point for the discovery of many important modern drugs. This fact has led to chemical and pharmacological investigations and general biological screening programs for natural products all over the world[3]. Liver is the important vital organ, regulating various physiological processes such as, metabolism, secretion, storage and detoxification of toxic substances. Therefore, damage to the liver inflicted by hepatotoxic agents is of great concern[4].

The hepatotoxicity is mainly caused by toxic chemicals such as carbon tetra chloride (CCl_4), alcohol, drugs such as paracetamol, antidiabetic drugs. Most of the chemicals damage the liver cell mainly by inducing lipid peroxidation and other oxidative damages. The reactive oxygen species such as superoxide anion radical ($.O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (.OH) have been implicated in the pathophysiology of various clinical disorders including ischemia, atherosclerosis, acute hypertension, liver

diseases and diabetes mellitus. With the shifting of attention from synthetic drugs to natural plant products[5], the use of plant extracts for the treatment for liver diseases are now on the increase. Plants that were once considered of no value are now being investigated, evaluated and developed into drugs with no side effects. One such potential plant is *Cassia fistula* Linn. (Hindi-Amaltas, English-Golden shower, Indian labrum), a member of *Fabacea* (alt. leguminasae) family. It is a native of India[6,7,8]. It is also grown in Mauritius, South Africa, Mexico, Brazil, China, Nepal, West Indies and East Africa as an ornamental plant due to its beautiful bunches of yellow flowers [9,10,11]. *C. fistula* has been used in the treatment of various ailments in ancient India, dating back to S*ushruta Samhita* and C*haraka Samhita*[12,13]. Both the leaves and pods were widely used in traditional medicine as strong purgatives and laxatives[14,15]. In ayurvedic medicinal system, *C. fistula* was used against various disorders such as haematemesis, pruritus, leucoderma, diabetes and other ailments[16.].

The leaves of *C.fistula* are known for their laxative, antiperiodic, ulcer healing and anti-rheumatic properties. Leaves of *C.fistula* were also found effective against cough and ringworm infections[17,18,19,20 .]

MATERIAL AND METHOD:

Plant material:

Cassia fistula were collected from Amravati (Amravati University campus), Maharashtra, India and authenticated by the taxonomist at the Department of Botany, Faculty of Science, Government Vidharbha Institute of science and Humanities Amravati University. A voucher specimen was deposited in the herbarium of Botanical Survey of India (BSI) for future reference with deposition number SADACAF9.

Preparation of extract:

Air dried and coarsely powdered root of the plant (1 kg) were soxhlet extracted with ethanol for 72 h. The ethanolic extract was then concentrated on a water bath and dried under reduced pressure to achieve a dark brown mass (95 g; yield- 9.5%).

Preliminary phytochemical screening:

Preliminary Phytochemical Screening of the extract was done by standard procedure (Khandelwal, 2000)[21].

Animals:

Inbred colony of adult Wistar albino rats (170 –200 g) of either sex were used for the pharmacological activities. They were kept in polypropylene cages at 25 ± 2° C, with relative humidity 45-55% under 12h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed (Treemurti feeds Nagpur,India.) and water *ad libitum.* The test extracts and the standard drugs were administered in the form of a suspension in water using 1% carboxymethylcellulose as suspending agent. The protocol of present study was approved by Institutional animal ethical committee constituted as per CPCSEA guidelines (1060/ac/07/CPCSEA/Dec2009).

Acute toxicity:

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method),). Wistar rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study[23]. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg / kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg / kg body weight.

Drugs and dosing schedule:

The animals were divided into six groups; group I (control), group II (CCl_4 treated), group III (CCl_4 + Silymarin treated), group IV and V (CCl_4 + extract). Animals of groups II, III, IV and V were administered 50% (v/v) CCl_4 in olive oil in a single dose of 2ml/Kg of body weight per day for 4 days via the S.C.(Sub-cutaneous) route. Simultaneously but at different hours of the day, animals of groups III, IV and V were fed with silymarin suspension (10 mg/kg body weight, I.P. (Intra-peritonial) in addition to ethanolic extract in doses of 100 mg/kg and 200 mg/kg body weight, IP for 4 days respectively. Animals of group I, were given distilled water in a volume of 10 ml/kg body weight.

