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Original article

THE EFFECT OF OXIDATIVE STRESS ON EPIGENETIC REGULATION OF GENE EXPRESSION IN INTESTINAL CANCER

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Abstract

Background. Cancer is caused by the accumulation of genetic and epigenetic changes in the oncogenes and tumor-substituting genes. The discovery of the epigenetic pathways that control gene expression has substantially progressed in understanding cancer biology.

Purpose. Investigation of epigenetic effects of oxidative stress on the regulation of gene expression in intestinal cancer.

Methods. DLD-1 colorectal adenocarcinoma cells were treated with the oxidant 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) and the antioxidant hydroxytyrosol. Cellular viability, apoptosis, reactive oxygen species (ROS) production, and expression of key genes (CDH17, CXCL12, TGFB1) were examined.

Results. Hydroxytyrosol treatment improved DLD-1 cell viability in a time-dependent manner. AAPH-induced oxidative stress decreased cell viability and increased apoptosis and ROS production. Combination treatment with hydroxytyrosol and antioxidant enzymes enhanced these effects. Oxidative stress altered the expression of CDH17, CXCL12 and TGFB1, with TGFB1 showing a dose-dependent increase.

Conclusion. Oxidative stress influences epigenetic regulation of gene expression in intestinal cancer cells. Combination therapies targeting both oxidative stress

pathways and epigenetic mechanisms may be a promising approach for colorectal cancer treatment.

Keywords: epigenetic; gene expression; DLD-1; AAPH; oxidative stress; hydroxytyrosol

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Introduction

The discovery of the epigenetic pathways that control gene expression has substantially progressed in understanding cancer biology [1]. Cancer is caused by the accumulation of genetic and epigenetic changes in the oncogenes and tumor-substituting genes. Epigenetic variations influence human appearance, behavior, stress responses, disease susceptibility, and even longevity by affecting the individual's gene expression [2]. Cancer is a broad and heterogeneous term that defines a wide range of diseases affecting any part of the organism [3]. The disorders are known as multi-phase diseases characterized primarily by abnormal cells in tissues whose unconstrained development is beyond their limits. They can spread to other body parts by invading organs [4]. A lack of balance between reactive Oxygen Species Production (ROS) and antioxidant enzyme responses is known as oxidative stress [5].

Colorectal cancer (CRC) has a rising prevalence and mortality rates in various countries as a global public health issue. Different figures show that in countries with inadequate healthcare, the CRC incidence is rising. More complementary strategies have been used to recognize genes usually methylated for colon cancer. Fifty-six lines were analyzed for colon cancers, 22 non-cancerous colonic humans, and 14 lines of human colon cancer cells [6].

CRC is the third leading cause of male and female cancer mortality [7]. The human colorectal mucosa has been shown to have high rates of various OS markers, such as reactive oxygen species (ROS) that may also be involved in CRC formation [8]. OS is known to have a pathogenic function in producing ulcerative colitis (UC) and CCC [6]. Sub-oxide radicals (O_2), peroxide (H_2O_2, H_2O), and hydroxyl radical (HO) are formed in such inflammatory conditions (ROS) at a higher concentration that they accumulate protein dysfunction and DNA damage that cause gene mutations [9].

The third most prevalent disease is Colorectal Cancer (CRC), accounting for around 9% of all cancer incidences worldwide. Additionally, the 4th most

common cause of cancer mortality is CRC [1]. The three central routes are the CRC pathway, the microsatellite instability path, and the clamped pathway; pathogenesis depends on different ways [10]. Most chromosomal chromosome segregation, telomere stability, and damage response characterize the CIN pathway. Various risk factors contribute to the onset and development of colorectal cancer, including environmental conditions, inactivity, smoking, drinking, diet, and obesity [6].

A tumor suppressor gene that plays a crucial role in regulating and propagating the intestine is a candidate tumor-specific transcription factor, caudal type homeobox-1. In colorectal cancers, colon cancer-derived cell lines are aberrantly dysregulated by encouraging hypermethylation [11]. The study explored epigenetic alterations in colorectal cell cancer that occur in reactive oxygen species (ROS) [12]. The effect of oxidative stress on tumor suppressor gene transcription is mainly unknown. The aimed of the study oxidative stress was examined for the epigenetic consequences of gene expression regulation in intestinal cancer.

Materials and methods

Materials

2 azobis poisons, 2(AAPH), 5-(- 6)- chloromethyl 20, 70-dichlorodihydrofuorescein ester, and vital reagents like Turf antibodies utilized. The changed Bird's medium (DMEM), fetal cow-like serum, and glutamine were used in this review, given in nitrogen.

