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Research Article

Effect of 50% ethanolic extract of roots of *Cissampelos pareira* on some bio-chemical parameters to investigate the hepato-protective study in animal model (Rats)

***Vinay Kumar[1], Dr. Anil Middha**

[1] Research Scholar, OPJS, University, Churu, Rajasthan, India

[2] Associate Professor, OPJS, University, Churu, Rajasthan, India

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ABSTRACT

In phytotherapeutic approach, the emphasis is on the development of a new drug whose extraction and fractionation have emanated on the basis of therapeutic activity. *Cissampelos pareira* Linn. (*C. microcarpa*, De Candolle) is a very variable, lofty, slender, dioecious, perennial, climber commonly distributed throughout topical and sub topical India, ascending up to an altitude of *c* 2,000m. The plant is very common in orchards, hedges, parks and gardens on moist soils, either creeping or twining around other plants. The roots of *Cissampelos pareira* (L.) Hirsuta (Menispermaceae) were collected and extracted with 50% ethanol solution. The selected plant extract was subjected for the preliminary acute toxicity studies in mice at different dose levels up to 2000 mg/kg. The results showed no abnormal symptoms either p.o or i.p and cause no mortality. Before the actual LD_{50} determination, a pilot study was made on a small group of mice mainly to select the dose ranges for the subsequent study. The 50% ethanolic extract of *Cissampelos pareira* were taken at various dose levels (200, 500, 1000, 1500, 2000 mg/kg b.wt.) dissolved in 1 % carboxymethyl cellulose administered orally to pairs of mice per dose level. The value of probability less than 5% ($P < 0.05$) was considered statically significant. Histological architecture of the *C. pariera* treated liver samples showed the ability of the *C. papeira* to prevent hepatocellular necrosis. In conclusion, the antitubercular drugs (isoniazid and rifampicin)-induced alterations on protein metabolism and hepatic antioxidant defense system were normalized by *Cissampelos pareira* co-administration, indicating a possible cytoprotective role of *Cissampelos pareira* against drug induced hepatotoxicity. Thus our studies give scientific evidences to support this plant's traditional uses as claimed in folklore medicine. It was concluded from the results that the alcoholic extract of *Cissampelos pareira* (L.) possesses hepato-protective effect in experimental animals

Key words: *Cissampelos pareira* Linn, *C. pariera,* dioecious

Introduction:

The use of traditional medicine and medicinal plants in most developing countries, for the maintenance of good health, has been widely observed (UNESCO, 1996). The big pharmaceutical companies, who made lot of money from synthetic medicines, did not rush out to disprove this misconception. In phytotherapeutic approach, the emphasis is on the development of a new drug whose extraction and fractionation have emanated on the basis of therapeutic activity. The standard fraction of an active extract or mixture of fractions may prove better therapeutically, less toxic and inexpensive compared to pure isolated compound drugs. However, crude plant preparation requires modern standards of safety and efficacy.

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbon, etc,) excess consumption of alcohol, infection and autoimmune disorder. Most of the hepatotoxic chemicals damage liver cells mainly by including lipid peroxidation and other oxidative damages in liver.

Antioxidant defense system (ADS) against oxidative stress is composed of several lines, and antioxidants are classified in four categories based on their function. *Cissampelos pareira* Linn. (*C.*

**Corresponding author: Vinay Kumar*

microcarpa, De Candolle) is a very variable, lofty, slender, dioecious, perennial, climber commonly distributed throughout topical and sub topical India, ascending up to an altitude of *c* 2,000m. Root stock woody, perennial; leaves usually peltate or orbicular-reniform, ovate-sub-reniform, with a trun-cate-cordate base, glabrous or hairy above 2.5-12 cm across; triangularly broad-ovate, or orbicular, obtuse, mucronate, base cordate or truncate, tomentose on both sides, ultimately becoming glabrous above and glaucous below; petiole pubescent. The flowering period is March to October (Anonymous, 1992). The detail pharmacognosy of *Cissampelos pareira* has been reported by Prasad et al., (1962). They have well differentiated the root and stem by studying various pharmacognostical parameters.

The plant is very common in orchards, hedges, parks and gardens on moist soils, either creeping or twining around other plants. It is also very common on the hilly tracts along water courses. It can also be propagated from root cuttings, planted at the beginning of monsoon. Some times it dies back in hot weather. The cultivation of the plant was attempted at Lucknow for the alkaloids, hayatine (Anonymous, 1966). All the parts of plants are used as medicine. The roots are the most valued part of the plant (Kirtikar and Basu, 2001, Chopra et al., 1958). It has been held in great esteem in the Ayurvedic system of medicine and has been recommended as a substitute for the costly imported drug, tubocurarine (Chopra et al., 1958). In India roots are edible and are employed in fermenting rice beer. The roots possess astringent, mild tonic, diuretic, stomachic, antilithic, analgesic, antipyretic and emmenagogue properties. They are frequently prescribed for treating cough, dyspepsia, diarrhea, dysentery, piles, dropsy and urinogenital troubles such as prolapsus uteri, cystitis, hemorrhage and menorrhagia and calcular nephritis. The juice is given to cattle also for curing diarrhea (Bhatnagar et al., 1961; Adesina, 1982). Two alkaloids were isolated from the leaves of *Cissampelos sympodialis*; a bisbenzylisoquinoline compound named warifteine ($C_{36}H_{36}N_2O_6$) and a novel 8,14-dihydromorphinandienone alkaloid named milonine (Basu et al 1970). The leaves and roots are used as a cure for dyspepsia, diarrhea, dropsy and in snake bite (Anonymous, 1992). Roots are employed in leucoria, gonorrhea and also in chronic inflammation of bladder (Feng et al., 1962).

MATERIALS AND METHODS:

Collection and authentication of plant material:

Cissampelos pareira and *Moringa oleifera* collected from in May 2013. *Both* plants were collected from Kheri Bawli Delhi India. The plants were identified by Prof. Dr. Anju Pal, Horticulture department, Pantnagar University, Pantnagar, Uttaranchal, India. (XC09/IPAV/04 for *Cissampelos pareira,* FC09/IPAB/04 for *Moringa oleifera* was deposited in the department for future reference.

