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Hepatoprotective activity of ethanolic extract of *tribulus cistoides* against CCL_4 induced hepato toxicity in wister male rats

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ABSTRACT

The liver performs diverse functions that are essential for life. In the absence of reliable liver protective drugs, a large number of natural medicinal preparations are used for the treatment of liver diseases. Therefore the present study aims to investigate the hepatoprotective effects of Salix *Tribulus cistoides* ethanolic extract against carbon tetrachloride (CCl4)-induced liver damage. Rats were divided into 6 groups of 6 animals each. Group I served as the normal healthy control, groups II rats were intoxicated with CCl4 i.p. (0.75ml of CCL_4 (25%) mixed with olive oil twice weekly for 9 weeks), Group 3: CCL_4induced hepatotoxicity rats treated with Silymarin (25mg/kg) by i.p. Group 4: CCL_4induced hepatotoxicity rats treated with ethanolic extract of *Tribulus cistoides (*100mg/kg) Group 5:CCL_4induced hepatotoxicity rats treated with ethanolic extract of *Tribulus cistoides* (200mg/kg) Group 6: CCL_4 induced hepatotoxicity rats treated with ethanolic extract of *Tribulus cistoides* (400mg/kg) and biochemical analysis, Determination of purity of *Tribulus cistoides* and histopathological studies was carried out. The administration of *Tribulus cistoides* ethanolic extract showed hepatic protection at an oral dose of 400 mg/kg. *Tribulus cistoides* ethanolic extract significantly reduced the elevated serum levels of intracellular liver enzymes as well as liver biomarkers in comparison to CCl4-intoxicated group. These findings were confirmed with the histopathological observations, where showed hepatic protection at an oral dose of 400 mg/kg. *Tribulus cistoides* ethanolic extract was capable of reversing the toxic effects of CCl4 on liver cells compared to that observed in CCl4-intoxicated animals. Our study show that *Tribulus cistoides* ethanolic extract has potential hepatoprotective effects at 400 mg/kg. These effects can be regarded to the antioxidant and anti-inflammatory properties of the extract.

Keywords: *Tribulus cistoides*, Hepatoprotective activity, Carbon tetra chloride, Silymarin

INTRODUCTION

Liver cirrhosis is one of the leading causes of death in the world, induced by a over alcohol consumption and heavy dose of drugs is a main reason for liver cirrhosis. Therefore, more effective approaches are urgently needed to treat this disease. On the basis of animal models established in experimental research, the mechanism underlying the development of liver diseases at the cellular and molecular level have been clarified and

stem cell therapy have been proposed, which have achieved satisfactory results.

In traditional medicine, natural or crude phytoextract are considered as alternative medicines, because some natural constituents present in them counterbalance the side effects of synthetic medicine. It is therefore obvious that the therapeutic potential and risk efficiency of traditional medicinal plants is based on the direct assessment of phytoextracts as well as effects of their purified compounds.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today we are witnessing a great deal of public interest in the herbal remedies. [1]

T. cistoides (Family: Zygophyllaceae) is an annual (sometimes perennial in warm climates) herb with a long, slender, branched tap-root. The greenish-red stems are up to 2 m long, branched, radiating from a central axis and covered with fine hairs. Though usually prostrate, the stems become more erect in shade or when competing with other plants. Leaves, 3-7 cm long, are in opposite pairs with one of the pair slightly smaller than the other. Each leaf consists of three to eight pairs of opposite, oblong-lanceolate leaflets, each leaflet being 5 to 15 mm long and 3 to 5 mm wide. The upper surface of the leaflets is darker than the underside. The flowers are yellow, 5-petalled, 7 to 15 mm in diameter, solitary and borne on short stalks in the axils of the smaller of each pair of leaves; they open in the morning and close or shed their petals in the afternoon. [2]

Many different compounds with a variety of biological properties and chemical structures have been identified from *T.Cistoides* leaf, including steroidal saponins, flavonoids, glycosides, phytosterols, tannins, terpenoids, amide derivatives, amino acids, and proteins. Among the different types of constituents, steroidal saponins and flavonoids are considered to be the most important metabolites with various bioactivities. Spirostanol and furostanolsaponins are considered the most characteristic chemicals in *T.cistoides* leaf.

