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In vitro hepatoprotective activity of ethanolic extract of *Coldenia procumbens* Linn

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ABSTRACT

An investigation has been carried out to evaluate the in vitro hepatoprotective effect of ethanolic extract of Coldenia procumbens Linn using antitubercular drugs as toxicant and silymarin as standard drug by MTT assay[(3-(4,5 dimethylthiazole –2 yl)-2,5 diphenyl tetrazolium bromide) assay.]Coldenia procumbens Linn has been widely used for a number of medicinal purposes especially in Siddha medicine. However, the information available on the pharmacological activity of the plant is very limited. Hence, it was proposed to carry out a preliminary in vitro analysis of the hepato protective activity of the plant, which gave promising results. This is the first report of the hepatoprotective activity of the plant.

Key words: *Coldenia procumbens* Linn, Isoniazid, Pyrazinamide, Rifampicin, MTT assay.

INTRODUCTION

Coldenia procumbens Linn (Boraginaceae) is distributed in India, Srilanka and other tropical countries. It is used for medicinal purposes in the codified Indian systems of medicine namely Ayurveda and Siddha .But it has not been explored properly and remains a silent drug in herbal medicine [1].

Liver plays a major role in detoxification. Any injury to it or impairment of its function may lead to many implications on one's health. Managenent of liver diseases is still a challenge to modern

medicine. The allopathic medicine has little to offer for the alleviation of hepatic ailments whereas the most important representatives are of phytoconstituents [2].

Hepatotoxicity is largely associated with the use of antitubercular drugs. In patients using Isoniazid there is an increase in liver aminotransferases and clinical hepatitis with loss of appetite, nausea and vomiting occurs[3]. Rifampicin may cause cholestatic jaundice and hepatitis. Major adverse effect of pyrazinamide is hepatotoxicity. Oxidation stress has been found to be the most important mechanism in hepatotoxicity of antitubercular drugs. So for this study marketed formulations of isoniazid, rifampicin, pyrazinamide were selected. Galactosamine hydrochloride was also used as a hepatotoxicant in this study [4]

The hepatoprotective activity of the extract was tested by MTT assay [(3-(4,5 dimethylthiazole – 2 yl)-2,5 diphenyl tetrazolium bromide) assay]. The principle involved is the cleavage of tetrazolium salt MTT into a blue coloured derivative by living cells which contains mitochondrial enzyme succinate dehydrogenase[5].

EXPERIMENTAL SECTION

Plant source:
Leaves of *Coldenia procumbens* Linn were collected from west Tambaram, Chennai, India and were authenticated by Prof. P. Jayaraman of Plant Anatomy Research Centre (PARC), Chennai. These were freed from earthy material, washed, shade dried and powdered.

Extraction:
One kg of powdered ariel parts of *Coldenia procumbens* Linn was taken and 2500 ml of 95% methanol was added. It was refluxed for 2 hours and filtered through muslin cloth while hot. Filtrate was concentrated, evaporated and standardized.

Preparation of solutions:

Toxicants:
10 mg of antitubercular drugs Isoniazid, Rifampicin and Pyrazinamide (1:2:5) were dissolved in 1 ml DMSO and diluted to 10 ml with minimum essential medium.1000, 500,250 and 125 µg/ml solutions were prepared by diluting with water.

10 mg of D (+)-Galactosamine was dissolved in 1 ml DMSO and diluted to 10 ml with MEM.50, 40 and 20 µg/ml solutions were prepared by diluting with water.
Silymarin at a concentration of 250µg/ml was used as standard

Sample Solution:
10 mg of *Coldenia procumbens* Linn was dissolved in 1 ml of DMSO and diluted to 10 ml with minimum essential medium. 1000,500,250 and 125 µg/ml solutions were prepared by diluting with water.

Cell lines used [5]:

The cell line BRL 3A used for screening hepatoprotective activity of the plant extract was obtained from National Centre for Cell Sciences, Pune, India. The description of the cell line is as follows.

Source : Rat
Strain : Buffalo
Tissue/Organ: Normal,Liver
Morphology: Epithelial

Method[6]:

The monolayer cell culture was trypsinated and the cell count was adjusted to 1.0 x 10^5cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microlitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100µl of different drug concentrations was added to the cells in microtitre plate. The plate was then incubated at 37ºC for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out and the observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50 µl of MTT was added to each well. The plates were gently shaken and incubated for 3 hours at 37ºC in 5% CO_2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilise the formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm.

