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Research Article

# CYTOTOXIC EFFECT OF *CORCHORUS DEPRESSUS* AGAINST HEPG2 AND HLE HUMAN LIVER CANCER CELLS

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**ABSTRACT**

**Objective:** The present study was designed to examine the cytotoxic effects of methanolic extract of aerial parts of *Corchorus depressus* and hexane, chloroform, ethyl acetate, and aqueous fractions of the same extract in the human hepatocellular carcinoma (HCC) (HepG2) and invasive hepatocellular carcinoma cell lines (HLE).

**Methods:** Anti-proliferative effects were evaluated using 3-(4,5-dimethythiazol2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and neutral red uptake **(**NRU) assay. Human HCC (HepG2) and invasive hepatocellular carcinoma cell lines (HLE) were treated with different concentrations of methanolic extract (10, 25, 50, 100, 200, 300, 400, and 500 µg/mL) of aerial parts of C. depressus as well as hexane, chloroform, ethyl acetate, and aqueous fractions (200 µg/mL) for 24 and 48 h. The cell viability and the half maximal inhibitory concentration ($IC_{50}$) were determined.

**Results**: The maximum cytotoxic effect was noticed with a maximum dose of methanolic extract (500 µg/mL) and alkaloidal fraction (200 µg) in this study with an $IC_{50}$ value of about 200 µg.

**Conclusion:** The set of studies showed that methanolic extract of aerial parts of *C. depressus* and alkaloidal, chloroform and ethyl acetate fractions fractions was capable of inhibiting cell growth and **c**ell proliferation by inducing cytotoxicity of HepG2 and HLE cells.

**Keywords:** Liver cancer, HepG2 and HLE cell line, *Corchorus depressus*, Cytotoxicity, MTT, Neutral red uptake assay.

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## INTRODUCTION

Hepatocellular carcinoma (liver cancer; HCC) is the fifth most common cancer worldwide now and causes significant public health problems, especially in association with chronic hepatitis B or C [1]. The liver functions to absorb and store nutrients, remove toxic waste and create clotting factors that stop bleeding from injuries. When HCC is present, the body exhibits symptoms such as drastic loss of weight, swelling in the abdomen, or jaundice [2]. The risk of developing liver cancer increases with factors associated with hepatitis B/C virus, aflatoxin, iron storage disease, cirrhosis, alcoholism, or obesity [3-5]. The average survival rate for hepatic cancer patients is 3–5% [6].

*Corchorus depressus* (Family Tiliaceae) (Cd) has been used in the indigenous system of medicine as a tonic, cooling medicine in fevers; its mucilage is prescribed in gonorrhea. Root is rubbed on stone and smeared over the forehead to get relief in migraine [7]. It is also used to increase the viscosity of the seminal fluid, to set-up menstrual disorder [8]. An extract of the plant is applied as a paste in the healing of wounds [9]. It has been used as antibacterial, antifungal, anthelmintic drug in folklore medicine [10], as antimalarial [11], has cardiotonic activity [12], as tonic [13], in the treatment of gonorrhea [14], as veterinary medicine [15] and possesses diuretic activity [16]. The plant is sweetish hot sharp acrid; removes tumors and pain; cures piles. It is given as a cooling medicine in fevers. The leaves are emollient. The seeds in decoction with milk and sugar are given as a tonic in Mahal Kohistan (Khirthar National Park) Pakistan [17].

*C. depressus* is reported to contain sitosterol, sitosterol glucoside, apigenin, luteolin, oleanolic acid, cordepressic acid, cordepressin, cordepressinic acid, α amyrin, kaempferol, etc., [18,19].

Lack of scientific data regarding antitumor properties of *C. depressus* (L.) prompted us to evaluate the claimed medicinal action.

Hepatic cancer is traditionally difficult to treat. Hence, the objectives of the current study were to (1) determine the *in vitro* cytotoxicity of *Cd* methanolic extract of aerial parts and its hexane, alkaloidal, chloroform, ethyl acetate and aqueous fractions, and aqueous fractions on HepG2 and HLE cancer cells and (2) determine the cytotoxicity of actinomycin, an FDA-approved drug, for various cancers, against HCC.

In light of some reports [20,21], it is reasonable to assume that this plant could have anticancer potential. Hence, in this study, we investigated the cytotoxic potential of Cd methanolic extract of aerial parts and its hexane, alkaloidal, chloroform, ethyl acetate and aqueous fractions, and aqueous fractions against human Hep G2 and HLE cancer cell lines.