Flavonoids

The flavonoids of *T.Cistoides* are mainly derivatives of quercetin, kaempferol and isorhamnetin. Quercetin, isoquercitrin, rutin, quercetin-3-O-gent, quercetin-3-O-gentr, quercetin-3-O-rha-gent, quercetin-3-O-gent-7-O-glu are flavonoids with quercetin as the basic parent structure. Isorhamnetin, isorhamnetin-3-O-glu, isorhamnetin-3-O-gent, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-gentr, isorhamnetin-3,7-di-O-glu, isorhamnetin-3-O-p-coumarylglu, isorhamnetin-3-O-gent-7-O-glu, isorhamnetin-3-O-gentr-7-O-glu are flavonoids with isorhamnetin as the basic parent structure . Kaempferol, kaempferol-3-O-glu, kaempferol-3-O-gent, kaempferol-3-O-rutinoside, kaempferol-3-O-gent-7-O-glu.

Alkaloids

Tribulusamide C, tribulusterine, tribulusin A, harmine, harman, harmmol, tribulusimide C, terrestriamide, N-trans-coumaroyltyramine, N-trans-caffeoylyramine, terrestribisamide are the main alkaloids isolated from thestems, leaves, and fruits of *T.Cistoides*. [3]

This study aims to give an overview on the recent development of herbal medicine in the prevention and treatment of liver cirrhosis and covers the possible mechanism of action of ethanolic extract of *TribulusCistoides* in treatment of liver cirrhosis. Many herbal remedies have been employed in various medical systems for the treatment and management of different diseases. The ethanolic extract of *TribulusCistoides*in powder form contains various alkaloids, flavonoids, glycosides, tannins, saponin, steroids. Hence, in this study a herbal extract *TribulusCistoides* was investigated against liver cirrhosis.

METHODOLOGY:

Preparation of Plant extract

The leaf of *tribuluscistoides* was dried in the shade. Then the 3kg shade – dried plant were pulverized to get coarse powder, sieved under mesh no.60. The powdered whole plant where extracted

using ethanol solvent at room temperature using percolation apparatus for 72hrs. The extraction was continued for 50hrs with 70% ethanol and 30% distilled water. The extraction was continued for 12hrs with 20% ethanol and 80% distilled water using percolation method at room temperature. The solvent was removed in vaccum to provide a dry extract (9.8% w/w, as compared to the powdered material). 17.6gm Lite greenish brown residue was obtained. The chemical constituents of the Alhocolic extract were identified by qualitative analysis and pharmacological screening. Phytochemical test revealed the presence of steroidal saponins, flavonoids, glycosides, phytosterols, tannins, terpenoids, amide derivatives, amino acids, and proteins.[4]

Animals

Rats are particularly suitable for study of physiology of the heart since after partial damage, the organ regenarates almost completely in course of week. Albino wisterrat (170-225gm) were used for this study. They were housed in a room with control environment (temp 25^{o}C) and 12hrs dark/light cycle with standard laboratory diet and water ad libitum. They were fed on healthy diet and maintained in hygienic environment.

IN VIVO HEPATOPROTECTIVE ACTIVITY

The rats were randomly distributed to six groups (n=6). To liver cirrhosis rats, test drug *tribuluscistoides* dissolved in sterile water given orally through orally. [0.75ml of CCL_4 (25%) mixed with olive oil] Group 1: Normal control rats treated with distilled water (6ml/kg). Group 2: Rats treated with CCL4 (3ml/kg/day) by p.o. Group 3: CCL_4induced hepatotoxicity rats treated with silymarin (25mg/kg) by i.p. Group 4: CCL_4induced hepatotoxicity rats treated with ethanolic extract of *tribuluscistoides* (100mg/kg). Group 5:CCL_4induced hepatotoxicity rats treatedtreated with ethanolic extract of *tribuluscistoides* (200mg/kg), Group 6: CCL_4induced hepatotoxicity rats treatedtreated with ethanolic extract of *tribuluscistoides* (400mg/kg)[5]