Drug and chemicals:

Rifampicin and isoniazid were obtained from Lupin pharmaceuticals Ltd., Silymarin (sigma chemicals company, U.S.A.), Bovine serum albumin (BSA) (Sigma Chemicals St louis, USA), SGOT, SGPT, SALP, Serum bilirubin, Serum protein Qualigens (Glaxo Smithkline, India), and all the other chemicals used were of the analytical and highest purity grade from standard companies. Water represents the double distilled water; standard orogastric cannula was used for oral drug administration.

Animals used:

Studies were carried out using Sprague-dawley rats weighing 170-200 g and Swiss albino mice weighing 25-35 g. They were obtained from the Central Animal House Facility of Central Drug Research Institute, Lucknow. The rats were group housed in polyacrylic cages (38×23×10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 ^{0}C) and relative humidity 44 – 56 %, with a dark and light cycle of 12 ± 1 h. They were allowed free access to standard dry pellet diet (Amrut, India) and water *ad libitum*. All procedures described were reviewed and approved by the institutional committee for ethical use of animals (Zimmerman, 1983).

Extraction:

Preparation of 50% EtOH extract of *Cissampelos pareira:*

The freshly collected roots (4 kg) of *Cissampelos pareira* were washed with distilled water and shade-dried. Then dried in tray drier under

controled conditions and powdered. The powdered plant materials (1000g) was macerated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol for 3 days (3 X 5L). The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The yield obtained was 93.0 g of solid residue (yield 9.3 % w/w). The extract obtained was further subjected to Phytochemical screening and pharmacological investigations.

PHARMACOLOGICAL STUDIES:

Experimental design for acute toxicity studies:

The adult Swiss albino mice of both sexes selected for acute toxicity study. Before the actual LD_{50} determination, a pilot study was made on a small group of mice mainly to select the dose ranges for the subsequent study. The 50% ethanolic extract of *Cissampelos pareira* were taken at various dose levels (200, 500, 1000, 1500, 2000 mg/kg b.wt.) dissolved in 1 % carboxymethyl cellulose administered orally to pairs of mice per dose level. The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. For the actual LD_{50} determination, the extract of *Cissampelos pareira* were administered once orally at various dose levels (200 to 2000 mg /kg b. wt.) to group of 3 mice of which have been fasting overnight (about 18 h.). The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. The animals were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality recorded. Behavior of the animals and any other toxic symptoms also observed for 72 h. and the animals were kept under observation upto 14 days.

The effective dose (ED_{50}) of 50% ethanolic extract of *Cissampelos pareira* was decided 1/10 of maximum dose (2000mg/Kg). So I was used the dose of 50% ethanolic extract of *Cissampelos pareira* such as 100, 200 and 400 mg/Kg body weight, p.o. for the Anti-hepetotoxicity activity.

Experimental design for hepato-protective studies:

Experimental Set up was followed as reported earlier by: Ravinder pal et al. in 2006.

The animals (Sprague-Dawley rats weighing 170-200 g) were divided into 6 groups of 6 animals each.

Group I: Control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg b.wt.) orally and this served as solvent control.

Group II: Animal received Rifampicin + Isoniazide (RIF +INH) (50mg/Kg body wt. each, p.o). RIFand INH solutions were prepared separately in sterile distilled water, the pH of RIF solution was adjusted to 3.0 with 0.1 mol / L HCL. RIF+INH were administered orally for 28 days, (Ravinder pal et all, 2006). After 28 days hepatotoxicity was confirmed with the help of bio-chemical and histopathological studies and this group of animals was used for detailed investigation.

Group III: Animals received RIF +INH (50mg/Kg body wt. each, p.o) and 50% EtOH extract of *Cissampelos pareira* (100 mg/kg body wt. p.o) for 28 days.

Group IV: Animals received RIF +INH (50mg/Kg body wt. each, p.o) and 50% EtOH extract of *Cissampelos pareira* (200 mg/kg body wt. p.o) for 28 days.

Group V: Animals received RIF +INH (50mg/Kg body wt. each, p.o) and 50% EtOH extract of *Cissampelos pareira* (400 mg/kg body wt. p.o) for 28 days.

Group VI: Animals received (RIF +INH) (50mg/Kg body wt. each, p.o) and Silymarin (100mg/kg body wt. p.o) for 28 day After completion of the treatment, animals were weighed and sacrificed by cervical decapitation. Blood samples were collected and serum was separated. Liver was removed immediately, washed in saline, weighed and homogenized in Tris-HCl buffer 0.1M pH 7.4. The serum and liver homogenate were used for the biochemical analysis. One part of liver was preserved in 10% formalin for histological studies (Mark *et al*.,1985).

INVESTIGATATION OF BIO-CHEMICAL PARAMETERS:

Serum was analyzed for the following parameters Serum glutamic pyruvic transaminase **(SGPT),** Serum glutamic oxaloacetic transaminase **(SGOT)**, alkaline phosphatase **(ALP)**, Total bilirubin, total protein and Albumin, And enzymatic parameters like- lipid peroxidation **(LPO)**, catalase **(CAT),** superoxide dismutase **(SOD**) by liver homogenate.

Determination of Serum glutamic pyruvic transaminase (SGPT):

Reagents*:*

Reagent 1: Buffered alanine α-KG substrate, pH 7.4

Reagent 2: DNPH colour reagent

Reagent 3: Sodium hydroxide 4 N

Reagent 4: Working pyruvate standard, 2mM

Preparation of working solutions: Solution I: Dilute 1 ml of reagent 3 to 10 ml with purified water.

Table 1: Testing procedure for SGPT

Tube No.	1	2	3	4	5
Enzyme activity (units/ml)	0	28	57	97	100
Reagent 1: Buffered alanine, pH 7.4	0.5	0.45	0.4	0.35	0.3
Reagent4: Working pyruvate standard, 2mM	-	0.05	0.1	0.15	0.2
Purified water	0.1	0.1	0.1	0.1	0.1
Reagent 2: DNPH colour reagent	0.5	0.5	0.5	0.5	0.5
Mix well and allow to stand at room temperature for 20 min.					
Solution I	5.0	5.0	5.0	5.0	5.0

Mix well by inversion. Allow to stand at room temperature for 10 min. and measure the O.D. of all the five tubes against purified water on a colorimeter using a green filter.

