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INVESTIGATION ON SYNERGISTIC EFFECT OF DOXORUBICIN IN COMBINATION WITH HISPOLON AND D-LIMONENE AGAINST HUMAN COLON CANCER CELLS

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ABSTRACT

Background: Multi genetic alterations lead to cancer progression, it is seldom possible to treat with monotherapy. Thus, nowadays, clinicians are using combinational drug therapy for cancer. As a synthetic drug treatment displays several adverse effects, the combination of natural products with synthetic drug therapy gained its importance.

Methodology: In this current study, we investigated the combination anticancer effect of Doxorubicin, Hispolon, and D-Limonene against colorectal cancer cell lines was determined by MTT assay. The antioxidant enzyme activity of various combinations of tri-drug on COLO-205 and HCT-116 cells was performed. The activity of antioxidant enzymes, SOD, Catalase, Gpx and GST was measured. The free radical scavenging activity of DOX, HIS and D-LIM was performed on DPPH, ABTS and nitric oxide free radicals measured. The gene expression levels of NF-κB P65 and BAX upon induction of DOX, HIS and D-LIM to the COLO 205 & HCT 116 cells was determined by Western blotting method.

Results: The cell viability, enzymatic, time and dose-dependent cytotoxicity assays demonstrated the anticancer effect of our tri drug-combination against COLO-205, HCT-116 cell lines. Besides, western blot results of tri drug-combination treatment displayed significantly decreased the levels of NF-κB P65.

Conclusion: Therefore, the current study results revealed that, the tri drug-combination of DOX, HIS and D-LIM treatment beneficial in colon cancer therapy.

Keywords: Doxorubicin, Hispolon, D-Limonene, NF-κB P65, combination, synergy, colon cancer, Apoptosis, COLO-205, HCT-116

INTRODUCTION

Genetic alterations leading to dysregulation of biological pathways that can cause severe disease conditions. Cancer is one such dreadful disease challenging the world, even in the 21st century. Among the various cancer types colorectal cancer (CRC) is one of the alarming diseases, which is the third most commonly occurred cancer in humans. Chemotherapy is predominantly used treatment in CRCs as it is normally diagnosed in the late stages when patients frequently present with distant metastases [1-2]. Doxorubicin (DOX) has been the most frequently used chemotherapeutic drug that primarily inhibit the action of topoisomerase I and II and eventually induce programmed cell death [3]. However, long-term usage of DOX may lead to severe side effects such as myocardial damage, alopecia, severe hair loss, etc. [4]. Hence developing the anticancer drugs with no/minimum side effects would over-come these issues.

D-limonene (D-LIM), a non-nutrient dietary component that is a major constituent of several citrus and other essential oils has been demonstrated as a potential anticancer natural compound with minimum cytotoxicity. Dietary limonene is known to inhibit the development of ras oncogene-induced mammary carcinomas in rats [5-6]. In addition, D-LIM has shown to sensitize a variety of human cancer cell lines for apoptosis induced by different anti-cancer drugs [7]. Besides, several studies exemplified the anticancer activity of Hispolon (HIS) a

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natural compound in various types of cancers. HIS is known to display its anticancer effect via inhibition of cell growth, induction of cell cycle arrest, and suppression of metastasis [8-9]. It is also proven that, to potentiate the apoptotic effects of TRAIL through downregulation of anti-apoptotic proteins and upregulation of death receptors linked with CHOP and pERK elevation and is a potent suppressor of the NFkB pathway which promotes cell proliferation and inhibits apoptosis [10]. Current cancer therapies usually targeting two or more cellular processes, therefore, combination drug therapy is now a prominent approach in cancer treatment. A recent study using D-LIM in combination with NVP-BEZ-235 demonstrated the synergistic anticancer activity against colon cancer cell lines [11]. It has previously been demonstrated that the synergy of natural compounds, D-LIM and HIS can significantly increase the anticancer activity against colon cancer cell lines [12]. It is apparent that targeting more than one critical molecular process with a combination of a chemically synthesized compound with natural compounds, allowing the delivery of lower drug dosages with different modes of activity and may reduce the cytotoxicity.

Therefore, the current study is aimed to demonstrate the synergistic anticancer effect of DOX, HIS, and D-LIM (Tri-drug) in COLO-205, HCT-116 cell lines. The antiproliferative and antioxidant enzyme activity of our tri-drug displayed a significant decrease against colon cancer cell lines. Besides western blot analysis of NF-κB P65 and BAX revealed the anticancer effect of tri-drug.

