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Original Article

Protective Effects of Methanolic Leaf Extract of *Citrullus colocynthis* Combined with Kefir against 1,2-Dimethylhydrazine and High-Fat Diet-Induced Colorectal Cancer in Wistar Rats

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ABSTRACT

Background: Colorectal Cancer (CRC) is a major global malignancy strongly influenced by diet and lifestyle. Natural products and probiotics are being explored for their chemopreventive potential. **Objectives:** To evaluate the combined protective effects of Methanolic Leaf Extract of *Citrullus colocynthis* (MLECC) and kefir against CRC induced by 1,2-Dimethylhydrazine (DMH) and a High-Fat Diet (HFD) in Wistar rats. **Materials and Methods:** Forty-two male Wistar rats were divided into four groups: control, disease (DMH + HFD), probiotic (DMH + HFD + kefir), and treatment (DMH + HFD + kefir + MLECC). Hematological indices, liver enzymes (AST, ALT, ALP), C-reactive protein, microbial enzyme activity, oxidative-stress biomarkers, Aberrant Crypt Foci (ACF), Ki-67 expression, and histopathology were evaluated. Data were analyzed by one-way ANOVA followed by Dunnett's *post hoc* test. **Results:** DMH + HFD exposure caused anemia, hepatic injury, inflammation, oxidative imbalance, and high ACF incidence. Kefir partly reversed these effects, while the MLECC + kefir combination normalized biochemical and histological parameters, reduced ACF by ≈60%, and suppressed Ki-67 expression ($p<0.05$ vs disease). **Conclusion:** Co-administration of MLECC and kefir exerts synergistic antioxidants, anti-inflammatory, and antiproliferative actions, supporting their potential as adjuncts in colorectal cancer prevention.

Keywords: Colorectal cancer, *Citrullus colocynthis*, Kefir, 1,2-Dimethylhydrazine, High-fat diet, Oxidative stress, Chemoprevention.

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INTRODUCTION

Colorectal Cancer (CRC) remains a major global health concern, ranking among the top three most diagnosed malignancies and the second leading cause of cancer mortality worldwide. According to GLOBOCAN 2020, CRC accounted for ~1.9 million new cases and 0.94 million deaths. Although incidence in India is comparatively lower, it has shown a steady rise in urban populations, largely due to dietary changes, obesity, and reduced physical activity.

CRC develops through a multistep process that includes transformation of benign adenomatous polyps into invasive adenocarcinomas. This progression is driven by mutations in genes such as *KRAS* and *TP53*, and dysregulation of signaling cascades including Wnt/β-catenin, NF-κB, and PI3K/AKT. Aberrant Crypt Foci (ACF) represent early microscopic lesions predictive of tumorigenesis.

1,2-Dimethylhydrazine (DMH) is widely used to induce experimental CRC, as its metabolites closely mimic human oncogenic mutations. When combined with a High-Fat Diet (HFD), it promotes oxidative stress, inflammation, and gut dysbiosis, intensifying carcinogenic potential.

Natural products and probiotics have gained attention as complementary strategies for chemoprevention. *Citrullus colocynthis* (L.) Schrad. (bitter apple) is a traditional medicinal plant containing cucurbitacins, flavonoids, and glycosides with reported anti-inflammatory and antiproliferative activities.

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Kefir, a fermented milk drink rich in lactic acid bacteria and yeasts, modulates gut microbiota and suppresses inflammatory responses.

The rationale of this work was to evaluate whether combining the Methanolic Leaf Extract of *Citrullus colocynthis* (MLECC) with kefir could yield synergistic protection against DMH + HFD-induced CRC. The study examined biochemical, hematological, oxidative, microbial, and histological parameters to elucidate their potential mechanisms of chemoprevention.

MATERIALS AND METHODS

Chemicals and reagents

1,2-Dimethylhydrazine (DMH) and analytical-grade reagents were obtained from Sri GhaMa Enterprises (India). The high-fat diet was purchased from VRK Nutritional Solutions (India). Methanolic leaf extract of *C. colocynthis* was procured from Kshipra Biotech Pvt. Ltd., Indore, India. Phytochemical profiling by HPLC confirmed the presence of cucurbitacins and flavonoids.

Preparation of kefir

Kefir grains were cultured in sterilized whole milk at room temperature for 24 hr. The fermented beverage was filtered to remove grains and stored at 4°C until use. Microbial composition was confirmed by plating on selective media for *Lactobacillus, Lactococcus*, and *Saccharomyces* species.

Experimental animals and ethics

42 healthy male Wistar rats (130-140 g) were housed under controlled temperature (25±2°C), humidity (45-55%), and a 12 hr light/dark cycle, with free access to food and water. All procedures followed CPCSEA guidelines and were approved by the Institutional Animal Ethics Committee (CPCSEA Registration No: 516/01/A/CPCSEA).

Experimental design

Rats were randomized into four groups (n=10 each).

- **Control:** vehicle (0.5% CMC in saline) + normal diet.
- **Disease:** DMH (40 mg/kg twice weekly) + HFD.
- **Probiotic:** DMH + HFD + kefir (3.87 mg/mL orally).
- **Treatment:** DMH + HFD + kefir + MLECC (200 mg/kg orally).

