Anti-hepatotoxic Effect of Root Ethanol Extract of Cyclea peltata against Acetaminophen Induced Oxidative Stress in Wistar Rats and in vitro Primary Hepatocyte Culture

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Abstract

Cyclea peltata (Poir.) Hook. f. & Thoms. (Menispermaceae) locally called 'Padathaali' or 'Padakkizhangu' is used in traditional medicine of Kerala to treat jaundice. The Kurichiya tribe of Kerala, India uses the tuberous roots of this plant along with a little salt to treat gastric pain. In the present study the root ethanolic extract of C. peltata (CP) was scientifically evaluated for in vivo acetaminophen (APAP) induced liver damage in Wistar rats, in vitro free radical scavenging, in vitro primary hepatocyte culture and estimation of tetrandrine using high performance thin layer chromatography (HPTLC). The Results showed that pretreatment with CP 250 and 500 mg/kg produced significant reduction of serum biochemical parameters (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin (SB) and cholesterol in APAP treated rats which was almost comparable to the standard drug silymarin (100 mg/kg). Histopathological studies also substantiated the biochemical findings. Both the doses of CP significantly decreased the malondialdehyde (MDA) and increased the reduced glutathione (GSH) levels of APAP treated rats in a dose dependent manner. In vitro primary hepatocyte culture with 10mM APAP+CP showed significant increase in cell viability and decrease in ALT, AST leakage into the culture medium. In vitro antioxidant studies with CP showed significant antilipid peroxidative and free radical scavenging (hydroxyl, superoxide and DPPH) effects. Thus results of the present study shows that hepatoprotective property of C. peltata may be due to reduction of lipid peroxidation, prevention of GSH depletion and hepatocyte membrane stabilizing effects.

Keywords: Cyclea peltata (CP), Tetrandrine (TET), Glutathione (GSH), Malondialdehyde (MDA), Primary Hepatocyte Culture

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1. Introduction

Liver is the most important organ which plays a key role in the metabolism of ingested nutrients, drugs and environmental toxins. According to Wolf (1999) hepatic damage is associated with distortion of many metabolic functions. Earlier reports of Chattopadhyay (2003) shows that in spite of tremendous strides in modern medicine, there are no effective drugs which stimulate liver function, offer protection to the liver from damage or helps in the regeneration of hepatic cells. Scientific research of herbal drugs with hepatoprotective property may be of great benefit as an alternative therapy in liver diseases. The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicine.

*Cyclea peltata* (Poir.) Hook. f. & Thoms., (Menispermaceae) locally called ‘Padathaali’ or ‘Padakkizhangu’ is a much branched climbing shrub found throughout south and east India with tuberous roots, peltate leaves, greenish yellow flowers and drupaceous fruits. Ramachandran and Nair (1981) have reported that Kurichiya tribe of Kerala uses the tuberous roots of this plant along with a little salt to treat gastric pain. Shine et al. (2009) has reported the antisecretory and antiulcer property of *C. peltata* using the lead obtained from the Kurichiya tribe. According to Kumar et al. (1980) the Garo tribe of Balpakram sanctuary in Meghalaya use the crushed root extract as a remedy against small pox. It has been reported in the Wealth of India (2004) that the roots of *C. peltata* are used to treat jaundice in traditional medicine of Kerala. Herein, we report the in vivo hepatoprotective property of the ethanol extract of roots of *C. peltata* (CP) against Acetaminophen (APAP)-induced oxidative stress in Wistar rats, in vitro primary hepatocyte culture and in vitro free radical scavenging.