MATERIALS AND METHODS

Doxorubicin, D-Limonene, and Hispolon were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium dodecyl sulfate, Tris, Tween-20, MTT, Dimethyl sulphoxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). The human colon cancer cell lines (COLO 205) and (HCT 116) was gifted from Dr. Royal Suresh (IIT, Chennai). RPMI 1640, McCoy's 5a, Fetal bovine serum (FBS), Penicillin 5 mg/ml, Streptomycin 5 mg/ml, Neomycin 10 mg/ml antibiotic mixture 100 X, 0.25% Trypsin-EDTA was purchased from GIBCO Ltd (Life Technologies TM., Grand Island, NY). Enzyme standards were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd, India). Anti-rabbit polyclonal antibodies against NF-κB P65 p65, BCL2, GAPDH, and goat anti-rabbit IgG, goat anti-mouse IgG were procured from (Cell Signalling Technology, Danvers, USA). Protein assay kit from Bio-Rad Laboratories, Inc. and ECL prime Western blotting substrate were purchased from (GE Healthcare Life Sciences, MA, USA), Cell lysis buffer (10X) was purchased from Cell Signaling Technology, Inc. Other chemicals of analytical grade were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Determination of cell viability by MTT assay

The antiproliferative effect of DOX, HIS, and/or D-LIM based on the cell viability of colon cancer cells by MTT assay. Precisely, cells were seeded at a density of 1 X 10^4 cells per well in 96-well plates. The cells were further treated with DOX, HIS, and D-LIM independently and with different combinations, incubated for 24 and 48 hrs. To determine the combined effect, the cells were treated with the drugs concurrently as well as sequentially [13]. In concurrent treatment, the cells were treated with D-LIM and HIS, DOX with D-LIM, and HIS for 48 hrs continuously. While in sequential treatment, cells were pre-treated with either LIM or HIS or DOX for 12h, followed by the exposure to the other agent for a total of 48 hrs. Cells were treated with increasing doses of DOX (100-300 nM), D-LIM (100-3000 µM), and HIS (10-300 µM) for 48 hrs, and viability was determined. Further, the cells were incubated with the MTT reagent 5 mg/ml in DMEM for 3 hrs at 37^{o}C, followed by solubilization of the formazan crystals with DMSO for 10min. Absorbance was measured at 570 nm using a microplate analyzer (iMark Microplate Absorbance Reader, BioRad). The percent of cell viability was calculated using the following equation [13].

% of cell viability = (OD sample)/ (OD control) ×100

% Inhibition of proliferation = % untreated cell viability (100) - % drug-treated cell viability

Antioxidant enzyme assays

6 x 10^6 cells were plated in 100 mm cell culture plate, and the cell cultures were treated with DOX, HIS, and/or D-LIM independently and in different combinations, incubated further for 24 hrs. The compound concentrations as followed in the above MTT assays. Cells were treated like 1) Con. 2) DOX 80 nM, 3) 100 HIS, 4) D-LIM 1000, 5) HIS 100 µM + D- LIM 1000 µM, & 6) HIS 100 µM + D- LIM 1000 µM + DOX 100 nM above. The treated cells were collected and dissolved in phosphate buffer saline, pH 7.4, and sonicated. The sonicated samples were centrifuged (Eppendorf, 5804R) at 14000 rpm for 10 min at 40^{o}C. The resulted supernatant was used for enzyme assays. Catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) activities were determined by using standard protocols.

Catalase assay

The catalase enzyme activity was assayed using the standard biochemistry protocol described previously. Briefly, prepared a 1 ml solution containing 30 mM H_2O_2 in 50mM phosphate buffer pH 7.9, 10 µl of the sample. The change in the OD was monitored for three minutes at 30 secs interval. The activity of catalase in the diluted sample is calculated using the first-order reaction [14].

K30 = (2.303/30)* log (A1/A2), A1: highest OD value, A2: lowest OD value

The activity of catalase was expressed as K30/mg protein (one unit is the amount of enzyme that consumes

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

1µmole of hydrogen peroxide/min).

Glutathione S-transferase activity

In brief, the reaction was prepared using 924 µl of phosphate buffer, 33.3 µl of CDNB solution and 10 µl of the sample and the reaction was initiated by adding 33.3 µl of GSH solution. The change in the OD was monitored at 340 nm for 5 min at one min intervals. Phosphate buffer served as a blank. The activity of GST was expressed as µmoles of CDNB-GSH conjugate formed/min/mg protein [15].

