Wild Cherry (*Prunus microcarpa*) Ameliorates Azoxymethane-Induced Aberrant Crypt Foci *in vivo*: Depicted Molecular Mechanisms

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***Abstract*—Colorectal cancer is the third diagnosed cancer across the globe despite modern therapeutic interventions. *Prunus microcarpa* has been consumed as a therapeutic tea for several human disorders; therefore, the study investigates the chemoprotective effects of *P. microcarpa* against azoxymethane-induced aberrant crypt foci (ACF) in rats. Fifty Sprague-Dawley rats were divided into five groups: A normal control group and untreated rats given saline; a reference group treated with 5-fluorouracil; and two groups treated with 500 mg/kg methanolic extracts of stems and fruits (MEPMSF and MEPMS), separately, for 2 months. In addition, all rats, except the normal controls, were injected with azoxymethane twice a week for two consecutive weeks and consumed sodium dextran sulfate-mixed water for 7 days. The plant extracts exhibited significant resistance against azoxymethane (AOM)-induced colon carcinogenesis, as indicated by lower ACF formation, reduced glandular dysplasia, decreased hyperchromasia, and a higher organization of simple columnar epithelial cells compared to untreated rats. MEPMF treatment positively modulated apoptotic mediators, evidenced by higher Bax and lower proliferating cell nuclear antigen levels in colonic tissues. *Prunus* supplementation reduced oxidative stress and cellular infiltrations in colon tissues, as evidenced by increased endogenous antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and reduced inflammatory mediators (tumor necrosis factor-α and interleukin-6) and lower levels of peroxidation byproducts (malondialdehyde), while preserving organ functions, such as those of the liver and kidneys. This study presents an increased safety margin and chemoprotective effects of *P. microcarpa* against AOM-induced colon cytotoxicity, providing a possible viable source for nutraceutical and biopharmaceutical formulation.**

Index Terms*—Aberrant crypt foci, Antioxidant, Histopathological, Immunohistochemical, *Prunus microcarpa

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I. Introduction

Colorectal cancer (CRC) is the third in incidence and the second cancer-related death across nations. CRC is considered the most prevalent digestive tract malignancy, accounting for 10% of global cancer incidence and 9.4% of all cancer deaths in 2020. CRC exhibits a heterogeneous and sophisticated pathological pathway that begins with the transformation of the colonic epithelium into aberrant crypt foci (ACF) that potentially progress into CRC in 10–15 years (Espírito Santo, et al., 2023).

The present primary care for colon cancer is still surgery; however, because of a lack of obvious signs and the unavailability of early detection, late diagnosis of colon cancer poses a major obstacle to the treatment journey. Moreover, radiotherapy and chemotherapies (used to slow down tumor growth) have many downsides, including nausea, vomiting, appetite loss, dizziness, hair loss, etc. (Morton, et al., 2023).

Despite pharmaceutical innovations, present therapy has failed to achieve healthcare satisfaction because of limited efficiency, including low cytotoxic selectivity, drug resistance, side effects, and poor water solubility (Arafat, et al., 2022). Therefore, recent decades have witnessed a remarkable ongoing search for natural products as safer alternatives with more synergistic actions than chemical synthetics (Wen, et al., 2024).

Medicinal plants and their phytoconstituents can have effective chemoprotective potentials against CRC by modulating various pathophysiological processes (e.g., apoptotic or autophagic processes, and arresting the cell cycle) and other molecular pathways (oxidative stress and inflammation) (Esmeeta, et al., 2022).

Prunus microcarpa is a wild cherry species native to dry calcareous hills and the Rocky Mountains of the Kurdistan region, Iraq. *P. microcarpa* has been used as a folkloric medicinal plant (local name: Balaluk) by Kurdish peoples living in northern Iraq for decades. Fruits and stems of this species have been hydro-distilled and given as therapeutic tea for many health disorders, such as upper respiratory infection, common cold, renal calculi, hair loss, and digestive

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problems (Abdulrahman and Shahbaz, 2020; Ahmed, 2016). The present study is part of an ongoing research project on *P. microcarpa*, for which we recently declared its liquid chromatography-mass spectrometry phytochemicals (AbdulJabbar and Ismail, 2025) and their *in vitro* biological potentials (Jabbar and Abdul-Samad Ismail, 2025). In correlation with its traditional use, the present study investigates the *in vivo* toxicity and chemoprotective effects of MEPMS and MEPMF in azoxymethane (AOM)-mediated colonic toxicity.

II. Materials and Methods

A. Plant Collection and Extraction

The aerial parts of *P. microcarpa* were collected from Warte, Erbil, Iraq (36° 52.12" N, 44° 76.51" E). The plant extracts were obtained using a microwave-coupled extraction procedure; details are available in the supplementary file (Bahadori, et al., 2020).

B. Acute Toxicity

Thirty Sprague Dawley rats (7–8 weeks old and 180–200 g) from both genders were randomly allocated to 3 cages (10 rats each, with half male and half female) and fasted overnight (12 h). Group A rats received distilled water; group B received 5 g/kg of MEPMF; group C received 5 g/kg of MEPMS. After a 2-week observational process, rats were sacrificed for relevant laboratory analysis (Adane, et al., 2023). As per the above trial, doses fixed for the chemoprotective trial, 10% of 5 g/kg (500 mg/kg) of MEPMF and MEPMS separately.

