



# AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

## **IN VITRO HEPATOPROTECTIVE ACTIVITY OF A SELECTED SIDDHA MEDICINAL PLANT *SIDA CORDATA* USING CHANG LIVER CELLS.**

**Gnanasekaran D<sup>1\*</sup>, Umamaheswara Reddy C<sup>2</sup>, Jaiprakash B<sup>3</sup>, Narayanan N<sup>4</sup>, Hannah Elizabeth<sup>5</sup>, Ravi kiran Y<sup>6</sup>**

1. Dept. of Pharmacology, Bharathi college of Pharmacy, Bharathinagar;
2. Dept. of Pharmacology, Sri Ramachandra college of Pharmacy, Porur, Chennai;
3. Gayathri college of Pharmacy, Orissa;
4. Jaya college of Pharmacy, Chennai;
5. Dept. of Zoology, Thiruvallur University, Vellore, Tamilnadu.
6. Bharathi college of Pharmacy, Bharathinagara, Karnataka, India;

### ABSTRACT

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. Management 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. The work presented in this paper is on plant mentioned as Kayakarpam plants in published as well as unpublished palm leaf literatures. The study was aimed to evaluation of the hepatoprotective activity of the whole plant *Sida cordata* on the Chang cell line (normal human liver cells). The ethanolic extract was tested for its inhibitory effect on chang cell Line. The percentage viability of the cell line was carried out. The cytotoxicity of *Sida cordata* on normal human liver cell was evaluated by the SRB assay [Sulphorhodamine B assay] and 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 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.

**Keywords:** - Hepatoprotective, Chang cell line, SRB, MTT, Mitochondrial.

\*Corresponding Author Email: [gnanasekaran\\_99@yahoo.co.uk](mailto:gnanasekaran_99@yahoo.co.uk)

Received 5 January 2012, Accepted 16 January 2012

Please cite this article in press as: Gnanasekaran D *et al.*, *In Vitro* Hepatoprotective Activity Against CCl<sub>4</sub> Induced Toxicity of Some Selected Siddha Medicinal Plants. American Journal of PharmTech Research 2012.

## INTRODUCTION

Siddha system of medicine also known as Siddha vaidya in India, considered as the crown of all the traditional arts of the ancient world owing to its richness and simplicity, practiced by siddhars. Siddha medicine is in usage of herbs, metals, minerals as well as animals in preparing highly effective medicines, is the oldest medical system in existence. The hallmark of traditional Siddha system is KAYAKARPAM i.e., imparting immunity to diseases<sup>1</sup>. 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. Management of liver diseases is still a challenge to modern medicine. The work presented in this paper is on plant mentioned as Kayakarpam plants in published as well as unpublished palm leaf literatures. The Pharmacological experiments have been evaluated with special reference to their rejuvenation activity. The parameters chosen are also indicative of their potential use in Kayakarpam.

Siddha Healthcare, a holistic approach to wellness and vitality is based on the ancient and practical medical system from India known as Siddha Vaidya. Loosely translated as "Knowledge of Health," Siddha Vaidya offers timeless wisdom to address modern ailments and increase mental and physical energy<sup>1</sup>.

*Sida cordata* belongs to the family malvaceae is distributed in India, Pakistan and other tropical countries. It is used for medicinal purposes in the codified Indian systems of medicine namely Ayurveda and Siddha. The whole plant material rejuvenate and given for chronic liver diseases<sup>2</sup>. It is stated in traditional siddha literature under the author Bhava Mishra, 'Bhava Prakash Nigandu'<sup>3</sup>. Roots of this plant (nagapala) used in liver diseases, rejuvenation and anti ageing. But it has not been explored properly and remains a silent drug in herbal medicine. The present study carried out on the ethanolic extract *Sida cordata* (whole plant) to show the hepatoprotective activity *in vitro*, against CCl<sub>4</sub> induced hepato toxicity.

## MATERIALS AND METHODS

### **Plant source:**

The whole plant *Sida cordata* was collected in the surrounding areas of Bidadi, Ramnagar Dist, Karnataka. It was authenticated by Prof. Nagendra M, Dept. of Botany, Bharathi College of science and arts. Authentication number (DBP/122)

**Extraction:** One kg of powdered whole plant of *Sida cordata* was taken in a soxhlet and 2500 ml of 90% ethanol was added. It was refluxed for 72 hours and filtered through muslin cloth while hot. The alcohol extract was dried under vacuum<sup>4</sup>.

### Cell lines and growth media<sup>5</sup>

Chang liver cell lines (Human normal liver cells) were cultured in DMEM (Dulbecco's modified eagles medium). The medium also contains 10% fetal calf serum, penicillin (100 U) and streptomycin (100 µg).