Biochemical analysis

At the end of experiment, blood samples were taken from heart and preserved in 37^{o} C for 30min and centrifuged for 15min. The serum was collected and preserved in -20^{0}C until measuring levels of serum marker enzyme Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT), bilirubin, protein. [6]

Determination of AST and ALT

Activities of AST and ALT were assayed. In different tubes, 1ml of the buffered substrate was added to 0.1ml of sample and incubated at 37^{o}Cfor 30min. Then 1ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1ml of sample was added only after the addition of DNPH reagent. The tubes were kept aside for 15min, and then 10ml of sodium hydroxide was added and read at 520nm. [7]

Determination of ALP

Procedure Serum was obtained by centrifugation of freshly clotted blood. The serum (1 cc.) was removed with a pipette and diluted with 19 cc. of distilled water. 1 cc. of this diluted serum was placed in a 20 cc. test-tube and 5 cc. of the appropriately buffered substrate solution were added. In a separate test-tube, buffered substrate solution alone served as a control for non-enzymatic hydrolysis. The tubes were then incubated at 37.5” for 1 hour in the determination of alkaline phosphatase, and for 2 hours in the determination of acid phosphatase. In the latter determination 4 drops of 1 M sodium carbonate solution were added after the period of incubation, in order to raise the pH to the optimal level for coupling. 1 cc. of the solution of tetrazotizeddiorthoanisidine was added to each tube, and the tubes were agitated vigorously or inverted several times to insure thorough mixing. 3 minutes were allowed to elapse for completion of coupling. To each tube 1 cc. of 40 per cent trichloroacetic acid was added to precipitate protein and favor release of dye from its protein complex. 10 cc. of ethyl acetate were added from a burette and the tubes were shaken vigorously until an even emulsion was produced. The tubes were then centrifuged for 10 minutes at 2500 rpm, and 5 cc. of the organic layer were transferred with a pipette to a Klett tube. The color did not fade on standing, but evaporation of ethyl acetate in over 1 hour resulted in increased color density. This was

avoided by stoppering the tubes when such delay was unavoidable. The color density was measured in a photoelectric calorimeter through a green filter (540 nm). [8]

Determination of GGT

Serum was obtained by centrifugation of freshly clotted blood. The serum (1 cc.) was removed with a pipette and diluted with 19 cc. of distilled water. Pipette in to a clean dry test tube. Working reagent added with test (1ml) and incubates at the assay temperature for 1min and add sample 0.1ml and mix well and read the initial absorbance A_o after 1minute.wavelenth at 405nm. Calculation: delta A/min x 1158. [8]

Determination of bilirubin

Take two test tubes and label them test and control. Distilled water 1.8ml and serum 0.2ml and diazo reagent 0.5ml methanol 2.5ml with test. And distilled water 1.8ml and serum 0.2ml and diazo blank 0.5ml and methanol 2.5ml with control. Keep the test tubes in dark. Reading taken at 1minute is direct /conjugated bilirubin.

Calculation: S.Bilirubin (mg%) = [ODt-ODc/ODs] x 8.[8]

Histopathological studies

The rats are decapitated by using anesthesia and a part of fresh liver was dissect out. The liver tissue was fixed in 10% formalin, embedded in paraffin and finally cut into 5mm section. From each subject, 10section were selected randomly and stained with hematoxylin-cosin and of them, 5 random fields were captured by motic camera for double blinded histological assessment, carried out by two other academic members of anatomical science. The histopathological fatty changes were evaluated using the grading and staging system and degreed from 0 to 4 as follows: 0 = without steatosis , 1= steatosis response, 2=approximately <25% steatosis , 3= approximately 26-55 % steatosis, 4=<78% steatosis, 5=<92% steatosis. Microscopic examination allows the following grading: Grade 0: No change, Grade 1: steatosis response, Grade 2: <92% steatosis, Grade 3:<25 % steatosis, Grade 4: <26-55 % steatosis, Grade 5: <78 % steatosis, For each group the main grade is calculated with the standard deviation to reveal significant differences. [9]

Statistical analysis

The result of hepatoprotective activity is expressed as mean "Mean ± SEM from five animals in each group. Results were statistically analyzed using one-way ANOVA followed by Newman Keuls multiple range test for individual comparisons: $p<0.01$ was considered significant. GraphPadInStat version 3.00 of GraphPadSoftware, Inc. (san diego, CA), was used for statistical analysis.

