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PHARMACOLOGICAL EVALUATION OF CYAMOPSIS TETRAGONOLOBA L. EXTRACTS FOR ITS HEPATOPROTECTIVE ACTIVITY

Shrinivas Sarje[1*], Seema Bhalerao[1], Mahesh Kamble[1], Shagufta Farooqui[1], Arshad Ibrahim[1]

[1]Department of Pharmacology, Nanded Pharmacy College, Nanded, Maharashtra, India.

ABSTRACT:

The *In-vivo* hepatoprotective activity of C.T.EA and C.T.Mth extracts were estimated by using carbon tetrachloride induced hepatotoxicity model. The degree of protection was estimated by measuring levels of biochemical markers like SGOT, SGPT, Total Bilirubin and Total Protein. The histopathological study was also carried sout and compared with carbon tetrachloride treated group. The interpretations of results were done by subjecting the data to statistical analysis using mean ±S.E.M. The C.T.Mth extract at dose of 200mg/kg shows promising Hepatoprotective effect than C.T.EA extract. The C.T.Mth extract of plant shows significant antioxidant activity at the concentration of 125µg/ml than C.T.EA extract at the concentration of 125µg/ml. The results suggest that the pods of *Cyamopsis tetragonoloba L.* possess potential antioxidant and hepatoprotective activity.

Keywords: *Cyamopsis tetragonoloba L.* (C.T.) Ethyl Acetate (EA) and Methanolic (Mth.) extracts, Hepatoprotective activity.

INTRODUCTION:

Liver is vital organ which plays important role in metabolism, storage, detoxification, synthesis and regulation of various body processes. Liver is largest and heaviest gland of the body weighing about 1.4 kg. In the average adult it is second largest organ of the body located in the diaphragm and occupies most of right hypochondrium and part of epigastrium of the abdomen.The causes of liver disease are viruses, excessive drug therapy, environmental pollution, alcoholic intoxification etc. Liver receive blood supply fromhepatic artery(20%) and portal circulation (80%) up to 20-25% of total cardiac output. Toxin, infectious agent medication and serum inflammatory mediator enter into the liver through the blood, may result in diverse range of disease processes, causing the loss of normal histological architecture reduced cell mass and loss of blood flow this may lead to decline liver function. No effective hepatoprotective therapy is available. Conventional medicines used in liver treatments are often insufficient. Many chronic irreversible and acute hepatic disorders culminate in ultimately

death due to lack of adequate remedies in modern medicines. It is therefore necessary to search for alternative drugs for treatment of liver diseases to replace currently used drugs of controversial efficacy and safety. In this condition there is greater demand of herbal formulation to treat liver diseases in developed as well as in developing countries for primary health care.

MATERIAL AND METHODS:

Animal used:

For the study Wistar ratsof either sex, of weight 150-200gm were selected.

Test group:

For the study six groups of animals were made. Each group having six rat.

Route of administration: Oral route administration.

Housing Condition:

Animals were housed six groups in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups; each group consisting of three mice.

Hepatoprotective Activity:

Hepatoprotective study of whole plant of *vigna radiata* was carried out using CCl_4 induced hepatotoxicity in rats.

IAEC Approval

Wistar rats of either sex weighing 150 to 200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

1.Carbon tetrachloride induced hepatotoxicity

Experimental design:

Male and female Wistar rats with weighing 200–250 g will be used. Animal will be randomly divided into 11 groups containing six animals in each group.

1. **Group I** (Negative control) Animals will receive Dimethyl Sulfoxide/ Saline water orally
2. **Group II** (Positive control) Animal will receive Carbon Tetrachloride
3. **Group III** Animals will receive standard drug (Silymarin).
4. **Group IV** Test group1 (Ethyl acetate extract, dose 100 mg/kg) for seven days.
5. **Group V** Test group 2 (Ethyl acetate extract, dose 200 mg/kg) for seven days.
6. **Group VI** Test group 3 (methanol extract, dose 100 mg/kg) for seven days.
7. **Group VII** Test group 4 (methanol extract, dose 200 mg/kg) for seven days.

