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Effect of *Coccinia indica* leaf extract on angiotensin converting enzyme (ACE) inhibitor induced hepatotoxicity in wistar albino rats

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SUMMARY

Enalapril is angiotensin converting enzyme (ACE) inhibitor have been extensively used to treat cardiovascular disorders, including hypertension. *Coccinia indica* is indigenous plant wildly used in traditional medicinal system including Ayurvedic, Siddha and Unani. The aim of this study was to find out the hepatoprotective activity of *C. indica* leaf extract against enalapril induced toxic effects. The 24 experimental rats were divided into 4 groups. Group 1 was maintained as control. Group 2 and 3 were treated with single dose of enalapril (1.5g/kg of bwt/orally). After treatment, group 2 maintained as enalapril control. The group 3 was treated with leaf extract of *C. indica* (400 mg/kg bwt/orally/day) for 7 days. Separate *C. indica* alone supplementation for 7 days were also maintained. After end of the treatment, animals were sacrificed simultaneously. To determine the serum liver function parameters were estimated. The histophological study of liver tissue was also determined. The changes liver function parameters were observed in enalapril control rats along with histological changes. The *C. indica* leaf extract supplementation restored all the parameters significantly. In this study, the protective effect of *C. indica* leaf

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extract against enalapril induced hepatotoxicity proves, the plant has many medicinal properties.

1. Introduction

Hypertension (HTN) is an important public health problem in both economically developed and developing nations [1]. The world's leading risk factor for global disease burden, is expected to cause more than half of the estimated 17 million deaths per year resulting from cardiovascular disease (CVD) worldwide [2]. Liver is major metabolic organ previous studies shows in drug induced liver injury occur as an unexpected reaction to therapeutic dosages. More than 900 different drugs have been implicated in causing liver injury, with the type of injury and degree of severity highly varied [3], and liver injury accounts to an elevated grade of morbidity and mortality [4–7]. The association of liver injury with antihypertensive agents has been best characterized by angiotensin-converting enzyme inhibitors [8]. Angiotensin converting enzyme (ACE), is responsible for the synthesis of angiotensin II, the final product of the renin angiotensin cascade [9–12]. Enalapril is angiotensin converting enzyme (ACE) inhibitor have been extensively used to treat cardiovascular disorders, including hypertension. These drugs are generally well tolerated [13,14]. However, hepatic injuries, which are rare [15], have been reported in patients treated with enalapril [3,13,16–20], captopril [21–23] and lisinopril [24,25].

Coccinia indica is a type plant belonging to the Cucurbitaceae. It is commonly known as Scarlet-fruited gourd and locally known as 'Kovai,' it is natively found in India, Asia and Central Africa [26]. Indigenous people used various parts of the plant to vegetable and medicinal purpose. The fruit of *C. indica* is used as vegetable when it is green in colour and eaten fresh when ripened into bright scarlet color. The young leaves and shoot tips of *C. indica* are used in Asia for cooking purpose [27,28]. The plant has also been extensively used in Ayurvedic, Unani and Siddha practice in the indian subcontinent [29–31]. *C. indica* is a fast growing perennial vine that grows several meters long. It can form dense mats on lands that readily cover shrubs and small trees. Its leaves are arranged alternately along the stems, the shape of leaves varies from heart to pentagon shaped. The upper surface of the leaf is hairless, whereas the lower is hairy. There are 3–8 glands on the blade near the leaf stalk. Tendrils are simple [32].

