

RESEARCH ARTICLE

Role of *Quercus Infectoria* Galls Extract in Colitis-Induced Colorectal Cancer by AOM-DSS in Albino Rats

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ABSTRACT

Colorectal cancer (CRC) is one of the most prominent cancers in the world. Adverse drug reactions due to cancer chemotherapy are indeed a significant global problem. *Quercus infectoria* (QI) is a medicinal plant widely recognized for its extensive therapeutic properties. To effectively assess the anti-tumor potential of QIGE versus CRC, the present study employed an AOM/DSS-induced rat model. Six groups of thirty-six albino rats have been created: Group 1, is considered the control negative (CN), Group 2: rats were given AOM/DSS as the positive control (CP), group 3, 5-Flurouracil treatment was given to the rats, for groups 4, 5, and 6, the crude QIGE was administered to rats at 250, 500, and 1000 mg/kg body weight. Rat body weights and the consistency of their feces were measured for DAI scores. Blood samples were analyzed for alterations in hematologic and serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9) tumor markers. Immunohistochemical of β -catenin and SATB-2 evaluations were conducted for grades and regression of colonic cancer. The study results revealed that rats in the CP group developed anemia and leukocytosis, accompanied by diarrhea and elevated tumor marker levels significantly ($P \leq 0.5$). While 5-FU failed to restore normal blood parameters and fecal consistency, administration of a high dose of the QIG extract significantly ($P \leq 0.5$) improved these abnormalities, returning blood parameters and tumor markers to normal levels and restoring healthy fecal consistency. Moreover, it effectively regressed tumor invasion and restored normal patterns of key oncogenic markers; β -catenin and SATB-2. Our outcomes grant critical comprehension into the therapeutic impact of QIGE for CRC management and further reinforce its promising role in clinical applications.

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INTRODUCTION

Worldwide, colorectal cancer ranks second in terms of fatality and is the third most prevalent cancer. Colon cancer is currently one of the biggest issues facing the healthcare sector in various countries (Abedizadeh *et al.*, 2024). The adverse effects of chemotherapy for treatment of colon cancer might be avoided by using medicinal plants that contain numerous phytochemicals, particularly flavonoids and polyphenol compounds, that can trigger apoptosis and restrict the growth of tumor cells (Aiello *et al.*, 2019). Inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis, constitutes a major risk factor for the development of colorectal cancer. Chronic inflammation accelerates the formation and progression of tumors (Rawla *et al.*, 2019). Inducing colorectal cancer is

most commonly performed through chemically induced colitis-associated cancer (CAC), using Dextran sulfate sodium (DSS), Azoxymethane (AOM), or both together by intraperitoneal injections or drinking water. It has been shown to be very useful, low-cost, and highly reproducible (Dzhalilova *et al.*, 2023). The prognosis of colorectal cancer is greatly influenced by the time of diagnosis and the degree at which the disease is identified (Wu *et al.*, 2022). Faecal occult blood test (FOBT) has served as a clinical tool for CRC screening (Zou *et al.*, 2022). Blood-based markers for the initially detection of CRC have been the subject of intensive research since cancer cells have produced the glycoproteins carcinoembryonic Antigen (CEA) (Hall *et al.*, 2019), and Carbohydrate antigen 19-9 (CA19-9) on their cell surfaces, which contribute to the tumors' malignant features (Kildusiene *et al.*, 2024). The

protein SATB2 was observed to be extremely tissue-type-specific, especially in glandular cell types the colon, rectum, and appendix (Dum *et al.*, 2023). Moreover, SATB2 has been related to cancer. Almost all of the local and invasive CRC expressed SATB2, indicating that SATB2 could be utilized as a marker for diagnosis, to distinguish colorectal cancer and various kinds of cancer (Dragomir *et al.*, 2014). The potential use of beta-catenin immunomarkers to identify premalignant dysplastic colorectal lesions' malignant potential, which can assist in early therapy and stop a subsequent malignancy from developing (Bhattacharya *et al.*, 2019). The chemotherapeutic treatment of colorectal cancer based on using of 5-fluorouracil (Gill *et al.*, 2003). Chemotherapy side effects include nausea, vomiting, diarrhea, and myelosuppression, which reduce blood cell synthesis and causes anemia, thrombocytopenia, and neutropenia. These conditions raise the risk of bleeding and infection (Parment *et al.*, 2022). Herbs and their chemical constituents may provide significant chemoprotective potential without undesirable side effects (Jabbar *et al.*, 2023). *Quercus infectoria* is a member of the *Quercus* genus (Fagaceae), it is a small tree can reach a height of about 2.5 meters. Its leaves are 4-6 cm long, and its acorn fruits are narrow, scaly, and cylindrical. Grown extensively in Middle Eastern country such as Iran, Iraq, Cyprus, Syria, Egypt, Saudi Arabia, Turkey, Malaysia and some parts of India (Jain, 2019; Elham *et al.*, 2021). *Quercus infectoria* gall extract includes components with antioxidant and anti-cancer properties like gallic acid, ellagic acid, and tannins (Vermani, 2009). The knowledge gaps lead to a great research opportunity for examining QIG's phytochemical properties in relation to colorectal cancer. The goal of the present research is to evaluate the impact of the *Quercus infectoria* gall extract by serum tumor markers, histopathological examination and immunohistochemistry on the regression of adenocarcinoma in the colon, revealing its potential and effectiveness as an alternative or complementary therapeutic agent for colorectal cancer.