The study lasted 13 weeks. On day 91, animals were euthanized under CO_2 asphyxiation. Blood, serum, and colon tissues were collected for analysis.

Hematological and biochemical assays

Blood was analyzed for RBC, WBC, hemoglobin, and platelet counts using an automated hematology analyzer. Serum AST, ALT, ALP, and C-reactive protein were measured using commercial colorimetric and immunoturbidimetric kits.

Microbial enzyme activities

Colonic and fecal homogenates were assayed for β-glucuronidase, β-glucosidase, and mucinase activities following standard protocols, with results normalized to protein content (Bradford method).

Oxidative stress parameters

Colon tissue homogenates were evaluated for reduced Glutathione (GSH), Glutathione Peroxidase (GPx), Catalase (CAT), and Lipid Peroxidation (LPO) as Thiobarbituric-acid reactive substances (TBARS).

Aberrant Crypt Foci (ACF) assessment

Fixed colons were stained with methylene blue, and ACF were counted microscopically at ×10 magnification.

Molecular and histological analysis

Gene expression of *Ki-67* and *BCL-2* was quantified by qRT-PCR relative to *GAPDH*. Histopathology was performed on H&E-stained sections, and β-catenin localization assessed by immunohistochemistry.

Statistical analysis

Results are expressed as mean±SEM. Data was analyzed by one-way ANOVA followed by Dunnett's *post hoc* test using GraphPad Prism 8.0. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Body weight and survival

DMH + HFD exposure caused a marked reduction in weight gain and growth rate, with 40% mortality compared to the control group. Animals treated with kefir or the MLECC + kefir combination exhibited improved body weight and survival, with mortality decreasing to 30% and 20%, respectively.

Changes in body weight, growth rate, survival, and mortality across experimental groups are summarized in Table 1.

These improvements suggest that probiotic supplementation, particularly when combined with *C. colocynthis* extract, enhances resilience against carcinogen-induced metabolic stress.

Hematological parameters

The disease group showed anemia (decreased RBC and hemoglobin) and leukocytosis with elevated neutrophils, indicative of systemic inflammation. Kefir treatment partially corrected these alterations, while the MLECC + kefir combination

restored hematological parameters close to normal levels ($p<0.05$).

The effects of MLECC and kefir on hematological parameters including RBC, WBC, hemoglobin, platelet count, neutrophils, and lymphocytes are presented in Table 2.

The effect of MLECC and kefir on hematological parameters in DMH + HFD-induced colorectal cancer rats is illustrated in Figure 1.

This recovery reflects the combined antioxidant and immunomodulatory effects of MLECC and kefir, which are likely to protect erythrocytes from oxidative damage and normalize leukocyte function.

Liver enzymes and inflammatory markers

DMH + HFD caused significant elevation of serum AST, ALT, ALP, and CRP, reflecting hepatic stress and systemic inflammation. Both kefir and MLECC reduced these elevations, with the combination producing near-normal values ($p<0.05$).

Serum liver enzymes (AST, ALT, ALP) and C-reactive protein levels across experimental groups are shown in Table 3.

The observed hepatoprotection may arise from flavonoids and cucurbitacins in MLECC stabilizing hepatocyte membranes, while probiotic peptides from kefir suppress inflammatory mediators such as NF-κB and IL-6.

Microbial enzyme activity

DMH + HFD increased β-glucuronidase, β-glucosidase, and mucinase activity, which promote carcinogen reactivation and mucus barrier degradation. Kefir reduced these enzyme levels, and the combination with MLECC achieved the most pronounced reduction ($p<0.05$).

Alterations in fecal pH and fecal microbial enzyme activities, including β-glucuronidase, β-glucosidase, and mucinase, across experimental groups are presented in Table 4.

The changes in fecal pH and fecal microbial enzyme activities following MLECC and kefir treatment are depicted in Figure 2.

The effects of MLECC and kefir on colonic mucosal microbial enzyme activities are summarized separately in Table 5.

The modulation of colonic mucosal microbial enzyme activities by MLECC and kefir is shown in Figure 3.

These findings support a synergistic microbiota-modulating effect, where kefir alters bacterial composition and MLECC polyphenols exhibit prebiotic-like properties.

Oxidative stress markers

The disease group showed depleted GSH, GPx, and CAT, along with elevated lipid peroxidation (TBARS). Treatment with kefir and MLECC significantly enhanced antioxidant enzyme activities and reduced lipid peroxidation ($p<0.05$).

The effects of treatments on antioxidant status and lipid peroxidation in colon tissue are summarized in Table 6.

The effects of MLECC and kefir on antioxidant status and lipid peroxidation in colon tissue are presented in Figure 4.

The results indicate potent free-radical-scavenging activity and reinforcement of endogenous defense systems. Cucurbitacins and flavonoids in *C. colocynthis*, combined with probiotic metabolites, may act synergistically to counteract DMH-induced oxidative injury.

Table 1: Impact of treatment on body weight and survival in DMH + HFD-induced CRC rats.