C. indica contain important raw material for drug production like bioactive compounds such as secondary metabolite like Aerial part - Heptacosane, Cephalandrol, β -sitosterol, Alkaloids Cephalandrins A and B, Fruits- β- Amyrin Acetate, Lupeol, Cucurbitacin B, Taraxerone, Taraxerol, β-carotene, Lycopene, Cryptoxanthin, Xyloglucan, Carotenoids, β-sitosterol, Stigma-7-en-3-one. Root - Resin, Alkaloids, Starch, Fatty Acids, Carbonic acid, Triterpenoid, Saponin Coccinoside, Flavonoid Glycoside, Lupeol, β-amyrin, β-sitosterol, Taraxerol [33]. Previous scientific research showed that leaf extract of *C. indica* exhibits hepatoprotective [34–36], antioxidant [37,38], anti-inflammatory [39] anti diabetic [40], hypolipidemic [41,42], and anti-bacterial [43] activities. This study investigated how the use of *C. indica* leaf extract could interfere with hepatotoxicity caused by enalapril by means of pharmacological and biochemical approaches.

2. Materials and methods

2.1. Animals

A total of 24 female healthy wistar albino rats with body weight ranging from 150 to 200 g were purchased from Sri Venkateswara Enterprises, Bangalore. The experimental rats were quarantined in animal house for 10 days and were maintained at 12:12 h's light/dark cycle at 20 °C ± 2 for 7 days with

ad libitum of food and sterile reverse osmosis water. All procedures were performed according to the institutional animal ethics committee's approval.

2.2. Drugs and chemicals

All the drug and chemicals were purchased from Hi media and Sigma Chemical Co, USA.

2.3. Collection of plant materials

The leaves of *C. indica* were collected from near Tiruchirappalli, India. The plant leaves were cleaned of extraneous matter, and necrotic parts removed by rinsing in fresh water. The leaves were further repeatedly rinsed thoroughly with running distilled water for further analysis.

2.4. Plant identification

The plant was identified and confirmed as *C. indica* in the RAPINET Herbarium, St. Joseph's college, Tiruchirappalli, India.

2.5. Preparation of diethyl ether extracts

The fresh green leaves of *C. indica* were shade dried and made into powdered manually. The 150 gm of dried powder soaked in the solvent diethyl ether and kept aside for two days. After two days, the ethereal layer was decanted and process repeated till plant material was exhaustively extracted with diethyl ether. Total extract was distilled off and concentrated under reduced pressure and controlled temperature by using rotary evaporator. The extract was green sticky mass (yield 50 gm). The extract was stored in freeze at 4c for further studies. Suspensions of extract were freshly prepared using 5% Tween 20 and 0.9% saline, for experimental use.

2.6. Experimental design

Rats were divided into four groups with six animals each. Group I was maintained as control with food and distilled water for 7 days. Other animals were subdivided and maintained as following groups up to 7 days such as Group II (Enalapril control), Group III (Enalapril + *C. indica* leaf extract therapy), Group IV (*C. indica* leaf extract control). The respective *C. indica* leaf extract were administered simultaneously along with enalapril supplementation to group III animals for 7 days. enalapril were purchased from The Madras Pharmaceuticals, Chennai, Tamil Nadu, India, respectively. The enalapril (1.5 g/kg bwt/p.o) for single dose was selected according to Jurima romet and Huang, (1992) [44]. The *C. indica* leaf extract (400 mg/kg bwt/p.o) for 7 days therapy was selected according to Kumar et al., (2010) [35].

2.7. Sample collection

After end of the treatment, rats were anaesthetized by chloroform, sacrificed and the blood and liver samples were collected from aseptically. Serum was isolated after centrifugation at 3000 rpm for 15 min. The serum aliquots were immediately used to determination of serum protein and liver function parameters and liver sample used histopathological studies.

2.8. Methods

2.8.1. Determinations of Fourier transform infrared (FT-IR) spectroscopy analysis

Shade dried diethyl ether extracts of *C. indica* leaf samples were ground into fine powder using a mortar and pestle. About 2 mg of the sample was mixed with 100 mg potassium bromide (FT-IR Grade) and then compressed to prepare a salt-disc (3 mm diameter). The disc was immediately kept in the

sample holder and FT-IR spectra were recorded in the range of absorption between 400 and 4000 cm-1. All investigations were carried out with a Shimadzu FT-IR spectrometer.