Procedure:

- Albino Wistar rats of either sex [200–250 g] were used in the study.
- Animals were randomly divided into seven groups containing six in each six each namely.
- Food was withdrawn 16 hrs before administration to enhance the acute liver toxicity.
- **Group II, III, IV, V ,VI and VII** were treated with CCl_4 **was administered (1 ml/kg) diluted in olive oil (1:1) was administered on 7th day after 1hr** of test sample treatment and scarified 24 hours after administration of CCl_4.
- On 7th day the blood was collected by carotid artery or retro orbital under mild ether anesthesia, serum was collected by allowing the blood samples to coagulate for 30 min at 37^0C followed by centrifugation (3000 rpm for 15 min) and biochemical parameters like Serum Glutamate Pyruvate Transminase (SGPT/ALT), Serum Glutamate Oxaloacetate Transminase (SGOT/AST), Total bilurubin and Total protein were estimated in the blood serum using Autoanalyser. Animals were sacrified by overdose of diethyl ether (inhalation anesthesia) and livers from all animals were removed, washed, collected and preserved in 10% formalin for histopathological studies.

Evaluation procedure for biochemical parameter

The blood was collected by carotid artery or retro orbital puncture from the ether anesthetized rats. The blood was allowed to clot and then centrifuged at 3000 rpm for 15 min. The haemolysed free serum sample were used for determination of biochemical parameters The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit. (Autoanalyser).Evaluation was carried out by estimating parameters such as Total bilurubin, Total protein, SGPT, SGOT by using enzymatic kit.

Statistical Analysis

The data were expressed as mean + standard of mean (SEM).Statistical analysis were performed by one way analysis of variance (ANOVA).

RESULTS:

"Pharmacological Screening of *Cyamopsis tetragonoloba L.* pods Extracts Extracts for Hepatoprotective activity"

Table no 1. Total Bilirubin levels in all groups of animal

Sr. no	Groups	Treatment	Total Bilirubin (mg/dl)
1	Group I	Negative Control (vehicle)	0.40±0.03
2	Group II	Positive Control (CCl_4)	7.10±0.19
3	Group III	Standard(Silymarin)	0.58±0.01
4	Group IV	C.T. Eth. A. Extracts100mg/ kg	2.20±0.20
5	Group V	C.T. Eth. A. Extracts 200mg/ kg	0.70±0.01
6	Group VI	C.T. Methanol extracts 100mg/ kg	1.20±0.13
7	Group VII	C.T. Methanol extracts 200mg/ kg	0.40±0.04