MATERIALS AND METHODS

Plant collection and extraction: The *Quercus Infectoria* Galls (QIG) had been collected in October 2024 from Qaradagh Mountain in the Kurdistan region, northeast of Iraq. The galls were air-dried at room temperature ($25 \pm 2^\circ\text{C}$), then have been ground into a very fine powder. Methanol (80%) was added to the dried powdered material. After that, it was incubated in a shaking incubator (Labtek, Australia) at 25°C for two to three days, followed by filtration. A rotary evaporator (Labtek, Australia) was later used to evaporate the extract. The dry powder of QIG extract was obtained by lyophilizing the aqueous residual for two days. Finally, at the biochemistry laboratory, College of Agricultural Sciences, University of Baghdad, Iraq, High Performance Liquid Chromatography (HPLC) was employed to analyze the crude extracts (Obouayeba *et al.*, 2014).

Animals model: Thirty-six albino rats, between six and eight weeks of age, weighing (> 135 grams) separated into six groups, each consisting of six animals, maintained in the animal house of the College of Veterinary Medicine, University of Sulaimani. The rats resided in a room with

adequate ventilation and a 12-hour light/dark cycle at 27°C and acclimated to having unlimited access to food (standard pellets) and water. The animals were transported to the animal facility following approval from the Ethics Committee. All protocols concerning the handling, care, and sampling of animals at Sulaimani University in Kurdistan have been conducted under authorized permission (VMUS.EC. 030529, December 2024).

Induction of colorectal cancer: All the rats except group 1 ($n=6$) regarded as negative control, received azoxymethane (AOM) (biorbyt, United Kingdom), 15 mg/kg body weight intraperitoneally, twice doses at first month only (Tajasuwan *et al.*, 2023). Following a week, rats' drinking water was supplemented with 3% Dextran Sulfate Sodium (DSS) (biorbyt, United Kingdom) for five days in sequence, then replaced by 14 days of water, which was regarded as one cycle of DSS (Li *et al.*, 2025), then repeated cyclic stimulation of (DSS) results in long-term chronic inflammation. After the 24-week successful induction CRC period, five groups of rats were allocated ($n=6$). Group 2 (Positive control) includes rats with adenocarcinomas that did not receive any treatment. Group 3 (Conventional group) was injected with 5-fluorouracil (biorbyt, United Kingdom). Group 4 (Treated I group) received oral administration of low dose (250 mg/kg) of *Quercus infectoria* galls extract. Group 5 (Treated II group) was orally given a median amount (500 mg/kg) of QIG extracts. Group 6 (Treated III group) was provided a high dosage (1000 mg/kg) of QIG extracts (Ibrahim, 2021). To administer the QIG extracts treatment, force-feeding using ball-tipped needles was done for precisely eight weeks in a row.

Clinical disease score assessment: Throughout the investigation, the rats were monitored every day for clinical abnormalities, feed intake, and gross appearance. Additionally, rats' body weight, feces consistency, and bleeding had been observed. The body weight of each group was taken at 0 days and each month. Additionally, fecal sample observation was conducted to detect any abnormalities in the color or consistency of the feces, in addition to examining the feces for the existence of invisible blood by using rapid fecal occult blood test card kit (FOB) according to the kit's instruction (Boson Biotech, China).

