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EXPLORING THE THERAPEUTIC POTENTIAL OF RESVERATROL AND QUERCETIN IN GASTROINTESTINAL CANCER: A NUTRITIONAL INTERVENTION APPROACH

Sidra Noor [1*], Osama Basharat[2], Aaima Farooq[3], Anam Tariq[4], Saba Siddiqui[5], Mahreen Faqeer Hussain[6], Mahnoor[7], Amsa Fatima[8]

[1*]Visiting Lecturer, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan (sidranadeemrs92@gmail.com)
[2]MPhil Student, University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan (Osamajutt.2017@gmail.com)
[3]Dietitian and Nutritionist, Job Leading Nutrition Department, Chaudhary Muhammad Akram Teaching and Research Hospital, Lahore, Pakistan (draaimafarooq143me@gmail.com)
[4]Bachelor Student, University of Veterinary and Animal Sciences, Lahore, Pakistan (contactanamtariq@yahoo.com)
[5]MPhil Student, Kinnaird College for Women University, Lahore, Pakistan (Saba.siddiqui342@gmail.com)
[6]MPhil Student, University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan (maniyahussain006@gmail.com)
[7]Nutritionist, Lady Willingdon Hospital, Lahore, Pakistan (manozia321@gmail.com)
[8]Lecturer, University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan (Amsa.fatima92@gmail.com)

***Corresponding Author:** Sidra Noor
*E-mail: (sidranadeemrs92@gmail.com)

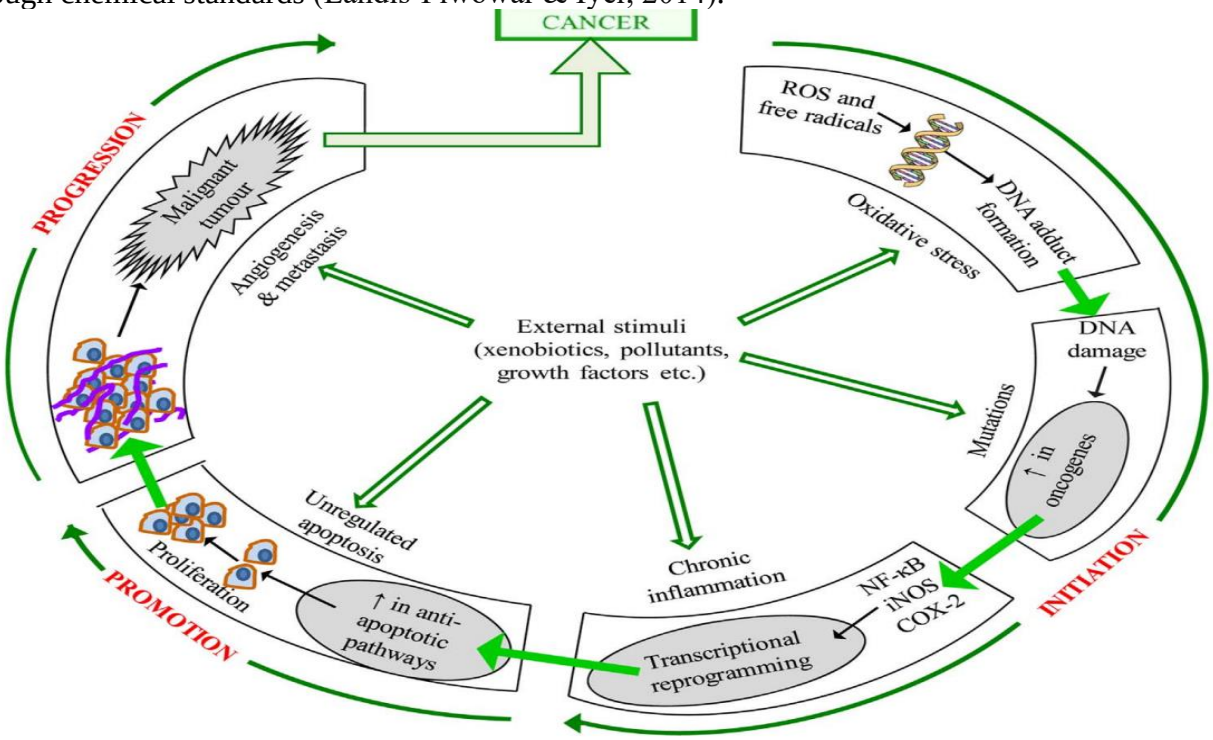
Abstract:
This study examines the impacts of resveratrol and quercetin, both individually and in combination, on different cellular irregularities and biomarkers in a carefully regulated experimental paradigm. Analyzed criteria included nucleus polymorphism, mucosal stratification, loss of nuclear polarity, decreased goblet cells, crypt abnormalities, M30 tissue level, AgNORs in the core, and beta-catenin expression. The results indicate substantial differences across the groups regarding nucleus variability, mucosal categorization, loss of nuclear polarity, decreased goblet cells, and crypt abnormalities ($p=0.001$). This suggests that the treatments have resulted in various degrees of cellular response. Furthermore, notable disparities were noted in the M30 tissue level, AgNORs in core, and beta-catenin expression, underscoring differential modifications in cellular processes and biomarkers associated with various therapies ($p<0.05$). More precisely, the concurrent use of resveratrol and quercetin resulted in decreased beta-catenin expression in comparison to the other experimental groups. These findings highlight the capability of resveratrol and quercetin, both separately and together, to regulate indicators of cellular health. This has implications for therapeutic treatments aimed at addressing cellular irregularities and associated diseases. Additional research is necessary to clarify the underlying mechanisms and improve therapeutic approaches using these natural chemicals.