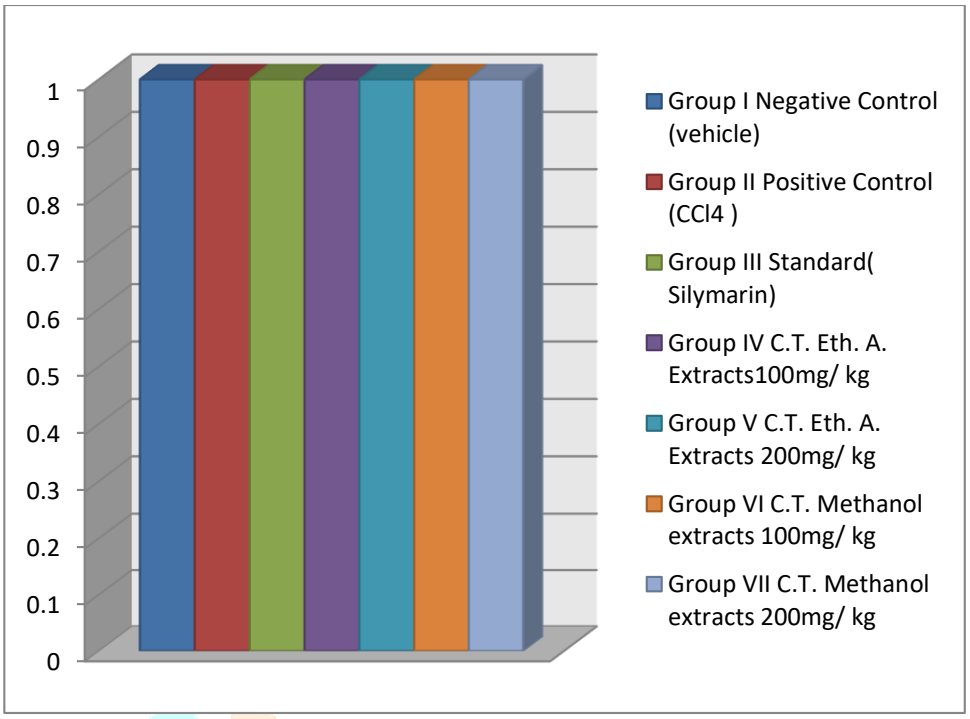


Chart 1. Total bilirubin level in all group

Table no14. Total Protein levels in all groups of animal

Sr. no	Groups	Treatment	Total Protein (g/dl)
1	Group I	Negative Control (vehicle)	7.72±0.19
2	Group II	Positive Control (CCl_4)	1.05±0.10
3	Group III	Standard(Silymarin)	7.55±0.15
4	Group IV	C.T. Eth. A. Extracts100mg/ kg	4.17±0.21
5	Group V	C.T. Eth. A. Extracts 200mg/ kg	6.29±0.12
6	Group VI	C.T. Methanol extracts 100mg/ kg	5.45±0.14
7	Group VII	C.T. Methanol extracts 200mg/ kg	7.41±0.12

Table no 15. SGOT levels in all groups

Sr.no	Groups	Treatment	SGOT(U/L)
1	Group I	Negative Control (vehicle)	29.78±1.80
2	Group II	Positive Control (CCl4)	98.09±5.44
3	Group III	Standard(Silymarin)	32.52±0.61
4	Group IV	V.R. Eth. A. Extracts100mg/ kg	64.01±0.12
5	Group V	V.R. Eth. A. Extracts 200mg/ kg	43.01±0.28
6	Group VI	V.R. ethanol extracts 100mg/ kg	46.01±0.23`
7	Group VII	V.R. ethanol extracts 200mg/ kg	36.01±0.12

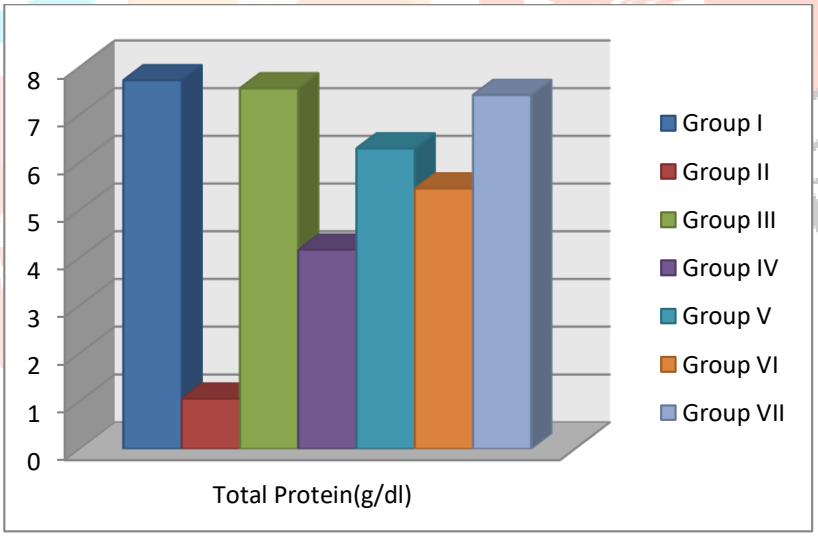


Chart 2. SGOT level in all group

Table no 3. SGPT levels in all groups of animal

Sr. no	Groups	Treatment	SGOT(U/L)
1	Group I	Negative Control (vehicle)	29.78±1.80
2	Group II	Positive Control (CCl_4)	98.09±5.44
3	Group III	Standard(Silymarin)	32.52±0.61
4	Group IV	C.T. Eth. A. Extracts100mg/ kg	63.01±0.12
5	Group V	C.T. Eth. A. Extracts 200mg/ kg	42.01±0.28
6	Group VI	C.T.Methanol extracts 100mg/ kg	45.20±0.23`
7	Group VII	C.T. Methanol extracts 200mg/ kg	35.35±0.12