Cell culture

Human colorectal cancer cells were grown in a single layer at 37 °C in a 5 percent CO_2 incubator from the National Cancer Research Centre. The cells were developed in DMEM with 10% FBS, two mmol L^{-1}, L-glutamine, and 25 mg mL^{-1}, as defined in [13].

Cell treatment

DLD1 cells have been treated at (50) μmol L^{-1} levels with AAPH for 24 and 48h; cells with 50 μmol L^{-1} AAPH have hatched for 24 hours for both Grass and catalase contemplates. The McCoy Adjusted 5A medium contains 10% FBS, 0.1 mmol superfluous amino acids, and 50 units for every mL of gentamycin. Fifty units for each mL of penicillin, undyingly non-harmful human colon epithelial CRL1807 developed. In a 5% CO_2 hatchery, cells were held at 37°C.

Cell viability assays

The suitability of the cells was estimated by the [13] strategy 3-(4,5-dimethylthiazol); momentarily, cells treated with a 50 mL MTT arrangement (5 mg mL^{-1}) were brooded with 5% CO_2 for four hours at 37°C toward the finish of AAPH

medicines. The MTT arrangement was isolated, and the organizing arrangement was disintegrated in the designing system utilizing 100 mL DMSO. The harvest retention was estimated using a 560 nm frequency multi-well spectrophotometer (Apparition Max 190; Atomic Gadgets, Sunnyvale, CA). As OD esteems, the reasonability of the cells communicated. The discoveries are estimated in the control societies as the level of retention.

Apoptosis analysis

As indicated by the maker definition to evaluate the level of apoptotic cells, the twofold shading of the Annexin V-fluorescein Isothiocyanate (FITC)/propidium iodide (BD Pharmingen). In DMEM well plates, the DLD1 cells were developed to convert 80 to 90 percent. Cells in this way go through AAPH, Turf, or catalase treatment. The cells were washed with PBS toward the finish of the hatching cycle, processed with 0.25% trypsin/EDTA, and washed with PBS. The limiting cushion for the cells was 500 mL, and Annexin V-FITC/PI was added. Cytometry of stream was utilized to count the cells at room temperature after 5 min incubation.

ROS assay

Carboxy-H2DCFDA and oxidized carboxy-DCFDA have been utilized for the diclorofuoresce in (DCF) test. The preloaded Fluorescence with Carboxy-H2DCFDA was standardized to screen cell count, shading, and ester cleavage contrasts between the distinctive treatments bunches by preloading Carboxy-DCFDA (H2DCFDA/DCFDA proportion) with the fluorescence of preloaded cells.

Extraction and Preparation of RNA

Total RNA was separated using Qiagen's RNAeasy units of the adenomatous polyps and ordinary mucosal cells (Valencia, CA). Without RNA, RNase tests have been treated with oligo (dT) and irregular preliminaries to dispose of sans genome DNA defilement using Superscript II (Invitrogen, Carlsbad, CA) (Invitrogen). The Applied Biosystems 5700 Grouping Locator Framework utilized 50 ng of cDNA from each example as a manual for enhancing the PCR utilizing explicit oligonucleotide groundworks (PE Applied Biosystems, Cultivate City, CA). PCR responses were completed using the SYBR Green PCR center unit coordinated by the maker (PE Applied Biosystems). Dissolving temperatures and dissocial bends affirmed the personality of the PCR items.

Gene Expression Assay

In view of past examinations, three critical qualities that are accepted to be adjusted in human colon disease articulation have been assessed. CDH17, CXCL12, and TGFB1 were made, and with Groundwork Express Programming, explicit preliminaries against every quality were planned. Groundworks have been created to develop just the cDNA layout further, but not the genomic

DNA format, as much as could be expected. The genomic DNA contaminations tried utilizing introductions for b-actin genomic DNA in all cDNA tests. The fluorescence of SYBR Green color attached to PCR items has been determined after each cycle to measure quality articulation. The cycle numbers are accounted for when the combined signs pass a self-assertive boundary (CT esteem). The CT esteems are utilized to assess the general significance of quality articulation.

Statistical analysis

An understudy T-test (for a correlation with two gatherings) and a single direction ANOVA measurable review were done (for a very long-time examination). The discoveries are viewed as medium±SEM. All the multivariate ANOVA tests depended on Wilks' standard and were completed utilizing Log' values for the articulation [14].