Group Name (n=6)	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Growth rate	No. of Initial animals	No. of deaths	% Mortality
Control	135±6.732	273±16.17	138±11.51	1.74	10	0	0%
Disease	133±5.971	210.6±5.56$^{\#}$	77.6±12.51$^{\#}$	1.02	10	4	40%
Kefir	134.33±8.787	239.6±2.36*	107.3±3.09*	1.27	10	3	30%
CC+Kefir	134.0±5.856	251.63±3.9*	117.6±4.52*	1.40	10	2	20%

Values are represented as Mean±SEM (n=10). *p* < 0.05 compared with disease control.*

Table 2: Effects of MLECC and Kefir on Hematological Parameters.

	RBC (×10^6/μL)	WBC (×10^3/μL)	HGB (g/dL)	Platelets (10^3/μL)	Neutrophil count	Lymphocyte count
Control	6.633±0.187	8157±132.8	13.63±0.3284	826.67±77.60	21.19±1.150	66.00±3.510
Disease	4.580±0.5003$^{\#}$	10890±315.1$^{\#}$	10.18±0.5829$^{\#}$	1493.33±52.11$^{\#}$	47.33±3.502$^{\#}$	34.67±2.160$^{\#}$
Kefir	5.893±0.1598*	8987±279.5 *	12.10±0.1256*	1369.52±48.25*	41.87±2.482 *	45.67±2.160*
CC+kefir	6.387±0.1846*	8436±253.6*	12.52±0.4983*	1221.39±50.13*	32.49±1.105*	49.89±2.585*

Values are represented as mean±SEM (n=10). *p* < 0.05 compared with disease control.*

Table 3: Effects of MLECC and kefir on liver enzymes and C-reactive protein.

Group Name (*n*=6)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Serum C-reactive protein (mg/dL)
Control	56.83±4.262	60.5±3.507	138.5±3.619	14.73±3.46
Disease	125.3±4.457$^{\#}$	120.7±4.010$^{\#}$	284.8±6.942$^{\#}$	36.12±2.97$^{\#}$
Kefir	103.7±4.546*	110.6±4.819*	248.1±6.128*	28.17±3.11*
CC+kefir	87.69±4.429*	86.54±3.509*	191.7±5.167*	19.01±2.53*

Values are represented as mean±SEM (*n*=10). **p* < 0.05 compared with disease control.*

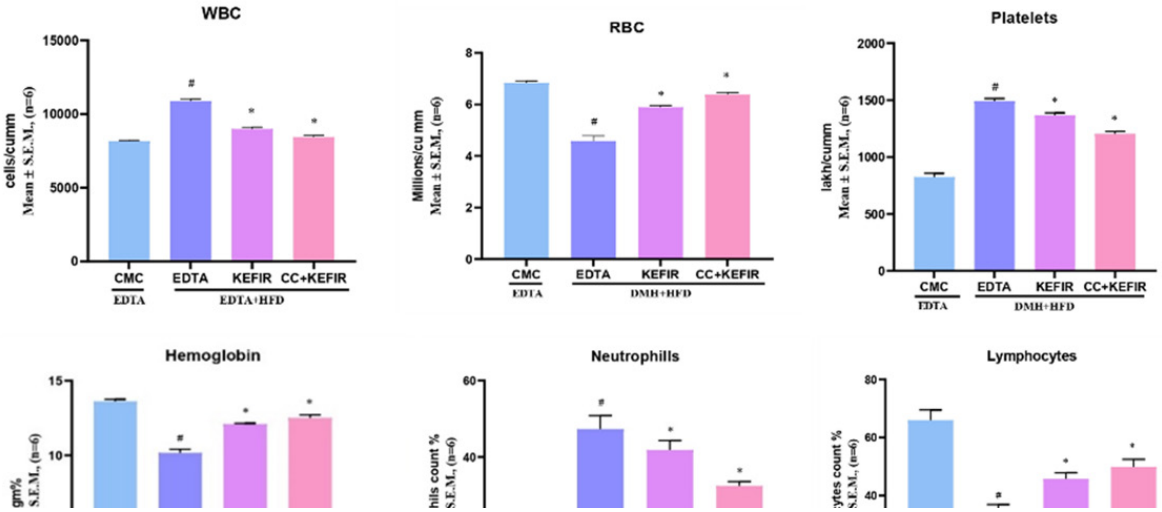

Figure 1: Effect of Methanolic Leaf Extract of *Citrullus colocynthis* (MLECC) and kefir on hematological parameters (WBC, RBC, hemoglobin, platelets, neutrophils, and lymphocytes) in DMH + HFD-induced colorectal cancer in Wistar rats. Bar graphs showing alterations in RBC, WBC, hemoglobin, platelet count, neutrophils, and lymphocytes among experimental groups. The disease group exhibited anemia and leukocytosis, whereas kefir and MLECC+kefir treatments significantly restored hematological indices toward normal values. Data are presented as mean±SEM (*n*=10). *p*<0.05 versus disease control.