2.8.2. Estimation of liver function parameters (LFT)

Serum liver function parameters (LFT) were determined by standard methods using spectrophotometrically. Total protein estimated by method of Lowry et al., (1951) [45]. The results were expressed as g/dl. Total Bilirubin level determined by method of Van den Bergh and Muller. (1916) [46]. The results were expressed as mg/dl. Serum glutamate oxaloacetate transaminase (sGOT, EC 2.6.1.1) determined by method of Raitman and Frankel. (1957) [47], Serum glutamate pyruvate transaminase (sGPT, EC 2.6.1.2) determined by method of Raitman and Frankel. (1957) [47], Serum alkaline phosphatase (ALP, EC 3.1.3.1) determined by method of King and Armstrong. (1934) [48], Lactate dehydrogenase (LDH, EC 1.1.1.27) and Gamma-glutamyl transferase (GGT, EC 2.3.2.2) were estimated using commercial kits purchased from Crest Biosystems, India. All the liver enzyme results were expressed as IU/L.

2.8.3. Histopathological studies

The same groups were maintained for histopathological study. The animals were perfused transcardially. The whole blood was cleared from the circulation by flushing normal saline till the draining fluid becomes clear and then 10% normal saline was flushed for fixation. The Bouin's fixed tissues were processed for routine paraffin sectioning and stained with Haematoxylin and Eosin (H&E) [49]. Briefly, liver tissues were hydrated, then dehydrated in graded alcohol series, briefly cleared in chloroform and xylene and then embedded in paraffin wax. Liver tissues were sectioned at 5 mm thickness using Rotary microtome and incubated overnight at room temperature. Then the sections were deparaffinized, rehydrated through descending alcohol series (100% alcohol, 90% alcohol, 70% alcohol and 50% alcohol) followed by distilled water. These sections were stained with H & E and then rapidly carried through ascending alcohol series. Morphology of liver tissues was analyzed and recorded by Nikon microscope (400).

2.8.4. Statistical analysis

Data were expressed as mean ± SEM. Statistical significance was evaluated by one way ANOVA using SPSS version 21. In all cases, $p < 0.05$ was considered as statistically significant.

3. Results

FTIR was used to analyze the functional groups of compounds. The absorption spectra of diethyl ether extracts of *C. indica* leaf are shown in Table 1. Diethyl ether extracts of *C. indica* leaf FTIR analysis confirms the presence of amines, alkanes and alcohol at 3412.53/cm peak value. The peak at 2921.54 and 2362.69/cm indicated the presence of alkane. The remaining peak at 2117.22, 1634.74, 1438.98, 1242.57, 1096.46, 665.86 and 601.60/cm represents the presence of Isothiocyanates stretch, Conjugated alkenes stretch, Bend carboxylic acids, Alcohols stretch, Ethers stretch, Alkyl halides stretch and Acid chlorides

Table 1
FTIR peak values and functional groups of *Coccinia indica* leaf extract.

Origin	Peak	Functional groups
N–H	3412.53	Amines stretch
C–H	2921.54	Alkanes stretch
C–H	2362.69	Alkanes stretch
N=C=S	2117.22	Isothiocyanates stretch
C=C	1634.74	Conjugated alkenes stretch
O–H	1438.98	Bend carboxylic acids
C–O	1242.57	Alcohols stretch
C–O–C	1096.46	Ethers stretch
C–Cl	665.86	Alkyl halides stretch
C–Cl	601.60	Acid chlorides stretch

stretch as a functional group, respectively. The major peaks and functional of dynamic compounds groups were analysed and results were compared with standard infrared chart by Coates. (2000) [50].

