Short communication

In vitro hepatoprotective activity of Corchorus depressus L. against CCl₄ induced toxicity in HepG2 cell line

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Aim: To evaluate in vitro hepatoprotective activity of ethanolic extract from Corchorus depressus (CDEE) against CCl₄ induced toxicity in HepG2 cell line.

Methods: In vitro cytotoxicity and hepatoprotective potential of CDEE were evaluated using HepG2 cells. Based on the cytotoxicity assay, CDEE (50, 100, 200 μg/ml) was assessed for hepatoprotective potential against CCl₄ induced toxicity by monitoring cell viability, aspartate aminotransferase (AST), alanine aminotransaminase (ALT), lactate dehydrogenase (LDH) leakage, lipid peroxidation (LPO) and glutathione level (GSH).

Results: The results indicated that CCl₄ treatment caused a significant decrease in cell viability. In addition, the toxin treatment initiated lipid peroxidation (LPO), caused leakage of enzymes like transaminase (AST & ALT) and LDH with a significant decrease in GSH levels in HepG2 cells. It was observed that CDEE effectively alleviated the changes induced by CCl₄ in a concentration-dependent manner.

Conclusion: Our study revealed that CDEE has potent cytoprotective effect against CCl₄ induced toxicity in HepG2 cell line and which may be attributed to decrease in CCl₄ induced reactive oxygen species levels and resultant oxidative stress.

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1. Introduction

Liver is a major organ of human that plays a crucial role in elimination and biotransformation of toxic substances. During the detoxification, reactive oxygen species (ROS) are generated within hepatocytes that result in oxidative damage, gross cellular changes and cell death causing hepatotoxicity or liver damage.¹ In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders. Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity.² Corchorus depressus (Linn.) (Family: Tiliaceae) commonly known as ‘bhaufali’ is perennial herb, woody 6–9 inches in length and the plant is regarded as good sand binder in the desert.³ It is distributed in arid and semi-arid regions of India and Pakistan to North and tropical Africa. Medicinal uses of this plant in general weakness, gonorrhea, diabetes, treachery troubles, improved sexual vigor, jaundice have been reported.⁴–⁶ Antipyretic and analgesic activities of a new triterpenic acid isolated from C. depressus have been reported.⁷,⁸ Ali and Ansari reported that application of the combined extract of Emblica officinalis, Lawsonia inermis, Nardostachys jatamansi and C. depressus prepared in the oil of Sesamum indicum diminished the falling of hair and gave them original color.⁹ In a previous study, the methanol extract of C. depressus has already been investigated for its hepatoprotective and in vivo antioxidant effects against carbon tetrachloride induced hepatotoxicity in rats.¹⁰ Chemical investigation of this plant has resulted in the isolation of various triterpenes,¹¹,¹² phenolics and sterols.¹³ However, to the best of our knowledge, the hepatoprotective effect of C. depressus in HepG2 cell line intoxicated with carbon tetrachloride (CCl₄) has not been demonstrated. Hence, this study was intended to investigate the in vitro hepatoprotective effect of ethanolic extract from C. depressus.

2. Materials and methods

2.1. Plant material

C. depressus (Linn.) was collected from Jodhpur district of Rajasthan, in month of August, 2011 and identified from Botanical
Survey of India, Arid Zone Regional Centre, Jodhpur 342 008 and a voucher specimen no. LMC/AP/001 was deposited in the college for future reference.

2.2. Preparation of extract

*C. depressus* (whole plant) was dried under shade at room temperature. After drying, plant was subjected to size reduction to a coarse powder by using dry grinder. The powder was packed into Soxhlet apparatus and defatted with petroleum ether (60–80 °C). The marc was dried and extracted with ethanol at 80 °C for 24 h. *C. depressus* ethanol extract (CDEE) was concentrated to dryness under reduced pressure in a rotary evaporator and stored in airtight containers in refrigerator below 10 °C. The percentage yield of the ethanol extract was found to 7.2% (w/w).