Keywords: *AgNORs, Beta-Catenin, Biomarkers, Cellular Health, Crypt Abnormalities, Goblet Cells, M30 Tissue Level, Mucosal Stratification, Natural Compounds, Nuclear Polarity, Nucleus Polymorphism, Quercetin, Resveratrol, Therapeutic Interventions.*

Cancer:

Cancer exerts a considerable global influence on health, mainly due to its across-the-board occurrence, resulting in human disability and early demise. In 2015, cancer was accountable for a total of 8.8 million fatalities, and it is expected to expand by almost 70% in the next twenty years. The most typical cancers in men are prostate gland, pulmonary, respiratory tract, and gastrointestinal cancers, whereas Lungs, breasts, and airways cancers are more dominant in female individuals. It is envisioned that breast cancer will account for 29% of all newly analyzed cancers in women (Choudhari *et al.*, 2020). Due to the substantial morbidity and mortality rates connected to cancer, there has been considerable scientific claim in finding unknown anticancer therapies derived from biological origins. Empirical data shows that a notable number of cancer subjects are attributed to the effect of environmental and lifestyle elements. In contrast, a little trace can be attributed to innate genetic anomalies (George, Chandran, & Abrahamse, 2021). An association exists between a quantity of reactive oxygen species and cancer initiation via many metabolic routes. The induction of DNA fluctuation by ROS can activate multiple harmful metabolic tracks that are essential in the mutation of typical cells into cancerous ones. Reactive free radicals and their byproducts tend to respond with DNA, forming cross-links and adducts. This procedure can induce alterations in the DNA commands, conversions, and, eventually, the beginning of cancer (Hanahan, 2022).

Cancer expansion applies a series of phases influenced by numerous signaling routes. Timely intervention at a premature stage can interrupt this cycle. Oxidative stress-induced DNA impairment, liver damage caused by xenobiotics and carcinogens, transformations in proto-oncogenes and tumor suppressor genes, constant inflammation, disrupted apoptosis, and unregulated cellular expansion are all supposed to be crucial elements in the upgrade of cancer (Li & Laterra, 2012). Chemopreventive medicines emanating from plants have demonstrated the ability to disrupt or prevent cancer growth at different junctures in its advancement. Plant-based remedies have been used as traditional therapies in the Ayurvedic therapy school for generations. Dietary phytochemicals can inhibit cancer evolution by disrupting one or more cellular ways, thereby recreating a vital function in controlling cancer through chemical standards (Landis-Piwowar & Iyer, 2014).



The expense and complexity of cancer medicine are both significant. Chemoresistance and the profound adverse consequences of ancestral chemotherapeutic medications are considered obstacles to the cure of cancer. Investigators have just started evaluating the impact of blending many anticancer drugs at reduced dosages to form combinatorial treatment strategies for ministering cancer. These analyses aim to underrate the adverse consequences of anticancer drugs while increasing their usefulness against tumors at more inferior dosages (Kabirai *et al.,* 2020).

Phytochemicals have lately been identified as crucial elements of combinatorial treatment. They are widely recognized as powerful supplementary healing agents because they improve antitumor effects and sensitivity to chemotherapy. Combinatorial medicines have garnered considerable claims in cancer examinations in recent times. While the anticancer effects of several phytochemicals are well-documented, a marked problem arises when multiple phytochemicals do not reveal their antitumor effects at more subordinate dosages due to fast metabolism or restricted bioavailability (Malarkey, Hoenerhoff, & Maronpot, 2013).Multiple clinical tests have been performed to assess the other therapeutic advantages of combining phytochemicals with conventional medications and develop creative combined therapy approaches for cancer. These studies have indicated that phytochemicals can improve the understanding of cancer cells to cancer treatment and raise the effectiveness of tumor therapy. As a result, phytochemicals have evolved as essential elements of combinatorial treatment (Blanquer-Rosselló *et al.*, 2013).

Cancer chemo preventive:

An innate trait of cancer cells is their capability to increase more rapidly than normal cells. The preliminary objective of most chemotherapy drugs is to selectively impede, eliminate, or hinder the proliferation of these rapidly separating cells. Nevertheless, these drugs impact standard cells, resulting in critical adverse responses and reduced therapeutic effectiveness. The carcinogenesis method advances through different molecular methods and signaling molecules. Cancer chemoprevention entails utilizing biological and artificial mechanisms to disrupt the operation of carcinogenesis by precisely targeting molecular signaling techniques. The mediators are mainly categorized as blocking and concealing agents (Hosseini & Ghorbani, 2015).

Chemoprevention, an advanced area of cancer analysis, involves using chemicals acquired from medicinal plants to control cancer through nutritional therapies. Transforming a fundamental biological observation into a clinically useful anticancer drug is challenging in creating chemopreventive mechanisms. The chemoprevention method must be uncontroversial and prolonged, and the implications must be suitable for extended management to those in good health, more aged patients, and those with comorbidities (Hermann *et al*., 2007). Multiple epidemiological examinations have documented that frequent consumption of fruits and vegetables dramatically decreases cancer. Dietary phytochemicals, bioactive mixtures derived from therapeutic plants or herbal diets, have newly demonstrated robust efficacy in cancer chemoprevention. This has resulted in the evolution of novel and alternative cancer prevention and therapy approaches. Several phytochemicals have been subjected to clinical trials to examine their potential for stopping cancer. The primary purpose of cancer chemoprevention is to lessen the risk of early-stage cancer and restrict tumor metastasis. Carcinogenesis is a complicated series of measures that involves several genes and other hereditary changes for a normal cell to transform into a cancerous cell. Cancer cell creation is generally categorized into stages: initiation, promotion, and advancement. The precise digit of genetic alterations in these phases has yet to be confirmed (Swetha *et al*., 2022).

