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Effect of *Momordica charantia* and *Ocimum basilicum* on *KRAS* expression in AOM-induced colon cancer in rats

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Abstract. *Okelola CA, Odufuwa KT, Ezima EN, Adegbesan BO, Bello TH. 2025. Effect of* Momordica charantia *and* Ocimum basilicum *on KRAS expression in aom-induced colon cancer in rats. Asian J Nat Prod Biochem 23: 93-100. KRAS* is one of the most frequently mutated oncogenes in colorectal cancer (CRC), contributing to tumor progression and therapeutic resistance. In light of increasing interest in phytotherapy, this study investigated the modulatory effects of aqueous extracts of *Momordica charantia* and *Ocimum basilicum* on *KRAS* gene expression in azoxymethane (AOM)-induced colon cancer in rats. Forty adult male Wistar rats were randomly assigned into five groups (n = 8 per group): three treatment groups (receiving 50, 100, and 200 mg/kg/day of the extract), an AOM-only negative control, and a distilled water-treated normal control. AOM was administered at 20 mg/kg/week for four weeks to induce colon carcinogenesis. After treatment, colon tissues were harvested for histological examination and *KRAS* gene expression analysis using quantitative real-time PCR (qRT-PCR). Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, and phenols in the extracts. Histological evaluation revealed dose-dependent protection in extract-treated groups, with restoration of mucosal structure and reduced atypical features. qRT-PCR showed significant downregulation of *KRAS* gene expression in the high-dose group (200 mg/kg), with a 2.8-fold reduction compared to the AOM-only group ($p < 0.01$). The medium-dose group showed a moderate 1.6-fold reduction, while the low-dose group exhibited slight upregulation of *KRAS* expression. These findings suggest that aqueous extracts of *M. charantia* and *O. basilicum* exert chemopreventive effects in AOM-induced colon cancer, likely through phytochemical-mediated downregulation of oncogenic *KRAS* expression and preservation of colon histoarchitecture. The study supports the potential use of these plants as complementary agents in colorectal cancer therapy.

Keywords: AOM, colon cancer, *KRAS*, *Momordica charantia*, *Ocimum basilicum*, qPCR

INTRODUCTION

Colon cancer is a leading cause of cancer-related morbidity and mortality worldwide, ranking third in incidence and second in cancer-related deaths (Ferlay et al. 2021). Most cases begin as adenomatous polyps, which may progress into malignant tumors through a series of genetic and epigenetic alterations (Taufik et al. 2019). Risk factors include dietary patterns, chronic inflammation, and genetic predispositions. Though colon cancer is traditionally a disease of older adults, recent trends show increasing incidence among younger populations.

A crucial factor in colon cancer pathogenesis is the activation of oncogenes and inactivation of tumor suppressor genes. Among these, the *KRAS* gene (Kirsten rat sarcoma viral oncogene homolog) plays a central role. It encodes a GTPase involved in cell proliferation, survival, and differentiation. Mutations in *KRAS* particularly in codons 12, 13, and 61 lead to continuous activation of the RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways, resulting in uncontrolled cell growth and resistance to apoptosis (Ryan and Corcoran 2018; Li et al. 2022). Approximately 40% of colorectal cancers harbor *KRAS* mutations, which are also predictive of poor response to anti-EGFR therapies like cetuximab (Canon et al. 2019). Kirsten rat sarcoma (*KRAS*) encodes a key signaling protein often mutated in colorectal cancer. While new targeted agents, such as *KRAS* G12C inhibitors, have shown efficacy in subsets of colorectal cancer patients, their applicability remains limited—fueling interest in plant-derived compounds, which provide low-toxicity, multi-pathway anticancer effects (Khan et al. 2023; Ros et al. 2024).

Phytochemicals such as flavonoids, terpenes, saponins, and alkaloids possess antioxidant, anti-inflammatory, and antiproliferative properties. These bioactives can modulate multiple signaling pathways and have shown potential in cancer prevention and treatment (Egbuna et al. 2021; Islam et al. 2022). Two plants of particular interest are *Momordica charantia* (bitter melon) and *Ocimum basilicum* (sweet basil).

Momordica charantia, widely used in traditional medicine, contains active compounds like charantin and momordicine that have been shown to inhibit tumor growth and induce apoptosis (Ahmad et al. 2016; La Torre et al. 2020). *Ocimum basilicum* contains essential oils and flavonoids, including eugenol and linalool, with demonstrated anticancer effects (Shenoy et al. 2016;

Eftekhar et al. 2019). Shenoy et al. (2016) reported that methanolic extracts of *O. basilicum* suppressed colon cancer cell growth by inducing oxidative stress and apoptosis. Minari et al. (2018) also found that combining these plants provided protective effects in chemically induced carcinogenesis models.

