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IN VITRO HEPATOPROTECTIVE ACTIVITY OF CHLOROFORM EXTRACT FROM AERIAL PARTS OF *HELICANTHES ELASTICA* (DESR.)DANSER

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ABSTRACT

Many plants possess hepatoprotective activity that exhibits additive or synergistic activity. In this study, a chloroform extract extracted from *Helicanthes elastica* was evaluated for in vitro hepatoprotective activity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against CCl_4 induced toxicity. The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. The study indicates positive hepatoprotective activity of the extract *Helicanthes elastica* (aerial parts) *in vitro* against CCl4 induced hepatotoxicity.

Key Words: MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), hepatoprotective, synergistic.

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INTRODUCTION

Helicanthes elastica (Desr.)Danser (mango mistletoe) is one of the less explored medicinal mistletoe found on almost every mango tree in India. It was intensively used by the women in Malenad region of Karnataka State to relive the pain during their menstruation. .It can calm a restless foetus or treat uterine bleeding during pregnancy and help prevent miscarriage. Traditionally, the leaves of this plant are used for checking abortion and for removing stones in the kidney and urinary bladder (1).

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotic from the body thereby

Providing protection against foreign substances by detoxifying and eliminating them. Liver cells possess the antioxidant defence system consisting of antioxidants such as GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death.

There are numerous plants and traditional formulations available for the treatment of liver diseases. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the

world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy. These active constituents with antioxidative, antifibrotic, antiviral and other properties may serve as primary compounds for further development as hepatoprotective drugs (2).

MATERIAL AND METHODS

Plant material

The plant *Helicanthes elastica* (Desr) Danser (*Loranthaceae*) growing on mango tree, [*Mangifera indica* (*Anacardiaceae*)] were collected from Kasaragod District. The plant material was taxonomically identified by the botanist Dr.Subrahmanya Prasad, Assistant Professor, Nehru Arts College, Kanhangad.

Extraction procedure

The plant material was cut in small pieces, air dried in shade for one week. The dried aerial parts of *Helicanthes elastica* undergone successive solvent extraction using solvents of increasing polarity viz. petroleum ether, n-hexane, chloroform, acetone, ethanol and water. Around 35grams of the dried powder was weighed, moistened with the respective solvent and packed in the soxhlet extractor and was then extracted with 500 ml each of the petroleum ether, n-hexane, chloroform, acetone, methanol and water. After each extraction the same dried marc was used for the subsequent extraction. Each extract was then filtered, the solvent distilled off and finally the dried extract was obtained (3, 4).

Preliminary phytochemical screening

The preliminary phytochemical screening of all the extracts was carried out by standard procedure (3-5). The results are shown in table-1. Chloroform extract was used for the hepatoprotective study based on the pilot study carried out (6, 7).

Hepatoprotective Activity (8)

Determination by MTT assay

Chang liver (human liver) cell line was maintained in Dulbecco's modified eagles media (HIMEDIA), both purchased from National Centre for Cell Sciences (NCCS), Pune, India (Gibco, Invitrogen). (The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) wasseeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of plant extracts and compound stock

1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. ccl_4 (0.1%) was added to induce toxicity.

Cytotoxicity Evaluation

After attaining sufficient growth, CCl_4 (0.1%) was added to induce toxicity and incubated for one hour, prepared extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable

changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize theformazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm.

The percentage of growth inhibition was calculated using the formula:

$$\textbf{\% of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The extracts were subjected for qualitative chemical analysis for the identification of various phytoconstituents, like alkaloids, glycosides, phenolics, flavanoids, carbohydrates, proteins and amino acids, terpenoids, sterols and saponins etc and the results of the chemical tests for each extract is recorded and tabulated in the following Table-1

Table-1 Results of qualitative phytochemical screening of petroleum ether, n-hexane, chloroform, acetone, ethanol and aqueous extracts of *H.elastica*

S.No	Phytoconstituents	Pet. Ether extract	n-Hexane extract	$CHCl_3$ extract	Acetone extract	Ethanol extract	Aqueous extract
1.	Alkaloids	-	-	-	-	-	-
2.	Glycosides	-	-	++	+	+	+
3.	Phenolics	-	-	++	+	++	+
4.	Flavones& Flavonoids	-	-	+	+	+	+
5.	Carbohydrates	-	-	-	-	+	+
6.	Proteins & Amino acids	-	-	-	-	-	-
7.	Terpenoids	-	-	++	+	+	-
8.	Sterols	+	+	-	-	-	-
9.	Saponins	-	-	-	-	-	+
10.	Gum & mucilages	-	-	-	+	+	+

Note- (++) indicate active constituents in high amount, (+) indicate active constituents in low amount (-) indicates the absence of active constituents.

***In Vitro Hepatoprotective* Effect**

Determination by MTT assay

The *in vitro* hepatoprotective activity of chloroform extracts at various concentrations was determined by MTT assay against CCl_4 induced toxicity. Chloroform extract at increasing concentrations showed much more hepatoprotective effect. The results are shown in table-2 and Fig-1.