C. Chemoprotection Procedure of P. microcarpa

Experimental design

Fifty male rats (7–8 weeks old and 180–200 g) were caged in 5 polypropylene cages (10 rats each) (Supplementary Figure 2) for a 1-week acclimatization period and then treated differently: (A) Normal control; (B) untreated; (C) reference 5-fluorouracil (5-FU) (35 mg/kg); (D) treated daily with 500 mg/kg MEPMS; (E) treated daily with 500 mg/kg MEPMF for 8 weeks. Moreover, rats in groups B-E were injected with two AOM (15 mg/kg, s.c.) injections in two subsequent weeks, and drank dextran sodium sulfate (DSS) mixed water DSS for 7 days to induce the ACF. At the end of the trial, rats were euthanized and colonic tissue and blood samples were taken for laboratory evaluations (Husain, et al., 2019).

D. Statistics

The ACF enumeration and other laboratory data were managed using multiple tests (the Statistical Package for the Social Sciences, Analysis of variance, *post hoc*, and Tukey's HSD test for multiple comparisons between groups. The figures were designed via GraphPad Prism (version 9.01, San Diego, USA). Results are designated as mean ± SEM, and the significance levels were set as $p < 0.05$.

III. Results

A. Acute Toxicity

The present animal supplementation with 5 g/kg of *P. microcarpa* extracts did not cause any morbidity or mortality in rats, even after a 14-day trial. Moreover, toxic signs, such as aggression, salivation, asthma, exophthalmos, rising fur, writhing, or tremors were not found in supplemented rats. Histopathological screening of the liver and kidneys unveiled comparable structural tissue layers between supplemented rats and normal control rats, indicating the safety of MEPMF and MEPMS supplementation up to 5 g/kg (Fig. 1).

B. Chemoprotective Effects of P. microcarpa against AOM-induced ACF

Gross evaluation

The gross views of colon tissues were obtained using methylene blue dye to compare proximal and distal colon parts and to estimate the number of crypts formed in each rat's colon (Table I). As shown in Table I, cancer control rats exhibited the highest crypt values regardless of colon parts (proximal and distal) and the highest bodyweight

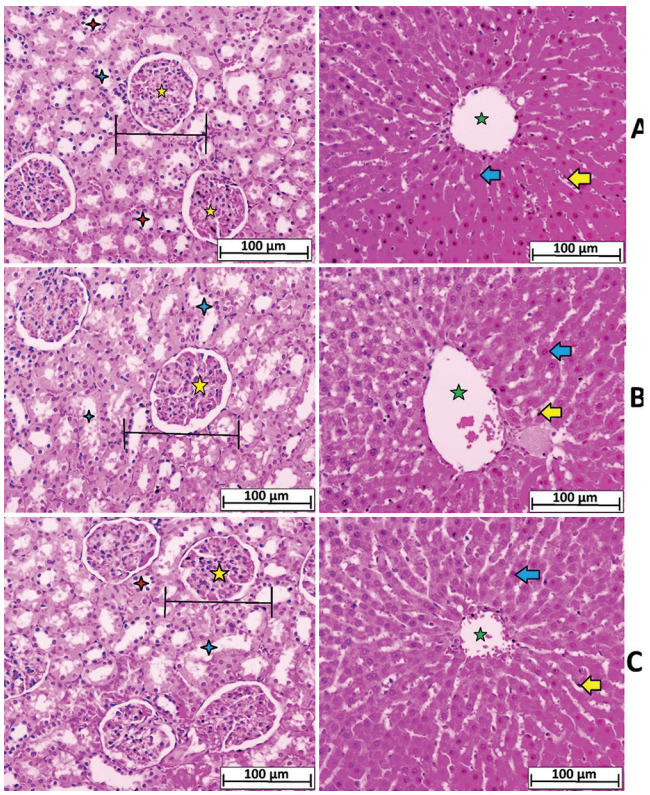

Fig. 1. Histological views (H and E stain, magnification, ×20) of organs from three experimental groups (10 rats each), Group A rats received distilled water. Group B and C rats ingested 5 g/kg of methanolic extracts of *P. microcarpa* fruits and stems, respectively. The results indicate comparable organ tissue arrangement between experimental rats. Green star, central vein; blue arrow, hepatocytes; yellow arrows, Kupffer cells; yellow star, glomeruli; black line, Bowman's capsule with glomerulus; red star, proximal convoluted tubule; blue star, distal convoluted tubules.

TABLE I
EFFECT OF PLANT EXTRACTS ON ACF INCIDENCE RATE AND BODY WEIGHT OF EXPERIMENTAL RATS

Groups	Crypt1	Crypt2	Crypt 3	Crypts≥4	Total ACF	ACF inhibition%	Body weight	Body weight inhibition%
A	N/A	N/A	N/A	N/A	N/A	N/A	327.3±7.2[a]	
B	7.4±1.7[b]	24.6±2.7[c]	27.2±4.1[c]	34.7±3.7[c]	93.9	0	210.2±5.8[c]	35.77
C	4.6±1.1[a]	6.9±1.5[a]	7.3±1.8[a]	16.6±1.3[a]	35.4	62.3[a]	273.4±4.5[b]	16.46
D	6.8±1.1[a]	12.3±2.3[b]	11.7±2.8[b]	27.3±2.5[b]	58.1	38.1[b]	251.6±8.2[b]	23.12
E	5.1±1.4[a]	7.4±2.1[b]	9.9±2.4[a]	19.2±3.3[a]	41.6	55.6[a]	264.3±9.5[b]	19.06