RESULTS

The result shows that the administration of *Tribulus cistoides* ethanolic extract showed dose dependent hepatoprotective activity, the plant showed significant hepatic protection at an oral dose of 400 mg/kg. *Tribulus cistoides* ethanolic extract significantly reduced the elevated serum levels of intracellular liver enzymes as well as liver biomarkers in comparison to CCl4- intoxicated group. The values were showed in Table: 1 & Figure 1-5.

Table: 1 Effect of *Tribulus cistoides* ethanolic extract on Biochemical parameters

S.NO	AST (UNITS/L)	ALT (UNITS/L)	GGT (UNITS/L)	ALP (UNITS/L)	BILIRUBIN (UNITS/L)
GROUP 1	43.23±1.51	52.75±1.2	45.4±2.32	127.1±1.5	0.42±0.02
GROUP 2	90.75±2.7	71.02±2.3	68.42±1.33	269.6±4.8	1.12±0.2
GROUP 3	46.78±2.3	55.01±0.1	48.15±1.2	152.1±2.3	0.63±0.02
GROUP 4	70.33±2.6	62.91±1.3	59.02±01	215.5±4.1	1.02±0.05
GROUP 5	60.98±1.9	61.23±2.1	56.23±1.02	211±5.7	0.83±0.04
GROUP 6	52.25±2.5	56.23±2.3	50.12±2.5	188.25±5.3	0.82±0.04

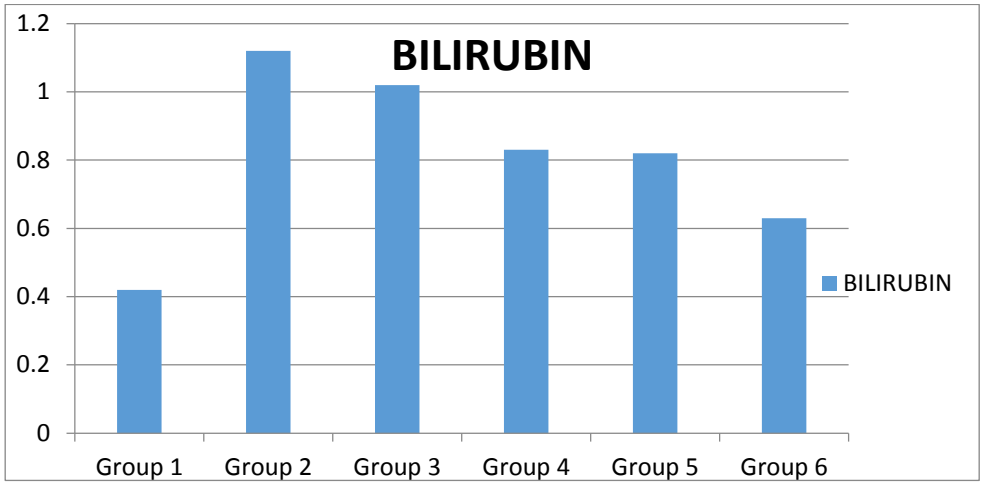

Figure: 1 Effect of *Tribulus cistoides* ethanolic extract on Bilirubin)