Glutathione peroxidase (GPx) activity

To perform the GPx activity, prepared a 1 ml reaction mixture with 660 µl of phosphate buffer, 1U of glutathione reductase, 100 µl of GSH solution, 100 µl of NADPH solution, and incubated for 5 min to allow H2O2 free oxidation of NADPH to obtain a baseline at 340 nm. The reaction was initiated by adding 10 µl of the sample and 100 µl of H2O2 and change in the absorbance was monitored at 340 nm for 5 min at one min intervals. The activity of GPx was expressed as µmoles NADPH oxidized/min/mg protein [16].

Superoxide dismutase (SOD) activity

The SOD enzyme activity was measured by preparing 1ml reaction mixture containing 984 µl of solution A, 16.6 µl of solution B, mixed well and the change in the absorbance were measured at 550 nm for 5 min at one min intervals. Water is used as a blank. The activity of SOD in the samples was determined by calculating the percent inhibition of cytochrome C reduction. The percent inhibition of cytochrome C was expressed in terms of the SOD activity using known amounts of SOD standards. One unit of SOD is defined as the amount of enzyme required to inhibit the rate of decrease of cytochrome C by 50% in a combined system with xanthine and xanthine oxidase at pH 7.8 in a 1 ml reaction volume. The activity of SOD in the sample was expressed as U SOD/mg protein [17].

Free Radical Scavenging Assay

6 x 10^6 cells plated in 100 mm cell culture plate were treated with DOX, HIS and/or D-LIM independently and in different combination as mentioned for the above experiments. After the 24 hrs of incubation, the cells were collected, dissolved in phosphate buffer saline, pH 7.4 and sonicated. The sonicated samples were centrifuged (Eppendorf, 5804R) at 14000 rpm for 10 min at 4ºC. The supernatant was used for DPPH, ABTS.+, and NO radical scavenging activity. Cells were treated like 1) Con. 2) DOX 80 nM, 3) 100 HIS, 4) D-LIM 1000, 5) HIS 100 µM + D- LIM 1000 µM, & 6) HIS 100 µM + D- LIM 1000 µM + DOX 100 nM.

DPPH radical scavenging

The activity of DOX, D-LIM, & HIS in case of scavenging of DPPH radicals was analyzed. The assays were performed using the reaction mixture containing freshly prepared 100 µM DPPH and different concentrations of DOX, D-LIM & HIS, and incubated at ambient temperature for 20 min in the dark and the absorbance was measured at 517 nm. DOX, D-LIM & HIS would donate an electron to DPPH radical and do scavenge it resulting in the bleach of the purple color to colorless [18].

ABTS.+ radical scavenging

To demonstrate the scavenging activity of model stable-free 2, 2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) (ABTS.+) radicals, ABTS.+ were generated by preparing a stock solution of 7 mM ABTS and 2.45 mM ammonium persulfate and incubated for 16 h in the dark at room temperature. Generated ABTS.+ stock solution was diluted slowly with PBS till the absorbance of 0.70 reached at 734nm. The working standard solution thus formed was mixed with different concentration of DOX, D-LIM, & HIS, incubated for 6 min at room temperature in the absence of light and optical density values were taken at 734nm, using PBS as a control [18].

Nitric oxide radical scavenging

A 100µl of reaction mixture containing different concentrations of DOX, D-LIM & HIS, and 10 mM sodium nitroprusside prepared and incubated for 150 min at room temperature. 100µl of Griess reagent (1 % sulphanilamide and 0.1 % N-naphthylethylenediamine dihydrochloride in 2 % H3PO4) were added to the reaction mixture. The absorbance of each reaction was measured at 550nm using a microplate analyzer (iMark Microplate Absorbance Reader, BioRad). A control sample was made with the equivalent amount of all the vehicles except the test compound [19].

Western blot analysis

Cell lysate preparation

The seeded colon cancer cells (1.5×10^6) cells were treated with DOX, HIS, and D-LIM independently and in different combinations as mentioned above, incubated for 48 h. The incubated cells were washed twice with cold PBS buffer (pH 7.4) and lysed using cold RIPA buffer (40-100 µl) (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 0.25% sodium deoxycholate) containing protease inhibitor cocktail (Roche, Indianapolis, IN, USA). The volume of RIPA buffer (50-100 µl) was adjusted according to the confluence of the cells, dropping it evenly over the whole plate and incubated for 10 min on ice. The cells were scraped from the plate and the cell lysate was centrifuged (Eppendorf, 5804R) at 14000 rpm for 10 min at 4ºC. The protein concentrations of the supernatants were determined using a Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany) kit method.