Preparation of samples for Biochemical studies:

On the day 5, after the treatment period all of the subject animals were anaesthetized and sacrificed and blood was withdrawn from heart and their serum was separated by centrifugation at 3000 rpm at 30^0C for 15 min. This was subsequently analyzed for various biochemical parameters including serum transaminases viz. SGOT[23], SGPT[23] total protein[24], total albumin, alkaline phosphatase[25,26] .

Histopathological evaluation of liver:

The formalin fixed liver tissues were embedded in paraffin wax and microtome sections of 5-6 μm were made from them. These thin sections were stained with haematoxylin and eosin for Light microscopy. Photomicrographs were subsequently made from these sections.

Statistical analysis:

The data obtained were analyzed using student's t-test and results expressed as Mean ± standard error of mean. Statistical differences between means were determined by ANOVA. Values of $p<0.05$ was considered significant.

RESULTS:

TABLE I: Phytochemical analysis of *Cassia fistula* ethanolic roots extract:

Sr.No.	Test	Result
1.	Alkaloids -	_ ve
2.	Glycosides+	+ ve
3.	Cardiac glycosides+	+ve
4.	Anthraquinones+	+ve
5.	Saponins-	-ve
6.	Flavonoids+	**+ve**

- Absent, + Present.

TABLE NO. II: Effect of *Cassia fistula* root extract and silymarin on biochemical parameters during CCl_4 induced acute liver damage in rats (n=6)

Sr.No.	S.G.O.T	S.G.P.T	A.L.P	Billirubin	CHL	ALB
Control (Normal)	44.50±1.51	36.83±2.63	25.48±1.16	2.17±0.12	101.3±2.40	3.98±0.14
CCl_4 Treated	145.50±40.02	128.00±11.10	89.82±7.36	12.52±1.34	213.8±27	1.06±0.28
Standard Silymarin	60.00±5.01***	43.50±5.99***	43.43±2.198***	5.25±1.34***	106.1±6.9*	4.35±0.21**
Ethanolic Extract(100mg)	62.67±6.60**	47.67±4.63**	43.43±2.04**	5.52±1.41	111.3±10.48*	3.95±0.36*
Ethanolic Extract(200mg)	70.33±5.47**	56.67±2.94**	57.15±9.80**	5.98±1.40**	115.30±2.50**	3.99±0.40**

Values in Mean ± S.E.M, N=6.
* Significant reduction compared to carbon tetrachloride (P<0.05)
** Significant increase compared to Carbon tetrachloride (P<0.05)

Phytochemical investigation:

The preliminary phytochemical investigation of the alcoholic extracts of the *Cassia fistula root* showed that it contains Alkaloids, Glycosides, carbohydrates, tannins, flavanoids, steroids, proteins and amino acids.The preliminary phytochemical analysis of the crude extracts (ethanol) of *Cassia fistula root* indicated the presence of Alkaloids, Glycosides, cardiac glycosides, tannins, flavanoids, ,saponins, proteins and amino acids as shown in table I.

Serum Analysis:

The administration of CCl_4 induced acute liver damage which is well indicated (Table II) by increased SGOT (Serum glutamate oxalate transaminase) Fig.AI, SGPT (Serum glutamate pyruvate transaminase) Fig.AII, ALP (Alkaline phosphatase) Fig.AIII, TPTN (Total protein) Fig.AIV, CHL (Cholesterol) Fig.AV and TBL (Total bilirubin) Fig.AVI when compared with normal control group. The highly significant ($p<0.0001$) reduction was observed in all the parameters in the ethanolic extract group in comparison with positive control treated group. Among these, ethanolic extracts shows maximum percentage reduction in SGOT, SGPT, ALP and TBL after administration of CCl_4. Though 200mg/kg alcoholic extract group also show significant results but less pronounced when compared with the 100mg/Kg ethanolic extract group. These results suggest the

possibility of the 200mg/Kg ethanolic extract to give very good protection against liver injury upon CCl_4 induction within 72 hrs.