Experimental rats included group A (received 10% Tween 20), group B rats received AOM injection+10% Tween 20, group C rats treated with AOM+35 mg/kg 5-fluorouracil, groups D and E rats treated with AOM+500 mg/kg of MEPMS and MEPMF, respectively

drop compared to other treated rats. The total foci number was found to significantly vary between experimental rats, with the highest values (93.6%) recorded for cancer control rats, followed by MEPMS (58.1%), MEPMF (41.6%), and the lowest values (35.4%) for 5-FU-treated rats. The 5-FU-treated rats showed the lowest total ACF values and the highest ACF suppression action (62.3%) compared to cancer control and other treated rats. The plant-treated rats (MEPMF and MEPMS) showed moderate inhibitory action (55.6 and 38.1%, respectively) on the ACF formation compared to cancer controls. Rats treated with 5-FU, MEPMS, or MEPMF resisted AOM-mediated body weight alteration, indicated by a moderate gain in their body weight (273.4, 251.6, and 264.3 g, respectively) and reduced body weight inhibition percentages compared to those of cancer control rats.

Histopathology of colon tissues

The histological observation of colonic tissues using hematoxylin and eosin revealed AOM effectiveness as an ACF inducer, indicated by glandular alterations in the submucosal tissue layers and glandular dysplasia, either aggregated near the lumen or rearranged along the colonic tissue basal lines with increased inflammatory cells. The cancer control rats exhibited colonic tissue damage, indicated by reduced mucin discharge, anisocytosis, hyperchromasia (increased mitotic activity), pleomorphic nuclei, fewer simple columnar cells (goblet cells), and reduced polar cells (Fig. 2). In contrast, 5-FU or *P. microcarpa* extracts delivery led to significant suppression of AOM-mediated ACF formation, denoted by more atypical epithelial cells, increased mucin formation, reduced modified nuclei, elevated goblet cells, normal mitotic activity, and fewer leukocyte infiltrations than cancer controls (Fig. 2).

Immunohistochemistry of colon tissues

The immunohistochemical estimation unveiled different levels of pro-apoptotic Bax protein expression in colon tissues because of AOM injection + treatment strategies (Fig. 3A-F). The cancer control rats exhibited reduced Bax protein levels (labeling index) in colon tissues, which explains increased mitotic actions, ACF formation, and colonic lesions in multilayered colonic tissues (Fig. 3B). In contrast, 5-FU, MEMPS, and MEPMF (500 mg/kg) showed increased Bax labeling index in the mucosal and sub-mucosal layers, parallel with less colonic tissue penetration (Fig. 3C-F).

The immunohistochemical proliferating cell nuclear antigen (PCNA) estimation revealed a lack of any PCNA expression

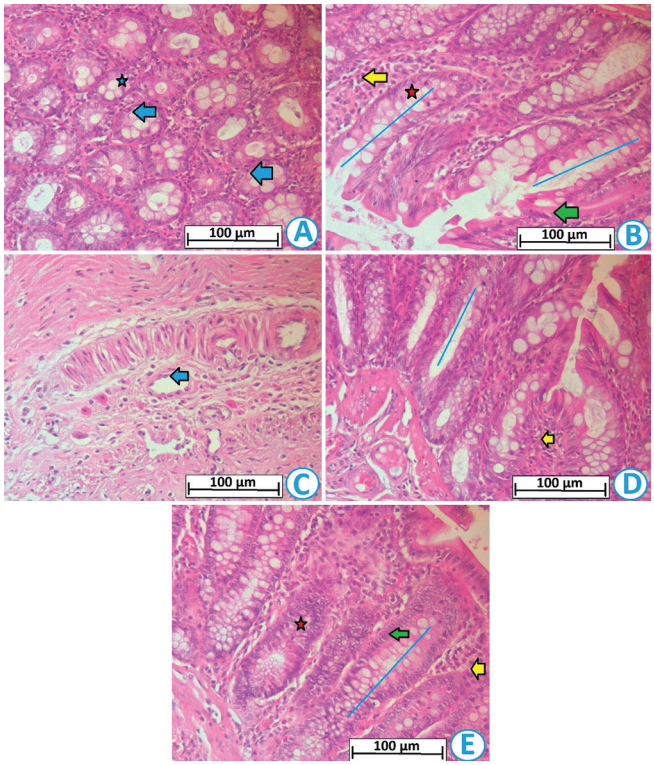

Fig. 2. Effects of *P. microcarpa* extracts on the azoxymethane (AOM)-mediated colonic toxicity in rats. Five experimental groups (10 rats each), Group A rats were treated with 10% Tween 20; Group B rats received an AOM injection and 10% Tween 20; Group C rats were treated with AOM + 35 mg/kg 5-fluorouracil; Group D and E rats had AOM + 500 mg/kg of methanolic extracts of stems and fruits, respectively. Group B rats exhibited elevated stratified cells with deformed nuclei near the lumen (blue line), increased ACF incidence (red star), elevated leukocyte (inflammatory cell) infiltration (yellow arrow); group C rats showed usual glandular arrangement with round to semi-elongated nucleated cells (green arrow) at the basal location; fewer multilayered cells, and reduced leukocyte infiltration (Hematoxylin and Eosin, Magnification, ×100).

in normal controls, indicating the absence of any apoptotic action or cellular renewal (Fig. 4A). The cancer control rats showed an elevated PCNA labeling index (98.1%) compared to 4.93% in the normal controls, indicating the continued dissemination of the AOM-mediated colon tissue damage (Fig. 4B). In contrast, rats treated with 5-FU or *P. microcarpa* extracts reduced PCNA labeling index (19.2, 30.3, 28.5 %, respectively) in colon tissues compared to cancer controls, indicating limited cellular proliferation (Fig. 4C-F).