The Disease Activity Index (DAI) is determined by evaluating three parameters percentage of weight loss, stool consistency, and rectal bleeding each rated on a scale from 0 to 4; alteration in the rate of body weight reduction (weight gain = 0; 5–10%, weight loss score = 1; 11–15%, weight loss score = 2; 16–20%, weight loss score = 3; 21–50%, weight loss score = 4; $>50\%$), stool consistency (normal and well-formed = 0; loose stool = 1; loose stool with mucins = 2; very soft (slight diarrhea) = 3; watery diarrhea = 4) and stool bleeding (normal color stool = 0; reddish color stool = 2; bloody stool = 4). The sum of these scores was used to determine the DAI score, which varied from 0 (healthy) to 12 (cancer) (Tajasuwan *et al.*, 2022).

Animal Sacrifice and Blood Specimens Collection: Rat was sacrificed under deep anesthesia with a ketamine and xylazine combination Ketamine-Xylazine administered intramuscularly at a dosage of 35 mg/kg ketamine combined with 5 mg/kg xylazine based on body weight.

(Hassan *et al.*, 2022). Blood samples were obtained through the cardiac puncture. After transferring three milliliters of the blood to an ethylenediaminetetraacetic acid (EDTA) tube and shaking it thoroughly, the sample was immediately examined, to determine complete blood count using an automated hematology analyzer (Swelab alfa, HE001, Sweden). The neutrophil lymphocyte ratio (NLR) was derived from the calculation of neutrophil absolute count divided by lymphocyte absolute count. In addition, the platelet- lymphocyte ratio (PLR) is computed by dividing the total platelet count by the total lymphocyte count from a complete blood count.

Serum Tumor Markers Measurement: Three milliliters of blood collected by heart puncture was placed in tubes (Aquisel) containing EDTA and centrifuged (at 4000 rpm for 8 minutes) for determining tumor indicators in serum (carcinoembryonic antigen and carbohydrate antigen 19-9) utilizing standardized kits and a fully automated analyzer Cobas 311 (Roche, Germa Serum Cytokine).

Tumor quantification and evaluation: Tumors were examined visually to determine their location in the caecum, as well as the proximal and distal parts of the colon, and were counted to evaluate tumor incidence, distribution, and multiplicity. Tumor incidence referred to the percentage of rats that developed tumors (such as adenocarcinomas or adenomas with mucin characteristics), while tumor multiplicity indicated the average number of tumors found in each rat that had tumors, reflecting the tumor count per rat. Measurements of tumor volume, length, width, and depth were taken using Amscope™. Tumor volume was calculated using the formula: $V \text{ (cm}^3\text{)} = (W^2 \times L)/2$, where W is the width and L is the length, based on the method for estimating rat mammary tumor volume with calipers (Tajasuwan *et al.*, 2022).

Histopathologic examination: At the end of the experiment, the cecum and the colon were taken out, and cleaned its fecal content then rinsed in normal saline. Segmented into sections approximately 0.5 cubic centimeters in size, then fixed for 48 hours in 10% neutral buffered formalin. Following ethanol processing in successive grades and xylene clearing, the samples were implanted in paraplast tissue-embedding material. Using a rotary microtome, tissue sections that were four micrometers thick were cut and placed on glass slides. Three sections were obtained; one was stained for a general histopathological analysis using hematoxylin and eosin, afterwards it was inspected using a full HD microscopic imaging device (Leica Microsystems GmbH, Germany). While the other two sections for IHC utilization.

Immunohistochemical analysis: Paraffin-embedded tissue ribbons, 4 μm thick, were deparaffinized using xylene. After dewaxing, the slides were rehydrated. For antigen retrieval, the sections were heated in a microwave oven for 25 minutes using a sodium citrate buffer (pH 6.5), then cooled in deionized water for 20 minutes. To block endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide for 12 minutes, followed by incubation with 3% bovine serum albumin for 20 minutes. The sections were then incubated at room temperature for one hour with

primary antibodies: B-catenin rabbit polyclonal antibody (diluted 1:600; Biorbyt, US) and SATB-2 rabbit polyclonal antibody (diluted 1:100; Biorbyt, US). The reaction was enhanced using streptavidin conjugated with horseradish peroxidase (Biorbyt, US), as per the manufacturer's instructions. Diaminobenzidine was used to visualize the immunoreactivity, and after counterstaining with hematoxylin, the slides were dried and mounted with coverslips.