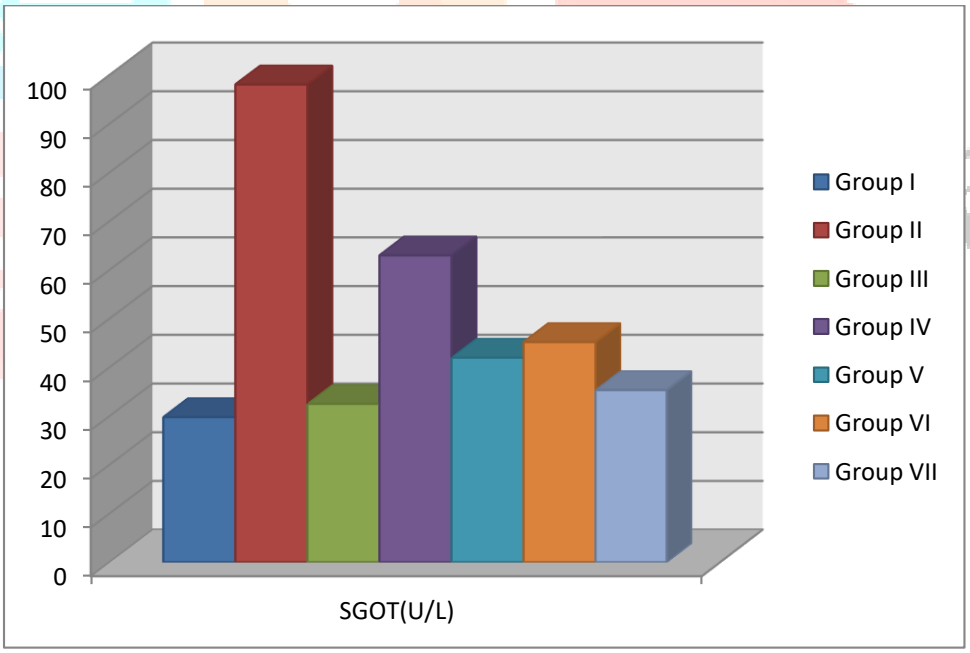


Chart 3. SGPT level in all group

Table no 4. All Biochemical parameter

Sr. no	Groups	Treatment	SGPT(U/L)
1	Group I	Negative Control (vehicle)	32.04±0.45
2	Group II	Positive Control (CCl_4)	98.01±0.89
3	Group III	Standard(Silymarin)	32.16±0.19
4	Group IV	C.T. Eth. A. Extracts100mg/ kg	40.28±1.48
5	Group V	C.T. Eth. A. Extracts 200mg/ kg	32.98±0.23
6	Group VI	C.T. Methanol extracts 100mg/ kg	38.36±0.49
7	Group VII	C.T. Methanol extracts 200mg/ kg	35.14±0.19

Each value represents the mean ± S. E. M. (n=6), P < 0.05: when compared to control (One way ANOVA followed by Dennett's test),*- Significant difference (P<0.05), when test and standard compared with positive control; **-Highly significant difference (P<0.001), when test and standard compared with positive control;#- No significant difference, when test is compared with standard,$- Significant difference, when test is compared with standard but more activity than standard.