Studies have shown that *M. charantia* exhibits antiproliferative, pro-apoptotic, and anti-inflammatory effects in various cancer models. Specifically, bioactive constituents such as momordicin, charantin, and triterpenoids have demonstrated the ability to inhibit cancer cell growth and modulate oncogenic signaling (Kaur et al. 2016; Lin et al. 2021). Similarly, *O. basilicum* is rich in flavonoids, phenolics, and essential oils like eugenol and methyl chavicol, which possess cytotoxic, antioxidant, and anti-inflammatory properties (Kooti et al. 2020; Morshed et al. 2022). These compounds have been shown to suppress tumor growth in colorectal and other gastrointestinal cancer models.

However, despite individual reports on the anticancer effects of these plants, there is limited evidence on their combined use, particularly in relation to *KRAS* gene modulation in colorectal cancer. The current study addresses this critical gap by evaluating the synergistic effect of aqueous extracts of *M. charantia* and *O. basilicum* on *KRAS* gene expression, histopathological changes, and weight trends in an AOM-induced colon cancer model in rats. By investigating their combinatory potential, this study aims to advance the understanding of plant-based gene modulation strategies in colorectal carcinogenesis.

MATERIALS AND METHODS

Study area

This study was conducted at the Animal Research Laboratory and Molecular Biology Unit of the Department of Biochemistry, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The area is located in the humid tropics of southwestern Nigeria with average ambient temperatures of 25-30°C, suitable for maintaining laboratory animal models.

Collection and identification of plant materials

Plants of *M. charantia* and *O. basilicum* were collected in April 2024 from local farms in Sagamu and Ijebu-Ode, Ogun State, Nigeria. The plant materials were authenticated at the Department of Botany, Olabisi Onabanjo University, Ago-Iwoye, Nigeria, and voucher specimens were deposited for future reference.

Preparation of aqueous extracts

Plants were washed, shade-dried for 14 days, and ground into fine powder using an electric blender. The powdered samples were soaked in distilled water (aqueous extraction) in a ratio of 1:10 (w/v) in stoppered glass containers. The mixtures were stirred intermittently and kept at room temperature for 48 hours. They were then filtered through muslin cloth followed by 200 mm mesh filtration. The resulting filtrates were concentrated using a rotary evaporator at 45°C and dried to a paste-like consistency. Extracts were stored at 4°C and reconstituted into appropriate doses for experimental use.

Phytochemical analysis

Qualitative and quantitative phytochemical analyses of the aqueous extracts were carried out according to standard methods described by Vimala et al. (2012), Ejikeme et al. (2016), and the AOAC (2015).

Animal material and ethical compliance

Forty adult male Wistar rats (weighing 180-220 g) were obtained from the Nigerian Institute of Medical Research (NIMR), Lagos. The animals were housed in standard plastic cages in a well-ventilated room at 25°C, with relative humidity between 61-95% and a 12-hour light/dark cycle. They were fed standard laboratory chow and had access to water ad libitum. Animal care and use conformed to institutional ethical guidelines and the World Medical Association (WMA) Declaration of Helsinki (2008) on the ethical treatment of laboratory animals.

Experimental design

Azoxymethane (AOM) was used to induce colon cancer in rats (Table 1). AOM was administered orally at a dose of 20 mg/kg/week for 4 consecutive weeks. Concurrently, rats were administered daily oral doses of the aqueous plant extracts at 50, 100, and 200 mg/kg for four weeks likewise. The extract doses were selected based on previously published LD50 data (Arthur et al. 2011).

At the end of the experimental period, rats were sacrificed via cervical dislocation. Colon tissues were collected; portions were fixed in 10% neutral buffered formalin for histological evaluation, while others were frozen for gene expression analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from colon tissues using TRIzol reagent (Invitrogen). RNA concentration and purity were determined spectrophotometrically at 260/280 nm. Reverse transcription was performed using a commercial cDNA synthesis kit according to the manufacturer's protocol.