2. Main Research

2.1. Materials and Methods

2.1.1. Plant material and preparation of the extract

*Cyclea peltata* roots were collected from Thiruvananthapuram district of Kerala, India during January 2009 and authenticated by plant taxonomist of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI). A voucher specimen has been deposited at the JNTBGRI Herbarium (TBGT 13814 dated January 10, 2009). The roots were washed thoroughly with water, shade-dried and powdered. The powder (100 g) was extracted with 1000 ml of 95% ethanol overnight using a Soxhlet apparatus. The extract was then filtered and the filtrate was concentrated under reduced pressure to yield 20 g (w/w) of the crude extract. This crude extract was referred to as CP which was suspended in 0.5% Tween-80 to required concentrations and used for the experiments.

2.1.2. Animals

Wistar albino rats, males (200-250 g) and Swiss albino mice, both sexes (25-30 g), obtained from the Institute's Animal House were used for the present study. They were housed under standard conditions and fed commercial rat feed (Lipton India Ltd, Mumbai, India) and boiled water *ad libitum*. All experiments involving animals were done according to NIH guidelines, after getting the approval of the Institute’s Animal Ethics Committee.
2.1.3. Drugs and Chemicals
Silymarin, Acetaminophen (Paracetamol/ APAP), Tetrandrine (TET), Dimethyl sulphoxide (DMSO) were purchased from Sigma Chemicals Co., USA. 5,5 Dithiobis (2-Nitrobenzoic acid) (DTNB), 1-Chlororo 2,4 dinotrobenzene (CDNB), Nitroblue tetrazolium chloride (NBT), Ethylene diamine tetra acetic acid disodium salt (EDTA) Sodium cyanide (NaCN), Riboflavin, Deoxyribose, Bovine serum albumin (BSA), Hydrogenperoxide (H₂O₂), Sodium pyrophosphate, Phenazine methosulphate (PMS), Penicillin/Streptomycine, Ca²⁺- free Hanks balanced salt solution (HBSS), HEPES buffer and culture plates were purchased from Hi-media, Mumbai, India. Biochemical Kits were purchased from Crest Bio-systems India.

2.1.4. Phytochemical analysis
2.1.4.1. Preliminary phytochemical analysis
CP was subjected to preliminary phytochemical analysis and TLC studies following the methodology of Harborne (1984).

2.1.4.2. High performance thin layer chromatography (HPTLC) of Tetrandrine (TET) in CP
HPTLC of the CP extract was carried out using the solvent system toluene: ethyl acetate: diethylamine (7.2: 2: 0.8) modified method of Shine et al. (2009). The chromatogram was visualized by spraying with Dragendorff’s reagent. Co-HPTLC of CP and authentic tetrandrine (Sigma-Aldrich, USA) was also performed.

2.1.5. Acetaminophen (APAP)-induced liver damage
As per the method of Mitra et al. (1998), acetaminophen (Paracetamol -APAP) was suspended in 0.5% gum acacia and administered P.O., at a dose of 2.5 g/kg. This dose is known to cause liver damage in rats. Rats were divided into 5 groups (6 per group). Group 1, the normal control group was given a single daily dose of 0.5 % Tween-80, P.O., for 4 days. Group 2, the APAP control group received a daily dose of 0.5% Tween-80, P.O., for 4 days and 2 ml of APAP suspension (2.5 g/kg, P.O.) on day three, 30 min after Tween-80 administration. Group 3 animals received silymarin at a dose of 100 mg/kg P.O., on all the 4 days and 2 ml of APAP suspension (2.5 g/kg) P.O., on day three, 30 min after silymarin administration. Groups 4 and 5 received a daily dose of CP (250 and 500 mg/kg respectively) P.O., for 4 days and 2 ml of APAP suspension (2.5 g/kg, P.O.) on day three, 30 min after CP administration.

2.1.5.1. Assessment of liver function
On the 5th day of the APAP hepatotoxicity study (after 48h starvation), all the animals were sacrificed and blood samples were collected for evaluating the serum biochemical parameters (Alanine transaminase (ALT), Aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin (SB) and cholesterol) according to standard methods by Shine et al. (2014).