Histochemical studies:

Histopathological studies also provided supportive evidence for the biochemical analysis. Normal control group showed a normal liver architecture, hepatocytes very well arranged, central and portal veins without alterations (figure BI). The livers of rats treated with CCl_4 for 5 days showed total loss of hepatic architecture with extensive accumulation of connective tissue resulting in formation of continuous fibrotic septa, nodules of regeneration, fatty changes, noticeable alterations in the central vein, hepatic necrosis, vacuolization, congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein, pronounced inflammation from portal to portal tract bridges and apoptosis compared to the normal control (figure BII).

Oral administration of Silymarin shows recovery of hepatic architecture with uniform central vein dilation and precentral hepatitis with lymphomonocytes surrounding the vein. Some little foci were observed in portal triaditis and only central zone hepatitis as Silymarin treated (figure B III). There are thick inflammatory bridges between portal tracts with parenchymal collapse. Inflammation around portal tracts includes some loss of lamina limitans (figure BII), among these plant extract, treatment with ethanolic extract returned the injured live to quite normal. Less pronounced destruction of the liver architecture without fibrosis and minimal inflammation. Only peripheral zonal fatty changes were observed as in extract treated (figure B IV) and (figure BV).

DISCUSSION:

Reactive oxygen species (ROS) are causative factors of degenerative diseases, including some hepatopathies. Liver is an important organ actively involved in metabolic functions and is a frequent target of number of toxicants. Carbon tetra chloride has been widely used for inducing experimental hepatic damage due to free radical formation during its metabolism by hepatic microsome, leading to lipid peroxidation, and consequently, liver damage. The resulting hepatic injury is characterized by leakage of cellular enzymes into blood stream and by necrosis and fibrosis[27].

Carbon tetrachloride is selected as hepatotoxicant in inducing injury to the liver as it is known to cause hepatotoxicity in man and experimental animals when given in overdose. Popularity of herbal remedies is increasing globally and at least one quarter of patients with liver diseases use ethnobotanicals[28]. Many formulations containing herbal extract are sold in the Indian market for liver disorders[29].

Therefore the present study was aimed at evaluating the scientific basis for the traditional use of *Cassia fistula root* using invivo experimental model. Several studies shown that α-tocopherol (Vitamin E) and Silymarin are potent antioxidant that could protect the liver against CCl_4 hepatotoxicity indicating that oxidative stress could play a pivotal role in CCl_4 hepatic injury[30]. To confirm the effects of the herbal medicinal plants in this study, biochemical and histological parameters were used. Assessment of liver damage can be made by estimating the activities of serum ALT, AST, ALP, TB which are enzymes and proteins originally present in higher concentration in cytoplasm. The elevated levels of these entire marker enzymes observed in the CCl_4 treated group II rats in the present study corresponded to the extensive liver damage induced by toxins, the tendency of these marker enzymes to return towards a near normalcy in group 3(Silymarin), 4 and group 5 (ethanolic extract) treated rats was a clear manifestation of hepatoprotective effect of *Cassia fistula root.*

The protective effect of *Cassia fistula root* is also proved by histopathological examination of livers of rats treated with ethanolic extracts prior to CCl_4 administration which were almost normal in structure with slight changes. Although the exact mechanisms behind this protection are uncertain, many theories have been proposed. In the last decades, special attention has been paid towards edible plants, especially those that are rich in secondary metabolites and now days, there is an increasing interest in the antioxidant activity of such phytochemical present in diet.