Table 2: Testing procedure for SGPT

Reagent 1: Buffered alanine, pH 7.4	0.25 ml
Incubate at 37˚C for 5 min.	
Serum	0.05 ml
Mix well and incubate at 37˚C for 30 min.	
Reagent 2: DNPH colour reagent	0.25 ml
Mix well and allow to stand at room temp. for 20 min.	
Solution I	2.5 ml

Determination of Serum glutamic oxaloacetic transaminase (SGOT):

Principle: Transaminase is an enzyme catalyzing the transfer of amino groups from α-amino acid to α-keto acid as follows.

2-Keto glutaric acid + Aspartic acid

⇩

Glutamate + Oxalo acetic acid.

Oxalo acetic acid formed in the reaction is spontaneously converted to pyruvic acid. Rate of reaction is then determined by the estimation of pyruvic acid using dinitrophenyl hydrazine. Dinitropheny hydrazine formed is estimated at 520 nm. The unreacted α-keto glutarate also gives coloured product with color reagent but the intensity is much less than that of pyruvate and hence it is negligible.

Reagents: Phosphate buffer – pH 7.4, standard pyruvate (2 mM), α - keto glutarate-aspartic acid substrate for SGOT, 2,4 – dinitrophenyl hydrazine, and 0.4 M sodium hydroxide.

Table 3: Testing procedure for SGOT

Tube No.	Sodium pyruvate (ml)	Substrate (ml)	Water (ml)	SGOT units
1	0.5	0.5	0.2	0
2	0.4	0.6	0.2	22
3	0.3	0.7	0.2	55
4	0.2	0.8	0.2	95
5	0.1	0.9	0.2	150
6	0.0	1.0	0.2	215

Table 4: Testing procedure for SGOT

Solutions	Test (ml)	Control (ml)	Blank (ml)	Standard (ml)
Substrate	1.0	1.0	1.0	1.0
Keep for 5 min. in boiling water bath at 37˚C				
Serum	0.2	-	-	-
Incubate at 37˚C for 60 min				
Sodium pyruvate	-	-	-	0.2
Dinitrophenyl hydrazine	1.0	1.0	1.0	1.0
Serum	-	0.2	-	-
Allow to stand for 20 min. at room temperature				
Sodium hydroxide	10.0	10.0	10.0	10.0

Mix well and allow to stand at room temperature for 10 min. Estimated with the help of chemoanalyser and expressed as U/l.

Determination of serum alkaline phosphatase (SALP):

The alkaline phosphates level was estimated by King and Armstrong, (1965) method alkaline phosphatase (Span diagnostic reagent kit).

Principle: Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4- amino antipyrine in presence of the oxidizing agent

Potassium ferricyanide and forms an orange-red coloured complex, which can be measured at 510 nm.

Reagents

Reagents 1: Buffered substrate, pH 10.0

Reagents 2: Chromogen reagent

Reagent 3: Phenol standard, 10 mg%

Preparation of working solution: Reconstitute one vial of reagent 1, buffered substrate with 4.5 ml of distilled water.

Table 5: Testing procedure for SALP

Medium	Blank	Standard	Control	Test
Working buffered substrate Distilled water	0.5 ml 1.5 ml	0.5 ml 1.5 ml	0.5 ml 1.5 ml	0.5 ml 1.5 ml
Mix well and incubate for 3 min. at 37˚C				
Serum	-	-	-	0.05 ml
Phenol standard, 10 mg%	-	0.05 ml	-	-
Mix well and incubate for 15 min. at 37˚C				
Chromogen reagent Serum	1.0 ml -	1.0 ml -	1.0 ml 0.05 ml	1.0 ml -

Mix well and allow to stand at room temperature for 10 min. Estimated with the help of chemoanalyser and expressed as U/l

Determination of serum total bilirubin:

Total bilirubin level in serum was determined by modified DMSO method using Agappe diagnostic kit (Bombay) (Walter and Gerard 1970). Sulfanilic acid reacts with sodium nitrate to produce deoxidized sulfanilic acid. Total bilirubin couples with deoxidized sulfanilic acid in the presence of methylsulfoxide to produce azobilirubin which may be measured at 532-536nm. In the absence of methyl sulfoxide, only direct (conjugated) bilirubin forms the azobilirubin complex.

Reagents:

1. Total bilirubin reagent Sulfanilic acid Dimethyl sulfoxide Stabilizer
2. Activator: odium nitrite
3. Artificial standard =- 10 mg/dl Procedure:

Total Bilirubin test: To 1.0ml total Bilirubin reagent, 0.2ml of activator and 0.1ml of serum were added, mixed well and incubated for exactly 5 minutes room temperature.

Sample blank was prepared by 1.0ml total bilirubin reagent with 0.1ml of distilled water, mixed well and incubated for exactly 5 minutes at room temperature. Estimated with the help of chemoanalyser and expressed as mg/dl.

Determination of serum total protein and albumin: Principle: Protein reacted with cupric ions in alkaline medium to form a violet coloured complex. The intensity of the complex was measured at 530nm against reagent blank 0.01ml of the standard solution, which was treated in the same way.

Procedure for total protein: The reagents used were from Span diagnostics kit. 1ml of working reagent was mixed with 0.01 ml of serum and absorbed at 530nm. The reagent blank, 0.01ml of standard solution was treated in same way. Mix well after the addition of each reagent Estimated with the help of chemoanalyser and expressed as g/dl.

Procedure for albumin: *The reagent used was from span diagnostics kit and absorbed at 630nm. The reagent blank, 0.01 ml of standard solution was treated in same way. Mix well after the addition of each reagent Estimated with the help of chemoanalyser and expressed as g/dl.*

ENZYMATIC ANTIOXIDANTS:

Measurement of Catalase (CAT):

In animals, catalase is present in all major body organs, especially being concentrated in liver and erythrocyte. During β-oxidation of fatty acids by flavoprotien dehydrogenase, hydrogen peroxide is generated, which is accepted upon by catalase

present in peroxisomes. (Nichollas and Schonbaum. 1963).

