The Pharma Innovation Journal 2019; 8(1): 86-89

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 86-89

www.thepharmajournal.com
Received: 21-11-2018
Accepted: 26-12-2018

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Cytotoxic effect of *Hemidesmus indicus* R. Br. on HCT 116 human colon cell lines

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Abstract
Mammalian tumour cells display resistance to chemotherapy and its severe side effects diminishes the clinical usefulness of a huge variety of anticancer agents. Plant-derived compounds manifest many beneficial effects, furthermore be able to probably inhibit several stages of cancer. In the present study, we endeavored to take advantage of bioactive compounds of *Hemidesmus indicus*, so we investigated their antioxidant and antiproliferative properties on colon cancer cell lines HCT116. The preliminary phytochemical screening of ethanolic extract showed the presence of significant secondary metabolites. Our findings infer that the potential bioactive compounds of plant has significantly inhibited the growth of colon cancer cells and its extract can be applied as adjuvant medicines in combination regular chemotherapeutic agents.

Keywords: Anantmul, *Hemidesmus Indicus* colon cancer antioxidant cytotoxic activity, HCT 116

1. Introduction
Plant secondary metabolites display a myriad of chemical structures with accompanying activities that have pharmaceutical potential. In nature, these specialized secondary metabolites are involved in the interactions of plants with their environments in roles such as signaling hormones, conferring resistance against pests and diseases, attracting pollinators, and defending against pathogens or herbivores [1].

Plants used in traditional medicinal systems, ethnomedicine, folk medicine, and herbalism provide a rational and obvious source of candidates for targeted identification of lead substances with novel structures, combinations, and mechanisms of action. They also have the added advantage that, as drugs, their safety and efficacy profiles are well established through historical use or long-term human experience [2]. With time plant extracts have been understood to include the elements accounted not only for their aroma as well as flavor but also for their antimicrobial nature. Various natural plant extracts have identified antimicrobial as well as therapeutic effects suggesting their potential to be used as cytotoxic agents. Phytochemical extracts for instance *Curcuma longa* (turmeric) and *Allium sativum* (garlic) consists of active ingredients like Curcumin and Allicin respectively which have anti microbial, anti inflammatory and anti-oxidant properties [3, 4].

Hemidesmus indicus universally accepted as Indian sarsapilla, belonging to family Asclepiadaceae. Vernacular name "Anantmul" is a Sanskrit word which means 'endless root' [5]. Plant has two varieties namely black variety, also called as 'Krishna Saarivaa' and white variety which is called as 'Saarivaa' [6]. Hemidesmus indicus is accepted by Ayurvedic formulary as white variety whereas, *Cryptolepis buchananii* Roem. and Schytt as black variety. *Ichnocarpus fruitescens* is also used as black variety by the people of West Bengal and Kerala [7]

Fig 1: *Hemidesmus indicus* (L.) R.Br. plant.

H. indicus is a slender laticiferous, twing, sometimes prostrate or semi erect shrub, occuring in greater part of India. Anantamul can be distinguished by its slender, twisted, rigid, cylindrical and aromatic root. Its bark is rust-colored and corky, as well as furrowed with annular cracks. Its stems are numerous, slender, terete, thickened at the nodes. Leaves are opposite, variable, elleiptic oplong to linear lanceolate, often variegated with white above and pubescent beneath. Flowers are greenish outside and deep purplish inside, crowded in subsessile axillary cymes. Folicles are slender, four inches long, cylindrical, sometimes curved and divaricate. Its seeds are numerous, black flattened [8]. Phytoconstituents of *H. indicus* ranges from hydrocarbons, glycosides, oligoglycosides, and terpenoids to steroids [9].
H. indicus roots have been reported for a number of pharmacological activites, most notably antimicrobial activity [10], antioxidant [11], wound healing activity [12], antihyperglycemic, antidyslipidemic [13], anti-arthritic activity [14], Cytotoxic activity [15] to cite a few.