However we can assume that the protective factor in this study is due to presence of alkaloids, flavonoids, saponins, tannin and terpenoids. The probable mechanism is mediated by their higher quantity of

flavonoids or terpenoids in ethanolic extract or by their combination via antioxidant and free radicals scavenging activities[31]. Flavonoids, phenolic acids and some terpenoids have been reported to possess antioxidant activities by different mechanisms[32].The second probable mechanism is due to effective blocking of oxidative stress and cytokines production, ethanolic extract protected against lipopolysaccaride induced liver damage through decreasing the level of TNF- and IL6 and prevented cytotoxic effect of oxygen free radicals and cytokines[33].

In conclusion the present study demonstrated that ethanolic extract of *Cassia fistula root* in comparison with silymarin groups has better hepatoprotective effect in CCl_4 induced liver damage. However, it is necessary to determine other parameters such as oxidative stress markers and molecular biology assays to confirm our findings. However, further studies will be required on molecular level and isolation of active constituents to substantiate this effect.

ACKNOWLEDGMENT:

The authors are grateful to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, Ministry of Justice and Empowerment, Government of India and Institutional Animal Ethical Committee, Government Vidarbha Institute of Science and Humanities, Amravati for giving the permission for performing the experimental work on rats.

FIGURES

FIGURES A: Showing comparative account of different markers of each group with control.

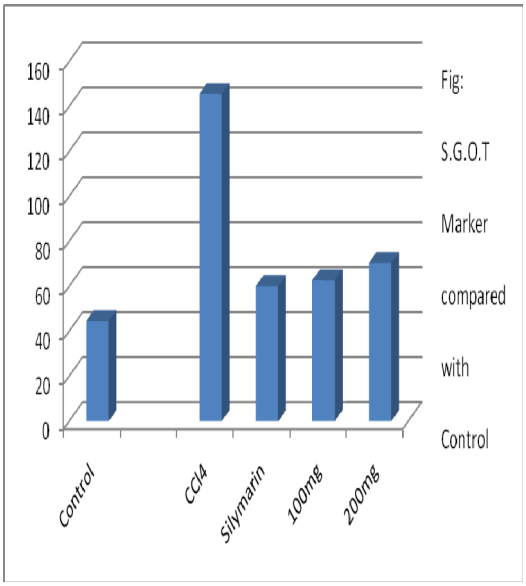

Figure: Serum Glutamate Oxaloacetate Transaminase.

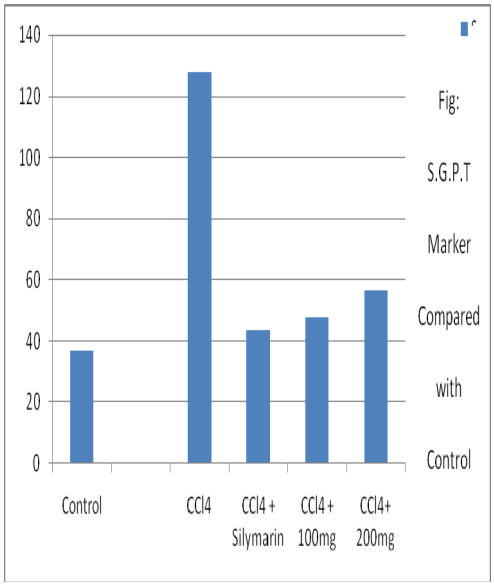

Figure: Serum Glutamate Pyruvate Transaminase.

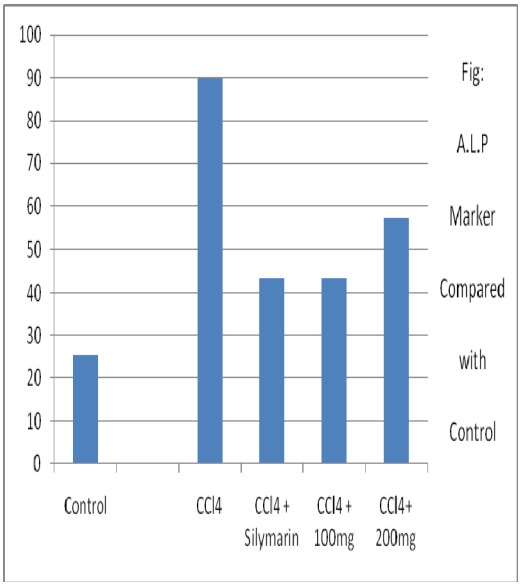

Figure: Serum Alkaline Phosphatase.

