ABSTRACT

Objective: The aim of this study was to investigate the in-vitro hepatoprotective activity of solvent extracts of *Albizia lebbeck*, *Cassia occidentalis*, and *Swertia chirata* on HepG2 cell line.

Methods: The methanolic, ethanolic, and acetone seed extracts of *A. lebbeck*, *C. occidentalis*, and leaves extract of *S. chirata* were used in this study. The different extracts of *A. lebbeck*, *C. occidentalis*, and *S. chirata* were assessed for their hepatoprotective activity on human liver hepatocellular carcinoma (HepG2) cell line against paracetamol (PCM) as a liver damage inducing agent. The cell line viability was assessed by 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide assay.

Results: The percentage cell viability was determined with respect to the normal control cells. Control cells showed 100% cell viability in all tested plant extracts. The PCM treated HepG2 cells showed a maximum cell viability (46.6±2.49%) in presence of all seed extracts of *A. lebbeck*. The silymarin and PCM treated HepG2 cells showed maximum cell viability (156.6±2.49%) in presence of leaves extract of *S. chirata*. The maximum cells viability of 131.6±9.39% was observed in methanolic seed extract of *A. lebbeck* (50 µg/mL), and the minimum cell viability of 107.3±3.68% was observed in acetone seed extract of *C. occidentalis* (50 µg/mL) comparatively.

Conclusions: The methanolic, ethanolic, and acetone extracts of seeds/leaves from *A. lebbeck, C. occidentalis*, and *S. chirata* were showed the hepatoprotective activity. Further in vivo and clinical studies are required to confirm their therapeutic efficacy.

Keywords: *Albizia lebbeck*, *Cassia occidentalis*, *Swertia chirata*, Paracetamol, HepG2, 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide assay.

INTRODUCTION

Herbal medicines have the ability to affect body systems. The effects are dependent on the chemical constituents present in the plant used. Scientists first started extracting and isolating chemicals from plants in the 18th century and since that time, we have grown a custom of looking at herbs and their effects in terms of the active constituents they contain. *Albizia lebbeck* (Shirish, *Leguminosae*) is a deciduous tree with compound leaves, flat an oblong fruit, round colored seeds, and grows wild. The plant is found throughout India, Bangladesh, tropical and subtropical Asia, and Africa [1,2]. Therapeutically bark is used in bronchitis; bark and seeds in piles; root in hemicranias; flowers in a cough, bronchitis, tropical pulmonary eosinophilia, and asthma [3]. The seeds contain e-chinocystic acid and β-sitotosterol quercetin. It has been reported that *A. lebbeck* has antibacterial, analgesic, anti-inflammatory, antioxidant, anti-inflammatory, and anti-anaphylactic, nootropic, anxiolytic, and anticonvulsant activities [4]. *Swertia chirata*, Indian name: *Chirayata* (*Gentianaceae*) is a robust annual herb which grows up to about 1.5 meters in height. It has leaves in opposite pair about 10 cm long, without stalks, pointed at the tip. The plant has numerous flowers, pale green in color, tinged with purple, with long white or pink hairs and minute sharp pointed fruits. The whole plant, collected in its flowering stage and dried, constitutes the drug. It is found in the Himalayan ranges of India from Kashmir to Bhutan at an altitude of 1200-3000 m. It is also found in the Khasi Hills of Meghalaya at an altitude of 1200-1500 m [5]. It has long been used by the Ayurvedic physicians as a bitter tonic. The plant contains a bitter glycoside chiratin, which yields on hydrolysis, two bitter principles, ophelic acid and chiratin. The ophelic acid is a brown hydrosopic substance which is soluble in water and alcohol. Chirata is an effective drug for reducing fever [6]. It has been widely used as herbal medicine in Asian countries particularly in India, Nepal, Myanmar, Arab and some parts of the European countries. Reported studies showed extracts of this plant has attributable properties as hypoglycemic, antipyretic, anti-inflammatory, antibacterial, antiviral, antimalarial, antithelotropic, and wound healing activity [7]. *Cassia occidentalis* belongs to the genus *Cassia* and the Family *Caesalpinioaceae*. It is called Stinking Weed. In Hausa, it is known as "Rai dore." In Igbo, it is called "Osiisi" while "Gaya" in Nupe [8]. It is an erect herb, commonly found by road sides, ditches and waste dumping sites. *C. occidentalis* has many applications in traditional medicine. All the parts of the plant have medicinal uses. Traditionally, its roots, leaves, flowers and seeds are used as laxatives and purgative. Phytochemically, the aqueous extract of *C. occidentalis* contains tannins, anthraquinone, sterol, cardiac glycosides, saponin, and alkaloids. The World Health Organization estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Medicinal plants play an important role in the discovery of novel drugs used in modern medicine [9]. Traditional medicines have been used for many centuries by a substantial proportion of the population of India. India has a rich heritage of traditional knowledge and is home to several important time-honored systems of health care like Ayurveda, Siddha, and Unani. It has been estimated that the proportion of medicinal plants in India (7,500 of the 17,000 higher plant species are medicinal plants) is higher than any country of the world with respect to the existing flora of that respective country [10]. There are a number of studies which highlight the uses of herbal drugs using the plant extracts of *S. chirata*, *Albizia*, and *Andrographis paniculata* to name a few in hepatoprotection [11,12]. These observations have drawn attention for the present study which is an attempt to explore the in-vitro hepatoprotective activity of seeds of *A. lebbeck* and *C. occidentalis* and leaves of *S. chirata* on human liver hepatocellular carcinoma cells.
METHODS
Collection and preparation of the plants extracts
*A. lebbeck* and *C. occidentalis* were collected from the area of Nakronda forest range, (30°41′19.60″N, 78°07′01.70″E), Dehradun, and *S. chirata* was collected from Chobattakhil (29°57′16.40″N, 78°52′41.20″E), Pauri Garhwal, Uttarakhand region of North India. The abovementioned plants were identified, authenticated and authenticated. The seeds of *A. lebbeck*, *C. occidentalis*, and leaves of *S. chirata* were air dried and pulverized into powder. About 30 g of the powdered sample of each medicinal plant were weighed and extracted in 100 mL each of methanol (ME), ethanol (ET) and acetone (AC) extract in a soxhlet apparatus separately and the process is carried out for 7 days at 40-45°C. The filtrate was evaporated to dryness at 40°C in a rotary evaporator. The above process was repeated for several times until the sufficient amount of extract is produced. The concentrated extract of each plant was stored at 4°C until when required for use.