Histopathological examination of liver section of control animals showed normal cellular architecture with distinct hepatic cells, prominent nucleus, sinusoidal space and central vein. There was no sign of inflammation, fatty change or necrosis in these animals (Fig. 6 A). Liver section of intoxicated with enalapril (1.5 g/kg bwt) treated animals showed severe hepatocyte degeneration and necrosis in the centrizonal area. Inflammatory cells were also observed in the portal triad (Fig. 6 B). Liver section of rat treated with diethyl ether extract of *C. indica* leaf (400 mg/kg bwt) and intoxicated with enalapril (1.5 g/kg bwt) showed reduction of necrosed area and inflammatory infiltrates in the centrizonal area with disappearance of inflammatory infiltrate around portal triad (Fig. 6C). Liver section of rat treated with diethyl ether extract of *C. indica* leaf (400 mg/kg bwt) showed normal cellular architecture with distinct hepatic cells, prominent nucleus, sinusoidal space and central vein. There was no sign of inflammation and necrosis in these animals (Fig. 6 D).

4. Discussion

Liver injury caused by toxins or chemicals is generally associated with acute or chronic damage. Injury by cytotoxins is characterized by necrosis, In this regard, the mechanism by which ACE

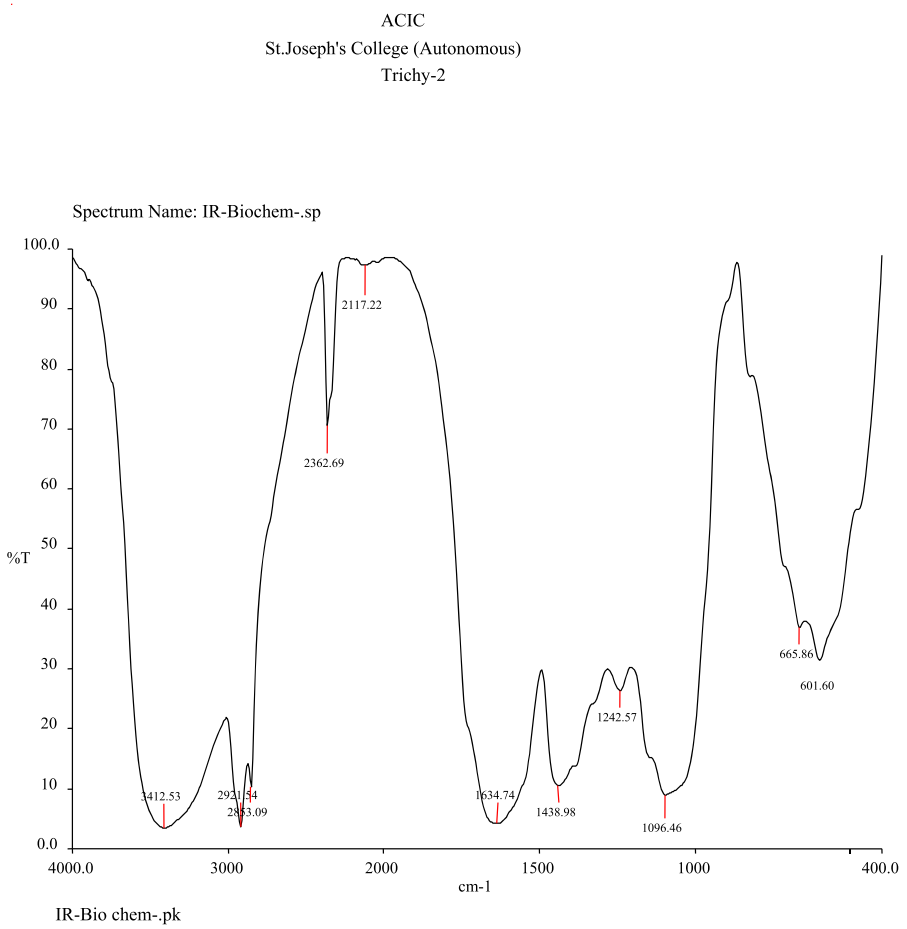

Fig. 1. Fourier transform infrared (FTIR) spectroscopy analysis of *Coccinia indica* leaf extract.