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

The protein samples were loaded at 10 % SDS acrylamide gel (Laemmli, 1970). The resultant SDS gels were equilibrated with transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3-8.5) for 10 min. The equilibrated gels were further used to transfer the proteins onto Polyvinylidene fluoride membrane (PVDF) by semi-dry electroblotting. The blotted PVDF membranes were blocked with fresh 5% non-fat dried milk (Cadburys, Marvel skimmed milk) in Tris-buffered saline (TBS) with 0.5% Tween-20 (Sigma) and washed in TBST (10 m Tris, 150 mM NaCl, 0.5% Tween-20, pH 7.4). The blocked membranes were further incubated with 10 ml of primary antibodies (Santa Cruz Biotech, 1:1000 dilutions in TBST/ 1:1000 dilutions in 5% BSA) overnight at 4ºC. The membrane was again washed and incubated with corresponding secondary antibodies (Santa Cruz Biotech, 1:5000 dilutions in TBST) for 1h at room temperature followed by washing with TBST.

Finally, the membranes were washed (3 X 5 min using 1X TBS and 3X5 min using 1X TBST) and bound antibody was detected using enhanced chemiluminescence (ECL, Bio-Rad) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was carried out using 'IBM SPSS'. All experiments were performed in triplicate and repeated three times. Values were expressed as mean ± standard deviation (SD) considered significant.

RESULTS AND DISCUSSION

In case of CRC treatment the clinicians frequently use anthracyclines like DOX, which induces life-threatening cardiomyopathy and congestive heart failure due to the increased uptake of this drug [4]. Therefore, current cancer treatment strategies favour combination therapies which would offer low toxicities to the cancer patients. Our previous studies with natural compounds such as D-LIM, HIS have proven to be the most reliable synergistic source of new and effective anticancer agents with minimum side effects [12]. Hence, the present study focused on combinational therapy of natural products (D-LIM, HIS), and a chemically synthesized compound (DOX) in colon cancer cells. Tumor resistance to apoptotic cell death is an important hallmark of cancer and contributes to increased survival of cells that have acquired oncogenic mutations, eventually leading to uncontrolled cell proliferation, invasion, metastasis, angiogenesis, and chemoresistance. To demonstrate the cell proliferation upon administration of our tri-drug we performed dose and time-dependent assays.

The independent IC_{50} values of COLO 205 for DOX, HIS, and D-LIM compounds recorded as 80.45 nM, 120.98 µM, and 1120.36 µM respectively; whereas in case of HCT- 116 cells, the IC 50 values are as follows, 90nM, 150 µM, and 1344 µM respectively. The combination of varying doses of HIS + LIM + DOX produced maximum antiproliferative activity at 48h when compared with independent compound treatment in both the cell lines such as shown in Fig. 1. The combination of HIS + LIM, HIS + LIM + DOX at a concentration of 100 µM + 1000 µM , 100 µM + 1000 µM + 100 nM respectively produced highest antiproliferative activity in COLO-205; whereas in HCT-116 the combination at a concentration of 130 µM + 1500 µM, 130 + 1500 µM + 130 nM respectively showed the highest activity. While testing combination affects the cells were exposed to drugs in different formats as mentioned above.

The increased antiproliferative effect was observed when the cells were exposed to both drugs simultaneously than sequential treatment (70% &84% vs. 60% & 70% or 55% & 65% for COLO-205) and (75% & 86% vs. 53% & 68% or 48% 62% for HCT-116) (Fig. 2). D-LIM, HIS, & DOX exhibited a significant suppressive effect on COLO 205 and HCT 116 viability in a dose-dependent manner. Such an inhibitory activity of cell viability was enhanced significantly when the COLO 205 and HCT 116 cells were exposed to D-LIM+ HIS, & DOX and D-LIM + HIS + DOX combinations.