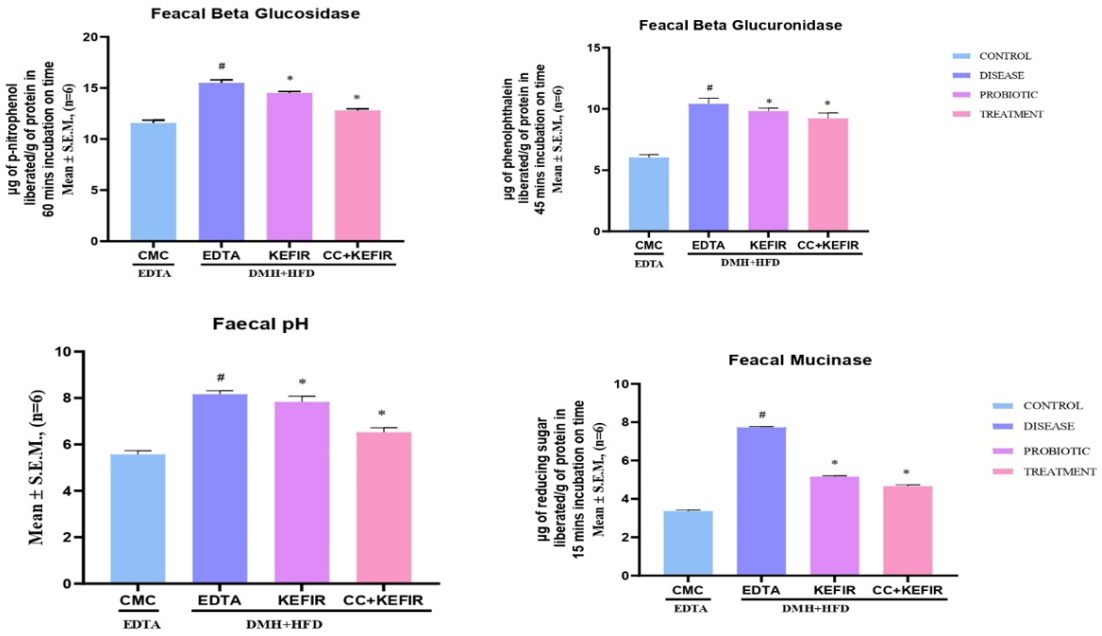

Figure 2: Effect of MLECC and kefir on fecal microbial enzyme activities (β-glucosidase, β-glucuronidase, and mucinase) and fecal pH in DMH + HFD-induced colorectal cancer in Wistar rats. Changes in β-glucuronidase, β-glucosidase, mucinase activity, and fecal pH in DMH + HFD-induced colorectal cancer rats. Disease induction reminds increased enzyme activities associated with carcinogen activation, whereas kefir and MLECC+kefir significantly reduced these microbial markers, indicating modulation of gut microbiota.

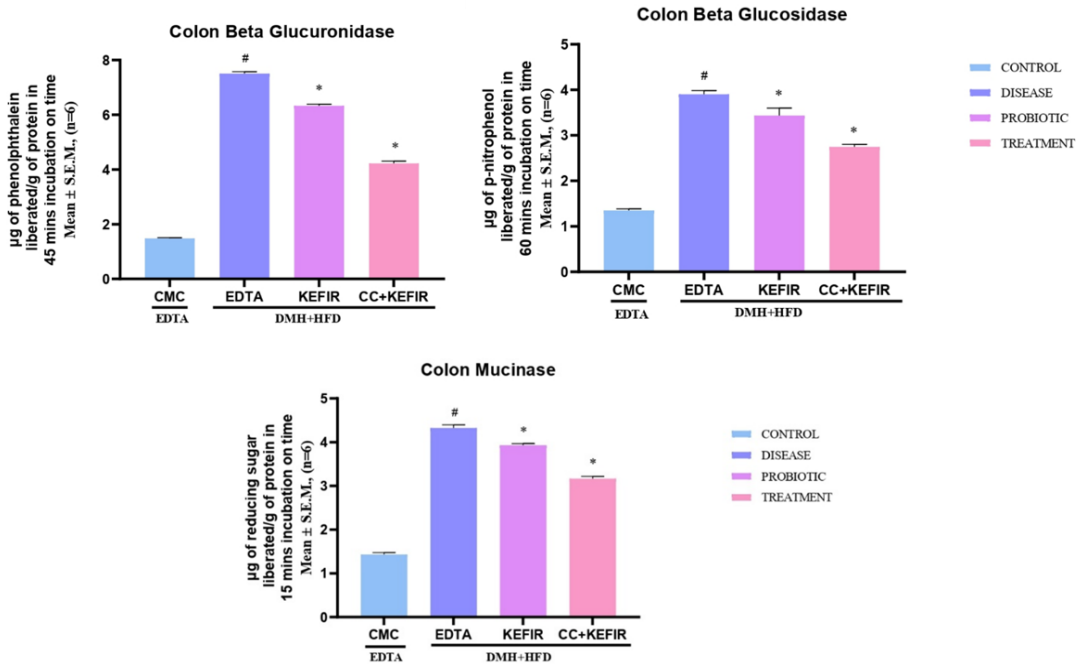

Figure 3: Effect of MLECC and kefir on colonic mucosal microbial enzymes (β-glucuronidase, β-glucosidase, and mucinase) in DMH + HFD-induced colorectal cancer rats. Enzyme activities of β-glucuronidase, β-glucosidase, and mucinase measured in colon tissue homogenates. Combined treatment markedly suppressed microbial enzyme levels compared with disease control, suggesting protection against mucosal degradation and carcinogenic metabolite production.

Table 4: Effects on microbial enzyme levels in fecal homogenates.