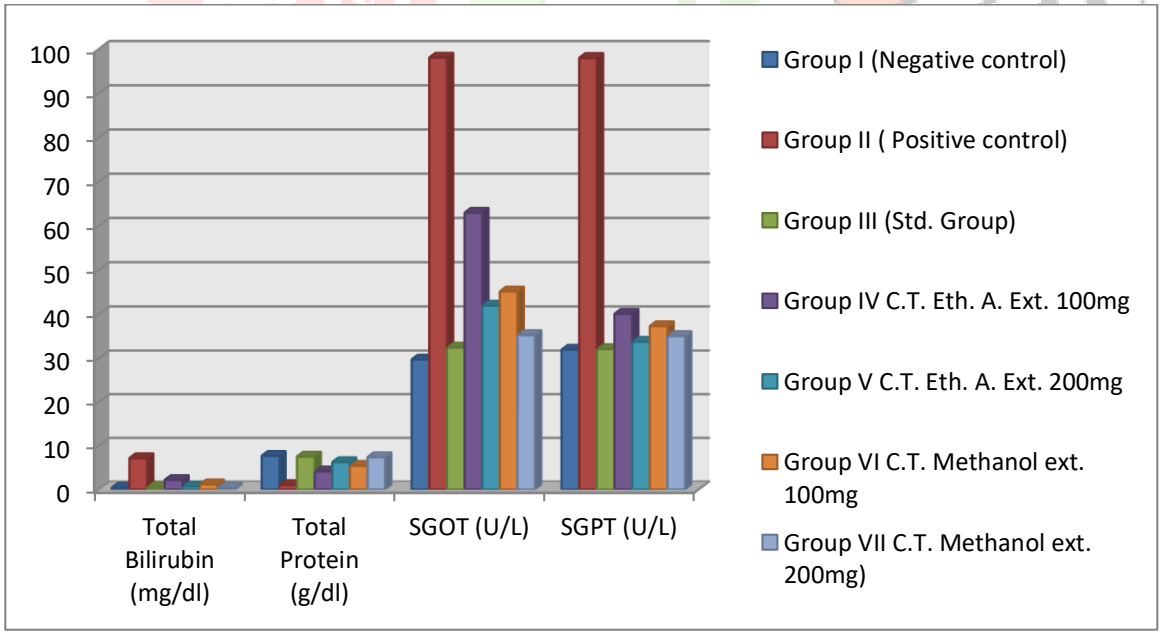


Chart 4. Biochemical Parameters of all groups

Table no.5- Effect of drug on the liver weight

Sr. no.	Dose(mg/kg)	Liver Weight (g/100g)
1	Negative control(Vehicle)	4.2
2	Positive Control(CCl_4)	5.3
3	Standard(Silymarin)	4.1
4	C.T. Eth. A. Extracts100mg/ kg	4.7
5	C.T. Eth. A. Extracts 200mg/ kg	4.5
6	C.T. ethanol extracts 100mg/ kg	4.3
7	C.T. ethanol extracts 200mg/ kg	4.10

Above observation table shows that liver weight increases due to CCl4 administration and it falls down with pretreatment of test or standard drug.

Gross anatomy of liver:

Group I: Negative control: Section shows normal architecture of liver (dark radish brown in colour) which was vehicle treated.

Group II: Positive control (CCl_4 treated): Section shows patches of liver cell necrosis with inflammatory collections.

Group III: Standard (Silymarin): liver shows almost near normal

Group IV: Test 1 (C.T. Eth. A. Extracts100mg/ kg): liver shows normal architecture with some damage to cell.

Group VI: Test 2 (C.T. Eth. A. Extracts 200mg/ kg): Test drug shows protection effect

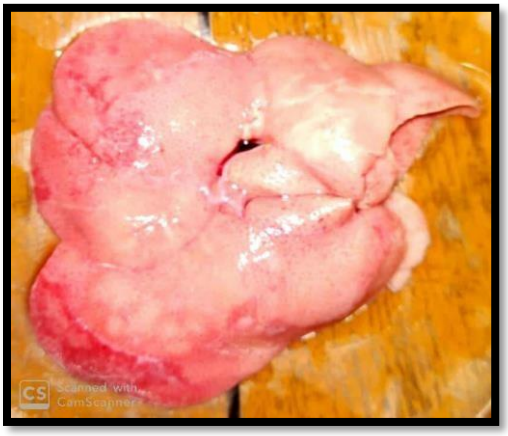
on liver**.**

Group V: Test 3 (C.T. ethanol extracts 100mg/ kg): liver shows normal architecture with moderately damaged cell.