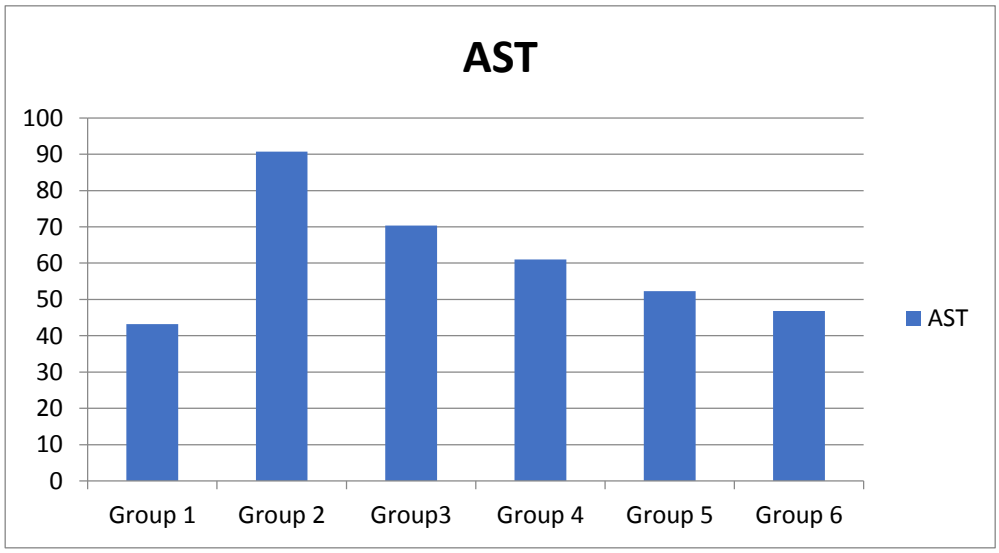

Figure: 2 Effect of *Tribulus cistoides* ethanolic extract on AST)

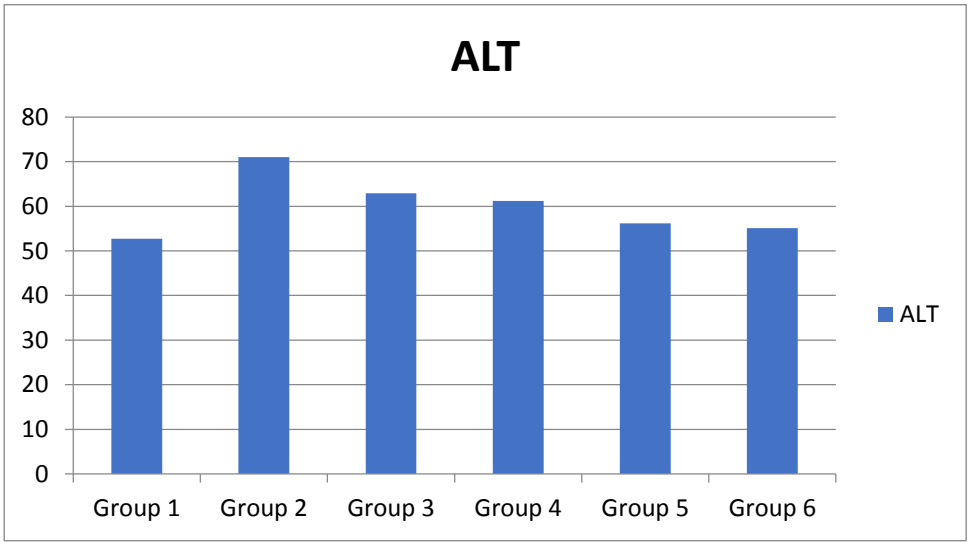

Figure: 3 Effect of *Tribulus cistoides* ethanolic extract on ALT)