inhibitors cause liver injury remains unclear, mainly because of the lack of a suitable experimental model [51]. Studies also show enalapril treatment and the first signs of liver damage suggest the possibility of a metabolic idiosyncrasy [52]. Based on literature in our present study enalapril was found to cause of hepatotoxicity in rat livers, high doses of enalapril elicited significantly decreased in total protein (Fig. 2) at same trend significantly increased levels of enzymatic biomarkers including serum sGPT, sGOT, GGT, ALP, LDH and total bilirubin of enalapril treated animals compared with normal group (Figs. 3–5). Histopathological changes were also observed in liver tissues in enalapril induced group such as severe hepatocyte degeneration and necrosis in the centrizonal area. Inflammatory cells were also observed in the portal triad (Fig. 6 B). We suggest that the elevated serum liver markers were released from the injured liver upon exposure to high doses of enalapril. In addition, serum enzymatic biomarkers and total bilirubin (Figs. 3–5) levels were markedly increased, especially at single dose of enalapril 1.5g/kg of bwt by orally, and this may be caused by excessive

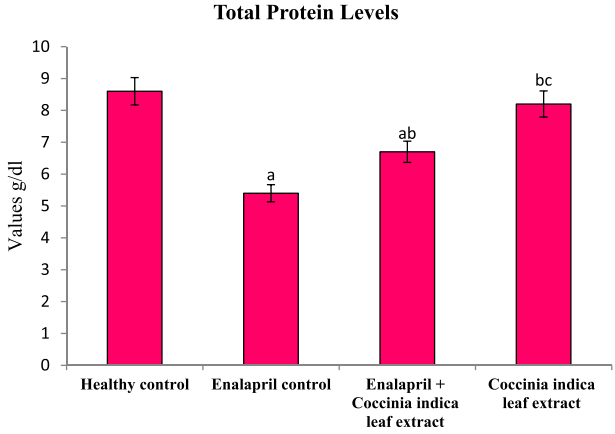

Fig. 2. Total protein values was expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of P < 0.05. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

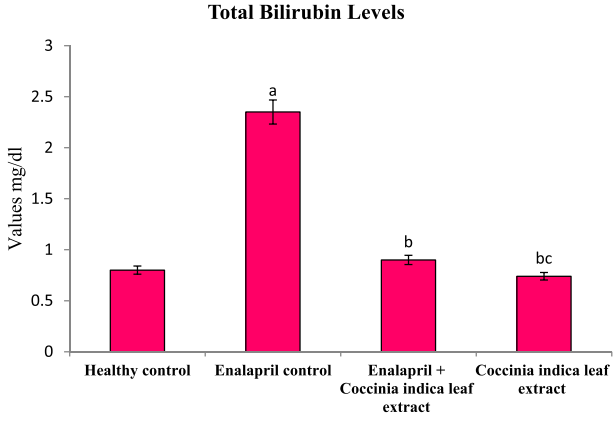

Fig. 3. Total bilirubin values was expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of P < 0.05. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

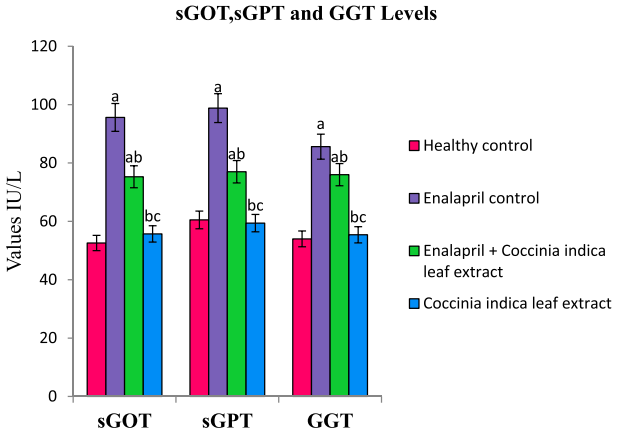

Fig. 4. Serum glutamic oxaloacetic transaminase (sGOT), glutamic pyruvic transaminase (sGPT) and gamma glutamyl transferase (GGT) values were expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of $P < 0.05$. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