## METHODS

### Authentication of plant material

The fully grown plants of Cd were collected from Adipur region of Kachchh district in the month of September. Their authentication was confirmed by Dr. Suman Chandra Sharma, Taxonomist, Department of Botany, Government Dungar College, Bikaner, Rajasthan, and its voucher specimen deposited this plant with the Department of Pharmacognosy, Ramanbhai Patel College of pharmacy, Changa (CHARUSAT).

### Cell culture

The Hep G2 and HLE cells were cultured in Dulbecco's Modified Eagle medium supplemented with 10% of fetal bovine serum and 1% penicillin-streptomycin. Cell cultures were maintained at 37°C in a fully humidified atmosphere containing 5% $CO_2$.

### Cell treatment

Cd dried aerial parts methanolic extract; hexane, alkaloidal, chloroform, ethyl acetate and aqueous fractions were dissolved in 0.1% dimethyl sulfoxide (DMSO) (v/v). HepG2 and HLE cells were plated at $1.2 \times 10^4$ cells/$cm^2$. 24 h later, cells were fed with fresh expansion culture medium

supplemented with different final concentrations of extract (10, 25, 50, 100, 200, 300, 400, and 500 µg/mL) or the corresponding volumes of the vehicle. After 24 h of treatment cells were collected after 0.05% trypsin application. Cell viability was also evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazoliumbromide (MTT) and neutral red uptake (NRU) assay [22].

**MTT assay**

According to the method described by Borenfreund *et al*. [23], the growth of cancer cells was quantified. Following a 24 h exposure period of drugs, cells were washed twice with phosphate buffered saline (PBS), and a 10 µL of MTT reagent (5 mg/mL in PBS) was added to each well including the blanks, which contained medium only. The plates were returned to the incubator for 4 h at 37°C. Subsequently, cells were washed twice with PBS, and 100 µL/well DMSO was added in each well as a solvent to dissolve the insoluble crystalline formazan products. The effect of plant extracts on cancer cells was quantified as the percentage of control absorbance of reduced dye at 550 nm on a microplate reader. For each treatment, five replicate wells were examined, and each experiment was repeated 3 times (n=3). Mean and standard deviation was calculated between three experiments. Results were calculated as percentage growth inhibition, untreated (control) cells versus treated cells according to the following formula:

$$\% \text{ Growth inhibition} = \frac{\text{Absorbance of positive control} - \text{Absorbance of test sample}}{\text{Absorbance of positive control}} \times 100$$

Where,

Positive control = absorbance of untreated cells after subtracting absorbance of media

Absorbance of test sample = absorbance of treated cells at a particular concentration of testing sample after subtracting the absorbance of media.

The MTT cell proliferation assay measures the cell proliferation rate and conversely when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT reagent yields low background absorbance values in the absence of cells. For each cell type, the linear relationship between cell number and signal produced is established, thus allowing accurate quantification of changes in the rate of cell proliferation [24].

**NRU assay**

The NRU assay was performed by removal of the medium after dosing cells, and 200 µL of neutral red solution (40 µg/mL) was added to each well (including the blanks, which contained medium only). After incubation for 2.5 h, the neutral red was removed, cells were carefully rinsed with pre-warmed PBS, and 200 µL of ethanol/acetic acid (1% glacial acetic acid in 5% ethanol) was added to all wells.

The plates were covered in foil and placed on a plate shaker for 30 min to extract neutral red from the cells and form a homogeneous solution. The absorbance of the wells was measured at 540 nm in a microplate reader within 60 min.

Cytotoxicity assays are widely used in toxicology studies. The NRU and 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assays are commonly used cytotoxicity assays to determine the cytotoxic properties of compounds. NRU assay has been used as an indicator of cytotoxicity in cultures of primary hepatocytes [25] and other cell lines [26]. Living cells take up the neutral red, which is concentrated within the lysosomes of cells [27]. MTT, a water-soluble tetrazolium salt, is converted to insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. It is then solubilized in DMSO, and the cell viability depicts from its absorbance values [28].

**Statistical analysis**

In both the *in vitro* tests, statistical analysis was done by Dunnett's *t*-test. The data were expressed as mean ± SEM. $p<0.05$ was considered as significant.

## RESULTS

**Cytotoxic assay**

Cd methanolic extract caused dose-dependent increase in cytotoxicity in HepG2 and HLE human liver cancer cells. The maximum cytotoxic effect was noticed with the maximum dose used in this study, i.e., 500 µg/mL (Fig. 1). The extract showed an inhibitory concentration ($IC_{50}$) is about 200 µg (Fig. 1). The anti-proliferative activity was significantly concentrated in three fractions (CHCl3, EtOAc and alkaloidal, n=3 for each fraction, mean ± sd) with similar results obtained with the NRU assay. The alkaloidal fraction had the maximum effect on the cells. (Fig. 2).