2.1.5.2. Histopathological studies
The liver samples from the above experiments were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using a rotary microtome (American Optical Co., USA), sections of thickness of about 5 µm were cut and stained with haematoxylin and eosin. These were examined under the microscope for histopathological changes such as necrosis, fatty changes, ballooning degeneration and infiltration of Kupffer cells and lymphocytes.
2.1.5.3. Malondialdehyde (MDA) estimation
The standard method described by Fong et al. (1973) was followed. The liver tissues were homogenized and suspended in thiobarbituric acid (TBA) and the optical density of the clear pink supernatant was read at 532 nm, after centrifugation. Malondialdehyde bis (dimethyl acetal) was used as standard.

2.1.5.4. Estimation of reduced glutathione (GSH)
The standard method described by Moron et al. (1979) was followed for the estimation of reduced glutathione (GSH). The samples were treated with dithio-bis-2-nitrobenzoic acid (DTNB) and the yellow colored complex developed was read at 412 nm.

2.1.6. In vitro primary hepatocyte culture
The operative procedure of Seglen (1973) was followed for the liver perfusion. A differential count of the initial cell suspension was routinely made with 100µl of the cell suspension diluted with 300µl of isotonic 0.6% trypan blue and counted in Burker chamber (hemocytometer). The hepatocytes obtained were suspended in Ca²⁺ free Hank’s balanced salt solution (HBSS) media supplemented with 15 mM HEPES, 10% FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml) and insulin (0.8 IU/ml) and plated in collagen type I precoated culture plates at a density of approximately 1.25X10⁵ cells/cm². After plating, the cells were incubated at 37⁰C (5% CO₂). Hepatocyte attachment to the culture dish was complete at 2 h after plating and the medium was changed to remove unattached or dead cells. At 18 h after plating hepatocytes were treated with CP/dimethyl sulphoxide (DMSO), after 24 hours hepatocytes were incubated with 10 mM APAP for 24 hrs as mentioned by Rousar et al. (2009) using 5% CO₂. In vitro hepatotoxicity was assessed by measuring levels of aspartate transaminase (AST), Alanine transaminase (ALT) leakage into the medium by fully automatic bioanalyser (Tulip) and cell viability was assessed using trypan blue exclusion method mentioned by Strober (2001).

2.1.7. In vitro free radical scavenging effect
2.1.7.1. In vitro anti-lipid peroxidation studies
The anti-lipid peroxidation effect of CP was studied by the method of Suja et al. (2004). Protein content was determined by the method of Lowry et al. (1951). Briefly, 2 g of rat liver tissue was sliced and homogenized with 150 mM KCl-Tris-HCl buffer (pH 7.2). The reaction mixture was composed of 0.25 ml liver homogenate, Tris - HCl buffer (pH 7.2), 0.1 mM ascorbic acid (AA), 4 mM FeCl₂ and 0.005 ml of various concentrations of CP. The mixture was incubated at 37⁰C for 1 h in capped tubes. Then, 0.1 N HCl, sodium dodecyl sulphate (SDS: 9.8%), 0.9 ml distilled water and 2 ml of thiobarbituric acid (TBA: 0.6%) were added to each tube and vigorously shaken. The tubes were placed in a boiling water bath at 100⁰C for 30 min. After cooling, 5 ml of butanol was added and centrifuged at 3000 rpm for 25 min. The absorbance of the supernatant was measured at 532 nm. The experiment was repeated twice.

2.1.7.2. Assessment of Hydroxyl radical scavenging activity:
As per the method of Ohkawa et al. (1979), hydroxyl radicals generated from Fe³⁺-ascorbate-EDTA-H₂O₂ were estimated by the degradation of deoxyribose, that resulted in thiobarbituricacid reacting substances, (TBARS) formation. The reaction mixture contained deoxyribose (2.8mM), FeCl₃ (0.1mM), KH₂PO₄-KOH buffer (20 mM, pH 7.4), EDTA (0.1mM), H₂O₂ (1mM), ascorbic acid (0.1 mM) and various concentration of CP extract, in a final volume of 1 ml. The reaction mixture was
incubated at 37°C for 1 h. Degradation of deoxyribose was measured by thiobarbaturic acid method and percentage inhibition was calculated. Curcumin was used as the standard reference compound.