Group Name (*n*=6)	Fecal Matter pH	ß-Glucuronidase	ß- Glucosidase	Mucinase
Control	5.567±0.16	6.033±0.23	11.56±0.17	3.365±0.14
Disease	8.163±0.15$^{\#}$	10.42±0.45$^{\#}$	15.5±0.71$^{\#}$	7.73±0.11$^{\#}$
Kefir	7.833±0.25*	9.82±0.24*	14.51±0.35*	5.158±0.11*
CC+kefir	6.524±0.35*	9.11±0.54*	12.80±0.36*	4.686±0.15*

Values are represented as Mean±SEM (n=10). *p* < 0.05 compared with disease control.

Aberrant crypt foci and proliferation markers

Aberrant Crypt Foci (ACF) were abundant in the disease group, confirming successful CRC induction. Kefir reduced ACF incidence to ≈ 57%, while MLECC + kefir reduced it further to ≈ 40%. *Ki-67* mRNA expression, elevated in the disease group, was significantly suppressed in the combination treatment (p<0.05).

The distribution and incidence of aberrant crypt foci among treatment groups are presented in Table 7.

Relative Ki-67 gene expression levels across experimental groups are summarized in Table 8.

The impact of MLECC and kefir on aberrant crypt foci formation and Ki-67 expression is illustrated in Figure 5.

Reduced ACF formation and proliferation marker expression confirm that both interventions hinder early tumor promotion. Comparable effects have been observed with other antioxidant plant compounds such as curcumin and quercetin.

Histopathology and β-catenin immunohistochemistry

Histological analysis revealed dysplastic lesions, loss of goblet cells, and inflammatory infiltrates in the disease group. Kefir treatment improved mucosal integrity, while the combination group exhibited near-normal colonic architecture with intact mucous-secreting cells. β-catenin immunostaining showed abnormal nuclear localization in disease samples, which was restored to membranous localization in the treatment group, indicating normalization of Wnt signaling.

Representative peripheral blood smear morphology across experimental groups is shown in Figure 6.

Histopathological changes and treatment-induced restoration of colonic architecture are illustrated in Figure 7.

Immunohistochemical localization of β-catenin in colon tissue and its modulation by MLECC and kefir treatment are shown in Figure 8.

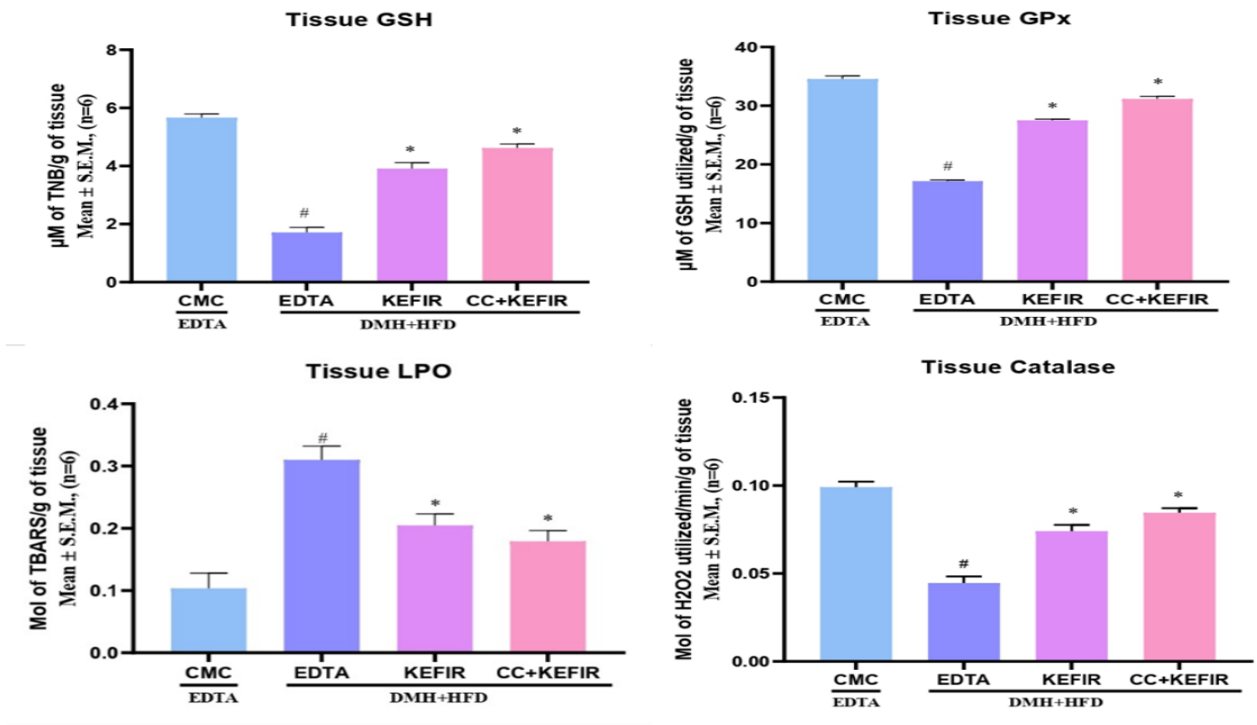

Figure 4: Effect of MLECC and kefir on oxidative stress biomarkers (GSH, GPx, LPO, and catalase) in colon tissue homogenates of DMH + HFD-induced colorectal cancer rats. Levels of reduced Glutathione (GSH), Glutathione Peroxidase (GPx), Catalase (CAT), and lipid peroxidation (LPO/TBARS) in experimental groups. DMH + HFD reduced antioxidant defenses and elevated oxidative damage, while combined treatment restored antioxidant enzyme activity and reduced lipid peroxidation. Data expressed as mean±SEM (*n*=10).