The initial stage implicates the creation of a perpetually altered cell, commonly caused by a changeover and numerous tools of start. In the second phase, the initially altered cells increase and develop into a noticeable cluster of cells, which is considerably likely a harmless lesion. During the promotion step, epigenetic variables impact the proliferation of the initiated cells. The intricate operation of the promotion phase needs to be nicely comprehended. The result of the upgrade often consists of nonmalignant or harmless cells, occasionally preneoplastic. These non-cancerous cells undergo further genetic transformations during the advancement step, forming cancerous cells. The

last phase, progression, entails changing nonmalignant gentle tumors into malignant tumors and is separate from the preceding steps (Mehta *et al*., 2010).
Stem cells are essential in the onset of carcinogenesis via various physical, chemical, biological, and viral influences. When initiated cells are revealed to a stimulating element, they can undergo a complete metamorphosis into cancerous cells. These successive steps are critical in diverting preneoplastic cells into malignant cells. A range of chemical, biological, natural, or genetic alterations in compartments causes carcinogenesis in multicellular organisms. While conversion significantly functions in carcinogenesis, other elements contribute to this process (Rudzińska *et al*., 2023).

Gastrointestinal Cancer:
Gastrointestinal cancer ranks as the most common malignancies in men and the second most prevalent among women in Iran, contributing to 50% of all fatalities caused by cancer in the population. Among gastrointestinal cancers, colon cancer stands as the fourth biggest cause of global cancer-related mortality, accounting for approximately eight percent of the total number of deaths caused by cancer. Early detection of colon cancer plays a pivotal role in enhancing survival rates. While chemotherapy drugs aim to hinder cell proliferation in specific tissues and induce apoptosis in tumor cells, the adverse effects of treatments for advanced colon cancer stages pose significant challenges. Traditional cancer treatments, such as systemic drug administration, not only target cancer cells but also affect healthy cells and tissues, leading to collateral damage (Núñez-Sánchez *et al*., 2015).
Despite advancements in anticancer drug development, treatment efficacy remains suboptimal, prompting the American Society of Clinical Oncology to underscore the urgency for novel cancer treatment approaches. Targeted utilization of cancer-preventing agents with specific chemical mechanisms emerges as a critical goal in colon cancer treatment (Hammoud, Cairns, & Jones, 2013). During the previous thirty years, numerous studies discovered extracts from plants, particularly phytochemicals, as potent suppressors or inhibitors of cancer development. Phytochemicals exert preventive effects toward cancer in each of the phases: avoiding carcinogens, identifying and eliminating pre-malignant lesions, and preventing cancer recurrence and tumor progression. Since dysregulated cell proliferation and apoptosis resistance are hallmark features of cancer cells, identifying natural plant components from edible sources is a safe approach to monitor cancer treatment. Resveratrol, a natural compound with known biological effects in cancer prevention and treatment, contains a phytoalexin compound those functions as a natural suppressor of cell growth. Quercetin, another phytochemical abundant in fruits and vegetables, is also recognized for its cancer-preventive properties. It is ascribed to its antioxidant action, suppression of carcinogen-activating enzymes in them, and modulation of intracellular communications pathways., and interaction with receptors and proteins (Buhrmann *et al*., 2018).
Studies suggest parallel administration of Quercetin and resveratrol may reduce restenosis and inhibit smooth muscle cell proliferation. Combining Quercetin with resveratrol holds promise for cancer control, as Quercetin may inhibit resveratrol sulfation, potentially increasing resveratrol's bioavailability and therapeutic efficacy. Thus, the present study aims to evaluate the effects of quercetin and resveratrol interventions, alone and in combination, on azoxymethane-induced gastrointestinal cancer in a rat model (Serini *et al*., 2018).

Resveratrol:
The compound, scientifically referred to as 3,4',5-trihydroxy-trans-stilbene, is a very promising nutraceutical that has the potential to improve cancer treatment and greatly progress cancer therapy.. Resveratrol, a natural phytoalexin, is synthesized by plants as a defense mechanism against environmental stress and pathogenic invasion. The compound was initially obtained in 1940 by isolating the Veratrum album's roots. Subsequently, in 1963, it was taken from the roots of Polygonum cuspidatum. While the compound's cardioprotective advantages were initially asserted in 1982, it acquired widespread recognition in 1992 when it was hypothesized that resveratrol in red wine could benefit heart health. In 1997, it was reported that applying resveratrol directly to the skin

prevented the formation of tumors in a mouse model of skin cancer, demonstrating its promise as a new and effective treatment for cancer (Udenigwe *et al.,* 2008).

Significantly, a thorough study has consistently reaffirmed its inhibitory function in cancer. Resveratrol acts as a chemo preventive agent in all four main stages of carcinogenesis, which are beginning, advancement, growth, and metastasis.. It has demonstrated effectiveness in treating cancer in laboratory settings (in vitro) and living organisms (in vivo). Resveratrol has the potential to be a valuable addition to traditional chemotherapy due to its antioxidant, anti-inflammatory, and direct antitumor effects. It has demonstrated effectiveness against obesity-related cancers such as liver, pancreatic, breast cancer after menopause, prostate, and colorectal cancer, as well as lung, skin, and blood cancers (Sengottuvelan & Nalini, 2006).

Several reviews have outlined the mechanisms and routes by which resveratrol produces its effects. These reviews indicate that resveratrol's antitumor activities are achieved by multiple mechanisms rather than a single mode of action. The capacity of this medication to affect various nodes in the development of tumors has made it valuable for usage in combination with other therapies. It can enhance the effectiveness of chemotherapy or make tumor cells resistant to treatment more susceptible to being killed. Furthermore, resveratrol can safeguard healthy cells against the detrimental impacts of conventional agents, such as xerostomia and mucositis. This attribute enhances its appeal as a potentially effective anticancer agent (Shukla & Singh, 2011).