Image analysis software (AHSQ) was employed to assess the slides, quantifying the proportion and intensity of positively stained immune cells using an H score. The scoring was independently verified in a blinded manner by two experienced pathologists, with any disagreements resolved through consensus. Immunopositive cells showed brown granules indicating B-catenin and SATB-2 expression. Staining was assessed separately for membranous, cytoplasmic, and nuclear patterns. The staining intensity for B-catenin and SATB-2 was graded on a scale from weak (+1), moderate (+2), moderate-strong (+3), to strong (+4). The proportion score, representing the estimated percentage of positive cells, was categorized as follows: 0 (0–5% positive), 1 (6–20%), 2 (21–40%), 3 (41–65%), and 4 (>65%). A total staining score, combining intensity and proportion, ranged from 0 to 16, defining the extent of positive reactivity (Fig. 10) (Zhu *et al.*, 2013).

Statistical analysis: This study's data was displayed as means $\pm$ standard errors (SE). Utilizing GraphPad Prism 7, the statistical significance between groups was identified using either one-way ANOVAs for treated and non-treated groups regarding haematological and biochemical parameters, then to compare the (mean $\pm$ SE) of body weight comparison of the groups for the two-tailed Student's t test, a mixed model two-way analysis of variance (ANOVA) was employed. Statistical significance was defined as P values below 0.05.

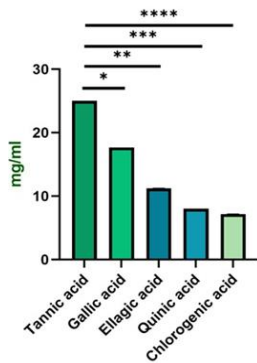
RESULTS

High Performance Liquid Chromatography (HPLC) analysis: An HPLC analysis was performed in order to quantify the overall concentration of phenolic acids and flavonoids present in QIG extract, which were 69.17 mg/ml, 18.66 mg/ml respectively. Tannic acid emerged as the dominant phenolic acid in the plant, with a notable concentration of 25.02 mg/ml. Compared to all other bioactive chemicals examined, this amount was considerably greater ($P < 0.05$), highlighting its prominent role and potential importance in the plant's biochemical profile (Fig. 1A). As well as, with 8.5 mg/ml, quercetin was the most abundant flavonoid in QIGE (Fig. 1B).

Impact of QIG on body weights: The rats in the control negative group progressively ($P < 0.05$) gained body weight during the experiment (Fig. 2A). Comparing to the positive control group, the rats that received (AOM+DSS) markedly decreased their body weight highly significantly ($P < 0.05$), followed by rats injected 5-FU had a considerably lower mean body weight ($P < 0.05$) Vs. the negative control groups. While the groups that were treated with the QIG significantly lost their body weight in a moderate-mild degree Vs. negative control, whereas it improved their weight Vs. the positive control and 5-FU groups,

particularly the treated group that received 1000 mg/kg QIG ($P < 0.05$) (Fig. 2B).

(A) Phenolic Acids in QIGE



(B) Flavonoids in QIGE

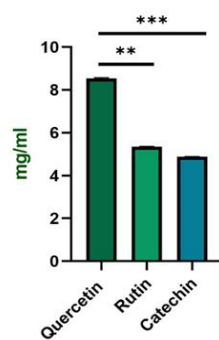
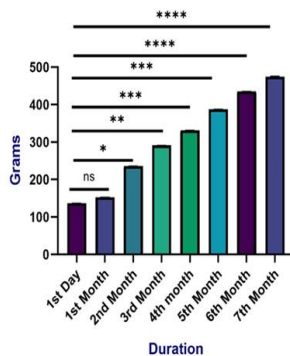


Fig. 1: Bioactive compounds in QIG extract. (A) Phenolic acids (Tannic acid, Gallic acid, Ellagic acid, Quinic acid and Chlorogenic acid) present in QIGE. (B) The flavonoids (Quercetin, Rutin and Catechin) amount that exist in QIGE.

(A) Weights of Control Negative Grp



(B) Weights in the Seventh month

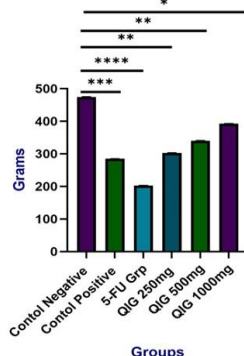


Fig. 2: Body weights of rats in the different studied groups. (A) Monthly body weight of the control negative group throughout the experiment duration. (B) Body weight variations among the studied experimental groups.