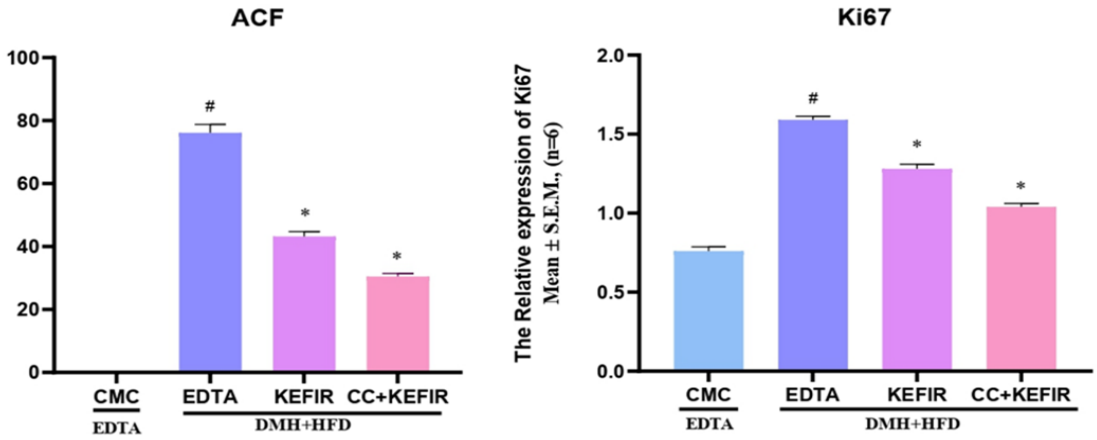

Figure 5: Effect of MLECC and kefir on Aberrant Crypt Foci (ACF) formation and Ki-67 expression in colon tissues of DMH + HFD-induced colorectal cancer rats. Quantitative analysis of ACF incidence and relative Ki-67 mRNA expression in colon tissue. Combination therapy significantly reduced ACF number and suppressed proliferation marker expression compared with disease control, indicating inhibition of early tumorigenesis.

Table 5: Effects on microbial enzyme levels in colon homogenates.

Group name (*n*=6)	ß-Glucuronidase	ß- Glucosidase	Mucinase
Control	1.482±0.0719	1.35±0.0874	1.435±0.0977
Disease	7.510±0.1657$^{\#}$	3.905±0.1945$^{\#}$	4.325±0.1734$^{\#}$
Kefir	6.332±0.1286*	3.435±0.3986*	3.932±0.0920*
CC+kefir	4.227±0.195*	2.749±0.128*	3.167±0.125*

Values are represented as Mean±SEM (*n*=10). *$p^{*} < 0.05$* compared with disease control.

Table 6: Effects on antioxidant markers in colon tissue.

Group Name (*n*=6)	GSH (*μ*M/g tissue)	GPx (μgm GSH utilized/ min/mg protein)	LPO (nmol MDA/mL plasma)	CAT (units/mg protein
Control	5.671±0.3074	34.56±1.245	0.1036±0.060	0.0991±0.0076
Disease	1.711±0.4296$^{\#}$	17.14±0.534$^{\#}$	0.3100±0.054$^{\#}$	0.0446±0.0092$^{\#}$
Kefir	3.910±0.5142*	27.50±0.529*	0.2048±0.045*	0.0741±0.0086*
CC+kefir	4.621±0.3360*	31.16±1.098*	0.1790±0.043*	0.0845±0.0065*

Values are represented as mean±SEM (*n*=10). *$p^{*} < 0.05$ compared with disease control.*

Table 7: Effects on ACF count.

Group Name (*n*=6)	Number of aberrant crypts per ACF				Total no. ACF	% of incidence
	1 crypt	2 crypts	3 crypts	4 crypts		
Control	NIL	NIL	NIL	NIL	NIL	NIL
Disease	22.80±5.2	17.33±3.44	14.0±1.18	22±1.58	76.13±6.53$^{\#}$	100
Kefir	10.98±2.8	9.21±1.63	9±0.98	14±2.0	43.19±3.94*	56.73
CC+Kefir	7.76±1.36	6.09±1.092	6.02±0.921	11.16±1.178	30.47±2.38*	40.02

Values are represented as mean±SEM (*n*=10). *$p^{*} < 0.05$ compared with disease control.*