Fig 2: Flowers of *Hemidesmus indicus*

Colorectal cancer is the third most common form of cancer and the second leading cause of cancer deaths in both men and women around the world. Alarmingly, increasing numbers of reported cases of colon cancer in recent years has made this form of cancer a major health concern [16]. An estimated 96,830 cases of colon and 40,000 cases of rectal cancer are expected to occur in 2014 [17]. The current treatment for colorectal cancer is generally surgical resection combined with chemotherapy by cytotoxic drugs and radiation. However, this therapy is just moderately successful especially for late stage cancers; therefore new approaches to the treatment of colorectal cancer are required. In recent years, interest has increased in using natural products for pharmacological purposes, as a form of complementary or replacement therapy [18].
In the absence of any transgenic models of colon cancer metastases, an *in vivo* model system that fulfils the rate limiting steps of metastasis (local invasion and distant colony formation) is needed the purpose of this study was to characterize the behavior of a human colon cancer cell line, HCT116, in an orthotropic model of colon cancer. HCT 116 is a human colon cancer cell line that is commonly used to study cancer biology. This is a growth factor independent cell line that has been shown to be invasive and highly motile in in-vitro studies. Subcutaneous xenograft experiments have demonstrated it to be highly tumorigenic However, subcutaneous xenograft implants uniformly fail to show invasion and metastases [19].
The aim of this study was to evaluate the antioxidant activities of *H. indicus* and its role in protecting against oxidative damage to DNA. The effect of the tea on the inhibition of proliferation of the colorectal cancer cell line, HCT 116 was also evaluated.

Fig 3: Roots of *Hemidesmus indicus*

2. Materials and Methods
2.1 Collection and Authentication of Plant Material
2.1.1 Sample collection:
Roots and rhizome powder of *Hemidesmus indicus* R.Br. (locally called Anantmul) was obtained and authenticated from NISCAIR-PUSA (Ref. no. NISCAIR/ RHMD/ consult/ 2013/2224/05).

2.1.2Preparation of Anantmul Extracts
Dried powder of *Hemidesmus indicus* (100 gm) was exhaustively extracted with 500 ml petroleum ether and then with methanol in Soxhlet apparatus for 24 hours and dark brown residue (3.7 gm) was obtained after evaporation of the solvent**.** The dried extract (HIME) was stored in an amber colored air tight container at 2.0°C temperature [12].

2.2.1 Preliminary Phytochemical Study
For the identification of various phytochemical constituents, the different extracts were subjected to qualitative tests as per the standard procedure [20, 21].

2.2.2. **Antioxidant Activity Assessment**
In-Vitro Antioxidant Activity conducted on *H indicus* extracts was DPPH (2, 2-diphenyl-picryl-hydrazil) test as per Silva [22] and H_2O_2 assay as per Yang [23] using ascorbic acid as standard. All the studies were carried out in triplicate.

2.3.1 Cell lines
HCT116 Cell lines were obtained from MCOPS, Mangalore, India.

2.3.2 Culture Media
The culture media for HCT116 (colorectal carcinoma cells; ATCC CCL247) cell lines were prepared by supplementing high glucose containing 'Dulbecco's modified eagle's medium (DMEM-high glucose)' (Hi-Media, Mumbai, India) with 10% (v/v) fetal bovine serum (FBS;Hi-Media, India) and 100 IU/mL Antibiotic antimycotic solution (100X liquid): (A002,Himedia, India). Cells were maintained and cultured in a 5% CO_2 in a humidified atmosphere at 37 °C [24].

2.3.3 *In-vitro* Cell Viability Study
Cell viability was determined by the Trypan Blue Exclusion Test [25]. Briefly, cells were treated for 48 h and collected in the exponential phase. 50µL of sample was mixed with 50µL of 0.4% trypan blue (TC193, Himedia, India) by gently

pipetting, and then 20µL of the mix were loaded into each chamber of the hemocytometer. Counts were performed in triplicate [26].

2.3.4 MTT Assay
HCT116 cell suspensions were dispensed (100 µL) in triplicate into 96-well culture plates at optimized concentrations of 1.5 X 10^5cells/ml. After a 24-hr recovery period, the cisplatin standard or HIME was diluted with distilled water were added. Seven dilutions of HIME were tested (100, 50, 25, 12.5, 6.25, 3.125 and1.5 µg/ml) and to control wells, only culture medium (100 µl) was added, followed by incubation period of 48 h.