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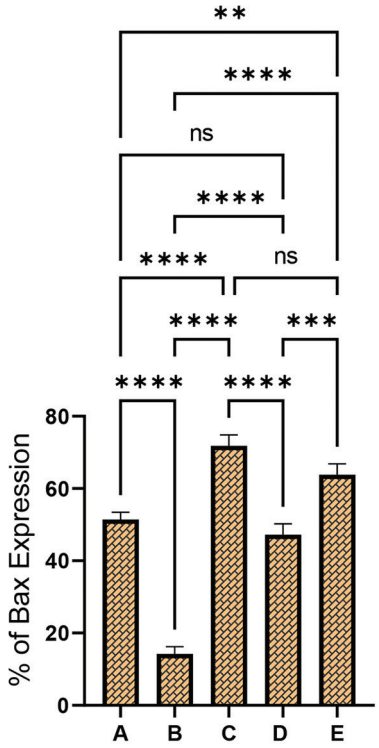

Fig. 3. The immunohistochemical Bax proteins' labeling index in the colon tissue of experimental rats (A-E). Group A rats had 10% Tween 20; group B rats received an azoxymethane (AOM) injection + 10% Tween 20 had lowest Bax protein expression; group C rats were treated with AOM + 35 mg/kg 5-fluorouracil; groups D and E rats had AOM + 500 mg/kg of methanolic extracts of stems and fruits, respectively, which showed up-regulated Bax protein in mucosal tissues, preventing further colonic mucosal penetration. ns, non-significant; **, p > 0.01; ***, p > 0.001; ****, p > 0.0001.

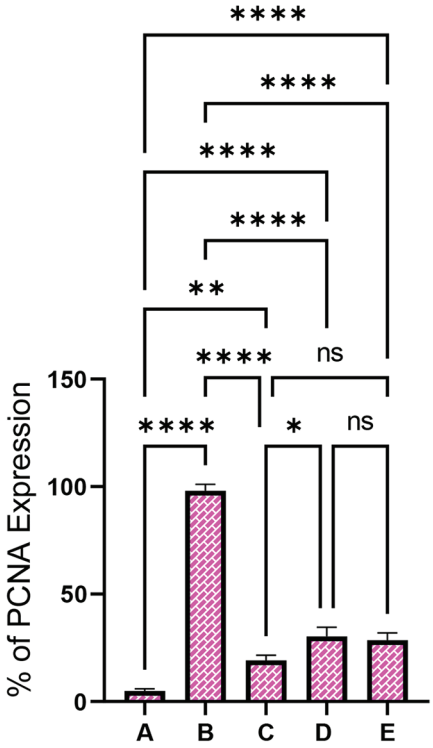

Fig. 4. The immunohistochemical Proliferating Cell Nuclear Antigen (PCNA) labeling index in the colon tissue of experimental rats (A-E). Group A rats were treated with 10% Tween 20; group B rats received azoxymethane (AOM) injection + 10% Tween 20 had increased PCNA protein in their mucosal tissues, parallel with elevated cellular proliferation and mitotic activity; group C rats were treated with AOM + 35 mg/kg 5-fluorouracil; groups D and E rats had AOM + 500 mg/kg of methanolic extracts of stems and fruits, respectively, which showed decreased PCNA protein appearance in mucosal tissues, preventing further cellular proliferation in damaged mucosal tissues. ns, non-significant; *, p > 0.05; **, p > 0.01; ***, p > 0.001; ****, p > 0.0001.

Effect of P. microcarpa extracts on Tissue Antioxidants in carcinogenic rats

The cancer control rats exhibited increased tissue oxidative stress, evidenced by a reduced amount of catalase (CAT) (21.3 µmol/min/mL), glutathione peroxidase (GPx) (1.6 U/mg), and superoxide dismutase (SOD) (53.8 U/mL) enzymes in their tissue homogenates compared to other treated rats. In contrast, 5-FU or plant treatment (MEPMS and MEPMF) limited AOM-mediated oxidative stress, denoted by increased CAT (84.5, 53.8, 65.7 µmol/min/ml, respectively), GPx (8.7, 3.9, 5.5 U/mg, respectively), and SOD (105.6, 86.4, 94.5 U/ml, respectively). As an indicator of lipid peroxidation, malondialdehyde (MDA) levels were estimated in the tissue homogenates, which were found to be superior (31.3 nmol/mg) in cancer control rats. Rats treated with 5-FU, MEPMS, or MEPMF displayed significant resistance against AOM-mediated lipid peroxidation, indicated by lower MDA (14.7, 22.8, 17.5 nmol/mg, respectively) levels compared to cancer control rats (Fig. 5A-E).

Effect of P. microcarpa extracts on the inflammation

The cancer controls (group B) showed increased pro-inflammatory mediators, including tumor necrosis factor-α (TNF-α) (129.8 P. pg/mL) and interleukin-6 (IL-6) (53.7 P.pg/mL), and decreased serum anti-inflammatory cytokine IL-10 (72.3 P.pg/mL), parallel with elevated colon tissue penetration. Rats receiving 5-FU, MEPMS, or MEPMF revealed a restoration of the AOM-mediated inflammation, represented by decreased pro-inflammatory TNF-α (38.2, 78.40, and 63.60 pg/mL, respectively) and IL-6 (14.5, 31.90, and 13.8 P.pg/mL) cytokines and higher IL-10 levels (584.8, 343.6, and 459.7 P.pg/mL, respectively) than cancer controls. The results indicated significant anti-inflammatory potentials of *P. microcarpa* extracts, ameliorating AOM-mediated colonic toxicity (Fig. 6A-E).