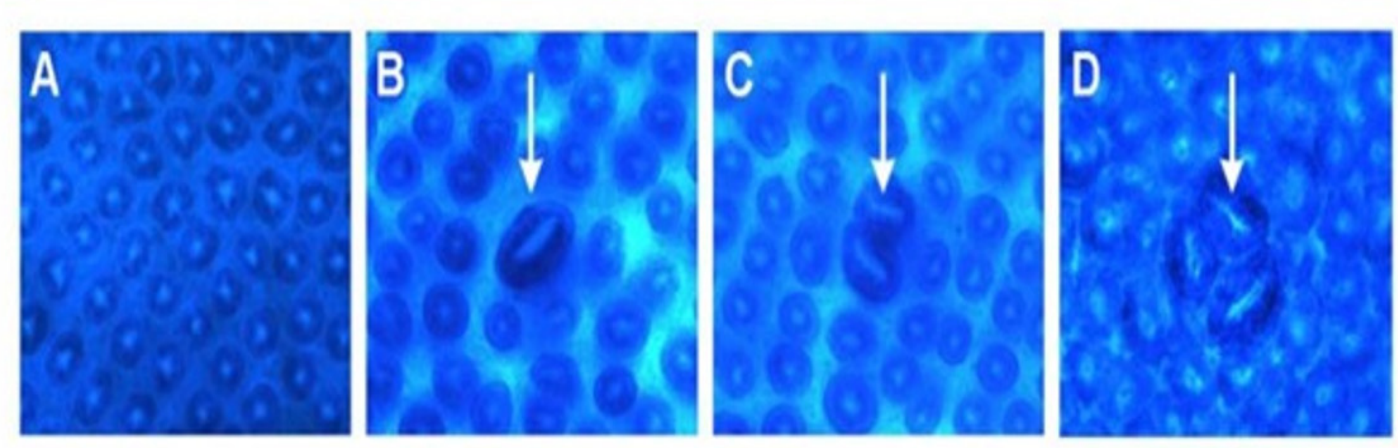

Figure 6: Representative blood smear morphology: (A) Normal control showing uniform erythrocytes; (B) DMH + HFD group showing abnormal cell morphology; (C) Kefir-treated group showing partial improvement; (D) MLECC + kefir group showing near-normal cell structure.

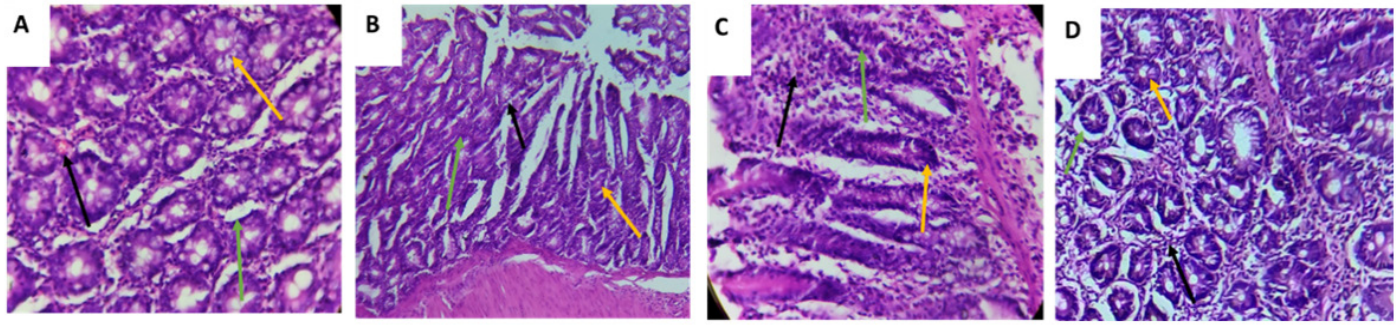

Figure 7: Representative histopathological sections of colon tissue (H&E, 40×): (A) Normal architecture in control group; (B) Distorted crypts and dysplasia in DMH + HFD group; (C) Partial restoration in kefir-treated group; (D) Improved mucosal and glandular structure in MLECC + kefir co-treated group.

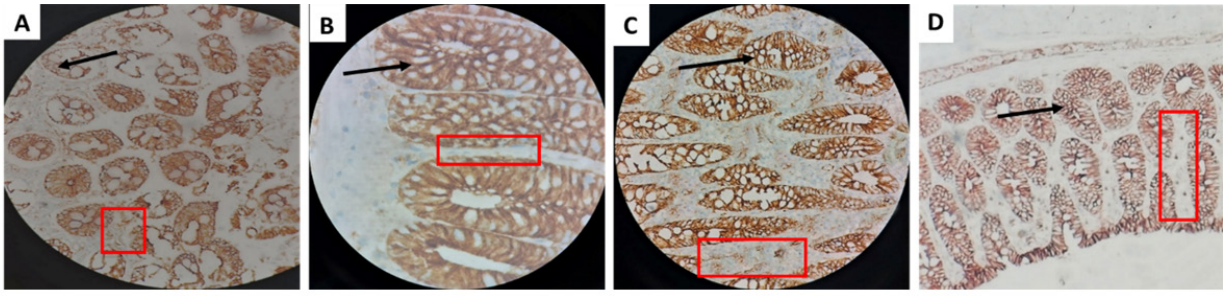

Figure 8: Immunohistochemical staining of β-catenin in colon tissue sections (40×). The disease control group (DMH + HFD) shows prominent nuclear and cytoplasmic accumulation of β-catenin, indicating enhanced proliferative signaling. Treatment with kefir and Methanolic Leaf Extract of Citrullus colocynthis (MLECC) restored predominant membranous localization, reflecting suppression of Wnt/β-catenin activation. Black arrows indicate β-catenin localization, and red boxes highlight preservation of cell junction integrity.

Table 8: Effects on *Ki-67* expression.