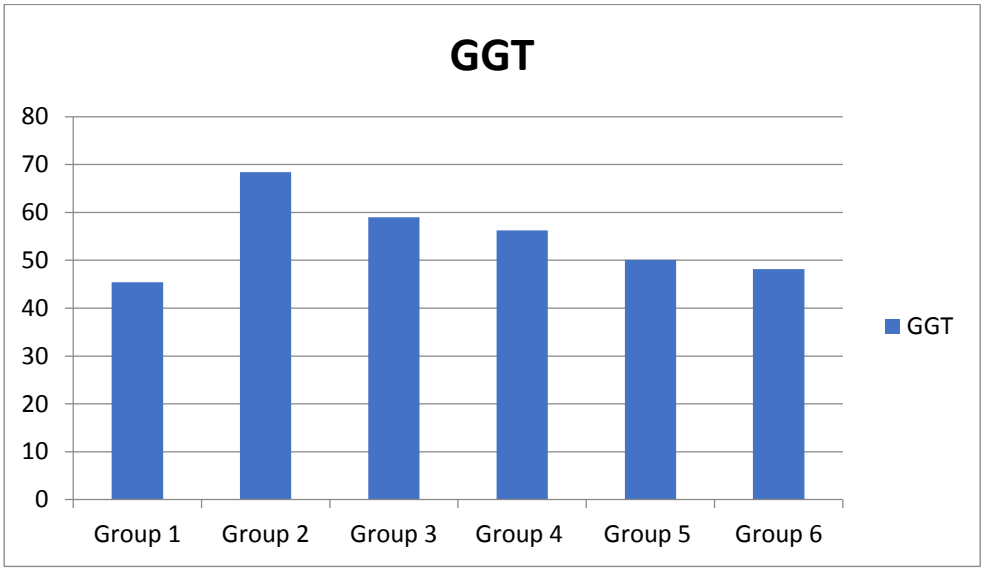

Figure: 4 Effect of *Tribulus cistoides* ethanolic extract on GGT)

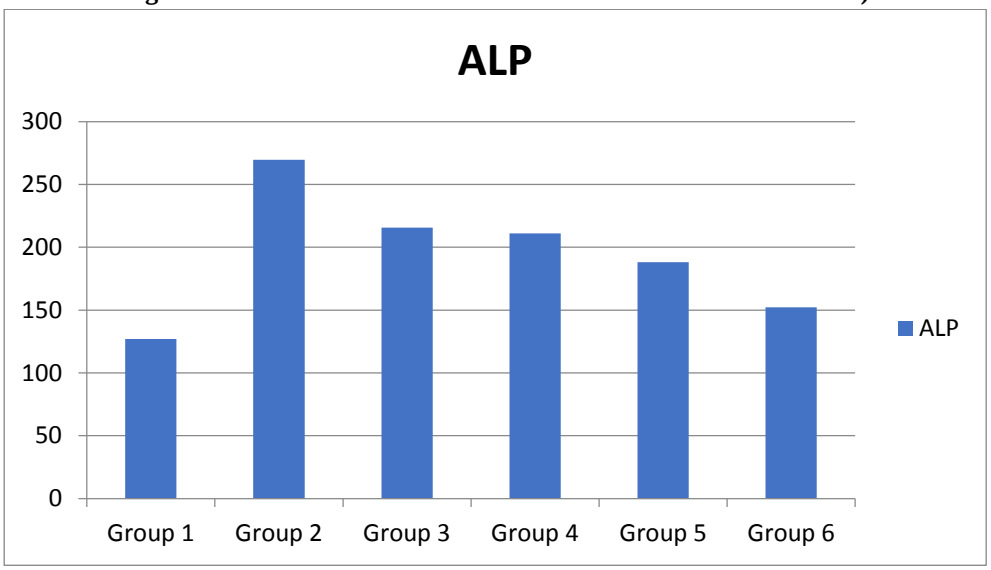

Figure: 5 Effect of *Tribulus cistoides* ethanolic extract on ALP)

HISTOPATHOLOGICAL STUDY

Hepatic histological analyses of *Tribulus Cistoides* and silymarin on CCl 4 -induced acute liver damage in mice. Liver tissues were subjected to hematoxylin and eosin staining (400×). (a) Control group; (b) animals treated with 25% CCl 4 ; displayed cell necrosis (long arrow) and vacuole formation (short arrow) (c) animals pretreated with silymarin (200 mg/kg) and then treated with CCl 4; (d–f) animals pretreated with Tribulus Cistoides (100,200,400gm/kg) and then treated with CCl 4. Figure:6