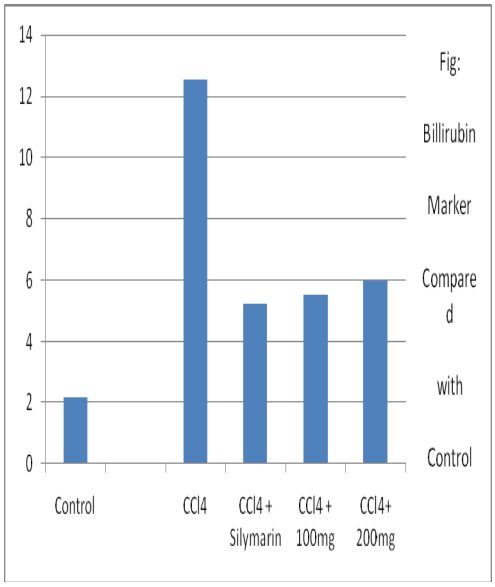

Figure: Serum Billirubin.

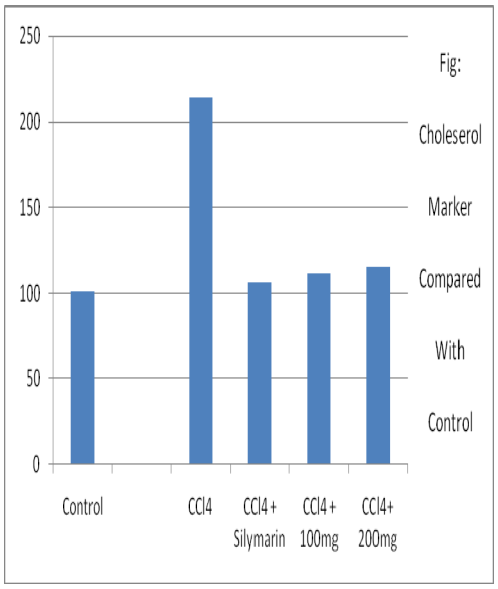

Figure: Serum Cholesterol.

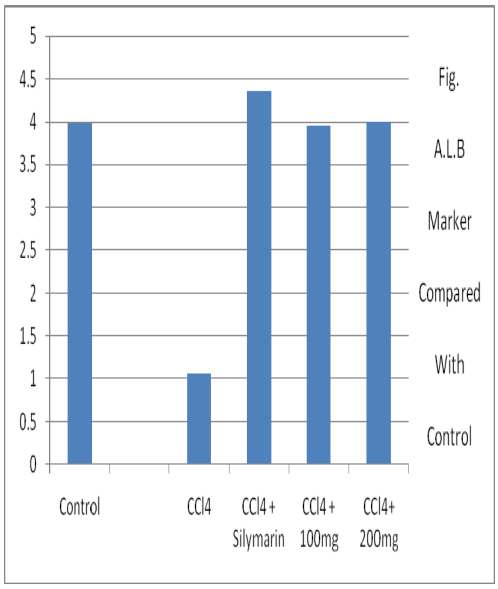

Figure: Serum Albumin.