2.3. Cell culture

Human liver hepatoma cells (HepG2) (obtained from National Centre for Cell Sciences, Pune, India) were seeded (1 × 10^5 cells/T25 Flask) and cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% Fetal Bovine serum (FBS) and penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in a humidified atmosphere of 5% CO2 at 37 °C until confluent. Cells were maintained in continuous passage by trypsinization of subconfluent cultures using TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock culture was grown in 25 cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.4. In vitro cytotoxicity assay

The CTC50 (50% cytotoxic concentration) was determined by estimating mitochondrial synthesis using tetrazolium assay.14

2.5. CCl4 induced toxicity in HepG2 cell line

The monolayer HepG2 cell culture was trypsinized and the cell count was adjusted to 1.0 × 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96-well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. The cells were treated with 100 μl of toxicant (medium containing 1% (v/v) CCl4, DMSO (medium containing 0.25% (v/v) DMSO); 1% (v/v) CCl4 + silymarin (200 μg/ml); CDEE (200 μg/ml); 1% (v/v) CCl4 + silymarin (50, 100, 200 μg/ml); 1% (v/v) CCl4 + CDEE (50, 100, 200 μg/ml) for 24 h. Cell viability, AST, ALT, LDH leakage, lipid peroxidation and glutathione assays were performed using standard methods as described in previous section.

2.5.1. Cell viability

Trypan blue exclusion test was carried out according to the method of Wu and Cederbaum with slight modification.15

2.5.2. AST, ALT and LDH leakage

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2.5.3. Lipid peroxidation

Malondialdehyde (MDA), the end product of lipid peroxidation, was measured using a thiobarbituric acid reactive substances (TBARS) assay.16

2.5.4. Glutathione levels

Total glutathione level was measured by DTNB-GSSG reductase recycling assay method. The total glutathione level was determined by the kinetic method from standard curve of GSH along with CDEE/Silymarin.17

2.6. Treatment with CDEE

HepG2 cells were plated in 96-well plates at a concentration of 1 × 10^5 cells/ml using DMEM medium containing 10% FBS incubated for 24 h at 37 °C under 5% CO2 to attain confluency. Cells were treated with 100 μl each of serum free culture medium containing 0.25% (v/v) DMSO; 1% (v/v) CCl4, silymarin (200 μg/ml); CDEE (200 μg/ml); 1% (v/v) CCl4 + silymarin (50, 100, 200 μg/ml); 1% (v/v) CCl4 + CDEE (50, 100, 200 μg/ml) for 24 h. Cell viability, AST, ALT, LDH leakage, lipid peroxidation and glutathione assays were performed using standard methods as described in previous section.

2.7. Statistical analysis

The results were expressed as mean ± S.E.M. The data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett’s test using GraphPad Prism 4.0 Software, San Diego, CA, USA to establish the statistical significance. Values of *P* < 0.05 were considered significant in all cases.

3. Results

3.1. In vitro cytotoxicity assay

CDEE evaluated for its cytotoxic activity by MTT assay. CDEE showed CTC50 value of 573.72 ± 11.83 μg/ml in HepG2 cell line.

3.2. CCl4 induced toxicity in HepG2 cell line

Time-dependent toxic effects of CCl4 in HepG2 cells were shown in Fig. 1. As compared to vehicle control, no significant change in cell viability, AST, ALT, LDH levels and lipid peroxidation as well as no significant change in GSH level was observed until an exposure period of 3 h, thus overruling any direct solvent mediated damage by CCl4 in HepG2 cells. There after a time-dependent significant (*p* < 0.01) increase in leakage of AST, ALT, LDH and significant loss of cell viability was observed compared to vehicle control. Similarly, a significant (*p* < 0.01) increase in lipid peroxidation with concurrent significant decrease in glutathione was noted compared to vehicle control.