The percentage growth inhibition and percentage cell protection was calculated using the formula

$$\% \text{ cell protection} = \left(\frac{\text{Mean O.D of individual test group}}{\text{Mean O.D of control group}} \right) \text{x100}$$

$$\% \text{ growth inhibition} = 100 - \left(\frac{\text{Mean O.D of individual test group}}{\text{Mean O.D of control group}} \right) \text{x100}$$

RESULTS AND DISCUSSION

The tetrazolium salt (3-(4, 5 dimethylthiazole –2 yl)-2,5 diphenyl tetrazolium bromide) is taken up into the cells and reduced in a mitochondria dependent reaction to yield a blue coloured formazan product. The product accumulates within the cell, due to the fact that it cannot pass through the plasma membrane. On solubilisation of the cells, the product is librated and can be readily detected and quantified by a simple colorimeric method.

The ability of cells to reduce MTT provides an indication of mitochondrial integrity and activity which in turn may be interpreted as a measure of viability and /or cell number. The assay has therefore been adopted for use with cultures of exponentially growing cells. Determination of their ability to reduce MTT to the formazan derivative after exposure to test compounds

compared to the control situation, enables the relative protection of test chemicals to be assessed. (Table 1 and 2)

Dose-response curves Figure 1&2 were calculated for the toxicants over a range of concentrations, enabling CTC_{50} to be calculated. (i.e. concentrations of hepatotoxicant required to reduce cell viability)This concentration of hepatotoxicants was used to test the protective effect of *Coldenia procumbens* Linn.

Since approximately 50 % inhibition was achieved with 500 µg/ml, CTC_{50} was taken as 500µg/ml for anti tubercular drugs on BRL 3-A cell lines. (Fig.1)

Since approximately 50 % inhibition was achieved with 40 µg/ml, CTC_{50} was taken as 40 µg/ml for Galactosamine HCl drugs on BRL 3A cell lines. (Fig.2)

Hepatoprotective activity of *C. procumbens* Linn against anti tubercular drugs by MTT assay

Anti tubercular drugs isoniazid, rifampicin, pyrazinamide in the ratio of 1:2:5 was used as hepatotoxicant to assess the hepatoprotective effect of *C.procumbens* Linn ethanolic extract. The percentage protection of plant extract was determined and presented in table3. Since the CTC_{50} of antitubercular drugs was approximately 500 µg/ml (Table1), this concentration was used to determine the hepatoprotective effect of the drugs against BRL 3-A cell lines by MTT assay.

Silymarin at the concentration of 250µg/ml showed highest protection (96.47%). C.procumbens Linn at 1000µg/ml showed 80% protection followed by 125µg/ml which showed least protection i.e. 56.47%

Hepatoprotective activity of C . procumbens Linn against galactosamine HCl by MTT assay

Galactosamine HCl was used as hepatotoxicant to assess the hepatoprotective effect of C.procumbens Linn. The percentage protection of plant extract was determined and presented in Table 4. Since the CTC_{50} of galactosamine HCl was approximately 40µg/ml (Table 2), this concentration was used to determine the hepatoprotective effect of the drugs against BRL 3-A cell lines by MTT assay.

Table1: Cytotoxicity of anti tubercular drugs on BRL 3-A cell line

Hepatotoxicant	Concentration (µg/ml)	Mean absorbance	% Growth inhibition
Anti tubercular drugs (INH+RMP+PYZ)	1000	0.098	61.57
	500	0.137	46.28
	250	0.178	30.20
	125	0.182	28.63
	Control	0.255	0

Silymarin at the concentration of 250µg/ml showed highest protection (97.14%). C.procumbens Linn at 1000µg/ml showed 86.93% protection followed by 125µg/ml which showed least protection i.e. 55.40%

Table 2: Cytotoxicity of galactosamine HCl on BRL-3A cell line

Hepato toxicant	Concentration (µg/ml)	Mean absorbance	% Growth inhibition
Galactosamine HCl	50	0.381	61.123
	40	0.502	48.78
	20	0.516	47.35
	Control	0.980	0

Table 3: Hepatoprotective activity of *C .procumbens* Linn against anti tubercular drugs by MTT assay