Later, the medium in each well was aspirated and replaced with 20 µl of MTT working solution (MTT) stock solution mixed with medium to attain a final concentration of 0.5 mg/ml. MTT powder was dissolved in Dulbecco's PBS to form a stock solution of MTT (5 mg/ml). The cells were incubated at 37 °C for 4 h, and then the medium was aspirated and replaced with 100 µl DMSO to dissolve the formazan crystals formed. The culture plates were shaken for 5 min and the absorbance of each well was read at 490 nm with 655 nm as the reference wavelength [27].

2.4.1 Statistical Analysis
Data are mean±SD of three independent experiments. Cell assays were analyzed by ANOVA followed by Dunnett´s test whereas IC50 values were analyzed by Student test using the SPSS software, version 21.0 (SPSS, Chicago, IL, USA). P value <0.05 was considered statistically significant.

3. Results
3.1 Preliminary Phytochemical Analysis
The results of the study showed a number of secondary metabolites (Table 1). It was observed that extracts of *H indicus* contained a higher concentration of secondary metabolites like Terpenoids, Saponins Flavonoids, Glycosides, Phytosterol, Tannins, which have already been reported to possess antioxidant as well as cytotoxic properties [28].

Table 1: Phytochemical Analysis of *Hemidesmus indicus*

S. No	Compounds	Analysis
1.	Alkaloids+	+
2.	Carbohydrate	-
3.	Fats & oils	-
4.	Flavonoids	++
5.	Glycosides	++
6	Protein & amino acid	+
7.	Phenols	+
8.	Phytosterol	++
9.	Resins	+
10.	Saponin	+++
11	Tannins	++
12	Terpenoids	+++

(+) - Indicates the presence of the phyto-constituent
(-) - Indicates the absence of the phyto- constituent.

3.2 Antioxidant Activity Assessment
The antioxidant effects of plant products must be calculated by combining two or more different *in vitro* assays to get appropriate data, because of the complex nature of phytochemicals. Each of these tests is based on one feature of the antioxidant activity, such as the ability to scavenge free radicals, or the metal ion chelation.

As a whole, radical scavenging activities of extracts were comparable ($P<0.05$; P = 0.0013) to that of the standard antioxidants, (ascorbic acid) for both the models. (Inset of Table 2)

Table 2: Free radical scavenging activity of *H. indicus* extract

Sl. No.	Dose µg/ml.	% Inhibitor by H_2O_2 Method	% Inhibitor by DPPH method
1	25	19.23	23.68
2	50	43.65	35.30
3	100	51.55	46.21
4	200	59.78	56.83
5	500	71.10	72.50
6	Standard	87.45	88.32

3.3 Cytotoxicity of HIME on Colon Cancer Cell Lines
The MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations, the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in-vitro cytotoxic effects of drugs on cell lines or primary patient cells [24].

In the present study, MTT assay showed that the incubation of cancer cells lines with *H. indicus extract (HIME)* reduced the viability of cancer cells and the dead cells were significantly increased with extract concentration (P<0.05).Also, the extract of *H. indicus* exhibited high cytotoxicity of 60.4%.

4. Conclusions
Cancer is affecting millions of people every year and our emphasis is to explore appropriate plant sources and to suggest a novel anti-cancer candidate that can combat colon cancer in a better way. Since plants have been proved to be a vital natural source of anti-cancer therapy for several years, in the present study, an attempt was made to determine and prove the anti-proliferation effect of methanol extracts of selected plant.

The results of the present study reveals the potentiality of *H. indicus* against colon cancer cell lines and supports the need of further studies to isolate it as an potential anticancer drug. Additionally, the study supports the anticancer property of medicinal plants used in the traditional Indian medicine system Therefore, assessment of medicinal plants used in the Ayurvedic system medicine could be an effective lead for exploration an of effective and safe anticancer drugs with minimal side effects.

However, there are limitations in this study. The experiments were performed not *in vivo*, but *in vitro*. Drug sensitivity can be different between *in vitro* and *in vivo*. Animal experiments and clinical trials ought to be done in the further study. Another limitation is that prescriptions of herb medicine are not standardized worldwide and difficult to use in West.

In conclusion, HIME extract maybe useful as adjuvant medicine in combination with standard chemotherapeutic agents to inhibit the growth of colon cancer cells. It could have growth inhibitory effects in combination with the conventional chemotherapeutic agents. Molecular study is needed to understand the mechanism of its growth inhibitory effect

6. References

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