FIGURES B: Showing the structure of Liver and Histological architecture.:

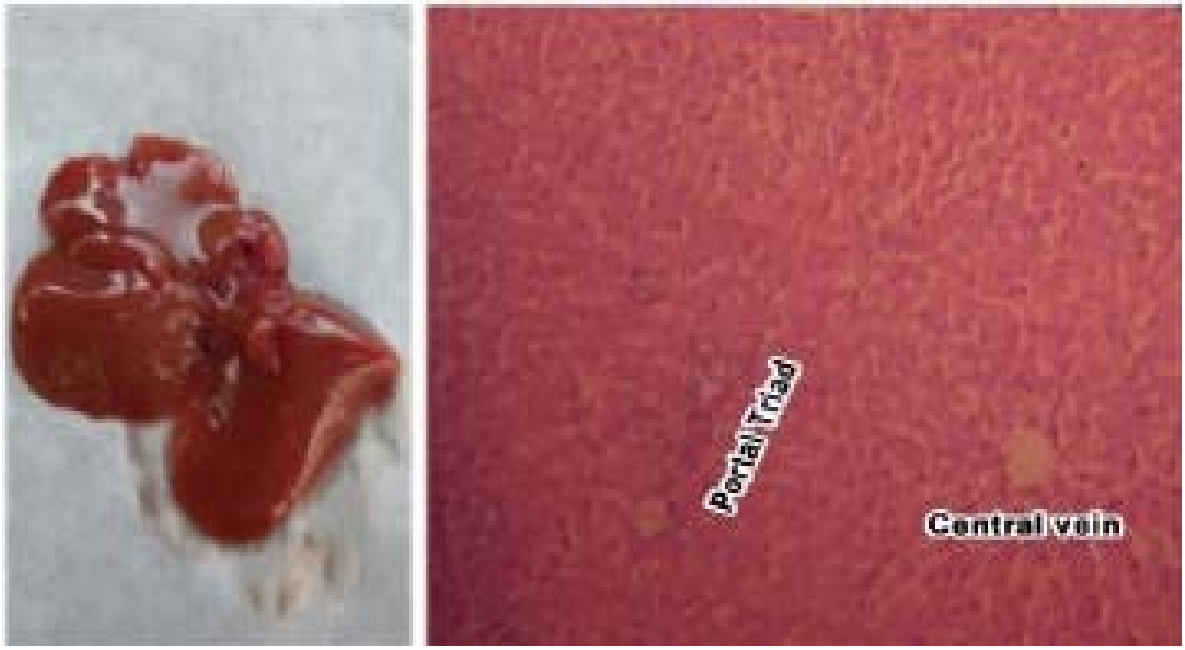

Fig.I : Control