Table-2 Result showing *in vitro* hepatoprotective activity of chloroform extract of *H. elastica* by MTT assay

S.No	Sample	Concentration (µg/ml)	Absorbance at 540nm	% cell viability
1	Control	-	1.225	100
2	Carbon tetrachloride	-	0.3374	27.54
3	Chloroform extract	6.25	0.5226	42.66
		12.5	0.6443	52.95
		25	0.7754	63.29
		50	0.9410	76.84
		100	0.9994	81.58

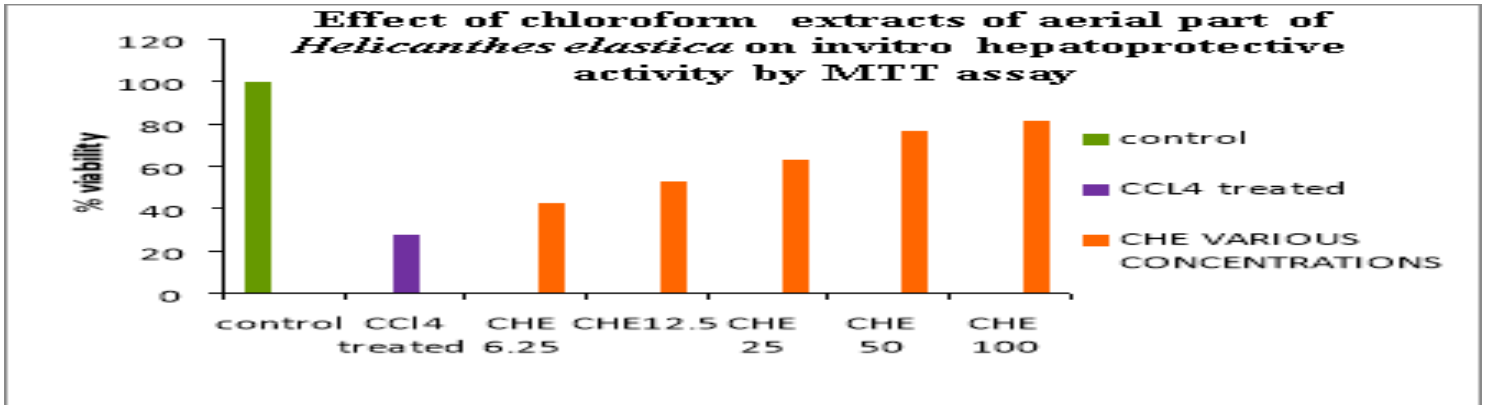
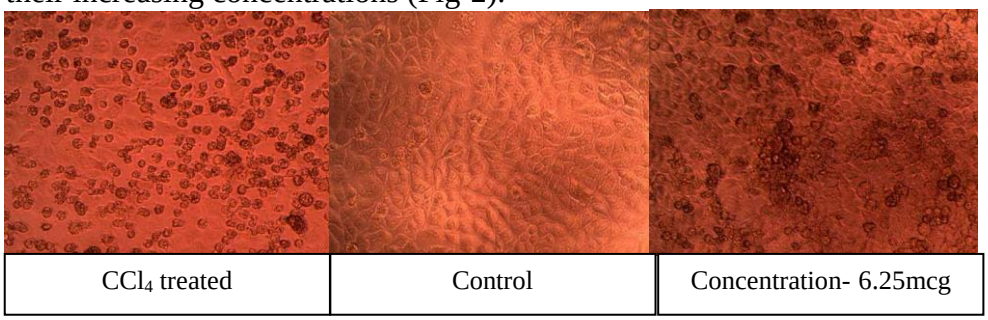
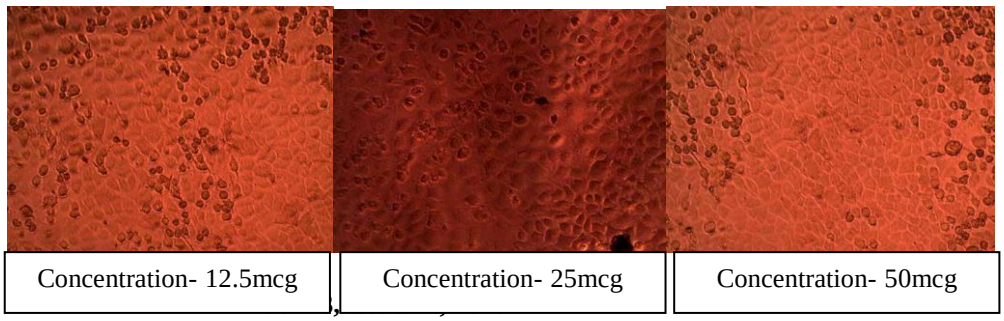


Fig -1 Result showing the percentage cell viability of the chloroform extract of *H.elastica* by MTT assay method.

Morphological Analysis of Chang Liver Cell Lines treated with extracts

All the chang liver cells (six) except one (control) were first undergone CCl_4 treatment, showed hepatotoxicity , Out of which the five cells were treated with the drug extracts of different concentration showed reduction in hepatotoxicity based on their increasing concentrations (Fig-2).





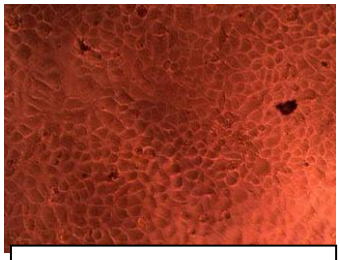


Fig -2 Results showing the difference of cell morphology upon treatment with control, CCl_4 and various concentrations of chloroform extract of aerial parts of *H.elastica*

CONCLUSION

The above study indicates positive hepatoprotective activity of the extract *Helicanthes elastica* (aerial parts) *in vitro* against CCl4 induced hepatotoxicity. Chloroform extract at a concentration of 100mcg showed higher hepatoprotective activity.

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