Table 1. Experimental design

The rats were randomly divided into five groups (n = 8 per group)
Group RA: AOM (20 mg/kg/week) + 200 mg/kg/day extract
Group RB: AOM (20 mg/kg/week) + 100 mg/kg/day extract
Group RC: AOM (20 mg/kg/week) + 50 mg/kg/day extract
Group NC (Negative Control): AOM only
Group PC (Positive Control): Distilled water only

Note: AOM: Azoxymethane

Table 2. Primer used in rt - qpcr

Genes	Primer	Bases	Annealing	Cycles
GAPDH	S: 5' -GAGAGCCTTGGCATCCCTAT 3'	20	55C	40
	AS: 5'GATGCGGAAGTTGGGTGTTT-3'	20		
KRAS	S: 5`GGTGAGTTTGTATTAAAAGGTACTGG 3'	26	55C	40
	AS: 5`-TCCTGCACCAGTAATATGCA-3'	20		

At the end of the experimental period, rats were sacrificed via cervical dislocation. Colon tissues were collected; portions were fixed in 10% neutral buffered formalin for histological evaluation, while others were frozen for gene expression analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from colon tissues using TRIzol reagent (Invitrogen). RNA concentration and purity were determined spectrophotometrically at 260/280 nm. Reverse transcription was performed using a commercial cDNA synthesis kit according to the manufacturer's protocol.

Quantitative real-time PCR (qPCR)

KRAS gene expression was quantified using SYBR Green-based qPCR on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) was used as an internal control (Table 2). Primers were validated for specificity and efficiency (95-105%). The 2^−ΔΔCt method (Livak and Schmittgen 2001) was used to calculate relative expression levels.

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) was used to assess statistical significance across groups, followed by Tukey's post hoc test for multiple comparisons. GraphPad Prism 9.0 software was used for analysis, and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The qualitative phytochemical screening of the aqueous extract of *O. basilicum* revealed the presence of ten bioactive constituents (Table 3). Alkaloids, saponins, and phenols were found in high abundance (++), while tannins, phlobatannins, terpenoids, cardiac glycosides, steroids, reducing sugars, and flavonoids were moderately present (+). Similarly, the extract of *M. charantia* tested positive for the same ten phytochemical classes (Table 4). Alkaloids and phenols demonstrated high abundance (++), with saponins showing a relatively strong positive reaction, consistent with their water solubility. The remaining phytochemicals exhibited moderate presence (+) across the extract.

Quantitative phytochemical analysis of *O. basilicum* extract (Table 5) showed that alkaloids (58.28 ± 0.01 mg/100 g), saponins (55.28 ± 0.02 mg/100 g), phenols (52.21 ± 0.02 mg/100 g), and tannins (51.24 ± 0.05 mg/100 g) were the most abundant compounds. Other constituents were present in the following quantities: terpenoids (40.87 ± 0.04 mg/100 g), steroids (38.79 ± 0.61 mg/100 g), reducing sugars (32.43 ± 0.02 mg/100 g), phlobatannins (32.68 ± 0.33 mg/100 g), cardiac glycosides (32.12 ± 0.04 mg/100 g), and flavonoids (25.12 ± 0.50 mg/100 g).

Table 3. Qualitative analysis of extracts of *Ocimum basilicum* plant

Phytochemicals	State
Alkaloid	++
Tannin	+
Phlobatannin	+
Saponin	++
Terpenoid	+
Cardiac glycosides	+
Steroid	+
Reducing sugar	+
Flavonoid	+
Phenol	++

Table 4. Qualitative analysis of aqueous extracts of *Momordica charantia* plant

Phytochemicals	State
Alkaloid	++
Tannin	+
Phlobatannin	+
Saponin	+
Terpenoid	+
Cardiac glycosides	+
Steroid	+
Reducing sugar	+
Flavonoid	+
Phenol	++

Table 5. Quantitative analysis of aqueous extracts of *Ocimum basilicum* plant

Phytochemicals	Quantity (mg/100 g)
Alkaloid	58.28 ± 0.01
Tannin	51.24 ± 0.05
Phlobatannin	32.68 ± 0.33
Saponin	55.28 ± 0.02
Terpenoid	40.87 ± 0.04
Cardiac glycosides	32.12 ± 0.04
Steroid	38.79 ± 0.61
Reducing sugar	32.43 ± 0.02
Flavonoid	25.12 ± 0.50
Phenol	52.21 ± 0.02

Table 6. Quantitative analysis of aqueous extracts of *Momordica charantia* plant