Effects of P. microcarpa extract on serum biochemicals

The normal control rats had the usual biochemical contents in their serum according to liver and kidney functional tests. Cancer control rats exhibited noticeable liver and kidney dysfunction, indicated by lowered albumin and total protein production, and increased creatinine and urea levels. In contrast, 5-FU or *P. microcarpa* supplementations restored AOM-mediated kidney and liver damage, shown by improved liver functional parameters (AST, ALT, and GGT), and reduced metabolic waste products (decreased urea

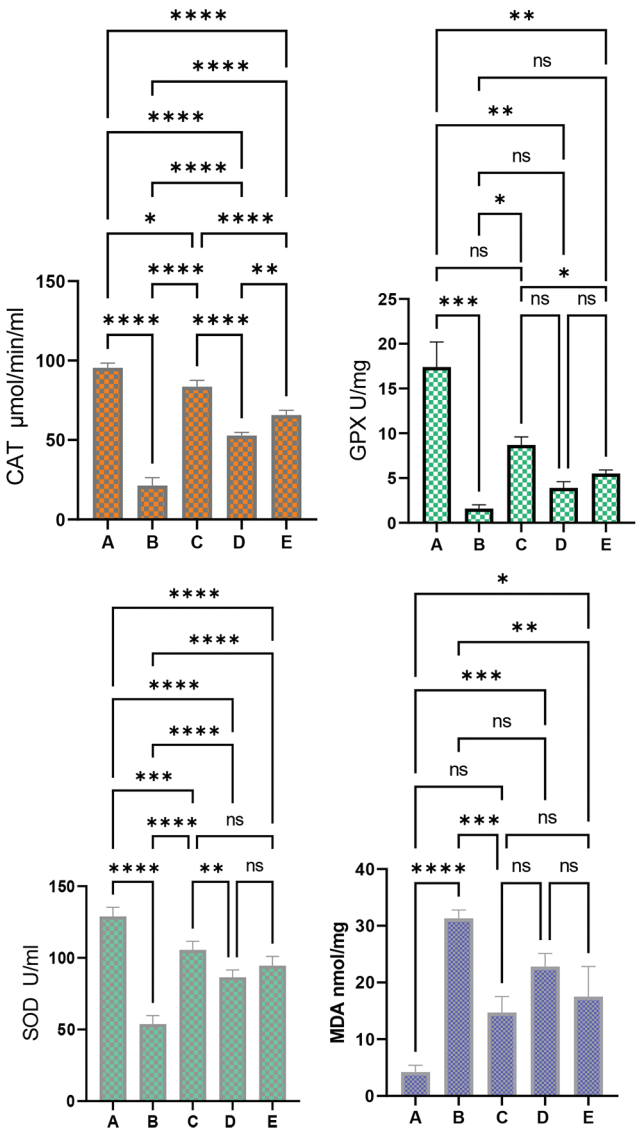

Fig. 5. Effect of *P. microcarpa* on antioxidants and MDA contents of experimental rats. Group A rats were treated with 10% Tween 20; group B rats received azoxymethane (AOM) injection + 10% Tween 20 exhibited sever AOM-mediated oxidative stress that worsen colonic tissue damage; group C rats were treated with AOM + 35 mg/kg 5-fluorouracil; groups D and E rats were treated with AOM + 500 mg/kg of methanolic extracts of stems and fruits, respectively, which had significantly higher antioxidant enzymes and lower MDA levels than group B. SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde. ns: Non-significant; *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$; ****, $p > 0.0001$.

and creatinine) as an indicator of efficient kidney filtration. The results indicate noticeable preserved potentials of *P. microcarpa* on liver and kidney functionalities in AOM-mediated carcinogenic rats (Table II).

IV. Discussion

Plant extracts can have numerous therapeutic potentials; however, their potential toxicity limits their application as curatives for various health issues (Amrani, et al., 2024). In our case, a single oral dosing of *P. microcarpa* extracts at 5 g/kg to rats did not cause any toxic signs or physiological abnormalities even after a 14-day trial. These results were found in parallel with our recently published study considering *P. microcarpa* as supplementation (5 g/kg) based on biochemical evaluation, but this time, further confirmation was obtained from histopathological results (Jabbar and Abdul-Samad Ismail, 2025). Similarly, researchers revealed non-toxic effects of *Prunus africana* in rats supplemented with 1600–5000 mg/kg methanolic extracts of its stem barks (Mwangi, et al., 2018). Accordingly, Mumefural (1250, 2500, and 5000 mg/kg), a bio compound extracted from the fruits of *Prunus mume* Sieb. et Zucc., didn't cause any toxic effects in animal models based on macroscopic, hematological, and biochemical indications (Kim, Han and Jeon, 2020).