Chemicals
3-(4,5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco’s Modified Eagles Medium (DMEM), trypsin, ethylenediaminetetraacetic acid (EDTA), glucose, dimethyl sulfoxide, and hydrogen peroxide were obtained from Hi-Media Laboratories Ltd., Mumbai.

HepG2 cell line
Human liver hepatocellular carcinoma (HepG2) cell lines were obtained from the National Centre for Cell Science (NCCS) Pune, India. The cells were regularly maintained in DMEM containing 10% FBS in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India). The cells were seeded at a density of 1×10⁵ cells/mL. The culture medium was changed twice a week.

MTT assay
The cells were seeded at density of 1.0×10⁵ cells/mL in 96-well flat bottomed plates, and incubated at 37°C in a humidified incubator with 5% CO₂. After 24 hrs, when partial monolayer was formed, the supernatant was flicked off and the monolayer was washed once. The final volume of all the treated and the control wells was kept same, i.e., 500 µL. Paracetamol (PCM) (1%), silymarin, (100 µg/mL), different concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL) of methanolic, ethanolic and acetone extract of *A. lebbeck*, *C. occidentalis* and *S. chirata* were added to specified wells. The silymarin and PCM treated HepG2 cells showed 156.3±1.69% cell viability in all extracts of *A. lebbeck*; 154±1.63% cell viability in all extracts of *C. occidentalis* and 56±1.24% cell viability in all extracts of *S. chirata* (Fig. 3). The methanolic seed extract of *A. lebbeck* at concentration 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL showed 140.6±1.69%, 154.3±5.90%, 105.3±2.49%, 87.6±4.02% cell viability, respectively. The ethanolic seed extract of *A. lebbeck* at concentration 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL showed 134±3.74%, 147.5±3.85%, 100±4.32%, 79.3±1.88% cell viability, respectively. The acetone seed extract of *A. lebbeck* at concentration 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL showed 136±3.36%, 136±7.71%, 94±5.09%, 79.6±1.88% cell viability, respectively. Out of all, 50 µg/mL concentration was selected for checking its protective efficacy in presence of PCM. 50 µg/mL methanolic extract (ME) and PCM showed 131.6±3.93% cell protection, 50 µg/mL ethanolic extract (ET) and PCM showed 120.3±7.03% cell protection and 50 µg/mL AC and PCM showed 115.3±8.77% cell protection (Figs. 4a and 5).