Phytochemicals	Quantity (mg/100g)
Alkaloid	50.47 ± 0.01
Tannin	42.39 ± 0.14
Phlobatannin	30.33 ± 0.01
Saponnin	48.29 ± 0.41
Terpenoid	36.14 ± 0.02
Cardiac Glycosides	41.42 ± 0.20
Steroid	39.76 ± 0.21
Reducing sugar	40.94 ± 0.01
Flavonoid	45.81 ± 1.00
Phenol	53.44 ± 0.10

Momordica charantia extract (Table 6) also exhibited considerable phytochemical richness. Phenols (53.44 ± 0.10 mg/100 g), flavonoids (45.81 ± 1.00 mg/100 g), and alkaloids (50.47 ± 0.01 mg/100 g) were notably high. Other components included saponins (48.29 ± 0.41 mg/100 g), reducing sugars (40.94 ± 0.01 mg/100 g), cardiac glycosides (41.42 ± 0.20 mg/100 g), tannins (42.39 ± 0.14 mg/100 g), steroids (39.76 ± 0.21 mg/100 g), terpenoids (36.14 ± 0.02 mg/100 g), and phlobatannins (30.33 ± 0.01 mg/100 g).

The QPCR analysis (Table 7) using the 2^−ΔΔCt method revealed distinct patterns in *KRAS* gene expression across the five treatment groups. Rats in the NC group (AOM only) showed significantly elevated *KRAS* expression relative to the PC group (distilled water). The RA group (200 mg/day) showed the greatest suppression, followed by RB (100 mg/day) and RC (50 mg/day). The difference between the RA group and the NC group was statistically significant ($p < 0.01$, Tukey's test).

Across the four-week period (Table 8), a dose-dependent increase in body weight was observed in the extract-treated groups (RA, RB, RC), with the RA group (200 mg/day) showing the most pronounced gain. Specifically, the RA group progressed from 114.75 ± 4.55 g in week 1 to 133.00 ± 3.08 g in week 4; conversely, the NC group (AOM only) showed a steady decline in weight from 125.25 ± 4.25 g in week 1 to 116.00 ± 2.71 g in week 4. The PC group (distilled water only) maintained a healthy weight gain trend, reaching the highest average weight by week 4 (135.25 ± 2.06 g).

Treatment description and experimental grouping (Table 9), Group PC (Positive Control): Animals received only distilled water throughout the experimental period and served as the healthy reference group. Group NC (Negative Control): Animals were administered 20 mg/kg body weight of AOM once weekly for four weeks, serving as the disease model without therapeutic intervention. Group RC (Low Dose Treatment): Animals received 20 mg/kg AOM weekly plus 50 mg/kg/day of the combined plant extract. Group RB (Medium Dose Treatment): Animals received 20 mg/kg AOM weekly plus 100 mg/kg/day of the combined plant extract. Group RA (High Dose Treatment): Animals received 20 mg/kg AOM weekly plus 200 mg/kg/day of the combined plant extract.

Table 7. Summary of *KRAS* gene expression across experimental groups

Group	Mean ΔCt ± SD	Fold Change (2^−ΔΔCt)	Direction of Modulation	p-value vs NC	Significant?
PC	4.89 ± 0.42	2.3	Upregulated	0.071	No
NC	5.94 ± 0.31	3.9	Baseline (AOM only)	–	–
RA	2.78 ± 0.33	1.2	Downregulated	0.003	Yes
RB	4.21 ± 0.37	1.8	Downregulated	0.021	Yes
RC	6.32 ± 0.55	4.6	Upregulated	0.045	Yes

Table 8. Weekly body weights of rats across all groups (g)

Week	Group RA (200 mg)	Group RB (100 mg)	Group RC (50 mg)	Group NC (AOM only)	Group PC (Distilled water)
1	114.75 ± 4.55	119.00 ± 2.16	122.75 ± 3.30	125.25 ± 4.25	128.75 ± 3.30
2	121.00 ± 2.94	120.00 ± 2.16	121.75 ± 1.71	122.00 ± 2.71	130.75 ± 1.50
3	127.00 ± 1.83	126.75 ± 3.96	123.75 ± 3.59	120.75 ± 3.30	131.25 ± 2.99
4	133.00 ± 3.08	129.25 ± 2.06	125.25 ± 1.50	116.00 ± 2.71	135.25 ± 2.06

Note: Values are presented as Mean ± Standard Deviation

Table 9. Group definitions and treatment description

Group	Treatment Description
PC (Positive control)	Rats that received distilled water only (normal control, not induced with AOM)
NC (Negative control)	Rats that received 20 mg/kg/week AOM only (cancer-induced, no treatment)
RA	Rats that received 20 mg/kg/week AOM + 200 mg/kg/day aqueous extract of *M. charantia* and *O. basilicum*
RB	Rats that received 20 mg/kg/week AOM + 100 mg/kg/day aqueous extract of *M. charantia* and *O. basilicum*
RC	Rats that received 20 mg/kg/week AOM + 50 mg/kg/day aqueous extract of *M. charantia* and *O. basilicum*