The catalase activity was assayed by the method of Sinha (1972).catalase catalyses the rapid decomposition of hydrogen peroxide to water.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The decomposition of hydrogen peroxide by catalase proceeds at one of the highest rates known for enzymatic reactions. (Forman and Fischer 1981).

Reagents:

Dichromate-acetic acid reagent: Five % potassium dichromate was prepared with acetic acid (1:3 v/v in distilled water).

Phosphate buffer - 0.01M, pH 7.0: 173 mg of disodium hydrogen phosphate and 122 mg of sodium dihydrogen phosphate were dissolved in 61 ml and 39 ml of distilled water respectively and made upto 200 ml with distilled water.

Hydrogen peroxide – 0.2M: 2.27 ml h hydrogen peroxide was made upto 100 ml with distilled water. ***Procedure:*** 0.1 ml of liver homogenate was taken, to which 1.0 ml of each phosphate buffer and hydrogen peroxide were added and a timer started. The reaction was arrested by the addition of 0.2 ml dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µm were taken and treated similarly. The tubes were heated in a boiling water bath for 10 minutes. The green color developed was read at 570 nm in a Double beam UV-VIS spectrometer (Perkin Elmer), Germany. Catalase activity was expressed as U/l.

Measurement of Superoxide dismutase (SOD)

Superoxide dismutase scavenges the superoxide radical ($O^{\bullet}_2$) and thus provides a first line defence against free radical damage. Superoxide dismutase in the liver homogenate was assayed by the method of Misra and Fridovich (1972) based on the oxidation of epinephrine adrenochrome transition by enzyme.

Superoxide dismutase catalyses the dismutation of superoxide anions (O_2^-) to hydrogen peroxide and molecular oxygen in the following manner.

2H2O + 2O2$^-$ Superoxide dismutase 2H2O2 + O2 Superoxide anion (O_2^-) interacts with peroxide to form hydroxyl radical ($OH^{\bullet}$) which causes damage in the absence of superoxide dismutase activity ($R^{\bullet}$) (Thomas *et al*., 1978).

Reagents:

Carbonate buffer – 0.05M, pH 10.2: 1.14 g of sodium carbonate and 84 g of sodium bicarbonate were dissolved in 80 and 20 ml distilled water respectively.

Ethylene diamine tetra acetate – 0.49M: 14.3 g of EDTA was dissolved in 100 ml of distilled water.

Epinephrine – 3.0M: 54 mg of epinephrine was dissolved in 100 ml of distilled water.

Procedure: 0.5 ml of liver homogenate was diluted with 0.5 ml of distilled water. To this, 0.25 ml ethanol and 0.15 ml of chloroform, all reagents chilled, were added. The mixture was shaken for 1 minute and centrifuged at 2000 rpm. The enzyme in the supernatant was determined. To 0.5 ml of the supernatant, 1.5 ml of buffer was added. The reaction was initiated by the addition of 0.4 ml epinephrine and change in optical density per minute was measured a6t 470 nm in a Double beam UV-VIS spectrometer (Perkin Elmer), Germany. SOD activity was expressed as U/l. Change in optical density per minute at 50% inhibition to adrenochrome transition by the enzyme is taken as one enzyme unit.

Measurement of Lipid peroxidation (LPO):

The concentration of thiobarbituric acid reactive substances (TBARS) was measured (lipid peroxidation product maondialdehyde (MDA) was estimated) in liver using the method of Okhawa *et al*., (1979). 1 ml of the sample was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8% thiobarbituric acid (TBA, pH7.4). The mixture was heated in a hot water bath at 85˚C for 1 h. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. The concentration was expressed as *n* moles of MDA per mg of protein using 1,1,3,3,-tetra-ethoxypropane as the standard.

Histopathological studies:

Pieces of liver from each liver lobe were fixed in Bouin's fluid for 24 and washed in running tap water to remove the color of Bouin's fluid and dehydrated in alcohol in ascending and descending

order, embedded in paraffin and cut at 5µm (Automatic Tissue Processor, Lipshaw) in a rotary microtome. These sections were then deparaffinized in xylene, stained with hematoxylin-eosin dye (Merck, India) and mounted with Canada balasam. The histopathological slides were examined and photographs were taken.

Statistical Analysis:

All the data were expressed as mean □SEM (standard error of mean) for six rats. Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls Multiple Comparision Test. The value of probability less than 5% ($P < 0.05$) was considered statically significant.

RESULTS AND DISCUSSION:

Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on SGPT, SGOT, SALP, Total protein , Albumin and total bilirubin against control and RIF + INH induced hepatotoxicity in Rats.

It is clearly evident from the table 6 that RIF+INH caused significant elevation of liver serum markers. In the RIF+INH treated group, the level of SGPT (25.65±3.06-84.22±5.42, p<0.001), SGOT (172.44±6.40 – 375.44±8.46, p<0.001), SALP (226.60±10.24 – 388.64±7.96, p<0.001), Total protein (6.98±1.54 –3.22±0.46,p<0.001), Albumin (1.42±0.24-0.68±0.06,p<0.001), Total bilirubin (0.64±0.20–1.88±0.24, p<0.001). In contrast, the groups treated with 50% ethanolic extracts of ***Cissampelos pareira*** at dose of (100 - 400 mg/kg) once daily for 28 days prevented the hepatotoxicity in a dose related manner. The ranges of protection in the serum marker were found to be SGPT (76.55±4.20 – 54.44±3.34, p>0.05 to p<0.001), SGOT (256.32±11.44 – 196.66±6.94, p<0.001),
SALP (365.59±8.11 –244.44 ± 11.22, p>0.05 to p<0.001), Total protein (4.86±0.42–7.66±0.28, p<0.05 to p<0.001), Albumin (0.94±0.05-1.29±0.04, p<0.05 to p<0.001), Total bilirubin (1.46±0.20 – 0.64±0.20, p<0.001). The protection of silymarin ranged for SGPT (84.22±5.42– 45.99±4.24, p<0.001), SGOT (375.44±8.46– 167.34±7.02, p<0.001), SALP (388.64±7.96–226.24±8.44, p<0.001), Total protein (3.22±0.46–7.26±0.68, p<0.001), Albumin (0.68±0.06-1.30±0.22, p<0.001), Total bilirubin (1.88±0.24–1.26±0.04, p<0.001) and Direct bilirubin (0.92 – 0.24, p<0.001) respectively as shown in table 6 and figures 1 & 2.

Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on CAT, SOD, and Lipid peroxidation against control and RIF + INH induced hepatotoxicity in Rats.

Table 7 & figure 3 illustrated the lipid peroxidation and the enzymic and non-enzymic antioxidant level in liver of experimental animals. Administration of RIF+INH led to increase in the levels of LPO (0.44±0.06 -4.52±0.34, p<0.001), and decrease in enzymic scavenger viz. CAT (24.02±2.24 –7.21±0.44, p<0.001), SOD (112.96±2.42 – 53.66±3.44, p<0.001) levels in the liver homogenate. Treatment of rats with 50% ethanolic extracts of ***Cissampelos pareira*** at dose of (100 - 400 mg/kg) markedly prevented the RIF+INH induced alterations of various parameters LPO (3.32±0.34 – 0.95±0.04, p<0.01 to p<0.001), CAT (14.98±1.21 – 20.44±0.64, p<0.001), SOD (63.64±2.84 – 90.54±2.22, p>0.05 to p<0.001). The protection of silymarin ranged for LPO (4.52±0.34 – 0.78±0.16, p<0.001), CAT(7.21±0.44 – 22.06±0.56, p<0.001), SOD (53.66±3.44 – 100.29±2.48, p<0.001), respectively.

Table 6: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on biochemical parameter of RIF + INH induced hepatotoxicity in Rats.

Treatment/dose	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Control	25.65 ± 3.06	172.44 ± 6.40	226.60 ± 10.24	6.98 ± 1.54	1.42 ± 0.24	0.64 ± 0.20
RIF+INH (50 mg /kg)	84.22 ± 5.42 ^{z}	375.44 ± 8.46^{z}	388.64 ± 7.96 ^{z}	3.22 ± 0.46 ^{z}	0.68 ± 0.06^{z}	1.88 ± 0.24 ^{z}
C. pareira (100mg/kg)	76.55± 4.20	256.32 ± 11.44^{c}	365.59 ± 8.11	4.86 ± 0.42^{a}	0.94 ± 0.05^{a}	1.46 ± 0.20 ^{c}
C. pareira (200mg/kg)	68.24± 5.24	285.60 ± 14.24^{c}	304.47 ± 11.52 ^{c}	6.61 ± 0.75^{c}	1.02 ± 0.05^{a}	0.86 ± 0.24 ^{c}
C. pareira (400mg/kg)	54.44 ± 3.34 ^{c}	196.66 ± 6.94^{c}	244.44 ± 11.22 ^{c}	7.66 ± 0.28^{c}	1.29 ± 0.04^{c}	0.64 ± 0.20 ^{c}
Silymarin (100 mg/kg)	45.98 ± 4.24 ^{c}	167.34 ± 7.02^{c}	226.24 ± 8.44 ^{c}	7.26 ± 0.68^{c}	1.30 ± 0.22^{c}	1.26 ± 0.04 ^{c}

Values are expressed as Mean ± SEM of 6 rats in each groups, ^{z}p<0.001 when compared to respective control and ^{a}p<0.05 and ^{c}p<0.001 when compared to respective Rif+INH control.

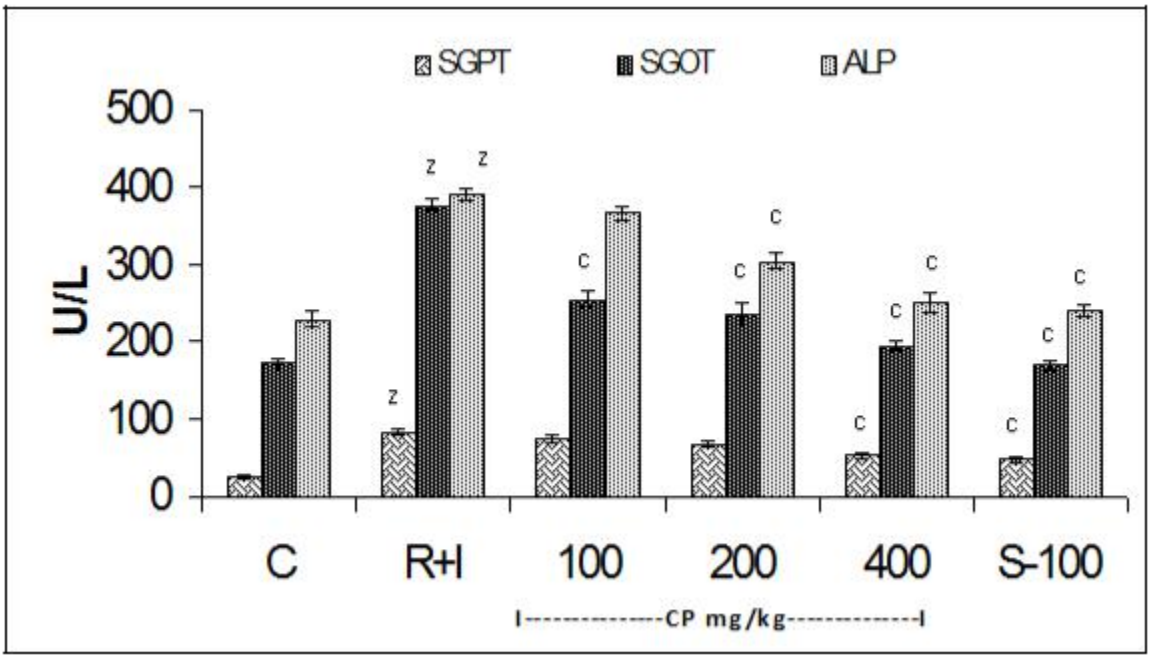

Figure 1: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on liver marker enzymes of RIF + INH induced hepatotoxicity in Rats. (Values are expressed as Mean ± SEM of 6 rats in each groups ^{z}p<0.001 when compared to respective control and ^{c}p<0.001 when compared to respective Rif+INH control.)