2.1.7.3. Assessment of superoxide radical scavenging activity:
Superoxide radical scavenging activity of CP was determined by nitroblue tetrazolium (NBT) reduction method described by McCord and Fridovich (1969). The reaction mixture contained EDTA (0.1M), 0.0015% NaCN, riboflavin (0.12mM), NBT(1.5mM), and various concentration of CP extract and phosphate buffer (67mM, pH 7.8) in a total volume of 3ml. the tubes were illuminated under an incandescent lamp for 15min and thereafter the optical density was measured at 530nm. The percentage inhibition of superoxide production was evaluated by comparing the absorbance of control and experimental tubes. Curcumin was used as the standard reference compound.

2.1.7.4. Assessment of DPPH radical scavenging activity
DPPH radical scavenging activity was measured by the spectrophotometric method described by Sreejayan et al. (1996). To a methanolic solution of DPPH (200µM), 0.05 ml of different concentrations of CP was dissolved in ethanol and added at different concentrations (10-500µg/ml). An equal amount of ethanol was added to the control. After 20 min, the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula given below.

\[
\text{Inhibition \%} = \frac{(\text{control-test}) \times 100}{\text{Control}}
\]

2.1.8. Behavioral and toxic effects
Five groups of 10 mice were administered P.O., 250, 500, 1000, 2000 and 4000 mg/kg of CP. They were observed continuously for 1 h for any gross behavioural changes like drowsiness, restlessness, writhing, convulsion, piloerection, symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again 24 h after dosing with CP.

2.1.9. Statistical analysis
The results were expressed as mean ± standard deviation of mean (SD). Analysis of variance (ANOVA) was done to compare and analyse the data followed by Duncan's multiple range test. Effects were considered significant at P ≤ 0.01 as per the method of Raghava (1987).

2.2. Results
Phytochemical screening of CP gave positive test for the presence of alkaloids, saponins, steroids and terpinoids. Co-HPTLC of CP with authentic tetrandrine confirmed the presence of tetrandrine in C. peltata (Fig. 1).

APAP treatment produced severe liver damage as indicated by a marked increase in AST, ALT, ALP, SB and cholesterol values of the toxin group and also the histopathological alterations when compared to the normal control. Pretreatment with CP (250, 500 mg/kg) produced significant reduction of these values which was almost comparable to the standard drug silymarin (100 mg/kg) and normal control (Table 1). Liver MDA level, a marker of lipid peroxidation increased and GSH, key antioxidant was lowered after APAP intoxication. Drug treatment with CP significantly
decreased the liver MDA levels and increased the liver GSH levels in APAP treated rats in a dose dependent manner (Table 2). Silymarin (100 mg/kg) pretreatment also showed significant reduction in MDA levels and increase in GSH levels. Histopathological studies of the normal group showed normal liver architecture with distinct hepatic cells, sinusoidal space and central vein. In the liver sections of rats intoxicated with APAP, degeneration of normal hepatic cells with intense centrilobular necrosis, broad infiltration of lymphocytes and Kupffer cells and loss of cell boundaries were observed. However, administration of CP/Silymarin to the rats intoxicated with APAP produced a marked degree of protection against the toxin-induced liver histological alterations (Figs. 2-7).

Isolated primary hepatocytes showed 90% viability whereas incubation of hepatocytes with 10 mM APAP showed reduction of cell viability upto 36%. The increase in marker enzyme (AST, ALT) levels in culture medium was an indication of hepatocyte damage. Pretreatment of hepatocytes with CP (25, 50, 100 µg/ml) and silymarin (25µg/ml) showed significant decrease in the leakage of these marker enzymes into culture medium and restored the viability of hepatocytes in a dose dependent manner. Pre-treatment with CP (100µg/ml) showed 60% viability and silymarin (25µg/ml) showed 81% protection (Figs. 8-10).