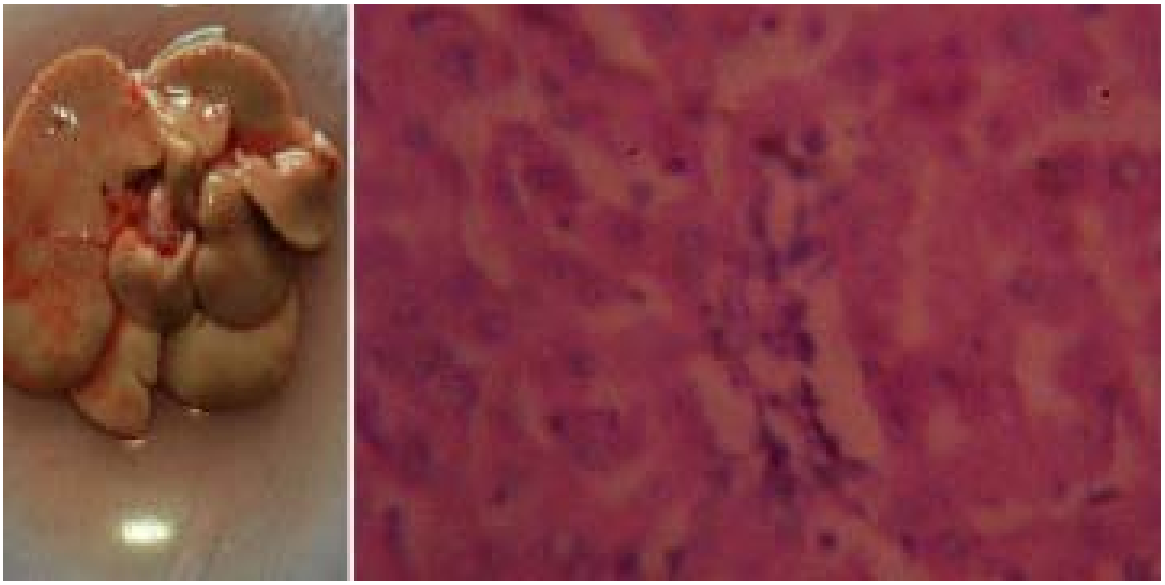

Fig.II : CCl_4 (400x)

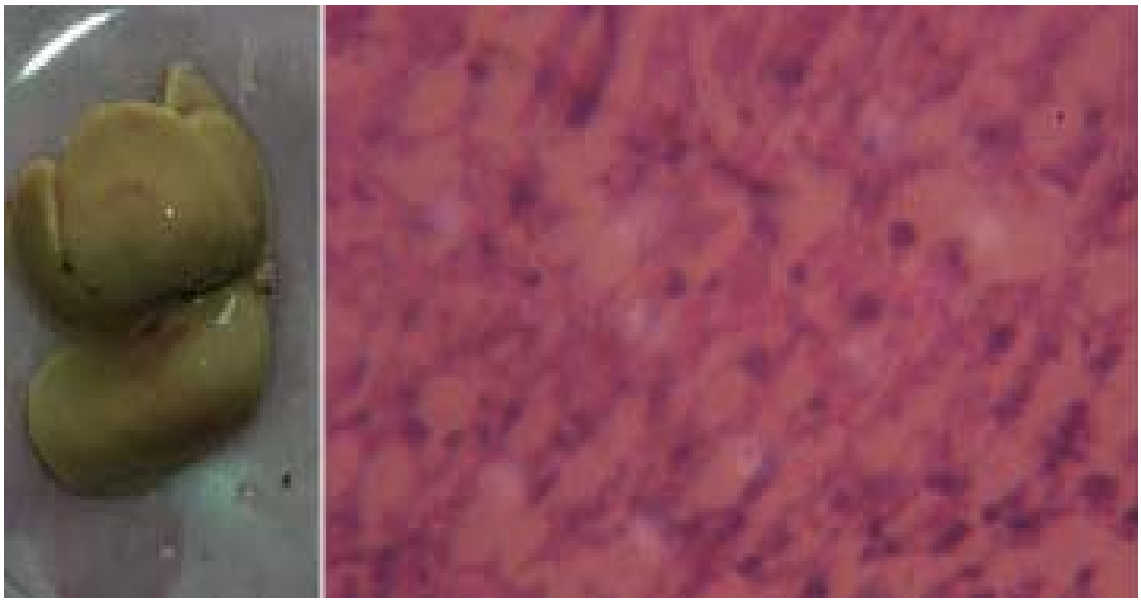

Fig.III : CCl_4 + Silymarin (400x)