Results

DLD-1 was exposure for performed Hydroxytyrosol 50 µmol for 24 or 48 hours. Hydroxytyrosol's effect on DLD-1 viability was 89.13 after 24 hr, and was 98.11 after 48 hr, while the control showed that viability was 114.27. Higher value was after 24 hr, then after 48 hr, followed by control, as shown in (Table 1) and (Figure 1).

Table 1.

Effect of Hydroxytyrosol on the viability percentage of DLD-1 cells

	24 hr	48 hr	control
(50) umol	89.13	98.11	114.27

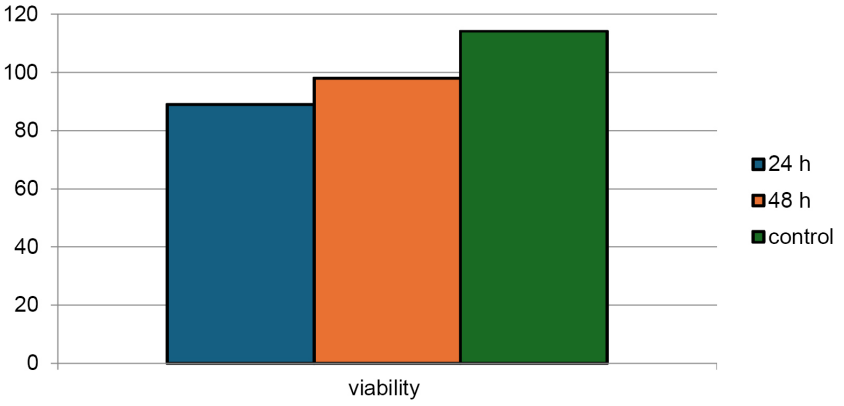

Fig. 1. The viability percentage of Hydroxytyrosol on DLD1 cells

After treatment, the effect of Hydroxytyrosol on cell viability was tested. The impact on the viability percentage of DLD1 on oxidative stress therapy cells, higher value was control, then SOD, catalase, hydroxytyrosol, HT + SOD, then HT + CAT, as shown in (Table 2) and (Figure 2).

Table 2.

Effect of oxidative stress treatment on % viability on DLD1 cells

Treatment	Viability %
SOD	92.15
Catalase	84.71
hydroxytyrosol	67.19
HT + SOD	46.18
HT + CAT	42.51
Control	100

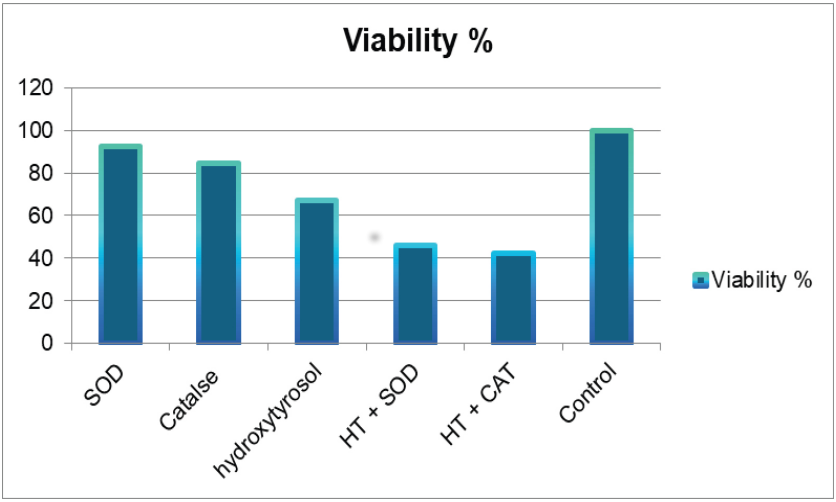

Fig. 2. Shows viability percentage on DLD1 cell lines

The effects of oxidative stress on DLD1 cells in producing H_2O_2 are showed 24-hour hydroxytyrosol treatment with H_2O_2 contributes to catalase activation. Also, the increased concentration of H_2O_2 and hydroxytyrosol activation was positive. Treatment of the cells with H_2O_2 has more activity than fold than untreated control cells hydroxytyrosol, as shown in (Table 3) and (Figure 3).

Table 3.