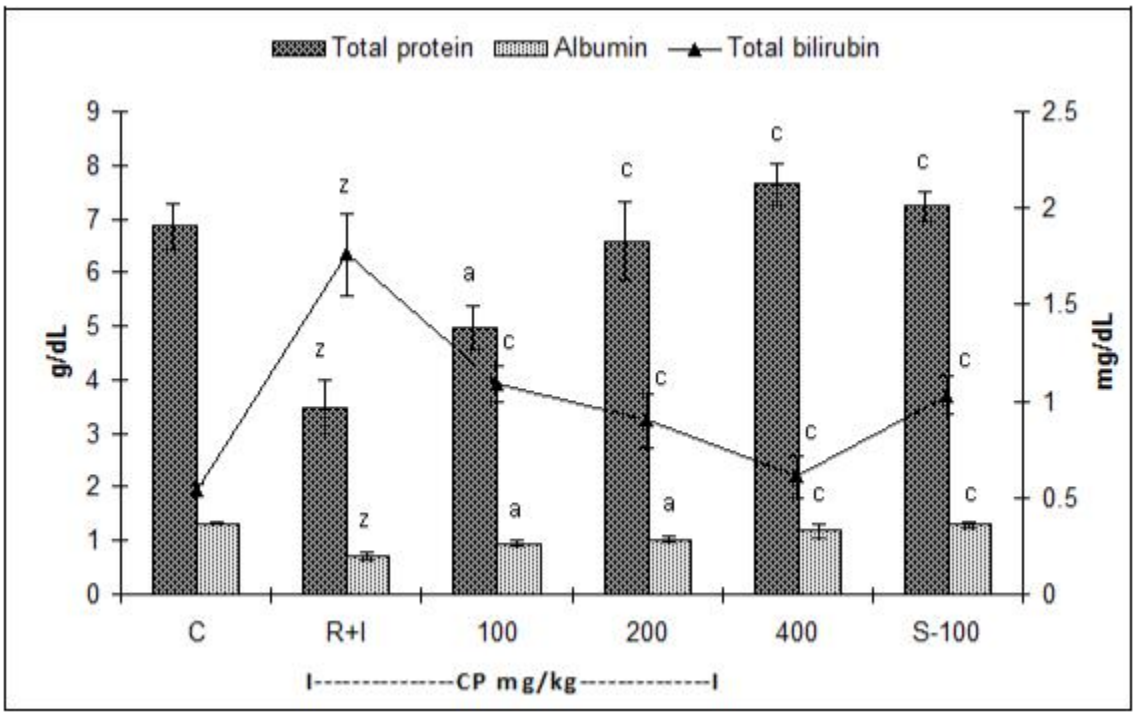

Figure 2: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on Total protein, albumin and bilirubin of RIF + INH induced hepatotoxicity in Rats (Values are expressed as Mean ± SEM of 6 rats in each groups, ^{z}p<0.001 when compared to respective control and ^{a}p<0.05 and ^{c}p<0.001 when compared to respective Rif+INH control.)

Table 7: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on antioxidant and lipid peroxidation in liver homogenate of RIF + INH induced hepatotoxicity in Rats.

Treatment/dose	*Catalase (U/mg)*	*SOD (U/mg)*	*LPO (MDA/g tissue/min)*
Control	24.02 ± 2.24	112.96 ± 2.42	0.44 ± 0.06
RIF+INH (50 mg /kg)	7.21 ± 0.44 ^{z}	53.66 ± 3.44 ^{z}	4.52 ± 0.34^{z}
C. pareira (100mg/kg)	14.98 ± 1.21	63.64 ± 2.84	3.32 ± 0.34^{b}
C. pareira (200mg/kg	17.35 ± 0.84 ^{c}	78.34 ± 4.19 ^{c}	2.68 ± 0.22^{c}
C. pareira (400mg/kg	20.44 ± 0.64 ^{c}	90.54 ± 2.22 ^{c}	0.95 ± 0.04^{c}
Silymarin (100 mg/kg)	22.06 ± 0.56^{c}	100.29 ± 2.48 ^{c}	0.78 ± 0.16^{c}

Values are expressed as Mean ± SEM of 6 rats in each groups (^{z}p<0.001 when compared to respective control and ^{b}p<0. 01 and ^{c}p<0.001 when compared to respective Rif+INH control)

