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Research Article

EVALUATION AND COMPARISON OF CYTOTOXIC EFFECT OF VILAZODONE HYDROCHLORIDE WITH 5-FLUOROURACIL IN HT-29 BOWEL CANCER CELL LINE

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ABSTRACT

Objectives: Vilazodone hydrochloride is a novel selective serotonin reuptake inhibitor (SSRI) used to treat major depressive disorders. There are only sparse data available to know about the SSRI's and its association with colon cancer. This study aims to evaluate and compare the *in vitro* cytotoxic effect of vilazodone with 5-fluorouracil (5-FU) in HT-29 cell line.

Methods: Cell viability was tested by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Mosmann, 1983). Test sample and standard drug in variable concentrations were added to the HT-29 cell lines for incubation over 24 h under ideal conditions. After washing the test and standard drug sample from the well with saline, MTT was added and incubated for 4 h. Dimethyl sulfoxide of 1 ml was added in all wells after incubation with MTT. The absorbance at 570 nm was measured with an ultraviolet - spectrophotometer.

Results: The values were tabulated, and the graph was plotted to find the IC-50 value (inhibitory concentration at 50%) which was struck at 28.5 µg/ml and12. 8 µg/ml for vilazodone hydrochloride and 5-FU, respectively.

Conclusion: The results show that vilazodone hydrochloride has good anticancer property comparable with 5-FU, which would probably play a role as a cytotoxic agent in tumor cells. The proposed mechanism of action could be by activation of caspase-3 enzyme, thereby increasing apoptosis and indicates its use in coexisting depression and colon carcinoma. Other mechanism includes suppression of oncogene p53, which can be confirmed by future studies.

Keywords: Vilazodone hydrochloride, Colon cancer cell line, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, Selective serotonin reuptake inhibitor.

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INTRODUCTION

As per the WHO epidemiology data, "Cancer is a leading cause of death worldwide, accounting for 8.8 million deaths in 2015. The second leading cause of cancer death is colorectal cancer, accounting for 774,000 deaths worldwide" [1]. The treatment of colorectal cancer includes surgery, radiotherapy, and chemotherapy with fluoropyrimidines especially 5-fluorouracil (5-FU) combined with folinic acid and oxaliplatin [2]. Apart from these available treatments, antidepressants are also considerably valued in cancer therapy. In the past two decades, the use of antidepressants principally selective serotonin reuptake inhibitors (SSRI's) in neoplastic diseases has noticeably increased because the majority of cancer patients suffer from depressed mood [3,4]. Distinctly, there is a query whether antidepressants promote or depress tumor growth in experimental animals and patients [5]. Citalopram and fluoxetine were found to suppress cell division in colonic tumor models (*in vivo*) of mice [6]. Paroxetine and fluoxetine were indeed found to induce apoptosis in lung cancer cells (A549), neuroblastoma cells (SKNAS), and medulloblastoma/rhabdomyosarcoma cells (TE671) [7,8]. On the contrary, it was studied that fluoxetine and paroxetine induce carcinogenesis and promotes the growth of ovarian and mammary tumor [9-11]. Diversely, preclinical studies in tumor implanted rats depict that SSRI's neither have stimulatory nor an inhibitory effect on the growth of tumor [12]. Henceforth, this inconsistent and contradictory data warrants the need for multiple studies to analyze the association of antidepressants and cancer.

Vilazodone hydrochloride: It is a novel SSRI and partial agonist of the serotonin (5-hydroxytryptamine) -1A receptor, used to treat major depressive disorders [13]. Vilazodone was approved in 2011 by the United States - Food and Drug Administration and in August 2015 by Central Drugs Standard Control Organization - India for the treatment of major depressive disorder [14]. Major and most common adverse effects are being acute pancreatitis, sleep paralysis, diarrhea, nausea, and headache [15]. This being a new drug needs to be explored for its association with colon cancer cells.

Aims and objectives

The aim of the study was to evaluate and compare the effect of vilazodone hydrochloride with 5-FU in the HT-29 colon cancer cell line *in vitro* using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Principle of MTT assay

This colorimetric assay measures the viable cells when the pale yellow MTT dye is acted on by the mitochondrial dehydrogenase enzyme which, in turn, cleaves the tetrazolium ring of MTT resulting in the formation of insoluble and impermeable dark purple colored formazan crystals [16].