### Method for Passaging the Cells<sup>5</sup>

#### Requirements

- Trypsin-phosphate-versene-glucose (TPVG) solution
- Tissue culture canted 25cm<sup>2</sup> flasks
- DMEM with antibiotics
- Cell line
- Haemocytometer
- Pasteur Pipettes
- Sterile Pipettes
- Cell Counter
- Fetal Calf Serum (FCS)

#### Procedure:

All the reagents were brought to 37<sup>0</sup>C before use.

- Trypsin-phosphate-versene-glucose (TPVG) solution was added to cover the monolayer, rinsed and discarded.
- Fresh TPVG solution was added and allowed to stand at room temperature for 2-3 minutes.
- TPVG solution was discarded and the monolayer was incubated at 37<sup>0</sup>C for 3-5 minutes and slightly tapped to free the cells from the surface.
- 10ml of DMEM containing 10% serum was added and pipetted to breakdown the clumps of cells.
- Total cell count was taken using a haemocytometer.
- Required amount of medium containing the required number of cells (0.5-1.0x10<sup>5</sup> cells/ml) was transferred into bottles.
- The flasks were incubated at 37<sup>0</sup>C, after the formation of monolayer, the cells were further utilized.

#### Cytotoxicity Screening

#### Determination of Mitochondrial Synthesis by Micro culture Tetrazolium (MTT) Assay<sup>6</sup>:

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based.

### Procedure

- The monolayer cell culture was trypsinized and the cell count was adjusted to  $1.0 \times 10^5$  cells/ml using medium containing 10% new born calf serum.
- To each well of the 96 well microtitre plate, 0.1ml 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 100 $\mu$ l of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37<sup>0</sup>C for 3 days in 5% CO<sub>2</sub> atmosphere, and microscopic examination was carried out and observations recorded every 24 hours.
- After 72 hours, the drug solutions in the wells were discarded and 50 $\mu$ l of MTT in DMEM was added to each well.
- The plates were gently shaken and incubated for 3 hours at 37<sup>0</sup>C in 5% CO<sub>2</sub> atmosphere.
- The supernatant was removed and 50 $\mu$ l of propanol was added and the plates were gently shaken to solubilize the formed formazan.
- The absorbance was measured using a microplate reader at a wavelength of 540nm.

### Determination of Total Cell Protein Content by Sulphorhodamine B (SRB) assay<sup>7</sup> (Table 1):

SRB is a bright pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in TCA (Trichloro acetic acid) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. When TCA-fixed and SRB stained samples are air-dried, they can be stored indefinitely without deterioration.

### Procedure

- The monolayer cell culture was trypsinized and the cell count was adjusted to  $1.0 \times 10^5$  cells/ml using medium containing 10% new born calf serum.

- To each well of the 96 well microtitre plate, 0.1ml 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 plates. The plates were then incubated at 37°C for 3 days in 5% CO<sub>2</sub> atmosphere, and microscopic examination was carried out and observations recorded every 24 hours.
- After 72 hours, 25µl of 50% trichloro acetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form a overall concentration of 10%.
- The plates were incubated at 4°C for one hour.
- The plates were flicked and washed five times with tap water to remove traces of medium, drug and serum, and were then air-dried.
- The air-dried plates were stained with SRB for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried.
- 100µl of 10mM tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes.
- The absorbance was measured using microplate reader at a wavelength of 540nm.

The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of Individual Test Group}}{\text{Mean OD of Control Group}} \times 100$$

***In vitro* hepatoprotective activity against CCl<sub>4</sub> induced toxicity<sup>8</sup> (Table2):**

**Methodology:**

- Below the CTC<sub>50</sub> value two dose levels were selected for each extract and used for further studies.
- Cell culture of Chang liver cells was trypsinized and the cell count was adjusted using medium containing 10% new born calf serum.
- To each well of the 96 well microtitre plate, 0.1mL 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; the monolayer was washed once and treated with 100µl of different drug concentrations for 24 hrs.

- After 24 hrs of pretreatment with the extracts, the cells were challenged with CCl<sub>4</sub> (15 mM) where 100µl of different drug concentration and 100µl of CCl<sub>4</sub> was added. The plates were then incubated at 37<sup>0</sup>C for further 24 hours in 5% CO<sub>2</sub> atmosphere.
- Microscopic examination was carried out and observations were recorded every 24 hours.
- After 72 hours, the drug solutions in the wells were discarded and 50µl of MTT in DMEM - PR was added to each well.
- The plates were gently shaken and incubated for 3 hours at 37<sup>0</sup>C in 5% CO<sub>2</sub> atmosphere.
- The supernatant was removed and 50µl of propanol was added to solubilize the formed formazan.
- The absorbance was measured using a microplate reader at a wavelength of 540nm.

