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**Research Article**

# ANTICANCER ACTIVITY OF CHITOSAN-ZINC OXIDE NANOCOMPOSITE FABRICATED WITH HERB

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**ABSTRACT**

Chitosan and its derivatives exhibit anticancer effects. Chitosan-zinc oxide nanocomposite fabricated with herb was studied for their anticancer activity using MCF7 cell line and its cytotoxic effects was studied on Vero cell line by MTT assay. The herb selected for this study is *cynodon dactylon*. The $IC_{50}$ and the selectivity index indicates that the nanocomposite is a potential anticancer agent.



## INTRODUCTION

Cancer is still one of the high ranking cause of death in the world, inspite of considerable progress in medical research. One of the main goal for scientists is investigating new anticancer agents. Anticancer chemotherapeutic agents generally produce severe side effects. Hence, it is important to find new, powerful anticancer agents that are highly effective, biodegradable and biocompatible. Chitin and chitosan biopolymers[1] have unique structural possibilities for chemical and mechanical modifications to generate novel properties and function. These biopolymers are biocompatible, biodegradable and nontoxic. Due to the unique properties, chitin and chitosan are excellent candidate for cancer cure.

Recently nanomaterials have been emerging as attractive pharmacological vehicles for drug delivery and cancer therapy. Nanomaterials have unusual physicochemical characteristics because of their small size, surface structure, solubility and shape [2]. Recently extensive studies using chitosan-metal oxide nanoparticles have been performed to enhance anticancer drug efficacy [3] and reduce the side effects of the drug.

The present work highlights the anticancer activity of chitosan-zinc oxide nanocomposite fabricated with herb (CZH). Anticancer activity of the nanocomposite was studied using MCF cell line (Breast cancer cell line) and its cytotoxic activity was studied on vero cell line by MTT assay.

### *Experimental*

### *Synthesis of chitosan*

Chitosan was extracted from crab shells using NaOH and HCl [4]. In the current work, Chitosan (60% DDA) was selected for the synthesis of nanocomposite.

### *Synthesis of chitosan-zinc oxide nanocomposite fabricated with herb*

Chitosan-zinc oxide nanocomposite was synthesised using 1% chitosan [60% DDA] nano zinc oxide. Later herb was loaded to the synthesised nanocomposite by swelling method [5]. The synthesised nanocomposite is represented by CZH, where C denotes Chitosan, Z-Zinc oxide and H-Herb[5].

### *Characterisation*

The synthesized nanocomposite with herb was characterized by UV, FTIR, FESEM, UV and FTIR spectroscopy which confirmed the formation of nanocomposite, FESEM revealed the particle size. Antibacterial studies were carried by Agar well diffusion method and the result showed that the composite

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had excelled [5] antibacterial activity towards *S. aureus&E.coli.*

***MTT ASSAY***

***Anti-Cancer Activity (MTT assay)***

The MTT assay was performed as first described by Mosmann**[6]**with modifications suggested by Denizot**[7]** and Lang. Cells [1X$10^5$/well] were plated in 24 well plates and incubated at $30^0$C with 5% $CO_2$ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hours. After incubation, the sample was removed from the well and washed with phosphate-buffered saline [*pH* 7.4]. 100 µl/well [5mg/ml] of 0.5% 3-(4,5–dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) was added and incubated for 4 hr. After incubation, 1 ml. of Dimethyl Sulfoxide (DMSO) was addedin all the wells. The absorbance at 570 nm was measured with UV spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition ($IC_{50}$) was determined graphically. The % cell viability was calculated using the following formula:

***% Cell Viability = ($A_{570}$ of treated cells/$A_{570}$ of control cells) X100***

Graphs were plotted using the % of cell viability in Y axis and concentration of the sample in X axis. Cell control and sample control were included in each assay to compare the cell viability in cytotoxicity and anticancer assessments. The MTT assay is repeated for vero cell line to determine the cytotoxicity of the nanocomposite CZH.

## RESULTS AND DISCUSSION

The effect of nanocomposite on vero cell line and MCF line were expressed as the percentage cell viability. Cell viability % of vero cells and cell viability of cancer cells (MCF7) are listed in Table 1. Grapical representation of cell viability of Vero cells and cancer cells (MCF7) at different concentrations is given in Fig. 1. Calculated $IC_{50}$ value for Vero cells and cancer cells are 140 µg/ml and 11.87 µg/ml respectively. Photographs of CZH treated vero cells and cancer cells are represented in Fig. 2 and Fig. 3 respectively.