Group VI: Test 4 (C.T. ethanol extracts 200mg/ kg): Test drug shows protective effect

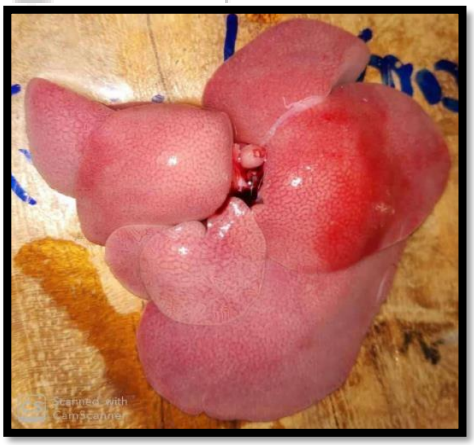
on liver**.**

Negative control **Positive control (Vehicle)** **(ccl_4 1ml)**

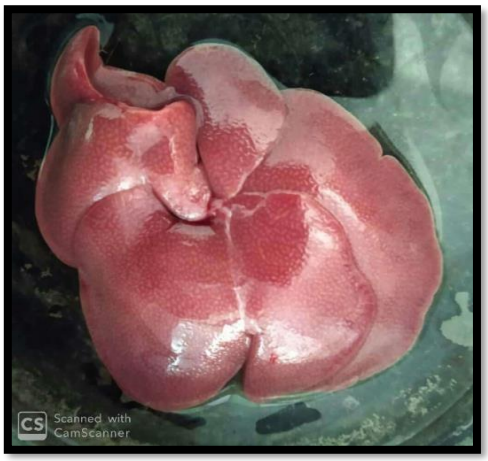


Standard (Silymarin 100 mg/kg)+CCl_4

C.T. Eth. A. E.100mg/ kg +CCl4 **C.T. Eth. A. E.200mg/ kg +CCl4**



C.T. Ethanol E. 100mg/ kg +CCl_4 **C.T. Ethanol E. 100mg/kg+CCl_4**



Histopathological evaluation of C.T. Ethyl acetate and C.T. Ethanol extract for Hepatoprotectiveactivity

Group I: Negative control: Section shows central vein surrounded by hepatic cord of cells (normal architecture).

Group II: Positive control (CCl4 treated): Section shows destruction of normal structure, liver cell necrosis and (ballooning) fatty changes.

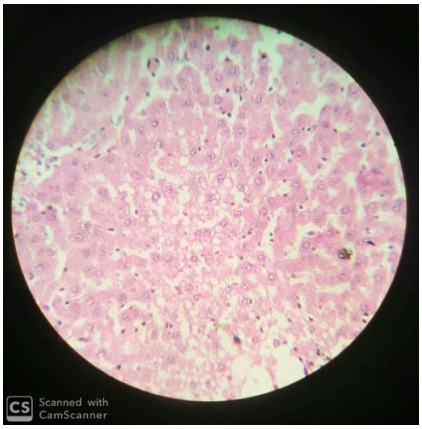
Group III: Standard (Silymarin): Section shows almost near normal

Group IV: Test 1 (C.T. Ethyl acetate 100 mg/kg): Section shows most of hepatocytes are distended with large lipid vacuoles with peripherally displaced nuclei, and necrosis.

Group VI: Test 2 (C.T. Ethyl acetate 200 mg/kg): Section shows normal architecture with mild necrosis**,** ballooning of cell and protective effect against toxicant.

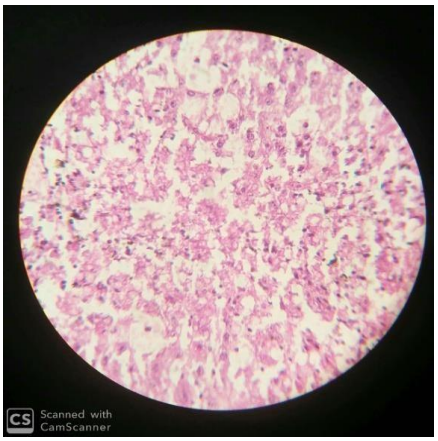
Group V: Test 3 (C.T. Ethanol 100 mg/kg): liver section shows liver abscess is commonly solitary and irregular cell wall with (ballooning) necrosis

Group VI: Test 4 (C.T. Ethanol 200mg/kg): liver section shows normal architecture of cell with irregular necrotic ballooning cell and protective effect againsttoxicant.