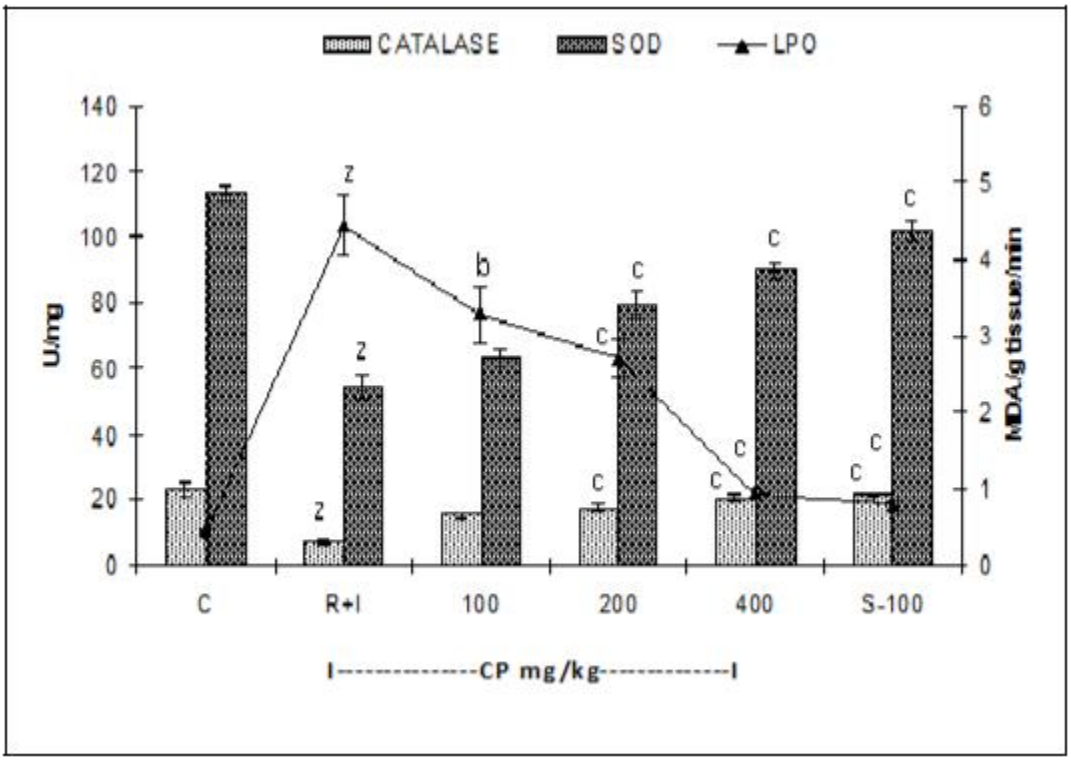

Figure 3: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on antioxidant and lipid peroxidation in liver homogenate of RIF + INH induced hepatotoxicity in Rats (Values are expressed as Mean ± SEM of 6 rats in each groups, ^{z}p<0.001 when compared to respective control and ^{b}p<0. 01 and ^{c}p<0.001 when compared to respective Rif+INH control)

Epidemiological studies have shown that fruits, vegetables, beverages, spices, tea and medicinal herbs rich in antioxidants and other micronutrients protect against diverse forms of chemically-induced hepatic damage, carcinogenesis, mutagenesis, DNA-damage and lipid peroxidation (Wattenberg, 1990). Liver, the key organ of metabolism and excretion, is constantly endowed with task of detoxification of xenobiotics, environmentally pollutants and chemotherapeutic agents. Thus, disorders associated with this organ are numerous and varied. Anti-tubercular drugs (ATDs) are the commonest agents causing serious, clinically significant drug induced liver disease in the developing countries (Acharya et al., 1996; Hwang et al., 1997). Most commonly used ATD like Isoniazid (INH) and Rifampicin (RMP) are hepatotoxic. Various factors predisposing to ATD hepatotoxicity, both genetic and acquired, are well delineated (Huang et al. 2002; Huang et al. 2003; Roy et al. 2001) but little is known about the cellular and biochemical mechanisms of ATD induced hepatotoxicity. While a perfect cure has not yet been found in modern medicine, the current usage of corticosteroids and immunosuppressive agents only brought about symptomatic relief (Handa *et al*., 1986). Furthermore, their usage is associated with risk of relapses and danger of side effects. On the other hand, Ayurveda, an indigenous system of medicine has long tradition of treating liver disorders with traditional knowledge (De *et al*., 1993).

In the past, several studies have reported that over 280 species belonging to more than 40 different genera as plants containing hepatotoxic pyrrolizidine alkaloids cause liver damage and cirrhosis (Anon., 1988). In spite of tremendous advances in medicinal plant research and rapid strides in modern medicine, there are hardly any drugs that can stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cells. There are however, a number of drugs employed in traditional system of medicine for liver affections. In recent years, there has been a shift towards therapeutic evaluation of herbal products in liver diseases by carefully synergizing the strength of the traditional knowledge with that of modern concept of evidence-based medicinal evaluation using

scientific tools (Oliveir *et al*., 2005)., but management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Therefore, the 50% Aq. EtOH extracts of *Cissampelos pareira* to assess the antihepatotoxicity in scientifically validated experimental models.

We have subjected 50% Aq. EtOH extract of *Cissampelos pareira* to assess the hepatoprotective effects on the tissue defense system in Rif+INZ and drug–induced hepatitis in rats. It is well established from the earlier studies that administration of isoniazid and rifampicin, the most common medication prescribed against tuberculosis, produces many metabolic and morphological aberrations in liver due to the fact that liver is the main detoxifying site for these antitubercular drugs. These antitubercular drugs induce hepatitis by a multiple step mechanism. It is characterized by a fall in serum albumin concentration and a rise in serum globulin concentration, which is related to the severity and duration of the disease. Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of isoniazid and rifampicin. Antitubercular drugs mediated oxidative damage is generally attributed to the formation of the highly reactive oxygen species, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane (Georgieva et al., 2004). Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components [reduced glutathione (GSH)] have been reported in isoniazid and rifampicin-induced hepatoxicity (Tasduq et al., 2005).

The 50% EtOH extract *Cissampelos pareira*, showed significant hepatoprotective activity in our study as evident from the protection provided as compared to the serum marker levels i.e SGOT, SGPT, ALP and Bilirubin in the isoniazid and rifampicin treated rats (table 6 & figures 1 and 2).
SGOT, SGPT, ALP and Bilirubin are intracellular enzymes present abundantly in the liver. Under normal conditions, In the case of hepatocellular damage, these enzymes will leak out from the damaged hepatocytes, causing an increase in serum enzyme activities. In tissues , SGOT and SGPT are found in higher concentrations in cytoplasm SGOT particular also exists in mitochondria. In liver injury, the transport function of the hepatocytes is disturbed resulting in the leakage of plasma membrane (Zimmerman and Seef, 1970), thereby causing an increased enzyme levels in serum. If injury involves organelles such as mitochondria, soluble enzymes like SGOT normally located there, will also be similarly released. Here the elevated activities of SGOT and SGPT in serum are indicative of cellular leakage and loss of functional integrity of cell membranes in liver. Alkaline phosphate (ALP) is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum. Hyperbilirubinaemia is also a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate.