Percentage inhibition of hydroxyl, superoxide and DPPH radical by CP was increased in a dose dependent manner, showing IC50 value of 52±1.63, 53±1.43 and 48±2.25 µg respectively. Curcumin showed better inhibition than CP with IC50 values of 2.89±0.28, 5.89±0.45 and 2.26±0.51 µg respectively (Table 3). In vitro anti-lipid peroxidation studies revealed a significant increase in malondialdehyde levels in FeCl2-AA control group when compared to normal control. CP treatment significantly reduced MDA levels in rat liver microsomes, at 100 and 200 µg/ml concentrations (Table 4).

In the toxicity study, no mortality occurred within 24 h with the 5 doses of CP tested. The LD50 value was therefore greater than 4000 mg/kg P.O., in mice (Data not shown).

3. Discussion

The burden of metabolism, exposure to various drugs, toxic chemicals and viral infections make the liver vulnerable to a variety of disorders such as acute or chronic inflammatory conditions like hepatitis and cirrhosis. Factors determining toxicity include the pharmacokinetics and metabolic fate of the compound and the ability of target organ to respond to the toxic insult. A few drugs exhibit curative effects in liver disorders but their adverse side effects cannot be ignored especially in chronic disease conditions where long term drug treatment is needed. In traditional medicine there are a number of herbal drugs that have been claimed to have curative effects on liver disorders without any side effects. The study of traditional drugs against liver diseases has resulted in the isolation of a number of active principles which were subjected to pharmacological, biochemical and clinical evaluation. In this process safety remains the most important starting point and the efficacy becomes a matter of validation. Zimmermann et al. (2007) have suggested that drug discovery need not be confined to discovery of a single molecule but multi-target approaches should be in main stream with renewed interest in multi ingredient synergistic formulations. Herbal extracts can deal with multiple targets simultaneously and may give synergistic effect due to their diversity of structures.
Acetaminophen (Paracetamol) is one of the most commonly used non-narcotic analgesic, antipyretic agents which is metabolised in the liver, being conjugated to give the glucuronide or sulfate. According to Pable et al. (1992) APAP is bioactivated to a toxic electrophile N-acetyl-p-benzoquinoneimine (NAPQI) which binds covalently to tissue macromolecules and probably oxidizes lipids or critical sulphydryl groups (protein thiols) and alters the homeostasis of calcium. If this free radical NAPQI is not inactivated by conjugation with glutathione it reacts with cell proteins and kills the liver cells which cause liver damage. In such higher oxidative stress, drug that can increase antioxidant defense system like glutathione can prevent liver damage. Zimmerman and Seeff (1970) has reported that due to liver injury the transport function of the hepatocytes get disturbed resulting in the leakage of plasma membrane which causes an increased enzyme level in the serum.

In the present study, the hepatic damage produced by APAP was evident by increased levels of enzymes (AST/ ALT/ ALP), bilirubin and cholesterol in serum, decreased tissue GSH, increased tissue MDA levels and histopathological changes. Treatment with CP offered significant protection against APAP-induced hepatic damage which was evidenced by the results of biochemical and histopathological parameters investigated. Moreover, Shine et al. (2014) has reported earlier the significant hepatoprotective effect of C. peltata alkaloids by similar anti-hepatotoxic study.