Effect of oxidative stress on the production of H_2O_2 on DLD1 cells

Treatment	% H_2O_2
SOD	109.11
Catalase	88.45
hydroxytyrosol	143.18
HT + SOD	127.41
HT + CAT	116.72
Control	100

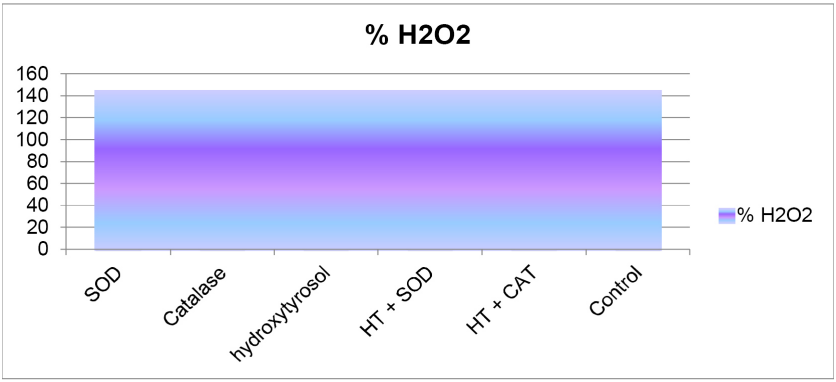

Fig. 3. Shows hydrogen peroxide percentage on DLD1 cell lines

The effect of programmed cell death DLD1 on human colon cancer cells is demonstrated in (Table 4). Colon cancer is the world's leading cause of cancer death, with few patients surviving. HT + SOD presented at higher colon cancer death levels as shown in (Table 4) and (Figure 4).

Table 4.

Effect of oxidative stress on programmed cell death on DLD1 cells

Treatment	% Death
SOD	34.07
Catalase	5.17
hydroxytyrosol	57.19
HT + SOD	94.52
HT + CAT	54.65
Control	3.33

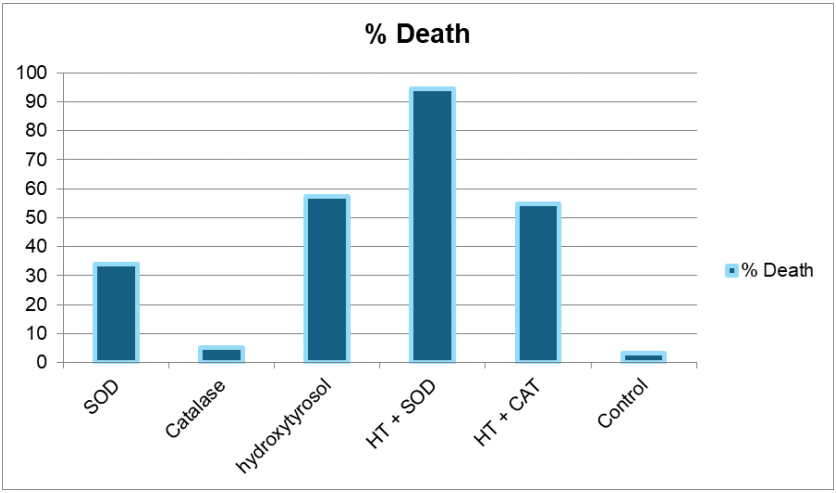

Fig. 4. Shows death percentage of the DLD1 cell lines

The effect of selected gene expression on human colon cancer (DLD1) cells of oxidative stress has been demonstrated that the expression levels CDH17, CXCL12, and TGFB1 of the three main proteins were examined to confirm proteins' participation. The results showed that TGFB1 expression increased by dose-dependent treatment (Table 5) and (Figure 5).

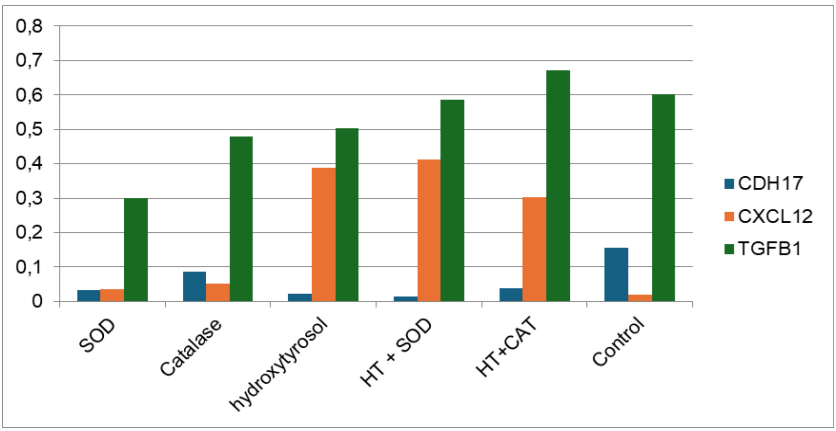

Fig. 5. Shows effect degree of the oxidative stress on selected gene expression on DLD1 cells

Table 5.