In Figure 1, the histological examination of colon tissue sections revealed marked architectural disruption of the mucosal lining. There was a visible reduction in inflammatory cell infiltration (yellow arrow), and areas containing atypically shaped malignant cells (red arrow) appeared significantly fewer. This suggests a decrease in neoplastic proliferation or the induction of apoptosis. Figure 2 sections showed mild distortion of mucosal structure with reduced inflammatory infiltrates (yellow arrow). The diminished presence of atypical malignant cells (red arrow) further suggests a decline in tumor progression and local immune activation. Moreover, in Figure 3, this sample showed less histological recovery, with multiple foci of intense inflammation (red arrows), suggestive of ongoing immune response. These may result from unresolved neoplastic cells, necrotic tissue, or a pro-tumorigenic inflammatory microenvironment. While Figure 4 histological findings were consistent with healthy colon morphology. The glandular architecture was intact, with no evidence of neoplastic or pre-neoplastic changes. Mild physiological inflammation was observed. In addition, Figure 5 sections indicated pronounced dysplasia: loss of glandular organization, nuclear pleomorphism, hyperchromasia, increased mitotic activity, and disrupted epithelial polarity features characteristic of malignant transformation.

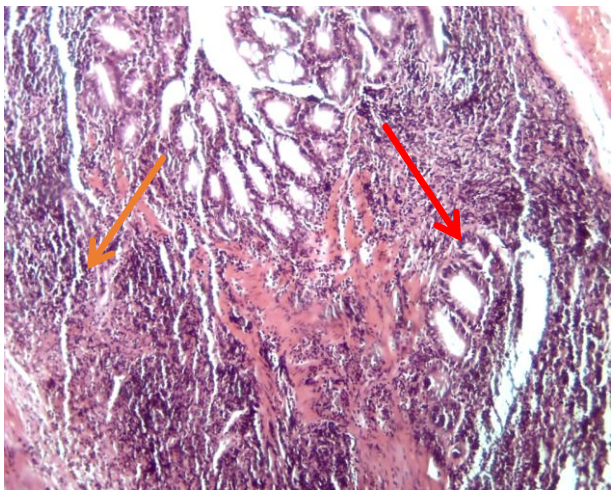

Figure 1. Histological section of colon tissue of rat from Group RA (AOM + 200 mg/day extract), H&E stain, ×400 magnification. Showing the restored crypt alignment, intact epithelial lining, and minimal inflammatory infiltration

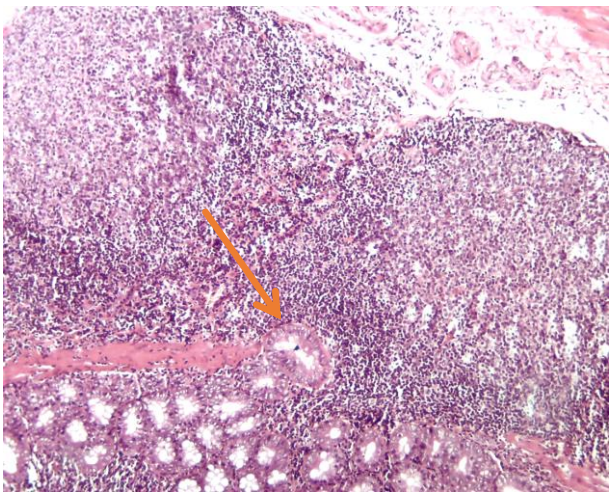

Figure 2. Histological section of colon tissue of rat from Group RB (AOM + 100 mg/day extract), H&E stain, ×400 magnification. It reveals slight distortion of the mucosal architecture, reduced inflammatory infiltrates and fewer atypical malignant cells