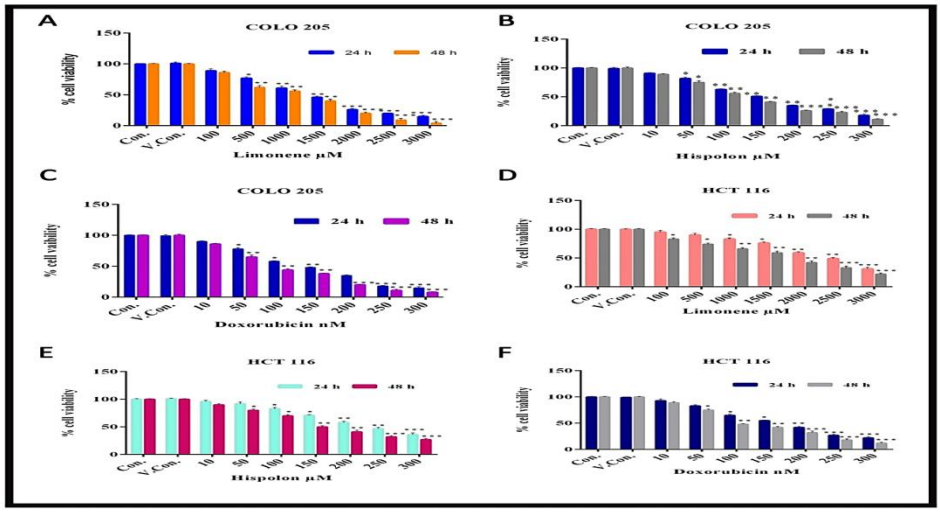

Figure-1: Effect of D-LIM, HIS, and DOX on cell viability of COLO 205 cells at 24h and 48h

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

The independent IC_{50} values of COLO 205 for DOX, HIS, and D-LIM compounds recorded as 80.45 nM, 120.98 µM, 1120.36 µM, 90nM, 150 µM, and 1344 µM respectively.

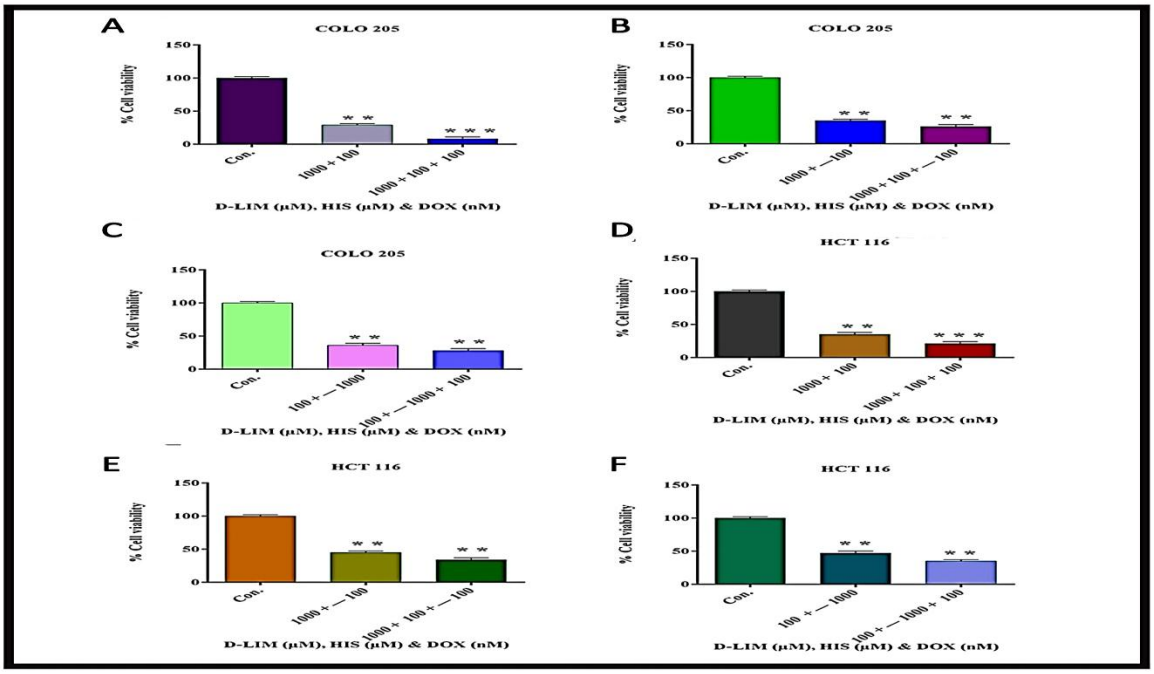

Figure-2: Combination effect of D-LIM, HIS, and DOX at 48 h

The combination of HIS + LIM, HIS + LIM + DOX at a concentration of 100 µM + 1000 µM , 100 µM + 1000 µM + 100 nM respectively produced highest antiproliferative activity in COLO-205. Both drugs simultaneously than sequential treatment (28% & 8% vs. 35% & 25% or 36% & 27% for COLO-205) and (35% & 21% vs. 45% & 34% or 48% & 35% for HCT-116).

Effect of D-Lim, His, and Dox on antioxidant enzymes

The antioxidant enzyme activity was performed to demonstrate the effect of various combinations of tri-drug on COLO-205 and HCT-116 cells. The activity of SOD, Catalase, GPx and GST was measured. The cells treated with D-LIM/HIS displayed significant raise in antioxidant activity, where as DOX treated cells displayed a significant fall compared to the control. However, the combination of HIS 100 µM + D- LIM 1000 µM showed increased antioxidant activity than tri-drug combination (HIS 100 µM + D- LIM 1000 µM + DOX 100 nM) due to the presence of DOX (Fig. 3A), in case of GPx, GST and SOD (Fig. 3 B,C, & D) same trend following like Fig. 3 A.