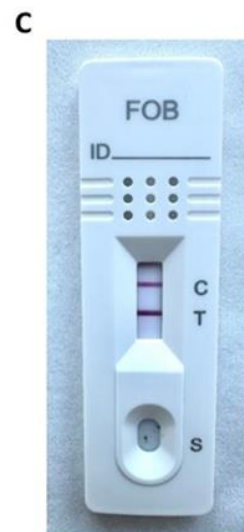
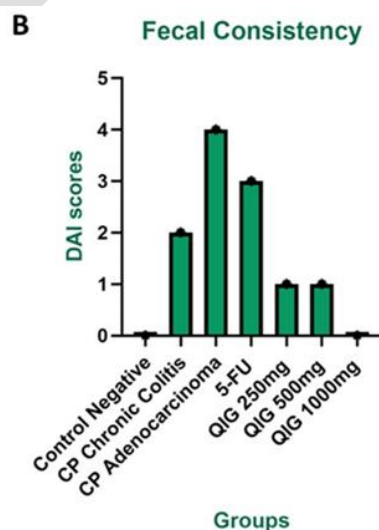


Fig. 3: The rats' fecal consistency. (A-a) Control negative group's normal fecal sample well formed (pellet-shape). (A-b) Many mucins are found in the feces of control positive group with chronic inflammation (A-c). Samples of watery diarrheal feces of rats with adenocarcinoma. (A-d) fecal specimens collected from the rats in 5-FU treated group. (B) DAI scores in all study groups. (C) The presence of two distinct bands is indicated by the positive (FOB) test result.

Fecal sample examination: In control negative rats the fecal sample was normal and well-formed (pellet-shape) score zero in disease activity index (DAI) and hemocult negative (Fig. 3A-a). Rats with chronic colitis showed a lot of mucins in their fecal samples during the third month (Fig. 3A-b) which resulted in a DAI score of two (Fig. 3B) and a hemocult negative. Fecal sample from rats with adenocarcinoma exhibit watery diarrhea (Fig. 3A-c) score four in (DAI) (Fig. 2B) as well as hemocult positive (Fig. 3C) suggesting a positive detection of blood in the fecal sample. Thin, very soft (slight diarrhea), and unformed feces (Fig. 3A-d) with a DAI score of three (Fig. 3B) and a hemocult negative result are noticed in the 5-FU treated group. Rats received 250mg and 500mg QIGE treatments had loose stools score one, and hemocult negative result, whereas, high dose QIGE group reported both normal consistency zero score as well as hemocult negative result (Fig. 3B).

Hematological measurements: Clear and significant differences in complete blood count (CBC) parameters were observed among the control- negative, adenocarcinoma, and treated groups, highlighting key physiological shifts related to disease and therapy. Notably, rats with adenocarcinoma tumors in the control positive (CP) group, in addition to those who received 5-FU (Fig. 4A-a) and QIG at 250 mg (Fig. 4A-b), exhibited marked reductions in red blood cell count (RBC), hemoglobin levels (Hb) (Fig. 4B), and mean corpuscular volume (MCV) (Fig. 4C). Conversely, these groups showed a pronounced increase in platelet count (PLT) (Fig. 4D). These hematologic changes underscore the profound systemic impact of adenocarcinoma and highlight potential biomarkers for monitoring disease progression. Notably, A significant ($P < 0.05$) increase in several critical parameters was spotted following treatment with QIG at 500 mg (Fig. 4A to C), whereas the high dose of 1000 mg effectively restored these measurements to their normal average ranges

(Fig. 4A to D). Furthermore, both the CP group and the QIG-treated groups at 250 mg and 500 mg exhibited considerably ($P<0.05$) elevated total white blood cell (WBC) counts (Fig. 5A- b, c) and neutrophil counts (Fig. 5B- b, c) when compared to the control negative (CN) group. In sharp contrast, the 5-FU group showed dramatically suppressed WBC and neutrophil counts (Fig. 5A-a, B-a), highlighting a strong immunosuppressive effect. Importantly, the high-dose QIG group, maintained WBC and neutrophil counts within normal average ranges (Fig. 5A-b, B-d), underscoring its potential role in immune system regulation and recovery. Lymphocyte counts significantly ($P<0.05$) decreased in the CP, 5-FU, and both low and median QIG dose groups compared to negative control (Fig. 5C-a, b, c), highlighting immune suppression in these groups. In contrast, monocyte levels were markedly elevated in these same groups, indicating an inflammatory response (Fig. 5D-a, b, c). Interestingly, the high-dose QIG treatment normalized both of lymphocyte and monocyte counts, suggesting a potential immunomodulatory effect at higher doses (Fig. 5C-d, D-d).