The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth Inhibition} = 100 - \left[ \frac{\text{Mean OD of Individual Test group}}{\text{Mean OD of Control Group}} \right] \times 100$$

### Statistical analysis

Data are expressed as means ± SEM. Mean difference between groups were analysed by Student 't' test. P value < 0.001 was considered to be statistically significant. Graph Pad Prism version 4.0 software was used for the statistical analysis

## RESULTS AND DISCUSSION

**Table 1: Determination of CTC<sub>50</sub> by using MTT and SRB assay in Chang liver cells (human normal liver cells).**

Extract	CTC <sub>50</sub> in (µg/ml)		
	MTT	SRB	Mean CTC <sub>50</sub>
<i>Sida cordata</i>	418.86 ± 10.07	436.27 ± 9.38	427.56

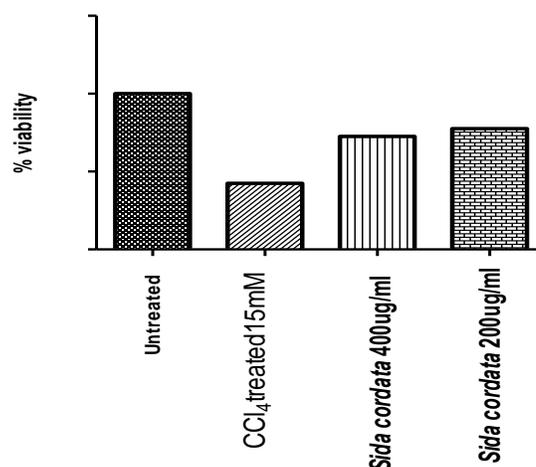
**Table 2: Protective effect of given extracts on CCl<sub>4</sub> induced toxicity in Chang liver cells**

Treatment	Concentration	% Viability of cells*
Control (untreated cells)	-	100
CCl <sub>4</sub>	15 mM	42.38 ± 0.94 <sup>+</sup>
<i>Sida cordata</i>	400 µg/ml	72.54 ± 1.09 <sup>++</sup>
	200 µg/ml	77.53 ± 1.15 <sup>++</sup>

\*Average of six independent determinations, values are mean ± S.E.M. + = P < 0.001, when compared to untreated cells. ++ = P < 0.001, when compared to CCl<sub>4</sub> intoxicated cells.

The longevity of an organism clearly depends on its individual parts and their effective organization. The screening for hepatoprotective activity was carried out to correlate with Kayakarpan description of the properties and shows a fairly good potential for use for the same. 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 liberated and can be readily detected and quantified by a simple colorimetric 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 (figure:1).



**Figure: 1: Histogram showing the Hepatoprotective activity (%cell viability Vs Concentration)**

The assay has therefore been adopted for use with cultures of exponentially growing cells<sup>9</sup>. 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. Under mild acidic conditions, SRB binds to protein basic amino acid residues in TCA (Trichloro acetic acid) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. From the above results it is confirmed that cells which are exposed only with toxicant CCl<sub>4</sub> showed a percentage viability of 42% while cells which are pretreated with extract showed an increase in percentage viability and the results were highly significant ( $P < 0.001$ , when compared to CCl<sub>4</sub> intoxicated cells). The percentage viability ranged from 72 to 78 % cell viability, when pretreated with the extracts.

**CONCLUSION:** The above study indicates positive hepatoprotective activity of the extract *Sida cordata* (whole plant) *in vitro*, against CCl<sub>4</sub> induced hepato toxicity. Earlier screening shows a positive Anti-stress activity also.

## REFERENCES:

1. <http://www.siddhaphysician.com/>
2. Agasthiar Pannendayiram Ennum Perunool Kaviyam, 2<sup>nd</sup> Edn. 1995, Thamarai Noolagam, Chennai, pp-322.
3. Bhava Mishra. Bhava Prakash Nigandu. 4<sup>th</sup> ed., 1985:585.
4. Kokate CK: Practical Pharmacognosy. 12<sup>th</sup> reprint, 9<sup>th</sup> ed New Delhi. Vallabh Prakashan.2005:107
5. Tsai MC, Song TY, Shih PH, Yen GC. Antioxidant properties of water soluble polysaccharides from *Antrodia cinnamomea* in submerged culture. Food Chemistry 2007; 104:1115- 1122.
6. Masters RW. Animal cell culture, Cytotoxicity and viability assays. 3<sup>rd</sup> ed., 2000:202-203.
7. Wilson AP. Cytotoxicity and Viability Assays in Animal Cell Culture: A Practical Approach. 3<sup>rd</sup> ed, Oxford University Press: Oxford 2000; 1.
8. Vijayan P, Kumar VS, Dhanraj SA, Badmani S, Suresh B. In Vitro Cytotoxicity and Anti-tumor Properties of the Total Alkaloid Fraction of Unripe Fruits of *Solanum pseudocapsicum*. Pharmaceutical biology 2002:406-456.
9. Beena P, Purnima S, Kokilavani R. In vitro hepatoprotective activity of ethanolic extract of *Coldenia procumbens* Linn. J Chem Pharm Res 2011; 3(2):144-149.