Although resveratrol has various advantages, its effectiveness in cancer treatment has only shown minimal advancement. The reason for the lack of progress in the clinical use of this highly promising nutraceutical, which has demonstrated significant potential in preclinical research, still needs to be discovered. The anticancer effects of resveratrol are achieved by critical pathways such as:

- Disrupting the long noncoding RNA MALAT1 suppresses the production of β-catenin and prevents its movement into the cell nucleus.
- It hinders the process of epithelial-mesenchymal transition and the activation of the transcription factor Snail, which is triggered by TGF-β/Smad.
- They are suppressing the production of IKK-induced TNF-β, which hinders the growth of cancer cells by deactivating NF-κB.
- They prevent the buildup of FOXO3a in the nucleus by inhibiting the p-PI3K/p-AKT pathway.
- It is inhibiting the phosphorylation of Src-STAT3 and promoting apoptosis in cancer cells.
- It suppresses the activation of HIF-1α caused by AKT/MAPK and enhances the breakdown of the HIF-1α protein by ubiquitination (Sengottuvelan & Nalini, 2006).

Quercetin:

Quercetin, known explicitly as 3,30,40,5,7-pentahydroxylflavone, is a type of flavonoid in various plants. It is recognized for its potential to inhibit the development of cancer. Flavonoids are a type of polyphenolic substance. Quercetin is a flavonoid found in fruits, vegetables, and plant-derived medicines. The anti-carcinogenic properties of Quercetin can be attested to its antioxidative activity, ability to inhibit carcinogen-activating enzymes, capacity to modify signal transduction pathways, and interactions with cellular receptors and proteins (Xavier *et al.,* 2011).

Quercetin's antioxidative capabilities arise from its capacity to contribute electrons and eliminate free radicals, the presence of hydroxyl groups at the 30- and 40-places in the B-ring and the third position in the C-ring is the primary reason. Quercetin can hinder the activity of enzymes involved in oxidation, including as xanthine oxidase, lipoxygenase, and NADPH oxidase, are responsible for reducing oxidative stress. (Rather & Bhagat, 2020).

Quercetin undergoes metabolic transformation, which impacts its capacity to be absorbed and its effectiveness. The substance undergoes quick metabolism and elimination, with its byproducts absorbed in the small and large intestines. The metabolites consist of several glucuronides and methylated versions distributed throughout the body. Regular consumption of foods high in Quercetin is essential for optimal levels of its metabolites in the bloodstream (Khandelwal *et al*., 2012). Although metabolic conversion often decreases Quercetin's action, many Quercetins exhibit

substantial activity. Activated macrophages can specifically take quercetin metabolites up during inflammation and then convert them back into the active aglycone form, which indicates that they have activities particular to the site of inflammation. In summary, Quercetin's ability is due to its antioxidant characteristics, ability to inhibit enzymes, and ability to regulate cellular signaling pathways. Sustained food consumption is crucial for sustaining therapeutic concentrations within the body.

Materials and methods:

Chemicals

- Quercetin
- Resveratrol
- Azoxymethane

The animals and Experimental Procedures

- A total of fifty-five male rats, with weights ranging from 200 to 220 grams, were obtained from the Animal Facility at FC College University. The rats were housed under controlled conditions, with lighting provided from 7:00 in the morning to seven in the evening and a temperature maintained between 25 degrees Celsius and 27°C. They were given unrestricted access to food and water.
- After one week of adaptation on a scientifically formulated food for laboratory animals, the rats were haphazardly divided into five groups (n=11 per group): healthy, Control, resveratrol, Quercetin, and a mixture of resveratrol and Quercetin.
- The university's veterinary ethics committee approved the animal experiments.

Tumor Induction

- In the fourth week, all groups except the healthy group received azoxymethane (15 mg/kg) subcutaneously once a week for two weeks as a carcinogenic agent.
- During the sixth week, a 2% solution of sodium sulfate dextran was administered to the rats via their drinking water for a duration of seven days in order to promote the development of colonic cancer.
- The healthy group subcutaneously received normal saline (15 ml/kg) to eliminate stress from AOM injections.

Intervention

- Resveratrol (8 mg/kg) and Quercetin (10 mg/kg) were administered via oral gavage from two weeks after carcinogenesis induction until week 19.
- Carboxymethyl cellulose was given to the healthy and Control groups via oral gavage to control stress caused by the administration process.
- At the conclusion of week 19, all animals were administered sodium thiopental (100 mg/kg) and their intestines were removed for examination.
- The results of resveratrol and Quercetin on apoptosis were examined using the M30 antibody, cell expansion via AgNOR staining, and histopathological and histomorphology assessments of AOM-induced colon cancer in rats. Beta-catenin levels were calculated microscopically.

Immunohistochemistry

- The avidin-biotin immunoperoxidase technique was used to perform immunohistochemistry on five μm tissue slices obtained from formalin-fixed, paraffin-embedded blocks.
- The sections were stained using a monoclonal mouse anti-rat Beta-catenin antibody, following the directions provided by the manufacturer.

Histopathological Modification

The colon samples were placed in a solution of 10% buffered formalin, embedded in paraffin, and cut into sections for histological investigation using Hematoxylin, Eosin coloring, and the stain used is Alcian Blue/Periodic Acid Schiff.. The stained sections were evaluated for five criteria related to colonic degeneration and neoplasia:

1. Nuclear diversity
2. Mucosal segmentation
3. Nuclear contradiction failure
4. Reduced number of goblet cells
5. Cryptic abnormalities

The antibody used in this study is M30 Cyto death antibody:

For the apoptosis study, samples were treated with freezing methanol and dyed with M30 Cyto death-FITC following the instructions provided by the research work.