Colon carcinogenesis models through AOM inoculation and putative pre-neoplastic ACF as the final point of marker lesion (cancerous polyps) have been established to evaluate the chemoprotective potentials of *P. microcarpa* extracts (Seetha, Devaraj, and Sudhandiran, 2021). ACF appears to be the earliest histological change at the beginning of the multi-step process of colorectal neoplasia initiation, which is recognized as microadenomas exhibiting epithelial changes resembling tubular and villous adenomas, including enlarged hyperchromatic nuclei, loss of polarity, numerous nuclear stratified/crowding, increased nuclear/cytoplasmic ratio, and elevated mitotic activity (Guo, Crossland and Crott, 2025). The present chemoprotective study conferred the beneficial effects of *P. microcarpa* extracts, suggesting increased potential of *P. microcarpa* extracts in halting colon cancer progression in the early stages. The mechanism of the chemoprotective actions of wild cherry is yet to be fully understood; however, we believe such biological potentials could be correlated to its nutritional components (phenolics, flavonoids, and terpenoids), mainly quinic acid, chlorogenic acid, and epicatechin in its fruit and stem extracts, as detailed in our recent study (AbdulJabbar and Ismail, 2025). The Quinic acid and chlorogenic acid, which are polyphenolic sources of *P. microcarpa*, have been confirmed as chemoprotective agents due to their positive effects on detoxification/metabolic processes, and modulating MAPK/Nuclear factor kappa B (NF-κB) and phosphatidylinositol 3-kinase/Protein Kinase B (PI3K/AKT) Pathways that suppressed the colonic ACF growth and reduced the conversion of pre-neoplastic into malignant neoplasia (Chojnacka, et al., 2022; Neamțu, et al., 2024). Similarly, chemoprotective potentials of *Prunus salicina* L. extracts on AOM-mediated crypt foci in rats have been linked to its polyphenol (chlorogenic/neochlorogenic acid) modulatory potentials on pro-inflammatory enzymes, NF-κB, vascular cell adhesion molecule 1 messenger RNA, nitric oxide synthase, and cyclooxygenase-2 (Banerjee, et al., 2016).

Cancer studies declared that cancerous lesions involve interference of the p53 pathway and apoptotic protein expression (Bcl-2 family protein and its related member, Bax protein), consequently regulating cellular apoptosis, cell cycle arrest, proliferation (PI3K/Akt mechanism), differentiation, and senescence (Hernández Borrero and El-Deiry, 2021). We found that supplementation with *P. microcarpa* extracts up-

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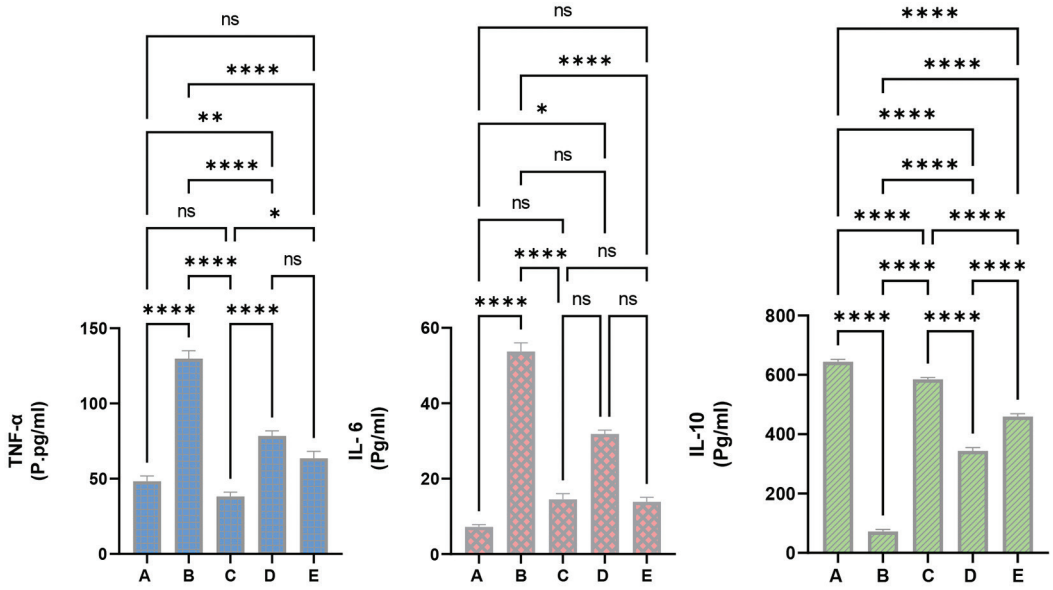

Fig. 6. Effect of *P. microcarpa* on serum inflammatory markers in azoxymethane (AOM)-mediated colon toxicity. Group A rats had 10% Tween 20; group B rats received an AOM injection + 10% Tween 20 had sever inflammatory condition; group C rats were treated with AOM + 35 mg/kg 5-fluorouracil; groups D and E rats received AOM + 500 mg/kg of methanolic extracts of stems and fruits, respectively, which had less AOM-induced inflammation, denoted by lower tumor necrosis factor-α and IL-6 cytokines than cancer controls. IL: Interleukin. ns, non-significant; *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$; ****, $p > 0.0001$.

TABLE II
EFFECTS OF *P. MICROCARPA* EXTRACTS ON SERUM BIOCHEMICALS IN CARCINOGENIC RATS

Groups	Total protein g/L	Albumin g/L	AST IU/L	ALT IU/L	GGT IU/L	Urea mmol/L	Creatinine umol/L
A	91.8±3.8[a]	28.4±3.8[a]	154.5±4.1[c]	68.5±4.8[a]	3.9±1.4[a]	5.3±1.9[a]	54.8±3.5[a]
B	55.8±3.2[c]	10.6±1.2[c]	268.3±6.4[d]	89.8±5.2[c]	6.8±1.6[c]	11.4±2.2[c]	81.5±6.3[c]
C	82.1±4.1[a]	21.7±3.0[a]	178.2±4.2[a]	72.4±3.1[a]	4.3±1.1[a]	6.9±2.2[b]	47.4±5.3[a]
D	63.9±3.9[b]	17.2±3.4[b]	223.5±4.1[b]	81.3±3.4[b]	5.5±1.3[b]	7.8±1.6[b]	62.7±4.8[b]
E	71.3±3.1[b]	19.4±4.3[a]	209.6±5.1[b]	79.8±4.1[b]	5.1±1.5[b]	7.4±1.9[b]	56.6±4.4[a]