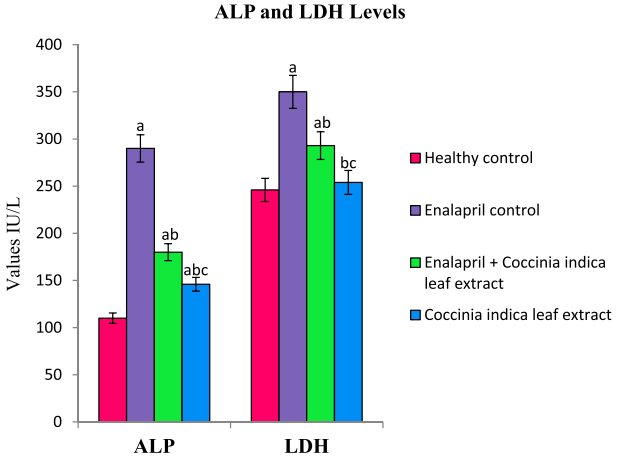

Fig. 5. Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) values were expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of $P < 0.05$. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

accumulation of enalapril in liver tissues. Evidence of enalapril induced liver injury has been demonstrated by various studies [3,13,16–20].

C. indica is traditional medicine system different parts of this plant namely the roots, leaves and fruits have long been considered as valuable sources of medicine for treating variety of diseases and ailments [31]. Recent experimental and clinical studies have shown the importance of *C. indica* leaf extract as a hepatoprotective [34–36], antioxidant [37,38], anti-inflammatory [39] anti diabetic [40], hypolipidemic [41], and anti-bacterial [43] activities. Preliminary phytochemical study showed the presence of Carbohydrates, Glycosides, Alkaloids, Tannins and Flavonoids in the ethereal extract of *C. indica* leaves [35]. The acute toxicity study LD_{50} values suggested that aqueous and diethyl ether leaf extract of *C. indica* is safe and nontoxic in healthy wistar rats and mice up to a dose of 2.00 g/kg of bwt