In the present investigation we have also observed decreased protein contents in plasma of hepatotoxic bearing animals appears to be attributed to the impaired hepatic function resulting from infiltration with heatotoxicity. The liver is an important site of protein synthesis and it has the highest rate of synthesis of tissue proteins. Recycling of amino acids has been decreased in hepatotoxic conditions resulting in enhanced efflux of these amino acids from the tissues. Thus, the host responds to increased hepatotoxicity load by increasing tissue protein breakdown. In hepatotoxic condition they exhibit hypoproteinemia. The administration of ***C. pareira*** (100, 200 and 400 mg/kg) to the hepatotoxic bearing group resumed the protein level to near normal (Table 6) and also in comparision with the standard drug silymarin, it shows the antihepatotoxic activity of the ***C . pareira*** plant extract (50% ethanolic) on isoniazid and rifampicin induced hepatotoxicity. Alterations in protein metabolism have been considered for decades to be one of the conditions associated with hepatic dysfunction. Our results showed decreased levels of protein in the serum of isoniazid and rifampicin-administered rats as compared to the Group I controls. Also significant declines in the serum albumin were observed. The disaggregation of polyribosomal profiles induced by antitubercular drugs is also associated with the inhibition of

protein synthesis, which may be partially responsible for the fatty liver, probably not necrosis although it contributes to disabling of the cell. Consumption of isoniazid and rifampicin increased the bilirubin level in the serum of Group II, antitubercular drugs administered rats as compared to that of control rats.

The elevated levels of SGOT, SGPT, ALP and Bilirubin observed in isoniazid and rifampicin - induced group may be due to the leakage of plasma membrane and loss of functional integrity of cell membranes in liver (Singh *et al*., 1999). Howevre on treatment with 50% ethanolic extract of the plant (100, 200 and 400 mg/kg) showed significant results, reducing the levels of these elevated levels in a dose dependent manner, indicates that the restoring of serum marker enzymes (Table 6 and figures 1 & 2).

RIF + INH induced hepatitis is due to their biotransformation to reactive metabolites that are capable of binding to cellular macromolecules. As an alternative to inducing cellular damage by covalent binding, there is evidence that these antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system. The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al. (2000). Our findings confirm the same pattern and show significant increase in the level of lipid peroxidation in the liver tissue of Group II (antitubercular drugs administered rats) as compared to that of Group I control rats (table 7 & figure 3). Increase in the level of lipid peroxides in liver reflected the hepatocellular damage. The depletion of antioxidant defenses and/or raise in free radical production deteriorates the prooxidant-antioxidant balance, leading to oxidative stress-induced cell death (Sodhi et al., 1997). The higher lipid peroxidation level indicated the increased production of O_2^- level was thought to increase the concentration of cellular radical level. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins (Fridovich, 1986).

These observations are in association with a significant increase in hepatic MDA level and decrease in the activities of hepatic antioxidant enzymes, such SOD and CAT with hepatocellular damage in isoniazid and rifampicin treated rats agrees the previous finding that a free radical-mediated process is involved in the development of liver damage. Cytoprotective enzymes which are located within both hydrophilic and hydrophobic compartments of the cells and the antioxidants in intra and extra cellular fluids are involved in the scavenging of free radicals. Catalase catalyses the disproportionations of hydrogen peroxide to be the first line of defence against oxidative damage to have lower activity in hepatotoxic bearing animals. Liver catalase activity is depressed in neoplasm in correlation with the degree of hepatotoxicity (Sanz *et al.,* 1994). Super oxide dismutase (SOD) is widely distributed in the cells with high oxidative metabolism and has been proposed to protect such cells against the deleterious effects of super oxide anion (Fridovich., 1975). The 50% ethanolic plant extract on treatment, lowered the melanoaldehyde (MDA) levels and enhancement in antioxidant enzymes in comparison with hepatotoxicity bearing animals (table 7). Therefore it became convenient to suggest that the extracts have got definite beneficial effect on RIF+INH induced hepatotoxicity.

In agreement with previous studies, hepatocellular necrosis caused elevation of the serum marker enzymes which are released from the liver in to blood. Elevation of these enzymes in serum clearly indicates plasma membrane damage and loss of functional integrity of cell membranes in liver. The hepatotoxicity may be due to lipid peroxidation, depletion of glutathione/cytochrome P-450, an altered immunological system, induced by various chemical agents or direct damage to the cell (DeLeve *et al*., 1995). Irrespective of the mechanisms of injury, it is very clear that ultimate ultimate hepatic necrosis is brought about by increased lipid peroxidation or depletion of glutathione.

Treatment 50% EtOH extract of *C. pariera* at a dose of 400 mg/kg, showed highly significant activity, which is almost comparable to the group treated with silymarin, a potent hepatoprotective drug used as reference standard. In the present study, the oxidative injury induced by RIF+ INH could be prevented by *C. papeira*. Thus this study

represents a novel and an attractive idea to prevent RIF+ INH induced hepatic injury by co-administration of *C. papeira*.

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Additionally, it is the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine but there are not much drugs available for the treatment of liver disorders. Therefore, the efficacy of many traditional remedies employed in herbal drugs for the treatment of liver ailments studied against different drug-induced liver damage in experimental animals

The most important part is the evolution of experimental hepatotoxicity and its importance as an animal model in treatment of disease relating to human efficacy. RIF+INH been used as antituberculer drug and studied as drug induced Hepatotoxicity has been discussed in details. RIF+INH is been widely used as a study for drug induced hepatotoxicity and its mechanism of action is also well illustrated. Recent studies on hepatotoxicity inhibitory compounds of plant origin have yielded an impressive array of research on medicinal plant. The efficacy of *Cissampelos pareira* in experimental liver toxicity described in the present investigation offer the potential for reaching on understanding of antihepatotoxic potency. The administration of *Cissampelos pareira* extract and *silymarin* shown the decreased the liver weight, which shows the rehabilitating capability of extracts in respect with antihepatotoxic potency in comparison with the standard drug *silymarin*. Besides *Cissampelos pareira* is very much effective in preventing RIF + induced hepatotoxicity possibly through antioxidents which was confirmed by various liver injury and biochemical hepatotoxic markers enzymes and molecular events this holds great promise for future research in human beings. The antihepatotoxic properties of Cissampelos *pareirashould provide use full* information in the possible application in hepatotoxicity. In conclusion, the antitubercular drugs (isoniazid and rifampicin)-induced alterations on protein metabolism and hepatic antioxidant defense system were normalized by *Cissampelos pareira* co-administration, indicating a possible cytoprotective role of *Cissampelos pareira* against drug induced hepatotoxicity. Thus our studies give scientific evidences to support this plant's traditional uses as claimed in folklore medicine.

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