Carcinogenic Markers: The results of serum tumor markers in the sixth month revealed that the levels of both CEA, and CA19-9 in the CP group with adenocarcinoma, were much higher significantly ($P<0.05$) compared to the negative control group. Although there was a significant ($P<0.05$) reduction in CEA and CA19-9 levels in the group receiving 5-FU treatment. Both markers' levels in the QIG 250mg and 500mg treated groups markedly decreased as compared to the CP group, although they are still above the normal range, while both of the markers in QIG 1000mg group return to their normal range levels (Fig. 6 A and B).

Impact of QIGE on the regression of colonic adenocarcinoma: The microscopic section of the colon in negative control revealed normal histologic structures of 4 organized layers, including: mucosa that forms a fold-like feature (Simple columnar epithelium), lamina propria that contains intestinal crypts, submucosa, muscularis propria, and serosa (Fig. 7a-c).

The microscopic characteristics of poorly differentiated adenocarcinoma in the colon showed invasive adenocarcinoma, which usually invades the submucosa through the muscularis mucosae and is

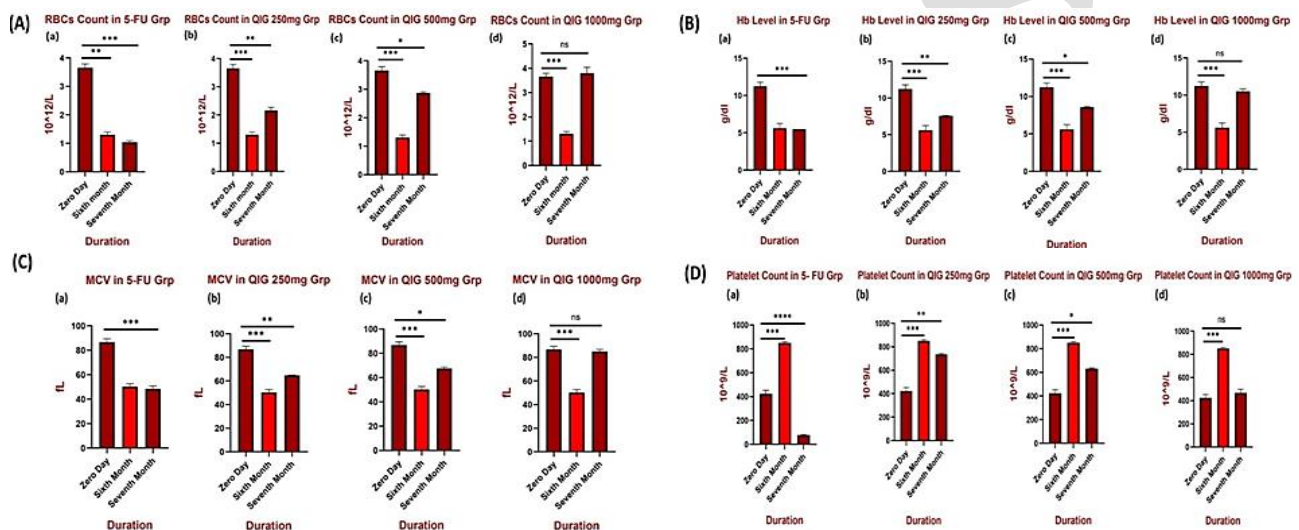


Fig. 4: (A) Red blood cells (RBCs) count in all study groups. (B) Hemoglobin (Hb) level in all study groups. (C) Mean erythrocyte volume across the different study groups. (D) Platelets count in each group.