Liver is the organ capable of natural regeneration of lost tissue and even 25% of the liver cells can regenerate into whole liver. This is predominantly due to the hepatocytes re-entering the cell cycle (from the quiescent G0 phase to the G1 phase and undergo mitosis). The in vitro primary liver system represents a better experimental approach to screen for potential hepatoprotective herbal drugs. Hepatocyte structural and functional integrity can be characterized in short-term primary monolayer cultures. APAP induced oxidative stress causes cell necrosis in cultured hepatocytes. According to the earlier reports of Bajt et al. (2004) the pathophysiology of APAP toxicity in hepatocyte culture was evident by increase in cellular and mitochondrial glutathione disulfide (GSSG) levels and the GSSG:GSH (Glutathione) ratio after APAP overdose. This results in increased level of free radicals which is correlated to decreased cell viability. In the present in vitro primary hepatocyte culture study, oxidative stress induced by APAP was reverted by CP as indicated by increase in cell viability and decrease in marker enzyme levels in the media. This shows that CP protects liver by stabilizing the cell membrane and inhibits the toxin induced free radical damage through its well proven antioxidant effect.

Reactive oxygen species including superoxide (O2•−), hydrogen peroxide (H2O2), hydroxyl (OH•), nitric oxide (NO) exerted oxidative stress in human body. When increased oxidative stress become higher than antioxidant defense of the cells, ROS starts attacking the cell proteins, lipids and carbohydrates and this leads to development of degenerative diseases. Antioxidants may prevent the formation of ROS or oxidative stress by interrupting ROS attack, scavenging the reactive metabolites and converting them to less reactive molecules. The antioxidants are used to treat diseases in which oxidative stress plays a major aetiological role.

In the present study, it was observed that CP significantly inhibited the liver MDA levels and increased the liver GSH levels in APAP treated rats. This may be due to the inhibition/scavenging of the free radicals formed during APAP metabolism. According to Recknagel and Lombardi (1961) in vivo system GSH plays a pivotal role in mitochondrial antioxidant defense. Yuan et al. (2007) has reported that GSH depletion increases the sensitivity of the hepatic tissue to free radical-mediated
damage caused by xenobiotic metabolism. In the present study, CP administration improved the glutathione status of the liver and consequently protected it from toxin induced damage.

Earlier reports of Chun et al. (1998) states that FeCl₂ - AA mixture is known to stimulate lipid peroxidation in rat liver microsomes and mitochondria in vitro. Decomposition of lipid membrane in the body leads to the formation of malondialdehyde (MDA) and other aldehydes. MDA reacts with thiobarbituric acid (TBA) to form pink coloured complex called thiobarbituric acid reactive substance (TBARS) and TBA-MDA is selectively detected at 532 nm. Measurement of MDA in biological fluid has been extensively used as the marker of oxidative stress and lipid peroxidation. Most of the hepatotoxic chemicals damage the liver mainly by inducing lipid peroxidation directly or indirectly. Chang et al. (1994) discovered that in higher animals, lipid peroxidation is known to cause destabilization of the cell membrane leading to liver injury. In the present study, it was observed that CP effectively reduced MDA the end product of lipid peroxidation in rat liver microsomes in vitro.

The effect of CP on the inhibition of free radical mediated deoxyribose damage was assessed by means of iron dependent DNA damage assay. The Fenton reaction generate OH- radical which degrades deoxyribose sugar of DNA using Fe²⁺ salt as an important catalytic component. The present study revealed the potent hydroxyl radical scavenging effect of CP.

Gulcin et al. (2010) has reported that in cellular oxidation reaction superoxide radicals are normally formed first and they produce other free radicals. Endogenously superoxide anion radicals are produced by flavoenzymes (xanthine oxidase) which are converted to hypoxanthine and subsequently to uric acid. In the present (Phenazinemethosulphate-NADH-Nitrobluetetrazolium) PMS-NADH-NBT in vitro system, superoxide anion is derived from dissolved oxygen by PMS-NADH coupling reaction and this reduces NBT (yellow dye) to blue coloured product called formazan. The superoxide scavenging drugs decreases the reduction of NBT. CP treatment significantly scavenged the superoxide in the PMS-NADH-NBT in vitro system.