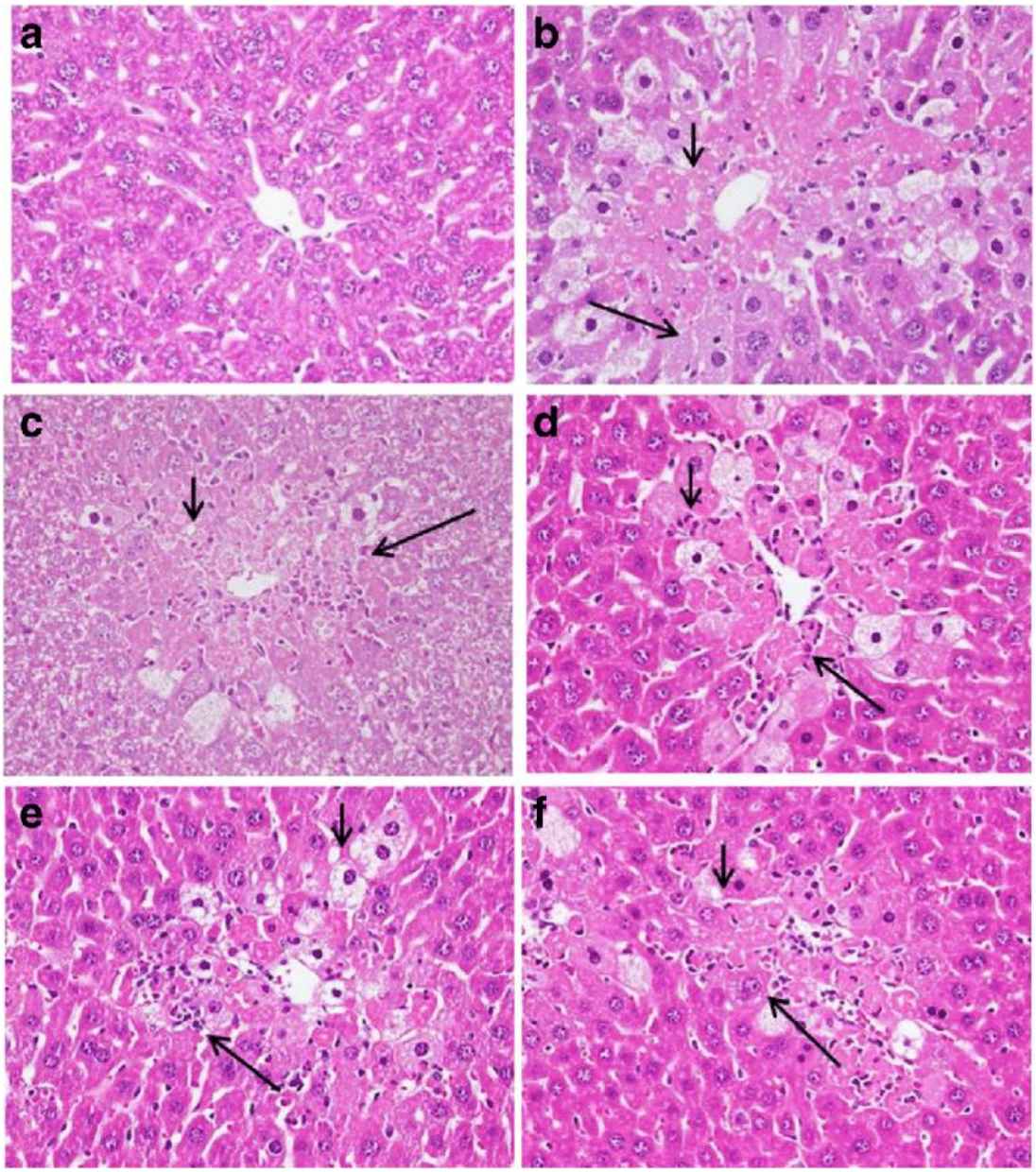

Figure: 6 Effect of *Tribulus cistoides* ethanolic extract on histopathology of liver