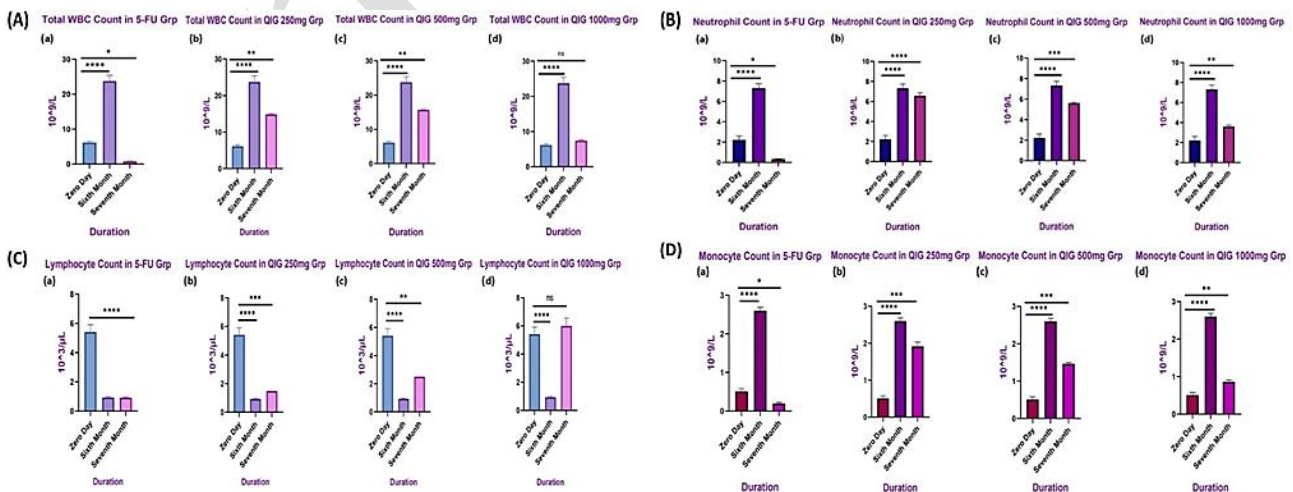


Fig. 5: (A) Total white blood cell (WBCs) count in all study groups. (B) Neutrophil count in all study groups. (C) Lymphocytes count among the different groups (D) Monocytes count in each group. Data are mean $\pm$ SE; one-way ANOVA with Dunnett's test was used. Significance: ($P>0.05$), * ($P\leq0.0332$), ** ($P\leq0.0021$), *** ($P\leq0.0002$), **** ($P\leq0.0001$).

Carcinoembryonic Antigen (CEA) Marker Carbohydrate Antigen 19-9 (CA 19-9) Marker

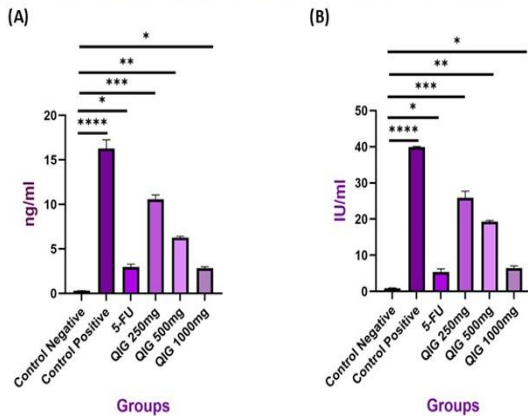


Fig. 6: Tumor markers level in control and treated groups. (A) Evaluation of CEA marker level in each group. (B) CA 19-9 marker levels compared among all study groups.

observed close to the serosa. This type of adenocarcinoma was most common in the CP, and groups that received 250 mg QIGE treatment, while in 5-FU and 500 and 1000 mg of QIGE groups showed the least case, microscopically presented also feature of desmoplasia reaction, a type of fibrous proliferation surrounding tumor cells secondary to invasive tumor growth. The neoplastic cells showed a typical atypia with intraluminal bridging commonly showing "dirty necrosis" consisting of necrotic cells with cytoplasmic fragments, crowded nuclei, hyperchromatism, and pleomorphism (Fig. 7d-f). While administration of *Quercus Infectoria Galls Extract* (QIGE), attenuates and regresses the invasion of colonic cancer from poorly to moderately differentiated and that recorded in 500 and 1000 mg QIGE with 5-FU, microscopically, the neoplastic cells forming tubular glands of the columnar cells have pseudostratified vesicular nuclei with crowded, most pencil-shaped cells,

and the neoplastic cells have a feature of hyperchromatism and pleomorphism (Fig. 7g-i). The high dose of QIGE significantly regressed the tumor invasion to well-differentiated adenocarcinoma, consisting of tubular, anastomosing, and branching glands in a desmoplastic stroma that was only found throughout the mucosa. The neoplastic cells showed abundant mitotic activity in addition severe degree of dysplastic changes seen in the non-neoplastic gland (Fig. 7j-l).