AgNORs

The colon was cleared of obstructions along its length, flushed with a solution containing standard saline, preserved in a 10 percent formalin solution, and embedded in paraffin. The updated techniques were used to perform AgNOR staining. The samples were exposed to the staining liquid for 60 minutes at room temperature under dark conditions. The PCNA-positive index and AgNORs number were determined by quantifying the number of PCNA-positive nuclei and AgNORs, respectively, in individual colon cancer specimens.

Statistical Analysis

Group comparisons were conducted using analysis of variance and Tukey's test. Dissimilarities in tumor incidence were studied using Fisher's exact test. The Chi-square test selected ratio comparisons. Values were expressed as mean ± SEM, with $P < 0.05$ deemed to have statistical significance.

Results

Nucleus Polymorphism

According to the information provided in Table 1, the lack of aberrant cells is associated with the group that is healthy. By comparison, the control cohort has the greatest occurrence and proportion of nuclear diversity scores, followed by the resveratrol, quercetin, and resveratrol + quercetin classes. The control group has the highest degree of anomalies, whereas the healthy group exhibits the lowest degree. The observed groups display a statistically significant variance in the frequency of nuclear polymorphism. Tukey's post-hoc test indicates important differences between the resveratrol, quercetin, and resveratrol plus Quercetin categories compared to the control team, with no significant distinctions among the resveratrol, quercetin, and resveratrol plus quercetin category.

Table 1: Nuclear Polymorphism Scores Across Treatment Groups

Group	Nuclear Polymorphism Score	Frequency	Percentage	Significant Difference	p-value
Healthy Group	Least Intensity	Lowest	Lowest	-	-
Control Group	Highest Intensity	Highest	Highest	Considerable difference from other groups	$p < 0.05$
Resveratrol Group	Lower than Control	Moderate	Moderate	Significant difference from Control Group	$p < 0.05$ (vs Control)
Quercetin Group	Lower than Control	Moderate	Moderate	Significant difference from Control Group	$p < 0.05$ (vs Control)
Resveratrol + Quercetin	Lower than Control	Moderate	Moderate	Significant difference from Control Group	$p < 0.05$ (vs Control)

The table summarizes the results of a study examining nuclear polymorphism scores across different groups, including a healthy group, control group, resveratrol-treated group, quercetin-treated group, and a group treated with both resveratrol and Quercetin. The control group exhibited the highest nuclear polymorphism frequency and percentage with the most intense abnormalities. In contrast, the healthy group had the lowest scores and intensity. The resveratrol, Quercetin, and combination groups showed significantly lower nuclear polymorphism scores than the control group ($p < 0.05$), indicating reduced abnormalities. However, no significant differences were found among the resveratrol, Quercetin, and combination treatment groups ($p > 0.05$).

Mucosal Stratification

According to the data presented in Table 2, the healthy group does not exhibit any aberrant cells. The Quercetin, resveratrol, and quercetin groups exhibit the most frequent and severe low-grade abnormalities. Conversely, the control class has the highest occurrence and intensity of abnormalities. There is no notable difference between the quercetin and flavonoid resveratrol plus quercetin categories. However, significant differences exist between these and the control groups ($P<0.001$). Tukey's post-hoc test confirms significant dissimilarities among the Quercetin, resveratrol plus Quercetin, and resveratrol class corresponding to the control category, with no considerable differences among the quercetin and resveratrol plus quercetin groups.

Table 2: Analysis of mucosal stratification in the groups under investigation

Group	High Grade (%)	High Grade (N)	Low Grade (%)	Low Grade (N)	Normal (%)	Normal (N)
Healthy	0	0	0	0	100	11
Control	72.63	8	36.46	5	0	0
Resveratrol	54.64	7	45.45	6	0	0
Quercetin	27.64	5	72.71	9	0	0
Resveratrol + Quercetin	27.36	6	72.65	9	0	0

ANOVA Test Result:

- P value = 0.001

Disruption of Nuclear Polarity

Table 3 demonstrates that the control group does not display any abnormalities. The group administered with quercetin exhibits the highest occurrence rate but relatively moderate intensity of abnormalities, whereas the group acting as a control has the highest frequency and intensity. The group that is in good health experiences the lowest frequency and lowest severity. Tukey's post-hoc test indicates significant distinctions between the resveratrol, quercetin, and resveratrol plus quercetin groups compared to the control group, with no significant variations within the resveratrol, quercetin, and resveratrol plus quercetin classes.

Table 3: Loss of nuclear polarity seen in the studied groups

Group	High Grade (%)	High Grade (N)	Low Grade (%)	Low Grade (N)	Normal (%)
Healthy	0	0	0	0	100
Control	81.72	8	18.27	3	0
Resveratrol	45.66	6	54.68	7	0
Quercetin	27.39	4	72.56	9	0
Resveratrol + Quercetin	36.47	5	63.78	8	0

ANOVA Test Result:

- P value = 0.001

Decreased Goblet Cells

According to the data shown in Table 4, the healthy group exhibits the highest frequency of standard cells. The group receiving a combination of resveratrol and quercetin shows the highest occurrence rate and least severe kind of abnormalities. On the other hand, the control group exhibits the highest level of both frequency and intensity of irregularities, which are comparable to the high-grade abnormalities observed in the quercetin group. The healthy category has the lowest frequency and severity of goblet cell dysfunction. Tukey's post-hoc test reveals significant disparities between the resveratrol plus quercetin group and the other groups, whereas there are no significant variations observed among the resveratrol, quercetin, and control categories.