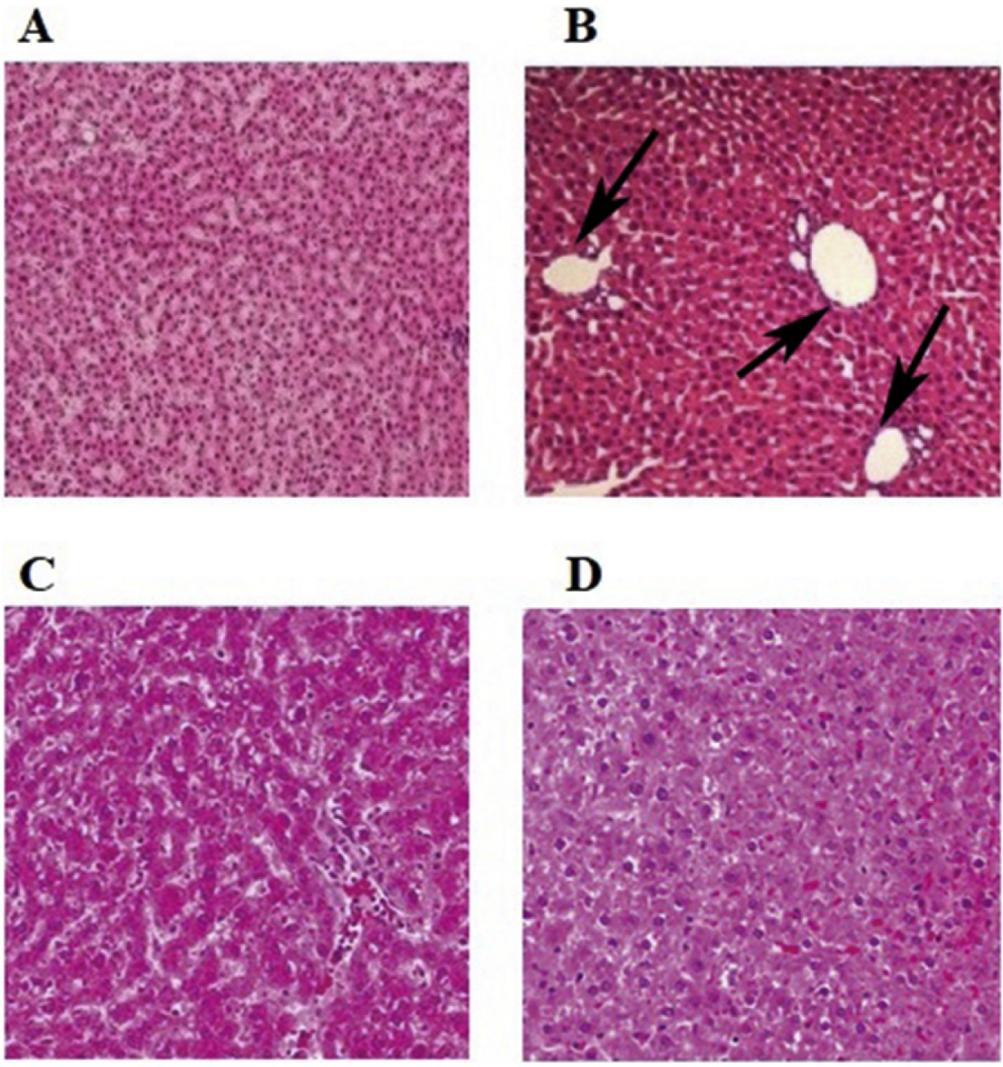

Fig. 6. Histopathological changes occurred in the liver after enalapril intoxication and prevention by the treatment with diethyl ether extract of *Coccinia indica* leaf. A) Normal, B) Enalapril control, C) Enalapril + *Coccinia indica* leaf extract therapy, D) *Coccinia indica* leaf extract control.

[35,53]. In our present hepatoprotective activity study dose (400 mg/kg of bwt) was selected according to Kumar et al., (2010) [35]. The FTIR spectral analysis showed (Fig. 1 & Table 1) the presence of Isothiocyanates stretch, Conjugated alkenes stretch, Bend carboxylic acids, Alcohols stretch, Ethers stretch, Alkyl halides stretch and Acid chlorides stretch as functional group. It indicates the medicinal property of *C. indica* leaf, which can be utilized for various pharmaceutical purposes. In this study, we also observed changes in serum liver function parameters and total protein levels (Figs. 2–5) in enalapril control rats along with histological changes (Fig. 6B) when compared to healthy rats. The *C. indica* leaf extracts supplemented group restored all the serum liver function parameters and total protein levels (Figs. 2–5) significantly. In histopathological studies reduced hepatic damage, lesser necrosis, inflammatory infiltrates, demonstrated a normal architecture of liver and no significant pathological

manifestations (Fig. 6C) like healthy group. *C. indica* leaf extract was found to be toxicologically safe as a potential hepatoprotective agent.

In conclusion based on the results of liver function parameters and histopathological studies, we found that diethyl ether extract of *C. indica* leaf therapy did not show any side effects in liver tissues of rats when compared to enalapril intoxication. Hence, this plant may be clinical usefulness of liver damage. However, further study is required to evaluate using a cardioprotective effect of *C. indica* leaves.

Conflict of interest

None of the authors declares a conflict of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yclnex.2019.01.004.

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