S.No	Con. of extract (µg/ml)+ hepatotoxicants(500µg/ml)	% Protection
1	*C.procumbens* Linn 1000 + toxicant	80.00
2	*C.procumbens* Linn 500 + toxicant	77.64
3	*C.procumbens* Linn 250 + toxicant	67.84
4	*C.procumbens* Linn 125 + toxicant	56.47
5	Silymarin250+ toxicant	96.47
6	Only toxicant	47.05
7	Control	100

Table 4: Hepatoprotective activity of *C. procumbens* Linn against galactosamine HCl by MTT assay

S.No	Con. of extract (µg/ml)+ hepatotoxicants(40µg/ml)	% Protection
1	*C.procumbens* Linn 1000+toxicant	86.93
2	*C.procumbens* Linn 500+toxicant	75.61
3	*C.procumbens* Linn 250+toxicant	72.34
4	*C. procumbens* Linn 125+toxicant	55.40
5	Silymarin250+ toxicant	97.14
6	Only toxicant	50.81
7	Control	100

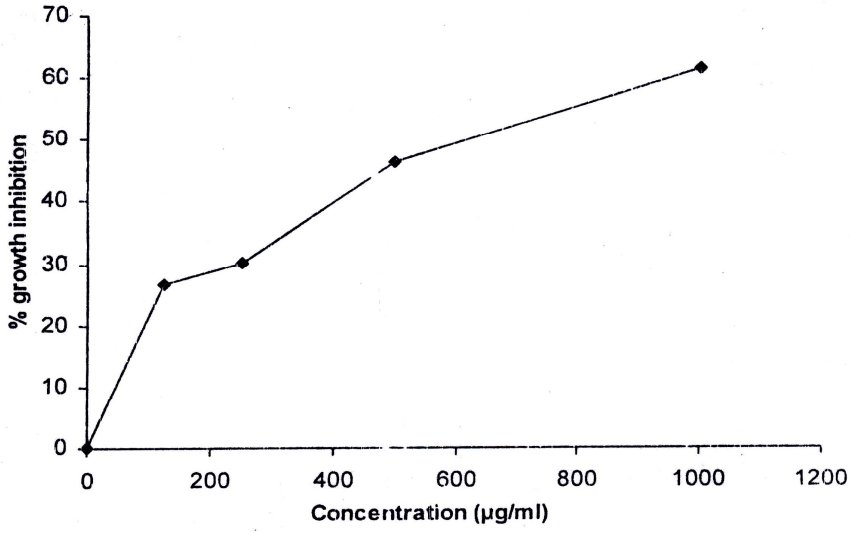

Fig.1. Dose response curve for anti tubercular drugs on BRL-3A cell line

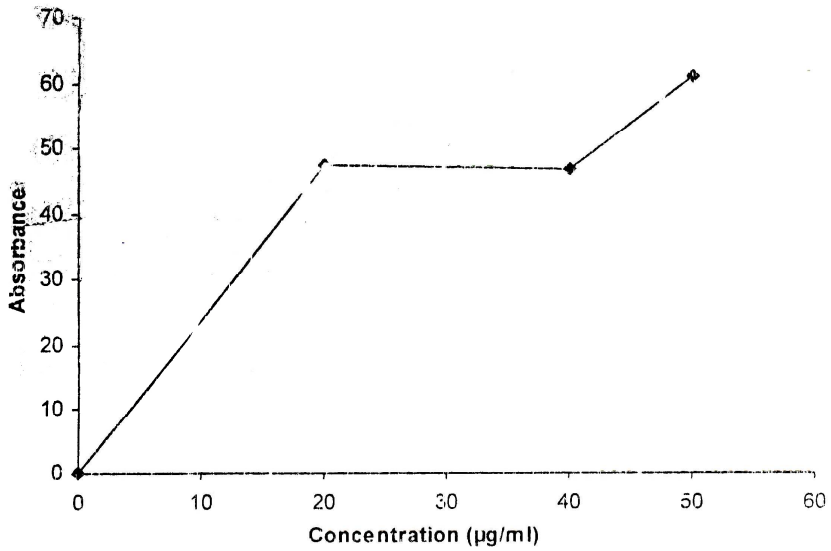

Fig.2. Dose response curve for galactosamine HCl on BRL-3A cell line

CONCLUSION

The CTC_{50} of anti tubercular drugs and galactosamine HCl, which were used as hepatotoxicants to assess the hepatoprotective effect of the plant extracts were found to be 500µg/ml and 40 µg/ml respectively against BRL-3 A cell lines. The plant extract showed over 80 % protection for both the toxicants which is promising for further *in vivo* studies.

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