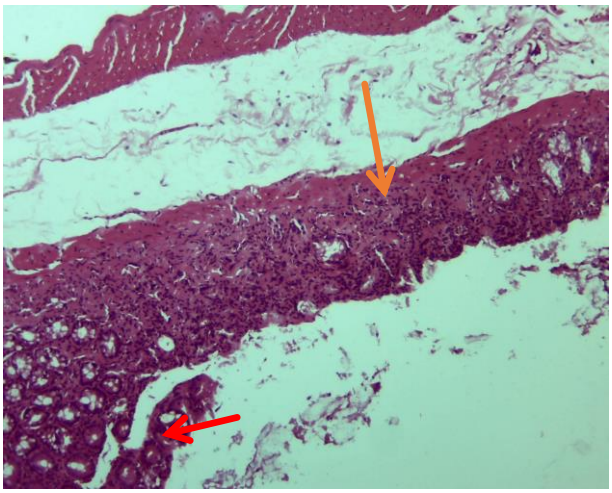

Figure 3. Histological section of the colon tissue from Group RC (AOM + 50 mg/day extract), stained with Hematoxylin and Eosin (H&E), viewed at ×400 magnification. The image shows less effective histological recovery

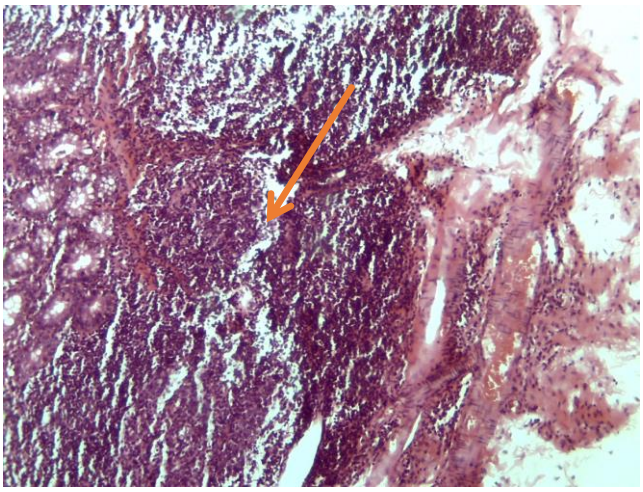

Figure 4. Histological section of the colon tissue from Group NC (AOM only), stained with Hematoxylin and Eosin (H&E), viewed at ×400 magnification. The image shows disrupted crypt architecture, mucosal erosion, and dense lymphocytic infiltration

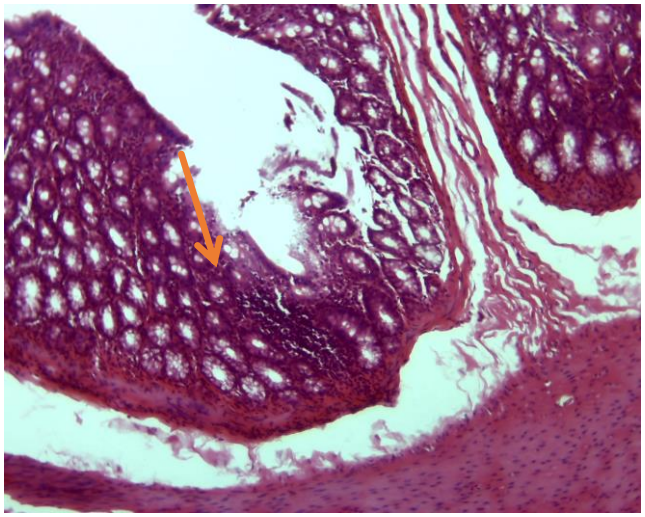

Figure 5. Histological section of colon tissue of induced colon cancer in rats from group PC (Water only) stained with Hematoxylin and Eosin (H&E), viewed at ×400 magnification. The image demonstrate preserved colonic glandular structure (no cancer or precancerous change) and mild physiological inflammation, typical of healthy tissue

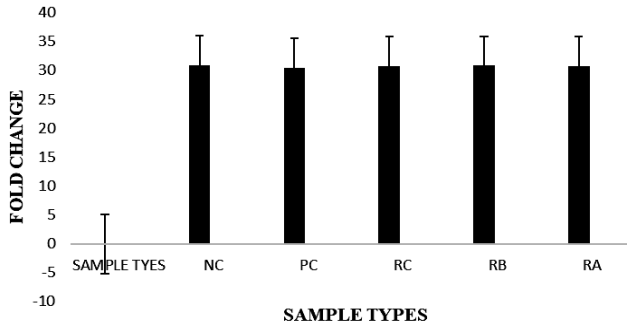

Figure 6. QPCR of *KRAS* mRNA expression in AOM induced colon cancer rats treated with different concentration of aqueous extract of *Momordica charantia* and *Ocimum basilicum* plant

