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RESEARCH ARTICLE

Acute toxicity and histo-pathological studies of liver of ethanolic extract of roots of cissampelos pareira in animals model.

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ABSTRACT

Cissampelos pareira Linn. 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.

KEYWORDS: *Cissampelos pareira Linn*, Hirsuta (Menispermaceae)

INTRODUCTION:

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. The use of traditional medicine and medicinal plants in most developing countries, for the maintenance of good health, has been widely observed (UNESCO, 1996).

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. Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999).

Cissampelos pareira Linn. 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 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

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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). 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:

The roots of *Cissampelos pareira* (L.) Hirsuta (Menispermaceae) were collected from herbal garden of MGIP. Lucknow. The plant material was identified and authenticated taxonomically at Mahatma Gandhi Institute of Pharmacy, Lucknow. A voucher specimen of the collected sample was deposited in the departmental herbarium for future reference.

Drug and chemicals:

Rifampicin and isoniazid were obtained from Lupin pharmaceuticals Ltd., Silymarin (sigma chemicals company, U.S.A.) 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-toxicity 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 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 days.

After completion of the treatment, animals were weighed and sacrificed by cervical decapitation. Some part of liver was preserved in 10% formalin for histological studies.

Histopathological studies:

Pieces of liver from each liver lobe were fixed in Bouin's fluid for 24 hr 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:

Acute toxicity studies:

The 50% ethanolic extract of ***Cissampelos pareira*** has shown 0 % mortality (Table 1) at a dose corresponds to 200, 500, 1000, 1500, 2000 mg/kg body weight after observing 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.

Table 1: Data showing the determination of acute toxicity of 50% ethanolic extract of *Cissampelos pareira* (CPE)

Treatment / Dose	Total mice	Mortality (After 72 hr.)
250mg/kg(25-30gm)	3	0
500mg/kg(25-30gm)	3	0
1000mg/kg(25-30gm)	3	0
1500mg/kg(25-30gm)	3	0
2000mg/kg(25-30gm)	3	0

Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on Body wt , Liver wt. and Kidney wt. in control and RIF + INH induced hepatotoxicity in Rats.

50% ethanolic extract of ***Cissampelos pareira*** at a dose of 100, 200 mg and 400 mg/kg once daily for 28 days and standard drug silymarin at a dose of 100mg/kg were subjected for studying the body weight and liver weight and kidney weight in hepatotoxic rats. The study showed that the body weights were significantly decreased from (195–185.5 $p<0.05$) in RIF+INH groups. However, 50% ethanolic extracts of ***C. pareira*** showed a dose dependent protection in body weight. Result for the highest dose ***(***400 mg/kg) is comparable to standard drug silymarin (100mg/kg) (Table 2).

The study showed that the liver weight was increased from 6.3 ± 0.07 to 7.53 ± 0.16 in RIF+INH groups. However, 50% ethanolic extracts of ***C. pareira*** showed a dose dependent protection in liver weight. Silymarin (100mg/kg) showed significant reduction in liver weight

compared to RIF+INH groups (Table 2). The study showed that the kidney weight was slightly increased from 0.98 ± 0.03 to 1.10 ± 0.08 in animals of RIF+INH treated groups. However, treatment with 50% ethanolic extracts of ***Cissampelos pareira*** revert the changes (Table 2).

Table 2: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on body wt. , liver wt. and kidney wt. of RIF + INH induced hepatotoxicity in Rats.

Treatment/dose	Body wt.	Liver wt.	Kidney wt.
Control	195.00 ± 4.84	6.30 ± 0.07	0.98 ± 0.03
RIF+INH (50 mg /kg)	185.50 ± 3.51	7.53 ± 0.16^{z}	1.10 ± 0.08
C. pareira (100mg/kg)	186.16 ± 4.30	7.20 ± 0.15	0.99 ± 0.05
C. pareira (200mg/kg	187.50 ± 4.97	6.60 ± 0.23^{c}	0.95 ± 0.05
C. pareira (400mg/kg	188.50 ± 3.36	6.46 ± 0.10^{c}	0.99 ± 0.06
Silymarin (100 mg/kg)	188.33 ± 3.34	6.31 ± 0.03^{c}	1.03 ± 0.06

Values are expressed as Mean ± SEM of 6 rats in each group
^{z}p<0.001 when compared to respective control 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.