**Cell viability**

We noticed that Cd treatment in HepG2 cells caused dose-dependent growth inhibition. Cytotoxic activity was determined by cell viability. The methanolic Cd aerial parts extract decreased the cell viability of HepG2 cancer cells prominently whereas in HLE the toxicity was quite less.

## DISCUSSION

Chemoprevention is a novel approach emphasizing on the prevention or delay of carcinogenesis by means of pharmacological, biological, and nutritional intervention and recently, cancer chemoprevention with strategies using foods and medicinal herbs has been regarded as one of the most visible fields for cancer control [29].

Palliative treatments for HCC are indicated if there is no curative treatment option, four palliative treatments are transarterial chemo-embolization, systemic chemotherapy, interferon, and hormonotherapy [30]. However,

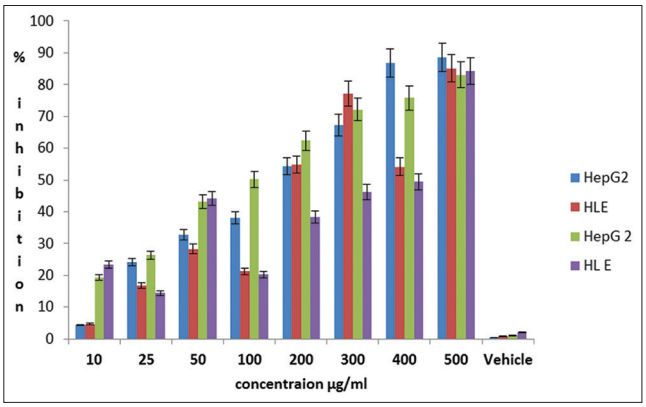


**Fig. 1: Cytotoxicity activity of a methanolic crude extract of *Corchorus depressus* using MTT and neutral red uptake assay with apparent $IC_{50}$ about 200 µg/mL after 24 h of treatment ± standard deviation**

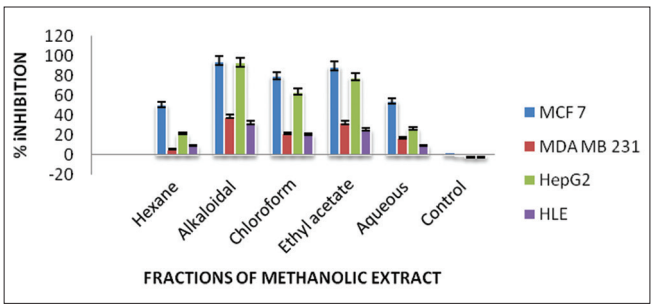


**Fig. 2: Cytotoxic activity of fractions (200 µg/mL) from *Corchorus depressus* methanolic extract using MTT and NRU assay indicating the most potent fraction after 24 h treatment**

palliative therapy of patients with HCC remains challenging as HCC is highly resistant to systemic therapies. More importantly, the incidence still nearly equals the mortality rate and more than 80% of patients present with advanced disease [31]. The overall disappointing results of both curative therapies and palliative treatments in advanced HCC patients support the research for other more active and specific treatments to be administered alone or in combination with the current therapy. Herbal compounds could affect all phases of HCC, including initiation, promotion, and progression [32]. The active development of innovative therapeutic approaches and molecularly targeted agents using herbal medicine could offer an opportunity to study the agents in HCC and gives new hope for the future.

*In vitro* cytotoxicity studies are commonly performed to evaluate the anticancer potentials of a drug or plant extracts. In this study, we noticed the cytotoxic potential of *Cd* in human liver cancer cell line.

Some of the mutagenic and carcinogenic effects of various carboline alkaloids have been related to their ability to intercalate into DNA [33,34] leading to altered DNA replication fidelity and enzymatic activities in DNA-repair processes [35,36]. They also significantly reduce cell proliferation [37]. In human neuroblastoma SH-SY5Y cells, some carboline alkaloids induced apoptosis as well as necrosis. Phytochemical evaluation of Cd has indicated the presence of β-carboline alkaloid/s so probably this cytotoxic effect of c*d* may be by above-cited mechanism.

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**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

**AUTHORS' CONTRIBUTION**

Purvi Kakrani, Harish Kakrani, and Manan Raval contributed to the design and implementation of the research, to the analysis of the results and the writing of the manuscript.

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