Table 4: Analysis of Goblet Cell Status in the Examined Groups

Group	High Grade (%)	High Grade (N)	Low Grade (%)	Low Grade (N)	Normal (%)
Healthy	0	0	0	0	100
Control	36.36	4	63.63	7	0
Resveratrol	45.45	5	54.54	6	0
Quercetin	36.36	4	63.63	7	0
Resveratrol + Quercetin	18.18	2	81.81	9	0

ANOVA Test Result:

- P value = 0.001

Crypt Abnormalities

Table 5 demonstrates notable variations in crypt abnormalities among the different groups. The healthy group exhibits the most prevalent state of normalcy. The group receiving a combination of resveratrol and quercetin exhibits the highest occurrence and slowest occurrence rate of crypt abnormalities, whereas the control group demonstrates the most elevated occurrence and rapidity. Tukey's post-hoc test reveals that the resveratrol, quercetin, and resveratrol plus quercetin classes differ significantly from the control group, but there are no notable variations among the resveratrol, quercetin, and resveratrol plus quercetin categories..

Table 5: Anomalies in Crypts Among Analyzed Cohorts

Group	High Grade (%)	Low Grade (%)	Normal (%)	Index (%)
Healthy	0	0	100	11
Control	72.72	27.27	0	0
Resveratrol	45.45	54.54	0	0
Quercetin	36.36	63.63	0	0
Resveratrol+Quercetin	27.27	72.73	0	0

The p-value obtained from the ANOVA test is <0.001.

M30 Tissue Level

Table 6 shows that the highest mean of M30 presentation is in the healthy group, and the most inferior is in the control group. The outcomes indicate an influential difference in M30 countenance among the groups, with significant distinctions between the healthy and all other groups except for the

resveratrol. Likewise, there were significant differences seen between the groups treated with resveratrol and quercetin, as well as between the control cohort and the individuals treated with quercetin and resveratrol. Tukey's post-hoc test demonstrates significant disparities between the resveratrol, Quercetin, and resveratrol plus Quercetin groups compared to the control group, with no significant differences among the resveratrol, quercetin, and resveratrol plus Quercetin groups.

Table 6: Analysis of M30 expression in the groups under study

Group	P value*	Median	SD	Mean
Healthy	P=0.001	6.18	0.53	4.65
Control		2.24	0.34	1.01
Resveratrol		3.54	0.67	1.38
Quercetin		1.12	0.89	2.65
Resveratrol + Quercetin		5.11	0.65	4.65

Ag NORs in Core

According to Table 7, the control class has the highest average AgNOR value, whereas the group of healthy rats has the lowest. The differences across the sets are statistically significant, with notable distinctions between the healthy group and the other groups, the Control group and the resveratrol category, and the resveratrol group and the resveratrol + Quercetin group. Tukey's post-hoc test reveals significant variations between the resveratrol, quercetin, and resveratrol plus quercetin groups compared to the control group, but no major variations within the resveratrol, quercetin, and resveratrol plus quercetin groups.

Table 7: AgNOR analysis was carried out on the studied groups.

Group	P value*	Median	SD	Mean	Number of Crypts
Healthy	P=0.001	1.19		1.29 1.10	25
Control		3.11		1.29 3.07	25
Resveratrol		2.0		1 1.99 2.01	25
Quercetin		3.		21 0.89 2.87	25
Resveratrol + Quercetin		1.		12 0.51 1.28	25

Beta-Catenin Expression

Table 8 displays the indication levels of Beta-catenin across different groups. Beta-catenin is a protein involved in cell adhesion and signaling pathways. The table presents the mean expression values for Beta-catenin in each group.

- The "Healthy" group shows a Beta-catenin expression level of 0.36.
- In contrast, the "Control" group exhibits a significantly higher expression level 5.74.
- The groups treated with Resveratrol, Quercetin, and a combination of both (Resveratrol + Quercetin) show intermediate levels of Beta-catenin expression, with values of 2.65, 3.00, and 1.29, respectively.

This table explains how treatments or conditions may impact Beta-catenin expression in the studied groups.

Table 8: Analysis of Beta-catenin expression in the groups under investigation

Group	Beta-catenin
Healthy	0.36
Control	5.74
Resveratrol	2.65
Quercetin	3.00
Resveratrol and Quercetin	1.29

Notes:

- The "Healthy" group consistently shows the highest percentage of normal cells across all variables.
- The "Control" group consistently shows abnormalities' highest frequency and severity.
- "Resveratrol," "Quercetin," and "Resveratrol + Quercetin" groups show intermediate values, often significantly better than the control group but not as good as the healthy group.
- The combined treatment of "Resveratrol + Quercetin" frequently results in lower severity of abnormalities than individual treatments, especially in beta-catenin expression.

DISCUSSION:

Colon cancer poses a significant health burden globally, demanding effective treatment strategies to halt its progression, especially in advanced stages. However, conventional chemotherapy often entails adverse side effects for patients. Therefore, there is a critical need for unexplored therapeutic approaches. Considering the promising anticancer properties of resveratrol and Quercetin, further investigation is warranted to explore their potential in colon cancer treatment (Thrift, 2016).

The current study aims to evaluate the comparative efficacy of resveratrol and flavonoid quercetin supplementation, both individually and in combination, in combating colon carcinoma in animals. This novel approach of combining natural antioxidants represents a groundbreaking strategy. Resveratrol and Quercetin offer several advantages as cancer inhibitors, including low toxicity at usable quantities, well-understood mechanisms of action, good patient acceptance as supplements, and cost-effectiveness.

The study findings reveal that resveratrol and Quercetin combined supplementation reduces nuclear polymorphism, stratification of mucosa, loss of nuclear contrariness, goblet cell reduction, crypt anomalies, AgNOR expression, and beta-catenin levels compared to individual supplementation. This indicates the synergistic efficacy of the combined infusion of resveratrol and quercetin in colon cancer treatment.