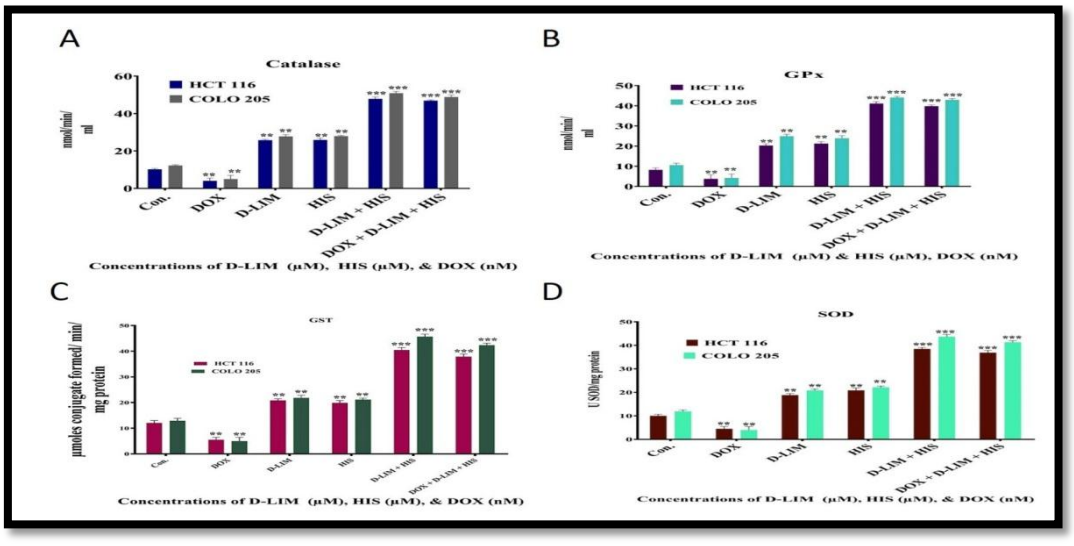

Figure-3: Effect of D-LIM, HIS and DOX on CAT, GPx, GST, & SOD in COLO-205 and HCT- 116 cells

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

Fig. 3 clearly indicated that when compared to untreated controls, there was a significant raise in enzyme activity of HIS and D-LIM treated cells, while there was decreases in DOX treated cells, but tri-drug combinations slightly fall compared to D-LIM + HIS drug combination due to presence of DOX in COLO-205 and HCT- 116 cells. Compared to HCT 116 cells, there was a significant raise in all above enzyme activities in COLO 205 cells.

Effect of D-Lim, His, and Dox on free radicals

Scavenging of stable-free radicals by D-LIM, HIS, and DOX was done by bleaching a model stable-free radical derived from ABTS i.e. ABTSþ and DPPH radical. The results showed that there was concentration-dependent bleaching of ABTSþ by D-LIM, HIS, and DOX. In case of DPPH assay, as shown in Fig. 4C, there was concentration-dependent bleaching of radical up to D-LIM + HIS concentrations of both the compounds after which there was an increase in the absorbance. Further, change in spectral behaviour showed that there is a formation of a new complex that has absorption maxima at 425 nm and a shoulder at 517 nm at which DPPH has absorption maxima. This possibly may be responsible for the increase in absorption after D-LIM + HIS + DOX concentration. Overall, antioxidant enzyme assays were significantly inhibited 75% with D-LIM+ HIS and 65% with D-LIM + HIS + DOX combination (Fig. 4).