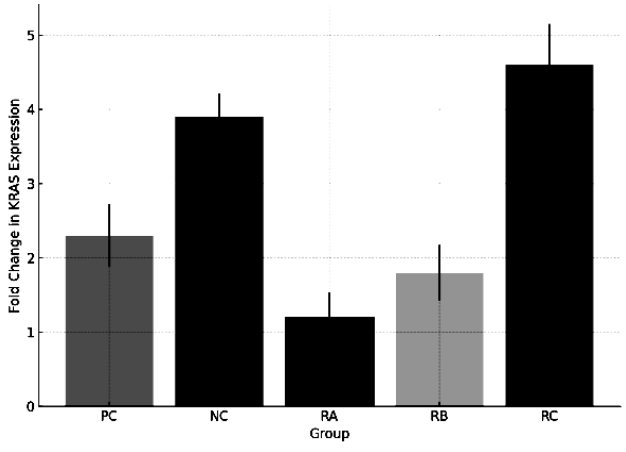

Figure 7. Relative *KRAS* gene expression levels (fold change, 2^−ΔΔCt) in colon tissue of rats across five groups

Figure 6 displays the relative expression levels of the *KRAS* oncogene across five experimental groups using the 2^−ΔΔCt method. It revealed a significant decrease in *KRAS* gene expression in Group RA compared to the AOM-only group (mean ΔCt = 2.78 vs. 5.94, p = 0.003, Cohen is d = 1.73, 95% CI [0.89, 2.57]). The large effect size suggests that the extract had a strong suppressive effect on *KRAS* activation. No significant difference was found between the PC and RB groups (p = 0.087), indicating a dose-dependent response. The highest upregulated *KRAS* expression is seen in Group RC, followed by Group PC. Group RC (low extract dose) shows fold changes above 2.0, suggesting a failure to suppress oncogenic signaling, likely due to insufficient phytochemical concentration. The PC group, which received no carcinogen or extract, unexpectedly displays moderate upregulation of *KRAS*. This may reflect baseline physiological variation or compensatory expression unrelated to AOM exposure.

Group RB (mid dose) and Group RA (high dose) showed significant downregulation of *KRAS* expression compared to both the NC and PC groups. Group RA, in particular, demonstrated *KRAS* fold changes close to 1.2, indicating effective modulation of oncogenic pathways and a potential return to near-normal expression levels. The NC group, exposed to AOM only, displayed moderate *KRAS* expression, suggesting a substantial, but not peak, activation of oncogenic pathways.

Figure 7 shows the relative *KRAS* gene expression levels (fold change, 2^−ΔΔCt) in colon tissue of rats across five groups: PC (normal control), NC (AOM only), and treatment groups RA (200 mg/kg), RB (100 mg/kg), and RC (50 mg/kg). Group RA showed the most significant downregulation of *KRAS* (p = 0.003), with expression approaching baseline. Error bars represent standard deviation (n = 8).

Discussion

Phytochemical screening of *M. charantia* and *O. basilicum* aqueous extracts confirmed the presence of several bioactive compounds, notably flavonoids, phenols, alkaloids, and saponins, which are widely documented for their antioxidant and anticancer properties. These secondary metabolites contribute synergistically to the extracts' ability to combat oxidative stress, modulate oncogenic signaling, and induce apoptosis in neoplastic cells.

Among these, flavonoids and phenolic compounds highly abundant in both plants play a pivotal role by scavenging reactive oxygen species (ROS) and stabilizing cellular redox states. These antioxidant actions are crucial in mitigating the oxidative DNA damage central to colon carcinogenesis. Flavonoids like vicenin and orientin from *O. basilicum* in colon cancer have also been shown to directly inhibit proliferation and induce apoptosis in colon cancer models (Shenoy et al. 2016), a trend echoed by the significant histological improvements in extract-treated rats in this study.

Histopathological findings revealed restoration of crypt architecture, reduced inflammatory infiltration, and

decreased nuclear atypia in treated groups compared to the AOM-only control, indicating effective suppression of tumor progression and enhancement of mucosal repair. These outcomes align with reports showing that compounds like momordicine, karavilagenin, eugenol, and methyl chavicol activate apoptotic pathways and enforce cell cycle checkpoints (Liu et al. 2019; Raina et al. 2020).

The body weight trends observed in this study confirm that *M. charantia* and *O. basilicum* extracts exhibit dose-dependent protective effects against AOM-induced systemic toxicity. These effects were reflected not only in gene expression and histology, but also in the maintenance of physiological growth and metabolism, further validating the chemopreventive potential of the combined extracts. These findings align with previous research demonstrating that flavonoid-rich plant extracts can improve appetite, enhance gut integrity, and reduce systemic inflammation, all of which contribute to weight stabilization in tumor-bearing animals (Kooti et al. 2020; Morshed et al. 2022).