Quercetin, known for its apoptotic induction properties, primarily operates through the mitochondrial-mediated pathway to induce apoptosis. It activates p53, upregulates pro-apoptotic molecules, and downregulates anti-apoptotic agents (e.g., survivin, Bcl-2). Additionally, Quercetin disrupts the cell cycle advancement at various phases and inhibits protein chaperones, leading to apoptosis induction. Its ability to regulate critical proteins entangled in cell cycle regulation and apoptosis underscores its potential as an adjunct to current chemotherapy regimens.

Resveratrol exhibits diverse antitumor effects relying on cancer cell kind and condition. It generates apoptosis and cell cycle detention in most tumor cells while protecting neurons and endothelial cells from oxidative stress-induced damage. The molecular mechanisms underlying its antitumor effects include regulation of mitochondrial enzyme systems, upregulation of tumor suppressor genes, and inhibition of survival proteins associated with chemoresistance. Additionally, resveratrol activates various signaling pathways in cell survival and proliferation, making it a promising candidate for cancer treatment (Fouad *et al.,* 2013).

Histopathological analyses demonstrate that the mixture supplementation of resveratrol and Quercetin exerts superior effects to individual supplementation, particularly in reducing nuclear polymorphism, stratification of mucosa, thrashing of nuclear oppositeness, and goblet cell reduction. These findings suggest a synergistic action of resveratrol and Quercetin in mitigating cellular changes associated with colon cancer progression.

Furthermore, the combination supplementation induces apoptosis and reduces cell proliferation more effectively than individual supplementation, as evidenced by decreased M30 tissue levels. Resveratrol and quercetin act in concert to modulate apoptotic pathways, leading to enhanced tumor cell death and reduced proliferation rates. This highlights the potential of combined resveratrol and quercetin supplementation as an effective therapeutic approach for colon cancer treatment.

Moreover, the combination supplementation significantly reduces AgNOR expression in colon cancer cells, indicating decreased cell proliferation. Resveratrol demonstrates a more substantial inhibitory influence on cell multiplication than Quercetin, further emphasizing its potential as a potent anticancer agent.

The study also reveals a significant decrease in beta-catenin expression and tumor incidence in the combination group corresponded to the control group. Beta-catenin activation is implicated in colon cancer initiation and promotion. The observed reduction in beta-catenin levels underscores the chemopreventive effect of combined resveratrol and quercetin supplementation in colon cancer.

In summary, the combination of resveratrol and Quercetin supplementing shows promising efficacy in inhibiting colon cancer progression in animal models. This novel therapeutic approach offers several advantages, including synergistic anticancer effects, reduced side effects, and cost-effectiveness. Additional research is warranted to explain analyze the fundamental processes and enhance restorative clinical application procedures. Combined resveratrol and quercetin supplementation holds great potential as a safe and effective strategy for colon cancer treatment, offering hope for improved patient outcomes and quality of life.

CONCLUSION:

In conclusion, the study underscores the potential of resveratrol and Quercetin, individually and in combination, as potential treatment option for treatment for cancer in the colon. The combined supplementation of these natural antioxidants exhibits synergistic effects, leading to significant reductions in various markers associated with colon cancer progression.

Quercetin emerges as a potent inducer of apoptosis, primarily through the mitochondrial-mediated pathway. It effectively activates essential apoptotic proteins while inhibiting anti-apoptotic factors, promoting programmed cell extinction in cancer cells. Moreover, Quercetin disrupts cell cycle progression at multiple phases, further impeding cancer cell proliferation. Its multifaceted action makes it a valuable adjunct to conventional chemotherapy, offering potential benefits in cancer treatment.

Similarly, resveratrol demonstrates diverse antitumor effects, including apoptosis installation, cell cycle detention, and inhibition of cell expansion. It exerts its actions by modulating various signaling pathways involved in cell survival and proliferation. Resveratrol protects normal cells, reducing oxidative stress and minimizing collateral damage from chemotherapy. These properties highlight its potential as a versatile and practical medicinal agent in cancer treatment.

Histopathological analyses reveal that combined supplementation of resveratrol and Quercetin significantly reduces cellular changes associated with colon cancer progression. The synergistic action of these compounds results in decreased nuclear polymorphism, stratification of mucosa, failure of nuclear oppositeness, goblet cell reduction, crypt anomalies, AgNOR expression, and beta-catenin levels compared to individual supplementation. These findings suggest that combining resveratrol and Quercetin offers enhanced efficacy in combating colon cancer progression.

Moreover, combined supplementation induces apoptosis and reduces cell proliferation more effectively than individual supplementation, highlighting its possibility as a treatment approach for cancer of the colon cure. The observed reductions in AgNOR expression and beta-catenin levels in

colon cancer cells further support the chemopreventive effect of combined resveratrol and quercetin supplementation.

Overall, the study findings support using resveratrol and Quercetin, alone or in combination, as promising therapeutic agents for colon cancer treatment. Further investigation is necessary for understanding the basic principles and enhance methods of treatment for clinical implementation. Combined resveratrol and Quercetin supplementation hold great promise as a safe and effective strategy for improving patient outcomes and quality of life in colon cancer treatment.