Effect of oxidative stress on selected gene expression on DLD1 cells

Treatment	CDH17	CXCL12	TGFB1
SOD	0.032*	0.035	0.301
Catalase	0.085*	0.052	0.480
hydroxytyrosol	0.023*	0.387	0.504
HT + SOD	0.015*	0.411	0.586
HT+CAT	0.038*	0.302	0.671
Control	0.157	0.019*	0.602

Values are the mean of three replicates, and * is significant at $p<0.05$

Discussion

Hydroxytyrosol is released from the colon during acidification treatment, with a median recovery of around 33 percent compared with the initial amount [15]. The colon is degraded in the big intestine by a Microflora that induces Hydroxytyrosol's biological activity for large intestinal cells [10]. Hydroxytyrosol is a colon metabolite made from olive products by chemical synthesis [16].

The directional passive diffusion process typical for Hydroxytyrosol absorbed into the intestine [2] and [17] determined the bioavailability and exclusion of Hydroxytyrosol in humans. Hydroxytyrosol decreases the ROS level (reactive gene species of oxygen) and avoids oxidant damage to the breast cancer lines of MCF10A [9].

The percentage viability of human colon cancer cell lines via cell reduction, apoptotic body development, chromatin condensation, cell proliferation suppression, and cell viability reductions in colon cancer cell lines has a significant influence [11]. A recent study of the colon in human hepatoma cells in oleuropein showed that the dose was diminished [5]. The cell viability activates the caspase pathway, inducing apoptosis and eliminating the gene's expression. N-acetylcysteine reversed autophagy defects, cell viability loss, and hydroxytyrosol induction. The therapy of SOD and Hydroxytyrosol for human colon cancer resulted in dose-dependent proliferation inhibition. Colon caused by Hydroxytyrosol as confirmed by the SOD, Catalase, HT+SOD, and HT+CAT ratios of flow. Many experiments have shown the presence of reactive oxygen species [12].

Our data show that those 24 hours of H_2O_2 cell treatment contribute to depletion. More data indicates the inducement of mitochondrial dysfunction by oxidative stress [6]. Oxidative damage to the mitochondrial membrane's thiols leads to the opening of the permeability pores, reducing membrane potential

and inhibiting synthesis [18]. Many studies have shown that depletion can lead to cell death. In this analysis, the deficit caused by H_2O_2 may be a significant factor regarding this evidence.

Oxidative stress refers to a disturbance of redox signaling and regulation and molecular damage in processing antioxidants and oxidants for later purposes. However, prolonged ovarian treatment can lead to the development of different aging diseases, including cancer, but can cause damage to proteins, lipids, and nucleic acids [16].

In the cytosol, nucleus, and inter-membrane space of mitochondria, SOD transforms superoxide into hydrogen peroxide (H_2O_2) and molecular oxygen. By maintaining low superoxide levels in cytosol, SOD protects cells from oxidative stress and subsequent cell death. Different cells of SOD inhibition increased superoxide, decreased antioxidant glutathione activity, and increased intracellular concentrations of H_2O_2 [12].

The study found that hydroxytyrosol induction was responsible for HT-induced growth inhibition. The findings show that after 48 hours of treatment, HT-induced DLD1 cells were dose-dependent. Throughout the procedure, the sum further increased. In this study, the combination of HT treatment with SOD increased the gene expression significantly [13].

Conclusion

This study demonstrates that the epigenetic effects of oxidative stress are associated with the regulation of gene expression in intestinal cancer. The findings indicate that oxidative stress can influence the epigenetic landscape, leading to altered expression of key genes implicated in colorectal cancer pathogenesis. Importantly, combination treatments utilizing antioxidants and targeting specific epigenetic pathways showed enhanced effects on cell viability, apoptosis, and gene expression. These results suggest that therapies addressing both oxidative stress and epigenetic mechanisms may be an effective strategy for the treatment of intestinal cancers. Further investigation into the interplay between oxidative stress and epigenetic regulation in colorectal cancer is warranted to develop more targeted and personalized therapeutic approaches.

Ethics committee conclusion. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee in Basic Sciences, Al-Qadisiyah University of (no. 490 in 1th Mar. 2023).

Conflict of interest information. The authors have declared no conflict of interest.

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