METHODS

Cell Line and Culture

HT-29 cell lines used in the experiment were obtained from National Centre for Cell Sciences, Pune. The cells were maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum,

penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

PROCEDURE - MTT ASSAY: MOSMANN, 1983

Cells (1 × 10^5/well) were plated and maintained at 37°C with 5% CO_2 condition. After attaining confluent stage, samples (Vilazodone and 5-FU) of various concentrations were added and incubated for 24 h. The sample was removed from the well after the incubation period, and the cells were bathed with phosphate-buffered saline (pH 7.4) or MEM without serum. MTT was added to the cells at a concentration of 100 µl/well (5 mg/ml) and incubated for 4 h. 1 ml of dimethyl sulfoxide (DMSO) was added after 4 h of incubation in all the wells. Keeping DMSO as the blank all the test samples were measured with an ultraviolet - spectrophotometer at an absorbance of 570 nm. Readings were plotted and the inhibitory concentration at 50% (IC50) was determined graphically [17]. The percentage (%) cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{of control cells}} \times 100$$

$$\% \text{ Cytotoxicity} = 100 - \% \text{ cell viability}$$

Graphs were plotted to represent percentage of cell cytotoxicity at Y-axis against the concentration of the sample in X-axis.

RESULTS

The percentage cytotoxicity of vilazodone hydrochloride and 5-FU at various concentrations is as shown in Tables 1 and 2, respectively. It implies that as the dose increases, the percentage viability decreases. Accordingly, the IC50 value of vilazodone hydrochloride was espied at 28.5 µg/ml and depicted in Fig. 1 and the IC50 value was 5-FU ascertained at 12.8 µg/ml and depicted in Fig. 2.

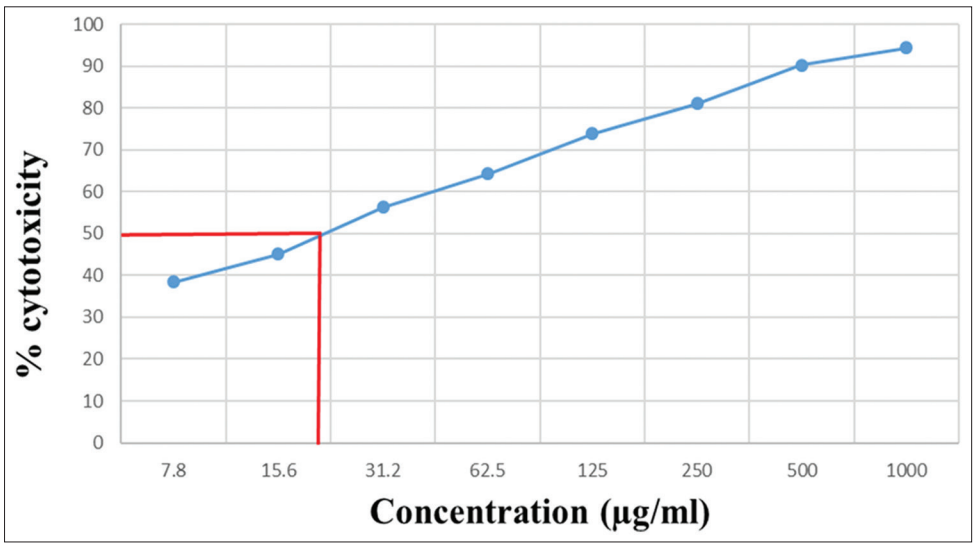

Fig. 1: IC-50 value (Inhibitory concentration at 50%) of vilazodone hydrochloride

Table 1: Cytotoxic effect of vilazodone on HT-29 cell line

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)	Cell death (%)
1	1000	Neat	0.029	5.66	94.34
2	500	1:1	0.050	9.76	90.24
3	250	1:2	0.097	18.94	81.06
4	125	1:4	0.134	26.17	73.83
5	62.5	1:8	0.183	35.74	64.26
6	31.2	1:16	0.224	43.75	56.25
7	15.6	1:32	0.281	54.88	45.12
8	7.8	1:64	0.315	61.52	38.48
9	Cell control	-	0.512	100	0