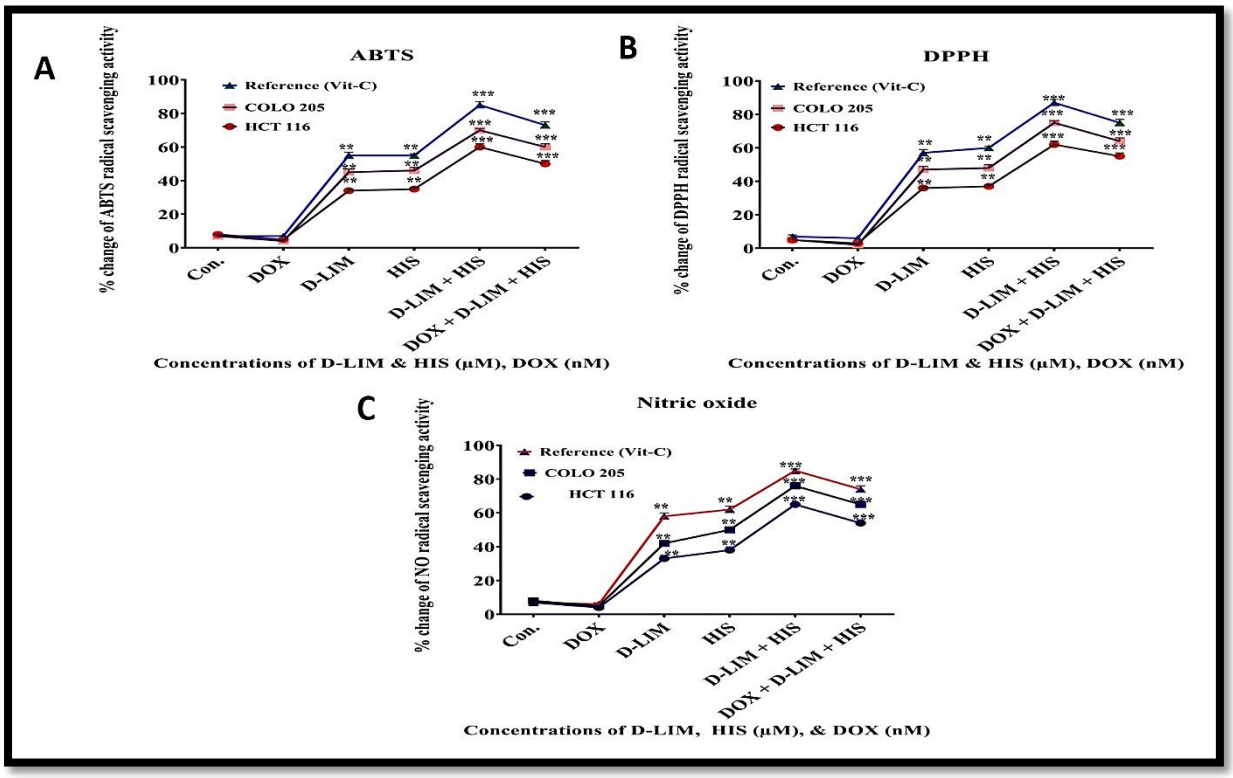

Figure-4: Effect of D-LIM, HIS and DOX on DPPH radical scavenging activity in COLO-205 and HCT-116 cells

Fig. 4 A clearly indicated that when compared to controls, there was a significant increases in radical scavenging activity of HIS and D-LIM treated cells, while there was more increases in DOX treated cells, but tri-drug combinations fall down compared to D-LIM + HIS drug combination due to presence of DOX in tri-drug combination, same trend following in case of ABTS, & DPPH (4B, & 4C) in COLO-205 and HCT- 116 cells. Compared to HCT 116 cells, there was a significant raise in all above nitric oxide, ABTS, & DPPH radical scavenging activity activities in COLO 205 cells.

DPPH radical scavenging assay

DPPH* scavenging assay, considered as one of the standards and easy colorimetric method is consistently used to assess the free radical scavenging potentials of antioxidant molecules. D- LIM, HIS, or/and DOX was assessed for its DPPH* scavenging activity. The antioxidant activity of D- LIM, HIS and D- LIM, HIS and DOX were found to increase in a concentration-dependent manner wherein, inhibition of 74.25±0.27 and 64.01±0.58 % were found Fig. 4A. The results revealed that combination exposure of both drugs at the same time point exhibited the highest inhibition in the free radical scavenging assay, and different protein analysis

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

like NF-κB P65 protein expression was gradual decreases and the BAX protein expression was gradually increases dose and combination dependent manner D-LIM, HIS or/and DOX.

ABTS radical scavenging assay

ABTS*+ is a well-known reactive radicals that can lead to cell damage. In this assay, ABTS•+ oxidizes to ABTS*+ chromophore on reaction with potassium persulphate and reduced by the antioxidant sample. The results justify that D- LIM, HIS, or/and DOX was assessed for its ABTS*+ scavenging activity. The antioxidant activity of D- LIM, HIS and D- LIM, HIS and DOX were found to increase in a concentration-dependent manner wherein, inhibition of 71.25±0.27 and 60.01±0.58 % were found Fig. 4B.