DPPH showed maximum absorption at 517 nm. In the presence of free radical scavenging compounds the deep violet colour vanished. The resultant bleaching of DPPH absorption represented the capacity of CP to scavenge free radicals independently.

Phytochemical studies by Rastogi and Mehrotra (1999); Reddi et al. (2005) showed that C. peltata roots contain alkaloids like fangchinoline, d-tetrandrine, dl-tetrandrine, d-isochondrodendrine, cycleapeltine, cycleadrine, cycleacurine and cycleanorine. Earlier report by Shine et al. (2009) indicates that Indian sample of root of C. peltata yielded tetrandrine as the major alkaloid. In the present study the presence of tetrandrine in CP was confirmed using HPTLC analysis.

Tetrandrine, the bis-benzylisoquinoline alkaloid is well known to possess activities including antioxidant, anti-fibrotic and anticancer as per the reports of Ng et al. (2006). According to Johnson (1998) tetrandrine also shows analgesic, antipyretic and immunomodulatory properties and useful in treating chronic inflammatory disorder. Huang (1999) found that tetrandrine could inhibit Ca²⁺ transmembrane movement and Recknagel (1983) has reported that lowering the calcium metabolism can reduce the pathological consequences of the attack by toxic metabolites.
Fernandes et al. (2006) reported that low concentrations of tetrandrine afford protection against liver mitochondria injury promoted by oxidative-stress events such as hydrogen peroxide production, lipid peroxidation, etc. Perhaps the alkaloid tetrandrine found in CP may be acting by a similar mechanism via inhibiting Ca²⁺ transmembrane movement, inhibiting inflammatory response and lipid peroxidation. In this scenario it is worthwhile to note that silymarin, used as a natural remedy for liver diseases contain active constituent silybin which slow down calcium metabolism, scavenge free radicals and inhibit lipid peroxidation according to the previous reports by Flora et al. (1998). The toxicity studies have also revealed the non-toxic nature of CP in mice. This is not surprising as C. peltata is extensively being used in medicinal practice of the Kurichiya tribe of Kerala. However, future studies are warranted in our laboratory to decipher the exact mechanism of action of CP in liver protection.

4. Conclusion

The results of the present study demonstrate the potent hepatoprotective effect of CP against in vivo and in vitro acetaminophen-induced oxidative liver injury which substantiates the ethnomedicinal claims of Cyclea peltata in the treatment of liver disorders and makes it an ideal plant for developing herbal drug to treat liver diseases devoid of side effects.

Table 1 Effect of ethanolic extract of root of Cyclea peltata (CP) and silymarin on serum biochemical parameters in Acetaminophen (APAP) - induced hepatic damage in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/I)</th>
<th>ALT (IU/I)</th>
<th>ALP (KA units)</th>
<th>Cholesterol (mg/dl)</th>
<th>S.B (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>37.50±7.30a</td>
<td>44.90±1.60b</td>
<td>71.40±2.20b</td>
<td>40.00±1.01b</td>
<td>0.25±0.04b</td>
</tr>
<tr>
<td>APAP Control</td>
<td>118.66±2.40c</td>
<td>263.20±1.80e</td>
<td>120.00±1.10e</td>
<td>60.00±.31e</td>
<td>0.95±0.02d</td>
</tr>
<tr>
<td>APAP+ CP (250 mg/kg)</td>
<td>72.33±1.70d</td>
<td>71.92±1.50d</td>
<td>85.00±2.30d</td>
<td>50.00±1.20d</td>
<td>0.42±0.03c</td>
</tr>
<tr>
<td>APAP+ CP (500 mg/kg)</td>
<td>62.84±2.60c</td>
<td>55.88±2.10c</td>
<td>80.00±2.00c</td>
<td>42.00±1.12c</td>
<td>0.30±0.01b</td>
</tr>
<tr>
<td>APAP+ Silymarin (100 mg/kg)</td>
<td>44.57±2.40b</td>
<td>50.22±2.30b</td>
<td>75.00±1.90b</td>
<td>35.00±1.32b</td>
<td>0.26±0.02a</td>
</tr>
</tbody>
</table>