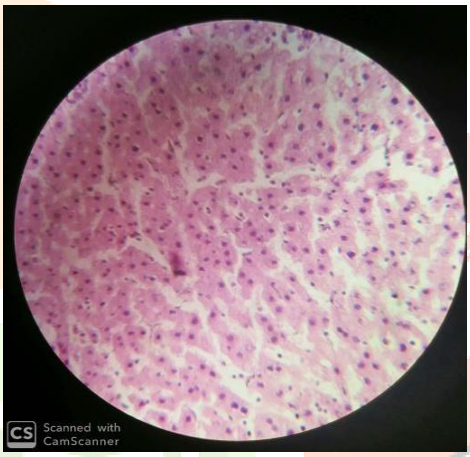


Negative Control

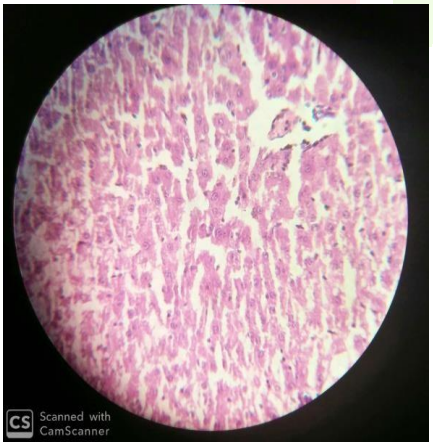
(Vehical)



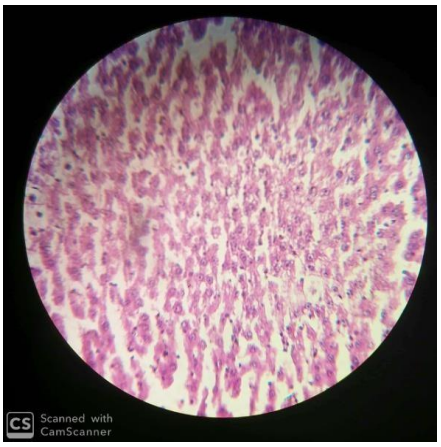
Positive control (CCl4 Treated)



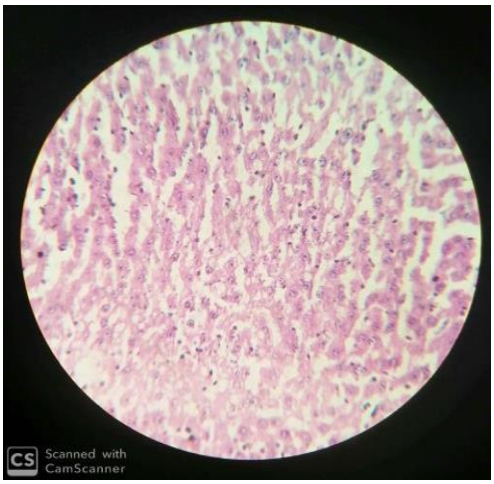
Standard (Silymarin 100 mg/kg +CCl4)