RESULTS
The percentage cell viability with respect to the normal control (NC) cell lines (HepG2) at different concentrations of methanolic, ethanolic and acetone seed extracts of *A. lebbeck*, *C. occidentalis* and leaves of *S. chirata* were determined. The NC cells showed 100±0.02% cell viability in all tested plant samples (Fig. 1). The PCM treated HepG2 cells showed 46.6±2.49% cell viability in all extracts of *A. lebbeck*; 44.3±1.69% cell viability in all extracts of *C. occidentalis* and 46.3±1.24% cell viability in all extracts of *S. chirata* (Fig. 2). The silymarin and PCM treated HepG2 cells showed 156.3±1.69% cell viability in all extracts of *A. lebbeck*; 154±1.63% cell viability in all extracts of *C. occidentalis* and 56±1.24% cell viability in all extracts of *S. chirata*. The results of hepatoprotective activity were expressed as the mean±SEM. A statistical analysis was performed with one-way analysis of variance followed by Tukey’s multiple comparison tests using Graph Pad Instat Software. p<0.05 was considered to be statistically significant (p<0.05).
100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL showed 119±7.87%, 134.6±8.9%, 90.3±10.3%, 71.3±7.71% cell viability, respectively. Out of all, 50 µg/mL concentration was selected for checking its protective efficacy in presence of PCM. 50 µg/mL ME and PCM showed 120±2.44% cell protection, 50 µg/mL ET and PCM showed 117±1.63% cell protection and 50 µg/mL AC and PCM showed 110±1.63% cell protection (Figs. 4b and 6).

The methanolic leave extract of S. chirata at concentration 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL showed 134.6±6.2%, 149.6±7.03%, 106.6±9.56%, 85.3±6.18% cell viability, respectively. The ethanolic leaves extract of S. chirata at concentrations 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL showed 120.6±8.05%, 144.3±3.09%, 108.6±10.6%, 71±5.71% cell viability, respectively. The acetone leaves extract of S. chirata at concentrations

**DISCUSSION**

Medicinal plants are crucial for about 80% of the world population in developing as well as the developed nations for their primary and basic health care needs owing to better tolerability, superior compatibility with human body and having lesser side effects. The herbal drugs are rapidly becoming popular due to the study of the extensive activity of different plant components and their therapeutic principles [14]. Among the plant species, A. lebbeck, C. occidentalis and S. chirata also seem to possess numerous pharmacological properties. Bioactive compounds such as saponins, alkaloids, flavonoids, and phenolic compounds are reported to be highly active against cytotoxicity in tumor cancer cells. They are widely used as anti-asthma, anti-septic,
and leaves 

107.3±3.68

against PCM induced cytotoxicity. Incubating 

100±0.02 

and 

110±1.63 .

showed the most 

in animal model. Pak J Pharm Sci  

100±0.02 

46.3±1.24 

154±1.63 

Percentage cell viability 

Linn. Int J Compr Pharm  

100±0.02 

46.6±2.49 

154±1.63 

120±2.44 

113.6±3.29 

44.3±1.69 

for the same time interval resulted in a dose-

ionic strength, pH and osmolarity which will impact the cell viability 

will significantly change thephysiochemical environment in terms of 

vivo

meaningful as it is unlikely that such concentrations will be reached 

and due to the probability that high concentrations of plant extracts 

involved in cell death. Indeed, the very nature of herbal extracts means 

extracts act at a common point involved in inhibiting distinct pathways 

present study, and it can only be speculated whether any or all of these 

addition to flavonoids [23]. Elucidating the exact cellular mechanism of 

action of these plant extracts on hepatic cells is outside the scope of the 

clinical studies are also required to confirm its therapeutic efficacy. 

CONCLUSION

ME of A. lebbeck, C. occidentalis and S. chirata showed the most 

efficient hepatoprotective activity against PCM on HepG2 cell lines 

with silymarin as control compared to ethanolic and AC of A. lebbeck, 

C. occidentalis and S. chirata, respectively. The above study indicates 

positive hepatoprotective activity of the above mentioned plants. 

Characterization and identification of active principles in ME of 

A. lebbeck are further required to evaluate its potential as a potent 

hepatoprotective agent for liver drug formulation. Further in-vivo and 

clinical studies are also required to confirm its therapeutic efficacy.

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