Values are the mean±S.D., n=6 Analysis of variance (ANOVA) followed by Duncan's multiple range test, means bearing different superscripts differ significantly (Ps 0.01)
Table 2  Effect of ethanolic extract of root of *Cyclea peltata* (CP) and silymarin on liver MDA and GSH levels in Acetaminophen (APAP) intoxicated Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (n mol/mg wet liver)</th>
<th>GSH (n mol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.524±0.040&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.623±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>APAP Control</td>
<td>0.832±0.050&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.287±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>APAP+ CP (250 mg/kg)</td>
<td>0.400±0.040&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.515±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>APAP+ CP (500 mg/kg)</td>
<td>0.314±0.050&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.618±0.003&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>APAP+ Silymarin (100 mg/kg)</td>
<td>0.300±0.060&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.826±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean±S.D., n=6 Analysis of variance (ANOVA) followed by Duncan's multiple range test, means bearing different superscripts differ significantly (P≤0.01)

Table 3  Inhibitory effect of ethanolic extract of root of *Cyclea peltata* (CP) on FeCl<sub>2</sub>-ascorbic acid-induced lipid peroxidation in rat liver homogenate *in vitro*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CP (µg/ml)</th>
<th>Malondialdehyde (MDA) (n mol/g wet liver)</th>
<th>MDA Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>1.35±0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;-AA</td>
<td>-</td>
<td>2.45±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;-AA+ CP</td>
<td>50</td>
<td>2.15±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;-AA+ CP</td>
<td>100</td>
<td>1.16±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;-AA+ CP</td>
<td>200</td>
<td>0.86±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean±S.D., n=6 Analysis of variance (ANOVA) followed by Duncan’s multiple range test, means bearing different superscripts differ significantly (P≤ 0.01)
Table 4 *In vitro* free radical (hydroxyl, superoxide and DPPH) scavenging effect of CP and curcumin

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration of 50% Inhibition of free radical (μg)</th>
<th>Hydroxyl</th>
<th>Superoxide</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. peltata</em> (CP)</td>
<td></td>
<td>52±1.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53±1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48±2.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin</td>
<td></td>
<td>2.89±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.89±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.26±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean±SD, n=6, Analysis of variance (ANOVA) followed by Duncan’s multiple range test, means bearing different superscripts differ significantly (P≤0.01)

Fig 1. Estimation of tetrandrine using HPTLC. **Track:- 4 Cyclea peltata** root ethanolic extract (CP). **Track:- 1-3 & 5-7:** (3 tracks before and 3 tracks after CP) different concentrations of Tetrandrine
Fig 2. Liver section of normal control rats showing hepatic cells with well defined nuclei and cytoplasm. Fig 3. Section of APAP treated rat liver showing marked necrosis, extensive vacuolation, loss of cell boundaries and disappearance of nuclei. Fig 4. Section of APAP treated rat liver showing vacuolation, broad infiltration of lymphocytes and Kupffer cells. Fig 5. Section of CP (250 mg/kg)+APAP treated rat liver showing marked improvement over APAP control. Fig 6. Section of CP (500 mg/kg)+APAP treated rat liver showing marked improvement over APAP control almost normal hepatic architecture. Fig 7. Section of silymarin (100 mg/kg)+APAP treated rat liver showing normalcy of hepatic cells (X 350).
Fig 8-10. Effect of ethanolic extract of Cyclea peltata (CP) and Silymarin on APAP induced cytotoxicity in primary rat hepatocyte (Fig. 8) ALT; (Fig. 9) AST and (Fig. 10) Hepatocyte viability. Significant P values ≤0.01, n =6, ANOVA followed by Duncan’s multiple range test.

5. Acknowledgements

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