Group Name (*n*=6)	Ki67
Control	0.76±0.066
Disease	1.59±0.057#
Kefir	1.28±0.072*
CC+kefir	1.04±0.052*

Values are represented as mean±SEM (*n*=10). **p* < 0.05 compared with disease control.*

These observations demonstrate normalization of Wnt/β-catenin signaling, consistent with cucurbitacin-mediated inhibition of oncogenic transcription and probiotic restoration of epithelial barrier function.

Overall interpretation

The combined administration of MLECC and kefir provided comprehensive protection against DMH + HFD-induced CRC. Improvements spanned hematological, hepatic, microbial, and oxidative parameters, along with reduced ACF and normalized histology. The synergy between plant-derived phytochemicals and probiotic constituents appears to target multiple hallmarks of cancer—sustained proliferation, inflammation, and oxidative stress.

CONCLUSION

The combined administration of Methanolic Leaf Extract of *Citrullus colocynthis* (MLECC) and kefir demonstrated marked chemo preventive effects against 1,2-dimethylhydrazine (DMH) and High-Fat Diet (HFD)-induced colorectal cancer in Wistar rats. The treatment corrected hematological disturbances, reduced hepatic enzyme elevation and systemic inflammation, suppressed microbial enzyme activity, restored antioxidant balance, and reduced aberrant crypt formation.

Histopathological and molecular findings, including normalization of β-catenin localization and downregulation of *Ki-67*, confirmed the reversal of carcinogenic alterations. The results underscore the synergistic antioxidant, anti-inflammatory, and antiproliferative mechanisms of MLECC and kefir. These findings provide a scientific basis for further exploration of plant-probiotic combinations as potential adjuvants in colorectal cancer prevention and therapy.

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ABBREVIATIONS

CRC: Colorectal cancer; **MLECC:** Methanolic leaf extract of Citrullus colocynthis; **DMH:** 1,2-Dimethylhydrazine; **HFD:** High-fat diet; **AST:** Aspartate transaminase; **ALT:** Alanine transaminase; **ALP:** Alkaline phosphatase; **CRP:** C-reactive protein; **ACF:** Aberrant crypt foci; **ANOVA:** Analysis of variance; **SEM:** Standard error of the mean; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **RBC:** Red blood cells; **WBC:** White blood cells; **HGB:** Hemoglobin; **CMC:** Carboxymethyl cellulose; **GSH:** Reduced glutathione; **GPx:** Glutathione peroxidase; **CAT:** Catalase; **LPO:** Lipid peroxidation; **TBARS:** Thiobarbituric-acid reactive substances; **qRT-PCR:** Quantitative reverse transcription polymerase chain reaction; **GAPDH:** Glyceraldehyde 3-phosphate dehydrogenase; **H&E:** Hematoxylin and Eosin.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

- **Santosh Kumar Ranajit:** Conceptualization, Visualization, validation, and final manuscript review.

- **Eswar kumar Kilari:** Data Validation, Original & Final review of Manuscript.
- **Satya Obbalareddy**: Data analysis, writing - review and editing.
- **Sanjay Samanth Manthena:** Conceptualization, methodology, data curation, analysis, writing - original draft, and review.

ETHICAL APPROVAL

All animal procedures were conducted according to CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC Approval No: IAEC-04/AU-Pharm/2024-25).

SUMMARY

The paper, which is titled "Protective Effects of Methanolic Leaf Extract of *Citrullus colocynthis* Combined with Kefir Against DMH + High-Fat Diet-Induced Colorectal Cancer in Wistar Rats," is aimed at exploring the chemo preventive potential of using a plant extract and a probiotic intervention in treating colorectal cancer.

Colorectal cancer is a global health burden resulting from dietary influences, oxidative stress, inflammation, and imbalance of microbiota. A hypothesized mechanism of synergy was provided whereby MLECC, rich in cucurbitacins and flavonoids, and a kefir-based supplement, a functional fermented milk product rich in probiotics, would offer synergistic protection against CRC progression.

Male Wistar rats were treated with 1,2-dimethylhydrazine (DMH) and High-Fat Diet (HFD) to induce carcinogenesis. Animals were divided into control, disease, kefir treatment, and MLECC + kefir combination treatment groups. The parameters examined were hematological, liver enzymes, inflammatory status, microbial enzymes, oxidative stress, and Aberrant Crypt Foci (ACF) along with Ki-67, histopathological changes, and β-catenin.

KEY FINDINGS INCLUDE

The co-exposures of DMH and HFD resulted in anemia, systemic inflammation, hepatotoxicity, oxidative imbalance, and increased incidence of ACF.

Kefir grains offered sole protection through their positive effects on microbiota-related parameters and inflammation.

This combination, MLECC + kefir, significantly restored the hematological and biochemical parameters, reduced oxidative stress, and inhibited the levels of microbial enzyme associated with the development of carcinoma.

Combination therapy resulted in a reduction in the formation of ACF by approximately 60%, and it also down-regulated the expression of Ki-67,

Likewise, histological analysis indicated restoration of colonic architecture, and β-catenin immunostaining indicated normalization of the Wnt signaling pathway.

The overall results indicate that the plant-probiotic combination has synergistic antioxidant, anti-inflammatory, microbiota-modulating, and antiproliferative activities that could play a role as adjunctive therapy for colorectal cancer prevention.

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