This study explored the chemopreventive effect of aqueous extracts of *Momordica charantia* and *Ocimum basilicum* on AOM-induced colon cancer in rats, with particular emphasis on the expression of the *KRAS* gene, a critical proto-oncogene implicated in colorectal tumorigenesis. Our findings show a dose-dependent downregulation of *KRAS* mRNA expression, accompanied by histological evidence of mucosal restoration and decreased inflammatory infiltration, especially in the high-dose group.

The modulation of *KRAS* expression is particularly significant given its central role in driving MAPK/ERK and PI3K/AKT signaling pathways, which are frequently dysregulated in colorectal cancer (CRC). *KRAS* mutations are not only associated with tumor initiation and progression but also with resistance to EGFR-targeted therapies. Therefore, the observed suppression of *KRAS* expression, particularly in the high-dose group (RA), offers novel insight into the potential molecular mechanisms through which these plant extracts may exert anti-tumorigenic effects.

One of the noteworthy strengths of this study is the clear dose-response pattern observed in *KRAS* expression across treatment groups. While the low-dose group (RC) showed negligible changes in *KRAS* activity, the high-dose group (RA) demonstrated a significant reduction ($p < 0.01$) in *KRAS* gene expression. This finding suggests that the phytochemical synergy of the extracts is both concentration-dependent and biologically effective, supporting earlier research that linked flavonoids, phenolics, and saponins to modulation of oncogenic signaling (Ganesan and Xu 2020; Islam et al. 2023).

Moreover, the findings in this study are consistent with those of Islam et al. (2022), who reported significant antiproliferative effects of polyphenol-rich plant extracts in CRC models via suppression of RAS and PI3K/AKT pathways. Shenoy et al. (2016) similarly demonstrated that *O. basilicum* extracts induced apoptosis and inhibited proliferation in HT-29 colon cancer cells, consistent with the *KRAS* downregulation and histological restoration observed in our high-dose treatment group. Additionally, compounds such as momordicine from *M. charantia* have been reported to suppress oncogenic signaling and induce G1-phase cell cycle arrest in colon cancer lines (La Torre et al. 2020; Raina et al. 2020).

The antioxidant capacity of these extracts, conferred by flavonoids and phenolic acids, may contribute to reduced oxidative DNA damage, a key event in colon carcinogenesis. These bioactives are known to scavenge reactive oxygen species, inhibit inflammatory mediators like COX-2 and NF-κB, and modulate apoptosis-related proteins such as BAX and caspases (Ganesan and Xu 2020). Such pleiotropic effects could underlie the observed normalization of colon architecture and reduced *KRAS* gene expression.

Importantly, phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, phenols, saponins, and terpenoids, which are well-documented for their antioxidant, anti-inflammatory, and antiproliferative activities. Flavonoids, in particular, have been reported to inhibit *KRAS* and downstream effectors by interfering with protein prenylation, membrane localization, or transcriptional regulation (La Torre et al. 2020). Our findings reinforce this potential and extend it to a novel botanical formulation combining *M. charantia* and *O. basilicum*.

Collectively, the findings support the chemopreventive and therapeutic potential of these extracts in colorectal cancer. By attenuating *KRAS*-driven signaling, reducing oxidative stress, and restoring histological integrity, *M. charantia* and *O. basilicum* demonstrate a multi-targeted action profile worthy of further pharmacological exploration.

In conclusion, this study demonstrated the chemopreventive potential of aqueous extracts of *Momordica charantia* and *Ocimum basilicum* in an azoxymethane (AOM)-induced colon cancer model in rats. The combined extracts downregulated oncogenic pathways—evidenced by suppressed expression of *KRAS*—and promoted mucosal recovery and reduced inflammation in a dose-dependent manner. The high-dose group (200 mg/kg/day) showed the greatest improvement across body weight, histoarchitecture, and gene expression. These results support the therapeutic relevance of the synergistic phytochemical composition of both plants, consistent with previous findings on the anticancer effects of flavonoids, phenolics, and terpenoids. However, gene expression data alone do not confirm protein-level modulation. Future studies should include protein validation via Western blot or ELISA, and explore broader targets and long-term outcomes to clarify mechanisms underlying the observed protective effects in colorectal cancer.

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