DISCUSSION

In the present study, the protective effects of *T.Cistoides* ethanolic extract of against CCL_4induced liver toxicity on rats were evaluated. The result showed that the ccl4 significantly increase the liver weight. The present study also showed that CCL_4induced liver cirrhosisthat should be considered as microsomal enzyme induction leading to increase storage of lipids peroxisome proliferation, and hyperfunction of the liver. Further the study demonstrated that *T.Cistoides* extract administration decreased the body and liver weight of the rats as well as reduction in the range of steatohepatitis. The state of liver function could be evaluated by blood assays, describing its functionality and its link with the biliary tract. *T.Cistoides* groups indicated that the extract impaired hepatocellular or secretory function of the liver in a dose dependent manner. The five biomarkers of liver damage are AST , ALT , ASP, GGT, bilirubin indicates the liver injury and the ratio of AST and ALT may be employed in disease diagnosis. An AST/ALT ratio greater than 1 suggests myocardial infaraction while, more than 2 is indicative of alcoholic hepatitis or cirrhosis and ranges from 1.2 to 1.4 is an indication of the abnormal functioning of the liver. In the present

study, the extract relieved hepatic damage, as revealed by the results of blood chemistry analysis and histopathological assesment; the significant alterations in the indicators of liver damage(ALT, ALP, AST,GGT,bilirubin)and steatosis ratio. The *T.Cistoides* extract contains flavonoids which are known to have insulin-like properties and also an inhibitory effect on the lipogenase enzyme. Flavonoids improved cell viability and inhibited cellular leakage of hepatocyte AST and ALT. further, its been shown that flavonoids can decrease the insulin resistance and insulin requierment in adipose and muscle tissue. Besides flavonoids, the plant contain alkaloid, glycoside, steroids saponin that have hypolipidemic properties. Alkaloids reduce carbohydrate absorption and metabolism. Thus, this component of the extract could reduce the absorption of CCL_4 and also interrupt the CCL_4 metabolization to lipids. Saponin is other component of the extract not only regulates lipid metabolism but also improve hypertriglyceridemia. Moreover, polyphenolic and flavonoids compounds of the plant having antioxidant properties are another explanation for antihepatosteatosis of the plant by blocking the second hit of the progression of the disease. [10]

CONCLUSION

T.cistoides extract administered improves the signs of liver in wister albino rats. The beneficial effects of *T.Cistoides* extract could be seen both in histopathological features and hepatic serum markers of the rats. At histopathological level, the hepatosteatosis was dimished in *T.Cistoides* extract groups in a dose dependent manner.

BIBLIOGRAPHY

[1]. Achenbach H, Hübner H, Reiter M. Cholestane- and pregnane-type glycosides from the roots of Tribulus cistoides. Phytochemistry. 41(3), 1996, 907-17

[2]. Neha Pandey. Protective effects of Pycnogenol on carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats. Food Chem Toxicol 46(1), 2008, 380-7.

[3]. Gauri Karwani. Hepatoprotective active of Mimosa pudica Linn. In carbon tetrachloride indued hepatotoxicity in rats. J of herbal medicine and toxicology. 5(1), 2011, 27-32.

[4]. Jagruti A. Patel Screening of Roots of BaliospermumMontanum For Hepatoprotective Activity Against Paracetamol Induced Liver Damage In Albino Rats, International Journal Of Green Pharmacy, 2009, 220-223.

[5]. Selvam ABD. Inventory of Vegetable Crude Drug samples housed in Botanical Survey of India, Howrah. Pharmacog. Rev., 2(3), 2008, 61-94.

[6]. Khare CP. Berlin, Heidelberg: Springer Verlag. Indian medicinal plants: An illustrated dictionary; 2007, 669–71.

[7]. Heidari MR, Mehrabani M, Pardakhty A, Khazaeli P, Zahedi MJ, Yakhchali M, et al. The analgesic effect of *Tribulus terrestris* extract and comparison of gastric ulcerogenicity of the extract with indomethacine in animal experiments. Ann N Y Acad Sci. 1095, 2007, 418–27.

[8]. Al-Ali M, Wahbi S, Twaij H, Al-Badr A. *Tribulus terrestris*: Preliminary study of its diuretic and contractile effects and comparison with Zea mays. J Ethnopharmacol. 85, 2003, 257–60.

[9]. Anand R, Patnaik GK, Kulshreshtha DK, Dhawan BN. Activity of certain fractions of *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats. Indian J Exp Biol. 32, 1994, 548–52.

[10]. Elias, H.; Bengelsdorf, H. "The Structure of the Liver in Vertebrates". *Cells Tissues Organs*. 14(4), 1952, 297–337. doi:10.1159/000140715’