O.D : Optical density

Table 2: Cytotoxic effect of 5-FU on HT-29 cell line

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)	Cell death (%)
1	1000	Neat	0.011	4.9	95.1
2	500	1:1	0.042	8.20	91.80
3	250	1:2	0.080	15.62	84.38
4	125	1:4	0.104	20.31	79.69
5	62.5	1:8	0.148	28.90	71.10
6	31.2	1:16	0.176	34.37	65.63
7	15.6	1:32	0.212	41.40	58.60
8	7.8	1:64	0.72	53.12	46.88
9	Cell control	-	0.512	100	0

5-FU: 5-flurouracil, O.D : Optical density

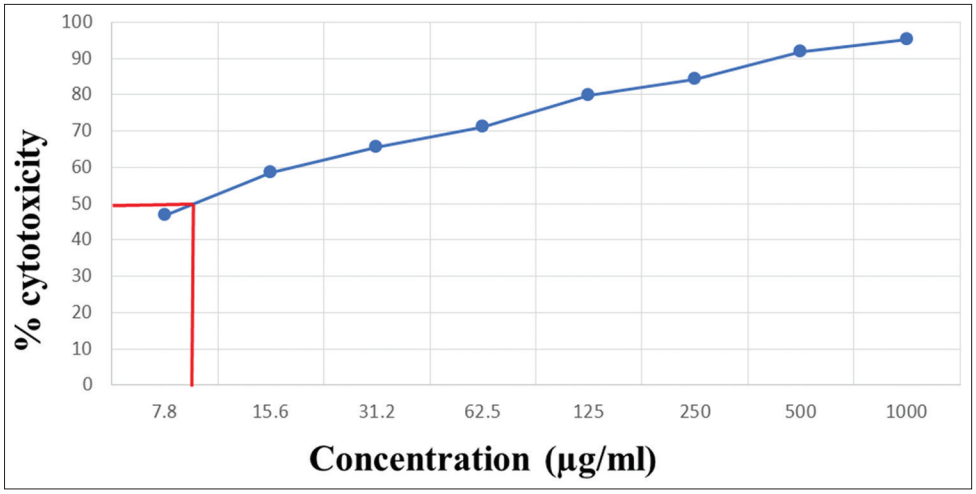

Fig. 2: IC-50 value (Inhibitory concentration at 50%) of 5-fluorouracil

DISCUSSION

Apart from modulating neurotransmission, antidepressant drugs have been noted to comport a range of effects, namely, immunomodulation, anti-oxidant, neuroprotection, analgesic, and anti-inflammatory activities, which are due to its influence on glial cell function [18,19]. At present, the scientific community has thrown its light to know about the possible anticancer activity of SSRI's and found that these drugs alter the pivotal cellular mechanism of carcinogenesis [20]. The SSRI's sertraline and paroxetine have been explorated to have anticancer property by arresting the cell division at G0 and G1 phase and by inflating caspase-3 activity in the colon cancer cells [20-22]. Fluoxetine exhibited features of cell death and anti-proliferation of *in vitro* neuroblastoma cells (SKNAS), Burkitt's lymphoma and breast cancer cells (T47D) [6,23,24]. Further, it was also added that fluoxetine-induced changes in the mitochondrial membrane permeability of human epithelial ovarian cells which resulted in the production of reactive oxygen species and ultimately cell death [24-27]. Correspondingly, our study also infers that vilazodone hydrochloride has got the good cytotoxic effect which is comparable and nearly equipotent with 5-FU in colon cancer cell lines which could be by activation of the Caspase-3 enzyme.

CONCLUSION

Our study advocates that the novel SSRI, vilazodone hydrochloride has potential cytotoxic effect against the colon cancer cells. As therapy with SSRI is usually indicated in cancer patients, the benefit of using vilazodone hydrochloride in colon cancer patients coupled with depression appears to be attractive.

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CONFLICTS OF INTEREST

No conflicts of interest.

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