NO radical scavenging assay

NO* is an effective pleiotropic mediator of physiological processes such as neuronal signaling, inhibition of platelet aggregation, smooth muscle relaxation, and regulation of cell-mediated toxicity. D- LIM, HIS, or/ DOX were assessed for its NO* scavenging activity, with Vit-C taken as a reference. Vit-C shows higher activity of 83.48 % at a final concentration, as illustrated in Fig. 4C. The Antioxidant activity of D- LIM+ HIS and D- LIM + HIS + DOX the samples were found to increase in a concentration-dependent manner wherein, almost comparable inhibition of 73.54±0.42 and 63.63±0.31 % were found in the case of D- LIM+ HIS + DOX and AA respectively at the highest evaluated final concentration.

The effect of tri-drug on apoptosis markers

NF-κB P65 is a key player in cancer cell progression, proliferation, invasion, angiogenesis, metastasis, and inflammation. To provide the effect of tri-drug on the key apoptosis markers we performed Western blotting to demonstrate the expression levels of NF-κB P65, and BAX upon induction of DOX, D- LIM, and HIS to the COLO 205 & HCT 116 cells **(**Fig. 5). We observed an increased level of BAXin cells treated with combinations of D-LIM+HIS and D-LIM+HIS+DOX, than individual treatments (Fig. 5). However, the expression of NF-κB P65 showed a decreased levels of expression was observed upon treatment either LIM+HIS or D-LIM+ HIS+DOX. Overall, our results have shown that cells treated with HIS, D-LIM, and DOX significantly inhibited the expression of p65 protein in COLO 205 cells, whereas, HIS, D-LIM, and DOX less significantly inhibited the expression of p65 protein in HCT-116 cells compared to COLO 205. HIS, D-LIM, and DOX gradually increased expression of BAX protein in COLO 205 cells, whereas, HIS, D-LIM, and DOX slightly increased expression of BAX protein in HCT-116 cells compared to COLO 205.

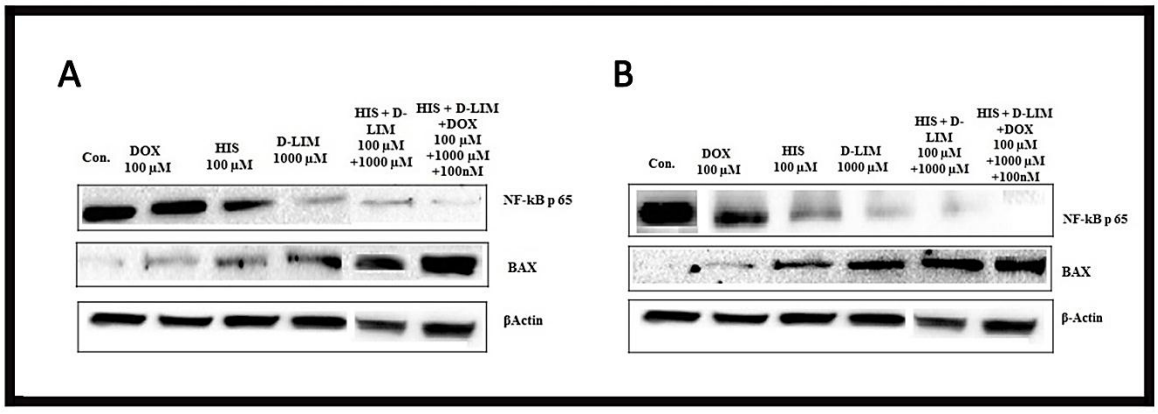

Figure-5: Effect of HIS, D-LIM, and DOX on p65 NF-kB & BAX expression

Fig. 5A & B. Western blot analysis of NF-κB P65 and BAX family mediated apoptosis markers protein expression, performed in COLO-205 and HCT-116 cells at 48 hrs. Cell lysates were prepared and the proteins were separated on SDS-polyacrylamide gel and transferred onto PVDF membranes. The membranes were probed with the indicated antibodies. β-actin was used as a control for protein loading. The results shown here were from three independent experiments.

Fig. 5A. Cells treated with HIS, D-LIM, and DOX significantly inhibited expression of p65 protein in COLO 205 cells, whereas, HIS, D-LIM, and DOX less significantly inhibited expression of p65 protein in HCT-116 cells compared to COLO 205.

Fig. 5B. HIS, D-LIM, and DOX gradually increased expression of BAX protein in COLO 205 cells, whereas, HIS, D-LIM, and DOX slightly increased expression of BAX protein in HCT- 116 cells compared to COLO 205.

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

CONCLUSION

In conclusion, these observations may be of value while carefully considering the combination of therapies in a clinical setting and beneficial in colon cancer therapy. Together, our results suggest that concurrent treatment of D-LIM, HIS & DOX shown a synergistic effect in human colon cancer COLO 205 & HCT 116 cells, and these drugs may have future clinical utility for treating colon cancer with minimum side effects.

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