To determine the cytotoxic selectivity of the substance tested, the selectivity index was calculated by using the equation

**Selectivity index = $IC_{50}$ of normal (vero) cell lines / $IC_{50}$ of cancer (MCF7) cell lines**

where SI $\geq$ 10 was considered to belong a selective compound according to Quispe and *et. al.*[8] and Valdes, Gracia *et. al*[9]. The selectivity index of CZH = 140/11.87= 11.79

The selectivity index reveals that CZHis potent anticancer agent. This anticancer study demonstrate a promising anticancer potential of the chitosan-zinc oxide nanocomposite fabricated with herb on breast cancer cell lines (MCF7)

**Table 1** % cell viability of CZH treated vero cells and cancer cells

| S.No | Concentration µg/ml | % cell Viability of Vero Cells | % cell Viability of cancer cells (MCF7) |
|---|---|---|---|
| 1 | 100 | 52.7 | 10.3 |
| 2 | 50 | 57.4 | 19.8 |
| 3 | 25 | 66.9 | 33.0 |
| 4 | 12.5 | 72.4 | 48.1 |
| 5 | 6.25 | 92.1 | 64.1 |
| 6 | 3.12 | 97.6 | 81.1 |
| 7 | Control cells | 100 | 100 |
| | | $IC_{50}$ = 140 µg | $IC_{50}$ = 11.87 µg |

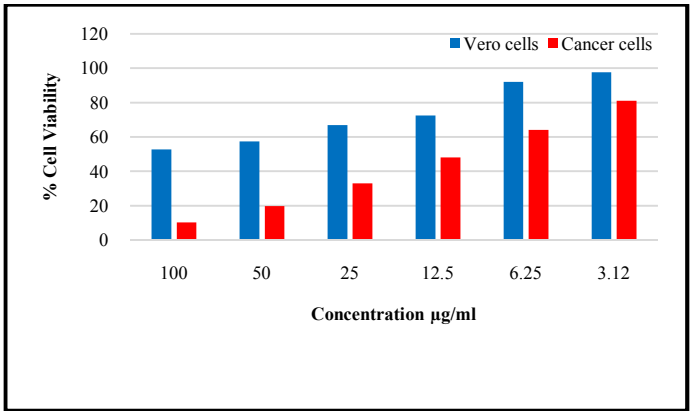


**Fig 1** % Cell viability of Vero and cancer cells

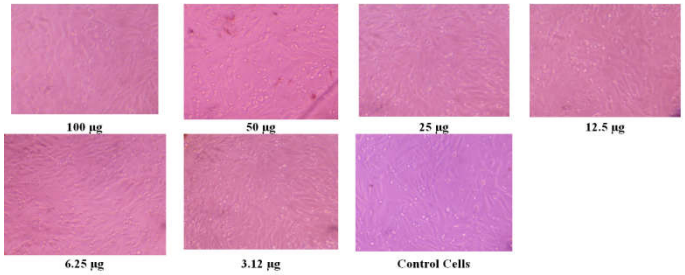


**Fig 2** Photographic images of CZH treated vero cells

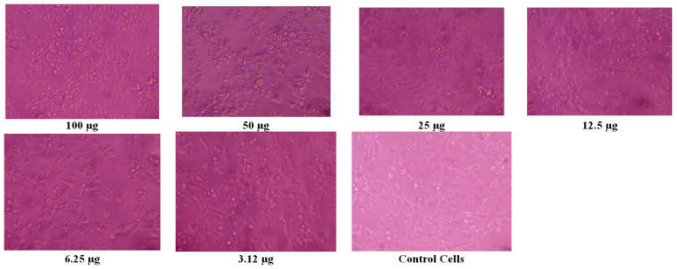


**Fig 3** Photographic images of $C_2ZH_2$ treated cancer cells (MCF7)

## CONCLUSION

The Chitosan-zinc oxide nanocomposite fabricated with herb is evaluated for their anticancer activity using MCF7 breast cancer cell line. MTT assay is used to study the cytotoxicity and anticancer activity of the nanocomposite. The $IC_{50}$and the selectivity index values reveals that the Chitosan-zinc oxide nanocomposite fabricated with herb is the potent anti caner agent.

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