3.3. Cytoprotective effect of CDEE in HepG2 cells

Table 1 depicts the results of cell viability, leakage parameters-AST, ALT, LDH, MDA and GSH levels in all experimental groups. A significant (*p* < 0.01) decrease in viability of cells and a significant (*p* < 0.01) increase in the levels of AST, ALT, LDH was observed in the HepG2 cells exposed to CCl4 as compared with Group 1 (normal control). These cells, when treated with CDEE (100 and 200 μg/ml) showed a significant restoration of the altered biochemical parameters towards the normal compared to CCl4 treated group and is dose dependent.

4. Discussion

The present study reveals the hepatoprotective effect of CDEE against CCl4 induced toxicity in HepG2 cells. In recent times *in vitro* cytotoxicity and hepatoprotective activity of plant extract and
bioassay guided fractions has gained importance for primary level screening. HepG2 cell line is a popular and an effective in vitro model for assessing hepatoprotective potential of phyto compounds and bioassay guided fractions due to its functional similarity to an intact liver. CCl4 is a hepatotoxic haloalkane whose mechanism has been studied intensively over the past years. It is one of the best studied solvent in regards to liver toxicity. It is well known that CCl4 undergoes metabolic activation by a cytochrome P-450 dependent step to free radical products which can initiate lipid peroxidation. The toxicity induced by CCl4 in vivo and in cultured hepatocytes, involves the stimulation of lipid peroxidation, detected as an increase in malondialdehyde (MDA) formation. Our results in conjunction with others reports, proved that CCl4 caused a time-dependent production of ROS and subsequent lipid peroxidation in HepG2 cells which was found to be maximum after an incubation period of 24 h. Therefore, HepG2 cells were incubated with CCl4 for 24 h to study the protective effects of CDEE against CCl4 induced toxicity. The loss of cell viability was measured as the end product of toxicity by means of trypan blue assay. The extent of cellular damage was measured in terms of release of leakage enzymes-AST, ALT and LDH. Increased release of these intracellular enzymes was observed at 24 h exposure to CCl4. This indicated membrane damage and instability owing to oxidative injury created by the hepatotoxin. Likewise, toxin treatment caused significant increase in MDA levels, with a concurrent decrease in glutathione content in HepG2 cells. These cells, when

Fig. 1. Time-dependent changes observed in (A) Cell viability (B) AST levels (C) ALT levels (D) LDH leakage (E) lipid peroxidation (MDA) and (F) GSH level after exposure to 1.0% (v/v) of CCl4 in HepG2 cells. Group 1 – Control; Group 2 – DMSO control 0.25% (v/v); Group 3 – 1.0% (v/v) CCl4 in 0.25% DMSO. Results are expressed as mean ± SEM (n = 6). Level of significance p < 0.05. *Compared to vehicle control.
treated with different concentrations (50, 100 and 200/ml) of CDEE showed a significant restoration of the altered biochemical parameters towards the normal compared to CCl4 treated group. The overall protective strategy followed evaluated the plausible protective nature of CDEE against CCl4 induced toxicity in HepG2 cell line. The mechanism involved was ascertained by selecting relevant parameters like leakage of enzymes, quantification of lipid peroxidation and measurement of intracellular glutathione levels. The possible underlying mechanism for the hepatoprotective effect of CDEE is because of its ability to inhibit lipid peroxidation and maintenance of glutathione in reduced state by virtue of its antioxidant powers. Several natural plants/plant products have been established as hepatoprotective against CCl4 induced toxicity in HepG2 cells. Moreover phytochemical analysis of CDEE has shown the presence of flavonoids and phenolic compounds9,9 which have been known for their antioxidant and hepato-protective activity.34–26 Hence, the hepatoprotective effect observed in the present study mainly due to the presence of any of these compounds present in C. depressus. However, further studies on the active compounds and their biochemical mechanisms responsible for the hepatoprotective effect of C. depressus will be necessary.

5. Conclusion

Overall, it could be concluded that CDEE protects against oxidative injury induced by CCl4 in vitro and it is also capable of enhancing the activities of hepatic enzymes implicated in combating ROS however further investigation should be carried out on the extract to identify the active constituents responsible for hepatoprotection.

Conflicts of interest
All authors have none to declare.

References