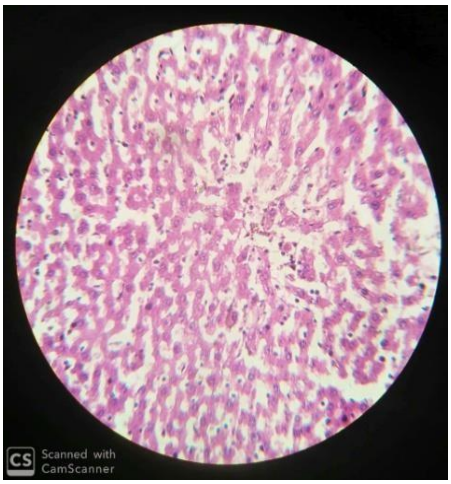
C.T. Ethyl acetate 100 mg/kg + CCl4



C.T. Ethyl acetate mg/kg. 200 mg/kg + CCl4



C.T. ethanol extracts + CCl_4



C.T. ethanol extracts 200mg/ kg 100mg/kg+ CCl_4

DISCUSSION

Liver injury induced hepatotoxicity and is commonly used model for screening hepatoprotective drugs. Liver injury due to carbon tetrachloride in the rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of $CCl_3O_3^-$, reactive oxidative free radical, which initiates lipid peroxidation. Administration of single dose of CCl_4 to a rat within 24 hours produces a centrilobular necrosis and fatty changes. The toxicant reaches its maximum concentration in the liver within 3 hours of administration.AST/SGOT predominantly found in mitochondria of hepatocytes. ALT/SGPT is more specific to the liver, is one of the most sensitive tests employed in the diagnosis of hepatic diseases and thus it is a better parameter for detecting liver injury. The AST/SGOT, ALT/SGPT, Total Bilirubin and Total protein levels are largely used as most common biochemical markers to evaluate liver injury. Administration of Carbon tetrachloride caused a significant elevation in enzyme levels such as AST, ALT, Total bilirubin and Total protein, this indicated the damaged structural integrity of liver, because they are cytoplasmic in the location and released into circulation after cellular damages indicating development of hepatotoxicity. SGPT has comparatively more activity in liver tissue. The increased activities in liver damage are due to necrotic or damaged hepatocytes and the enzyme is sensitive to hepatic dysfunction. Due to Carbon tetrachloride inducing agent the levels of SGOT, SGPT, Total bilirubin were elevated and the total protein levels was decline than normal. The pre-treatment of C.T.EA and C.T.Mth pod extracts at dose levels of 100 and 200 mg/kg were shown a restored the ALT, AST, Total bilirubin and Total protein levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg).

The serum bilirubin increased due to large number of chemicals, drugs and diseases. Carbon tetrachloride, as inducing agent causes increase in the bilirubin, that is not hyperbilirubinanemia, as the raise is much less than double. But C.T.EA and C.T.Mth extracts at 100 mg/kg and 200 mg/kg were restored the approximate elevated level.

Histopathological evaluation of livers revealed that the C.T.EA and C.T.Mth pods extracts reduced inflammation of hepatocytes, swelling, necrosis and no. liver lesions inducedby CCl_4. The above results suggest that the C.T.EA and C.T.Mth pods extracts inhibit CCl_4 induced oxidative hepatic damage.It protects tissue from the effects of CCl_4 and reduces insidious progressive inflammation leading hepatic cell necrosis. Phytochemical study of C.T.EA and C.T.Mth pods extracts shows the presence of flavonoid, p-coumarin, Saponins, tannin and triterpenes and thus both the extracts proved to be an antioxidant and herbal remedies.

SUMMARY AND CONCLUSION

Hepatoprotective activity was carried out by using carbon tetrachloride induced liver damage model in rats. Rats in (IV-VII) group were treated with C.T.EA and C.T.Mth extracts with the dose of 100 mg/kg and 200 mg/kg against Silymarin as standard. The study reveals that there is falls in biochemical parameters of rats. The result of hepatoprotective activity of test drug against CCl4 reveals, C.T.EA and C.T.Mth has promising and significant activity. So it was concluded that the *Cyamopsis tetragonoloba* pods extract can be one of the herbal remedies for hepatic injury.The Biochemical and Histopathological parameters were studied which indicates the status of structural and functional integrity of the cells which is further provide support to the suggestive mechanism of action.From our results, it can be concluded that increase in serum marker enzymes (SGPT,SGOT, and Total bilirubin) levels and where as decrease total protein level in carbon tetrachloride treated rats is compared with the Positive control group. C.T.EA and C.T.Mth pods extracts at different doses of 100mg/kg and 200mg/kg shows significant protective effect against CCl4 induced liver damage. Recently, much attention has been given to the protective biochemical functions of naturally occurring antioxidants in biological system and on their mechanism.

From our observation and result, it was concluded that *Cyamopsis tetragonoloba* pods extract has most pronounced hepatoprotective and antioxidant effect and thus it is proved to be an antioxidant & one of the herbal remedies for the treatment of liver ailment.

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