Group A rats were treated with 10% Tween 20, group B rats received an AOM injection+10% Tween 20, group C rats were treated with AOM+35 mg/kg 5-fluorouracil, groups D and E rats were treated with AOM+500 mg/kg of methanolic extracts of stems and fruits, respectively. Since it indicate similar letters means non-significant. In other word, different letters on values in the same column indicate significant at $p > 0.05$.

regulated the Bax proteins in the colonic tissues, resulting in less ACF incidence compared to cancer controls. Accordingly, the anticancer effects of pectinase-treated *P. mume* Fruit on SW480 human CRC cells were correlated with its positive modulation of Bax, caspase-9, cleaved PARP, caspase-3, caspase-8, and downregulation of antiapoptotic Bcl-2 that consequently increased apoptotic cell population, formation of apoptotic bodies, and cell shrinkage (Cho, et al., 2019). Moreover, quinic acid derivatives and chlorogenic acid, main compounds of *P. microcarpa*, have been postulated as anti-cancer agents because of their regulatory actions on cellular processes, inducing cell death, inhibition of migration, metastasis, cell cycle arrest, and angiogenesis through modulating, including β-catenin/T-cell factor, Bcl-2/Bax activity, which were closely related to Wnt signaling pathway (Neamțu, et al., 2024).

CRC exhibits abnormal epithelial cell proliferation as a result of up-regulated PCNA protein, a regulator of DNA replication and cell cycle that is tremendously increased in tumor cells, causing continuous metastasis and apoptotic dysfunctionality as occurred in our cancer control rats (Zhou, et al., 2018). In contrast, *P. microcarpa*-supplemented rats exhibited reduced PCNA expression, indicating less cellular proliferation and less labeling index (cells with abnormal cell cycle). Such modulatory actions of *P. microcarpa* extracts on immunohistochemical/apoptotic proteins could be linked with its phytoconstituents (phenolics and flavonoids, such as quinic acid, chlorogenic acids, and epicatechin), which were repeatedly postulated as chemoprotective agents (Chojnacka, et al., 2022; Neamțu, et al., 2024). Accordingly, researchers highlighted increased modulatory potentials of quinic acid (main compounds of *Andrographis paniculata* extracts) on Bcl-2/Bax and other apoptotic proteins as the main molecular mechanisms underlying its anticancer actions against numerous human cancer cells, HeLa, BT549, 293, MCF7, and A549 cells (Anoor, et al., 2022).

Oxidative stress is a well-known cellular process initiated by an imbalance between free radical formation and antioxidant generation. As a major initiator of many inflammatory processes, oxidative stress has been linked with numerous human diseases, including gastrointestinal diseases and colon carcinogenesis. Increased reactive oxygen specie (ROS) formation provokes chronic intestinal disease, which is considered a major risk factor for CRC (Wang and Fu,

2023). We found that supplementation with *P. microcarpa* extracts resisted oxidative stress in AOM-mediated carcinogenic rats, indicated by its positive modulatory effects on endogenous antioxidants and down-regulation of MDA levels. Such antioxidant potentials of *P. microcarpa* fruits and stems can be linked with its phytochemicals, as detailed in our recent study (AbdulJabbar and Ismail, 2025). Accordingly, researchers presented increased antioxidant potentials of *Prunus* phytochemicals (hydroxybenzoic acid, 9 hydroxycinnamic acids, 3 flavan-3-ols,7 flavonols, 3 isoflavones, 3 flavanones), which are mainly linked with their ameliorative actions on oxidative stress-related diseases, including CRCs (Condello and Meschini, 2021; Jesus, et al., 2019; Peixoto, et al., 2020).

The body's natural response to oxidative stress-mediated intestinal damage is inflammation. Intestinal tissues exposed to continuous inflammation will progress into chronic inflammation and stimulation of autoimmune action (Sahoo, et al., 2023). This condition led to an alteration of the cellular integrity and weakened immune system of the gut mucosa, facilitating pathogenic invasion or progressing into tumor formation. The fully mature tumors may worsen the inflammatory environment by stimulating further chemokine and cytokine release through a feedback loop. AOM inoculation provokes NF-κB mechanism and pro-inflammatory cytokine release (TNF-α, IL-6, and IL-1β). The latter inflammatory chemicals enhance the production of metalloproteinase enzymes (modulate the COX-2 overproduction) and stimulate the JAK/STAT pathway that mimics the apoptosis/angiogenesis process, and activate cellular proliferation (PI3K/Akt) (Aliyu, et al., 2022). In our study, *P. microcarpa* supplementation attenuated AOM-mediated inflammation in carcinogenic rats, indicated by reduced IL-6 and TNF-α levels and significantly increased IL-10 levels. Accordingly, the previous studies declared increased anti-inflammatory potentials of wild *Prunus* extracts, which were mainly linked with its phytoconstituents (phenylpropanoids, terpenoids, anthocyanins, and carotenoids) (Jyotshna and Shanker, 2025). Similarly, the phenolic-enriched fractions of *Prunus spinosa* L. fruits significantly reduced pro-oxidant and inflammatory mediators, evidenced by downregulated ROS molecules, TNF-α, neutrophil elastase, and slightly inhibited IL-8 and IL-6 productions in the cells provoked by fMLP, fMLP+ cytochalasin B, and bacterial lipopolysaccharides (Magiera, et al., 2022). The depicted chemoprotective mechanism of *P. microcarpa* extracts is summarized in Fig. 7.