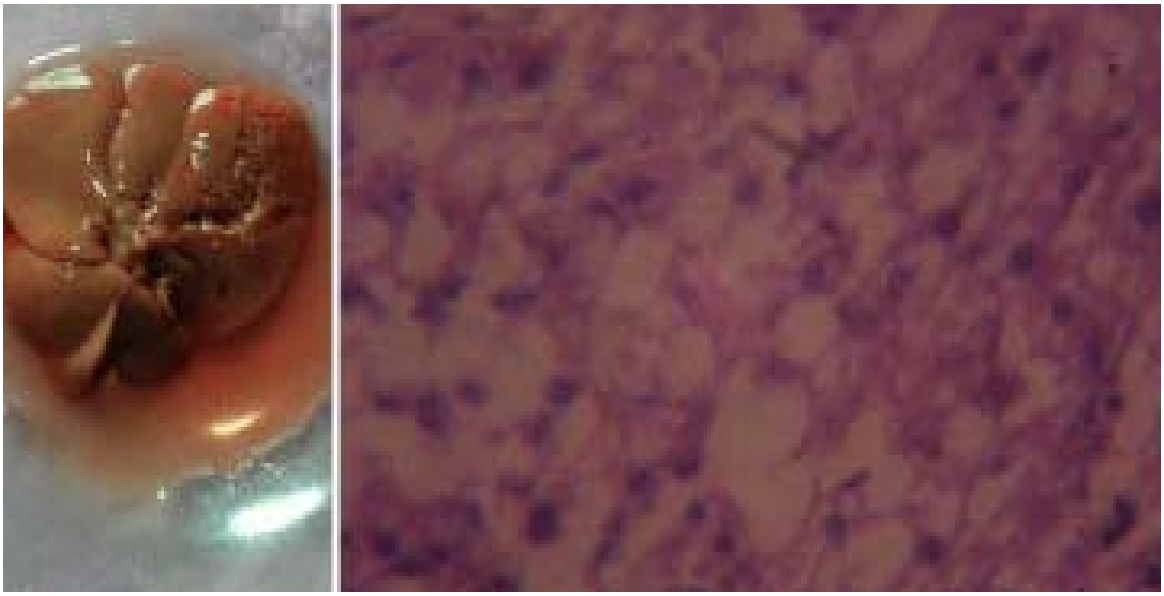

Fig.IV : CCl_4 + Extract 100mg/Kg (400x)

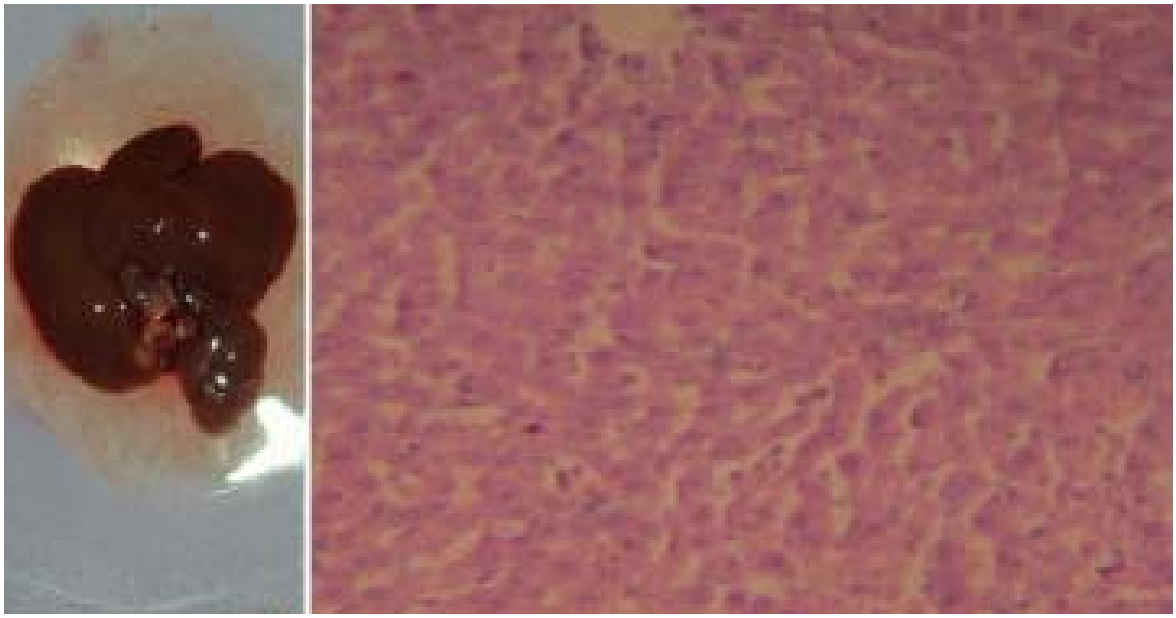

Fig.V : CCl_4 + Extract 200mg/Kg (400x)

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