References:

1. Blanquer-Rosselló, M. M., Oliver, J., Valle, A., & Roca, P. (2013). Effect of xanthohumol and 8-prenylnaringenin on MCF-7 breast cancer cells oxidative stress and mitochondrial complexes expression. *Journal of Cellular Biochemistry, 114*(12), 2785-2794.
2. Buhrmann, C., Yazdi, M., Popper, B., Shayan, P., Goel, A., Aggarwal, B. B., et al. (2018). Resveratrol chemosensitizes TNF-β-induced survival of 5-FU-treated colorectal cancer cells. *Nutrients, 10*(7), 888.
3. Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P., & Prakash, O. (2020). Phytochemicals in cancer treatment: From preclinical studies to clinical practice. *Frontiers in Pharmacology, 10*, 497776.
4. Fouad, M. A., Agha, A. M., Merzabani, M. M., & Shouman, S. A. (2013). Resveratrol inhibits proliferation, angiogenesis, and induces apoptosis in colon cancer cells: Calorie restriction is the force to the cytotoxicity. *Human & Experimental Toxicology, 32*(10), 1067-1080.
5. George, B. P., Chandran, R., & Abrahamse, H. (2021). Role of phytochemicals in cancer chemoprevention: Insights. *Antioxidants, 10*(9), 1455.
6. Hammoud, S. S., Cairns, B. R., & Jones, D. A. (2013). Epigenetic regulation of colon cancer and intestinal stem cells. *Current Opinion in Cell Biology, 25*(2), 177-183.
7. Hanahan, D. (2022). Hallmarks of cancer: new dimensions. *Cancer Discovery, 12*(1), 31-46.
8. Hermann, P. C., Huber, S. L., Herrler, T., et al. (2007). Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell, 1*(3), 313-323.
9. Hosseini, A., & Ghorbani, A. (2015). Cancer therapy with phytochemicals: Evidence from clinical studies. *Avicenna Journal of Phytomedicine, 5*, 84-97.
10. Kabirai, A., Chahar, A., Chahar, N., & Gupta, J. (2020). Chemical carcinogenesis: A brief review on mechanism & metabolism. *Journal of Oral Medicine, Oral Surgery, Oral Pathology, and Oral Radiology, 6*, 120-124.
11. Khandelwal, A. R., Hebert, V. Y., Kleinedler, J. J., Rogers, L. K., Ullevig, S. L., Asmis, R., et al. (2012). Resveratrol and quercetin interact to inhibit neointimal hyperplasia in mice with a carotid injury. *Journal of Nutrition, 142*(8), 1487-1494.
12. Landis-Piwowar, K. R., & Iyer, N. R. (2014). Cancer chemoprevention: Current state of the art. *Cancer Growth and Metastasis, 7*, 19-25.
13. Li, Y., & Laterra, J. (2012). Cancer stem cells: distinct entities or dynamically regulated phenotypes? *Cancer Research, 72*(3), 576-580.
14. Malarkey, D. E., Hoenerhoff, M., & Maronpot, R. R. (2013). Chapter 5 - Carcinogenesis: Mechanisms and manifestations. In W. M. Haschek, C. G. Rousseaux, & M. A. Wallig (Eds.), *Haschek and Rousseaux's Handbook of Toxicologic Pathology* (3rd ed., pp. 107-146). Academic Press.
15. Mehta, R. G., Murillo, G., Naithani, R., & Peng, X. (2010). Cancer chemoprevention by natural products: how far have we come? *Pharmaceutical Research, 27*, 950-961.
16. Núñez-Sánchez, M. A., González-Sarrías, A., Romo-Vaquero, M., García-Villalba, R., Selma, M. V., Tomás-Barberán, F. A., et al. (2015). Dietary phenolics against colorectal cancer–From

promising preclinical results to poor translation into clinical trials: Pitfalls and future needs. *Molecular Nutrition & Food Research, 59*(7), 1274-1291.
17. Rather, R. A., & Bhagat, M. (2020). Quercetin as an innovative therapeutic tool for cancer chemoprevention: Molecular mechanisms and implications in human health. *Cancer Medicine, 9*(24), 9181-9192.
18. Rudzińska, A., Juchaniuk, P., Oberda, J., Wiśniewska, J., Wojdan, W., Szklener, K., & Mańdziuk, S. (2023). Phytochemicals in cancer treatment and cancer prevention—review on epidemiological data and clinical trials. *Nutrients, 15*(8), 1896.
19. Sengottuvelan, M., & Nalini, N. (2006). Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1,2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. *British Journal of Nutrition, 96*(1), 145-153.
20. Sengottuvelan, M., & Nalini, N. (2006). Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1, 2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. *British Journal of Nutrition, 96*(1), 145-153.
21. Serini, S., Cassano, R., Corsetto, P. A., Rizzo, A. M., Calviello, G., & Trombino, S. (2018). Omega-3 PUFA loaded in resveratrol-based solid lipid nanoparticles: Physicochemical properties and antineoplastic activities in human colorectal cancer cells in vitro. *International Journal of Molecular Sciences, 19*(2), 586.
22. Shukla, Y., & Singh, R. (2011). Resveratrol and cellular mechanisms of cancer prevention. *Annals of the New York Academy of Sciences, 1215*, 1-8.
23. Swetha, M., Keerthana, C. K., Rayginia, T. P., & Anto, R. J. (2022). Cancer chemoprevention: A strategic approach using phytochemicals. *Frontiers in Pharmacology, 12*, 809308.
24. Thrift, A. P. (2016). The epidemic of oesophageal carcinoma: Where are we now? *Cancer Epidemiology, 41*, 88-95.
25. Udenigwe, C. C., Ramprasath, V. R., Aluko, R. E., & Jones, P. J. (2008). Potential of resveratrol in anticancer and anti-inflammatory therapy. *Nutrition Reviews, 66*(8), 445-454.
26. Xavier, C. P., Lima, C. F., Rohde, M., & Pereira-Wilson, C. (2011). Quercetin enhances 5-fluorouracil-induced apoptosis in MSI colorectal cancer cells through p53 modulation. *Cancer Chemotherapy and Pharmacology, 68*(6), 1449-1457.