HISTOPATHOLOGICAL STUDEIS:

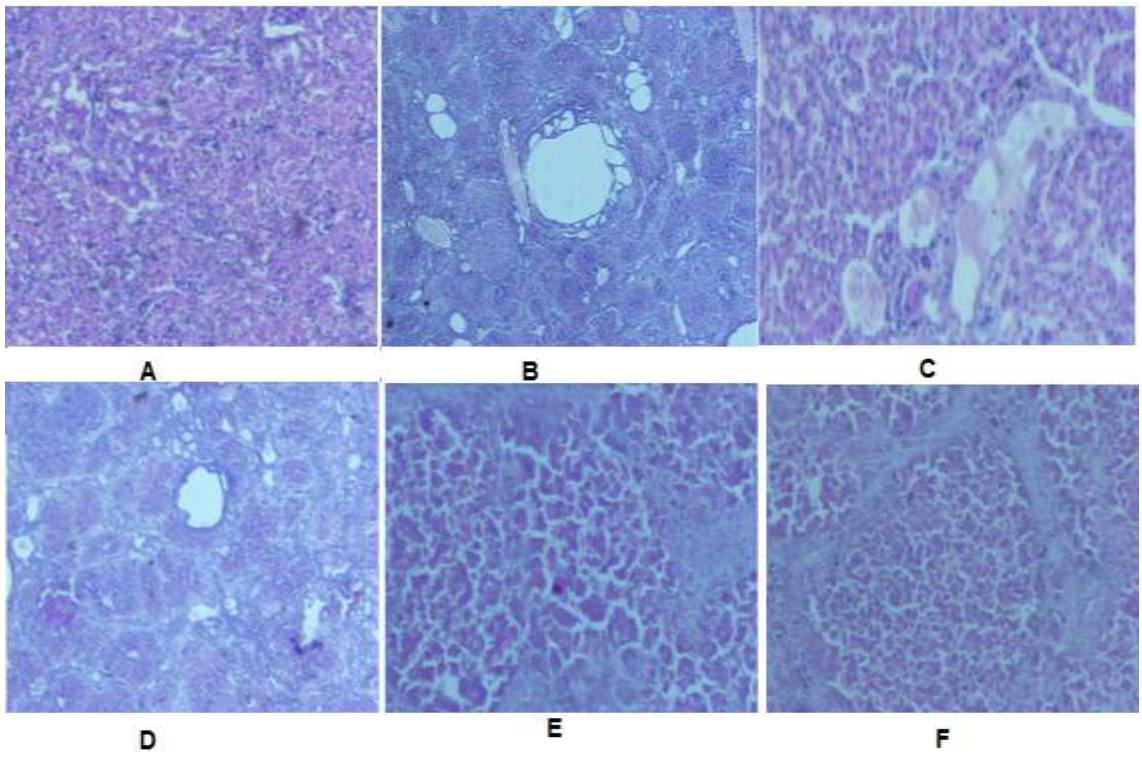

Figure 1: Effect of 50% ethanolic extract of *Cissampelos pareira* (CPE) on RIF + INH induced hepatotoxicity in Rats. **A; Group I:** Liver sections of normal control rats showing normal hepatic cells with well preserved cytoplasm; well brought out central vien; prominent nucleus and nucleolus. **B; Group II** Liver sections of RIF+INH (50mg/kg, each, p.o.) treated rats showing; massive fatty changes ,necrosis ballooning degeneration, and irritation of lymphocytes and kuffer cells around the central vien and the loss of cellular boundaries. **C; Group III** Liver sections of rats treated RIF+INH (50mg/kg, each p.o.) + C.pare-ira extracts (100mg/kg,p.o.) x 28 days , showing well brought out central vien ,hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus. **D; Group IV** Liver sections of rats treated RIF+INH (50mg/kg, each, p.o.) + C.pareira extracts (200mg/kg,p.o.) x 28 days , showing well brought out central vien ,hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus. **E; Group V** Liver sections of rats treated RIF+INH(50mg/kg, each, p.o.) + C.pareira extract (400mg/kg,p.o.) x 28 days ,showing well brought out cental vein,hepatic cells with preserved cytoplasm,prominent nucleus and nucleolus. **F; Group VI** Liver sections of rats treated RIF+INH (50mg/kg, each, p.o.) + silymarin (100mg/kg,p.o.) x 28 days , showing well brought out central vien ,hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus.

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.

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. However, several researchers reported that the plant drugs are safe and effective in treatment of inflammation and gastrointestinal disorders (Amresh, 2007c). Therefore, the 50% EtOH extracts assessed to understand the molecular defensive mechanism involved in safety and efficacy. Experimental and clinical research conducted at various research laboratories has confirmed that protection by plants is probably mediated through antioxidant mechanisms. (Amresh, 2007b).

Further 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).

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. Histological observations basically support the results obtained from serum enzyme essays. Histopathological studies (Figure 1) demonstrated that isoniazid and rifampicin (compared to normal) induces degeneration in hepatocytes (fatty hyrdropic changes), degeneration in hepatic cords, focal necrosis, congestion in central vein and sinusoids, infiltration of lymphocytes and kupfer cell proliferation along with scattered degeneration and bleeding areas. According to

microscopic examinations, severe hepatic lesions induced by isoniazid and rifampicin which are in good agreement with the results of the biochemical tests. Thus, histological architecture of the *C. pariera* treated liver samples showed the ability of the *C. papeira* to prevent hepatocellular necrosis.

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+INH induced hepatotoxicity possibly through antioxidant 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 pareira* should provide useful information in the possible application in hepatotoxicity. Thus our studies give scientific evidences to support this plant's traditional uses as claimed in folklore medicine.

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