CRC has shown an inverse relationship with liver function indicators circulating in the blood (ALT, AST, GGT, TP, and ALP). Colon cancer patients seem to have liver dysfunctionality according to abnormal liver estimations, including smaller liver size, reduced protein synthesis, less cell turnover, poor metabolic capabilities, and more vulnerability to toxins/bleedings, all of which disrupt gut homeostasis, subsequently initiating mucosal lesions and CRC (He, et al., 2021). Another early sign of CRC initiation is insufficient renal function, characterized by a reduced glomerular filtration rate and accumulation of metabolic

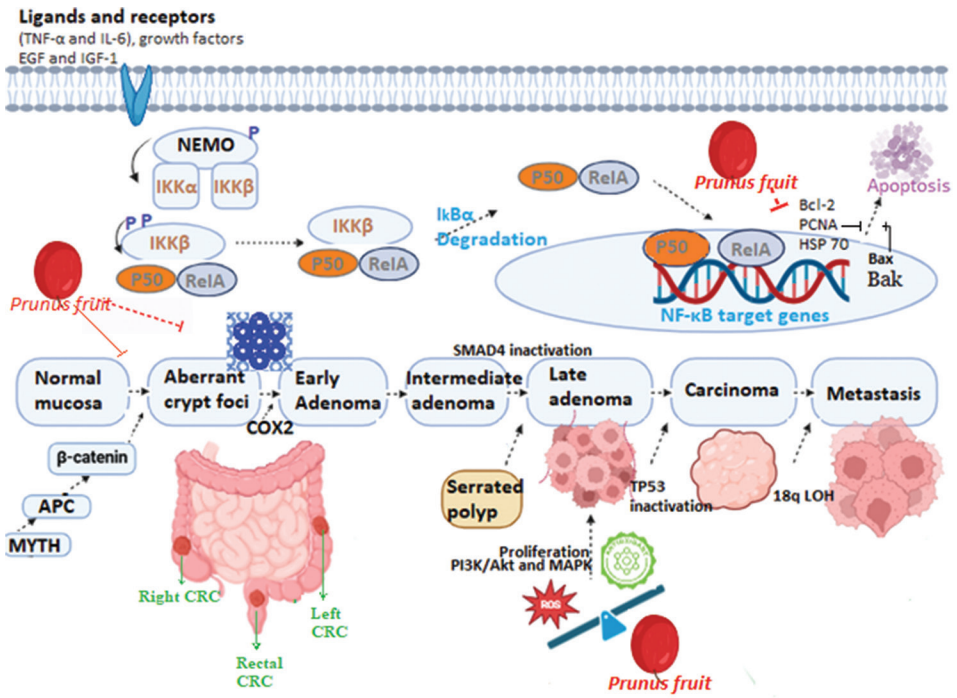

Fig. 7. Key regulator genes and their associated pathways underlie Colorectal cancer generation and development. Chromosomal instability causes 85% of all cancers, which is required to progress the adenoma-carcinoma axis. The *APC* gene is the major mutational target of MUTYH, which starts a carcinogenic process. The carcinogenic process in this axis is found in context with mutations in *TP53*/proliferating cell nuclear antigen genes that, in turn, modulate the nuclear factor-kappa B pathway, suppressing apoptotic actions and stimulating inflammatory processes and continuous cellular proliferation. The cancer cell proliferation is enhanced by the existing imbalance between reactive oxygen species formation and antioxidant generation.

byproducts (creatinine, bilirubin, and urea) (Al-Qudimat, et al., 2023). The present data analysis unveiled noticeable protective effects of *P. microcarpa* extracts against AOM-mediated biochemical alterations in carcinogenic rats, indicated by maintained albumin/protein levels and lower metabolic waste products compared to cancer control rats. Similarly, *Prunus armeniaca* L. extracts exhibited significant hepatoprotective actions in paracetamol-mediated liver injury in rats, shown by reduced sGOT, TBARS, GGT, sGPT, sALP, LDH, TP, and increased Albumin levels, almost comparable to the reference drug Ursodeoxycholic acid group. Such bioactivities of the *Prunus* species were mainly linked with its phytochemicals (Alkaloids, saponin glycosides, condensed tannins, volatile oil, steroids, terpenoids, and flavonoids) (Raj, et al., 2016).

V. Conclusion

The outcome of this study presents the *in vivo* chemoprotective role of wild cherry *P. microcarpa* against AOM-induced colon carcinogenesis in rats. Supplementation with the *P. microcarpa* extracts at 5 g/kg did not cause any toxic damage or mortality, even after a 2-week trial. Treatment with *P. microcarpa* extracts at 500 mg/kg significantly provoked apoptotic actions in the colorectal mucosal layer, evidenced by up-regulation of Bax proteins and reduction in the PCNA expression. Moreover, *P. microcarpa* fruits were found to be more effective than its stems in the alleviation of oxidative stress and inflammation in AOM-mediated carcinogenic rats, indicated by up-regulated SOD, CAT, GPx, IL-10, and reduced generation of MDA, TNF-α, and IL-6 chemicals, as well as maintaining liver and kidney functions. Altogether, it could be considered a modulatory action and the molecular mechanisms underlying the ACF suppression effects of *P. microcarpa* extracts. Limitations of the work include a small animal size, self-budget, and poor animal facility; therefore, future extended experimental trials on larger animal groups are suggested before considering it for clinical trials as potential nutraceutical and biopharmaceutical agents.

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