Regarding immunohistochemistry, β -catenin is expressed according to the differentiation of CRC. A high cytoplasmic-nuclear ratio is observed in grade III CRC in the positive control and 250mg/kg QIGE groups significantly ($P \geq 0.5$). Also, the strong staining and peak scores of positive cells (scores=12-16) were observed in the positive control group, followed by 250mg/kg QIGE group (Fig. 8 A- C). While the most common pattern of expression was cytoplasmic in grade II CRC at 500mg/kg and 5-FU, followed by 1000 mg/kg QIGE, the scores also decreased significantly ($P \geq 0.05$) compared to the positive control group (Fig. 8 A-C). In contrast, the normal membranous pattern was only detected in the group that was treated with 1000 mg/kg QIGE with grade I and score (scores=8-12) that improved the regression and differentiation significantly ($P \geq 0.5$) (Fig. 8A- C).

The SATB-2 CRC reliable marker showed diversity in expression in studied groups (Fig. 9A-C), for example, it was weakly expressed (Score 1-3, 40-45%) in the poorly invasive CRC (grade III) in three groups; positive control and 250mg/kg QIGE groups significantly ($P \geq 0.5$). In comparison to 500mg/kg QIGE and 5-FU, the intensity increased significantly ($P \geq 0.5$) as well as the scores were moderate expression (Score 3-9, 65%), in contrast, at 1000 mg/kg QIGE the proportion and intensity were raised significantly ($P \geq 0.5$) as strong expression (Score 6-16, 85%).

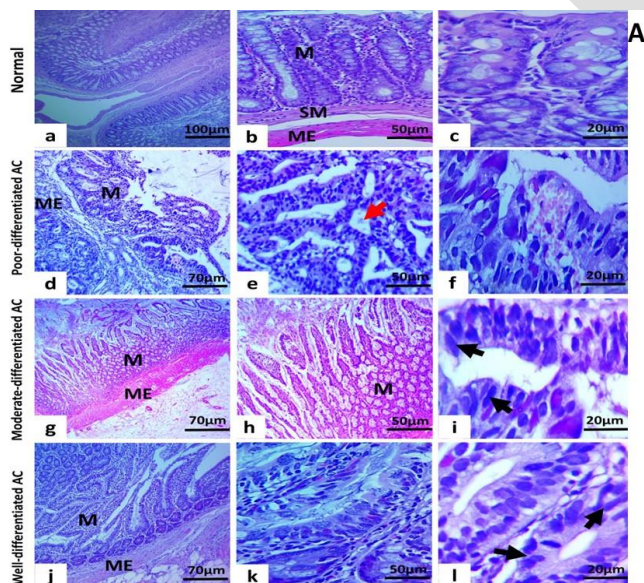
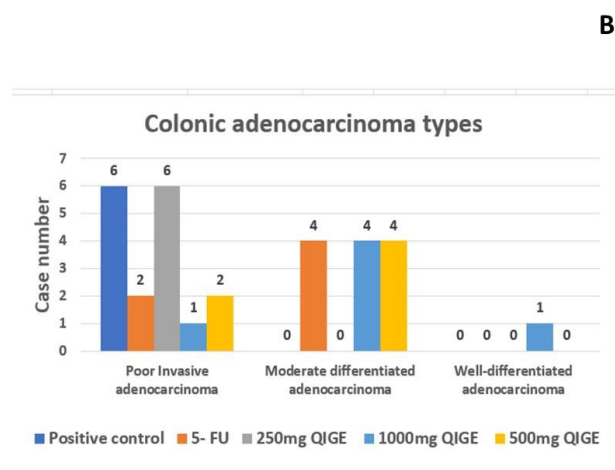


Fig. 7: A: Microscopic section of rat's colon revealed. A-c: Normal colonic layers from mucosa-serosa with intact morphology in the negative control group. d-f: Poorly differentiated adenocarcinoma (grade III), the neoplastic cells showed a glandular pattern with intraluminal bridging and necrotic debris (red arrows), having crowded nuclei with hyperchromatism and pleomorphism (black arrows). g-i: Moderately differentiated adenocarcinoma (grade II), the neoplastic cells form tubular glands of crowded, mostly pencil-shaped cells (black arrows), and the neoplastic cells have a feature of hyperchromatism and pleomorphism. j-i: Well-differentiated adenocarcinoma (grade I), the neoplastic cells form a gland only found throughout the



mucosa that had pleomorphism and hyperchromatism (black arrows) features (H&E stain). B: The classification of colonic adenocarcinoma among the studied groups.

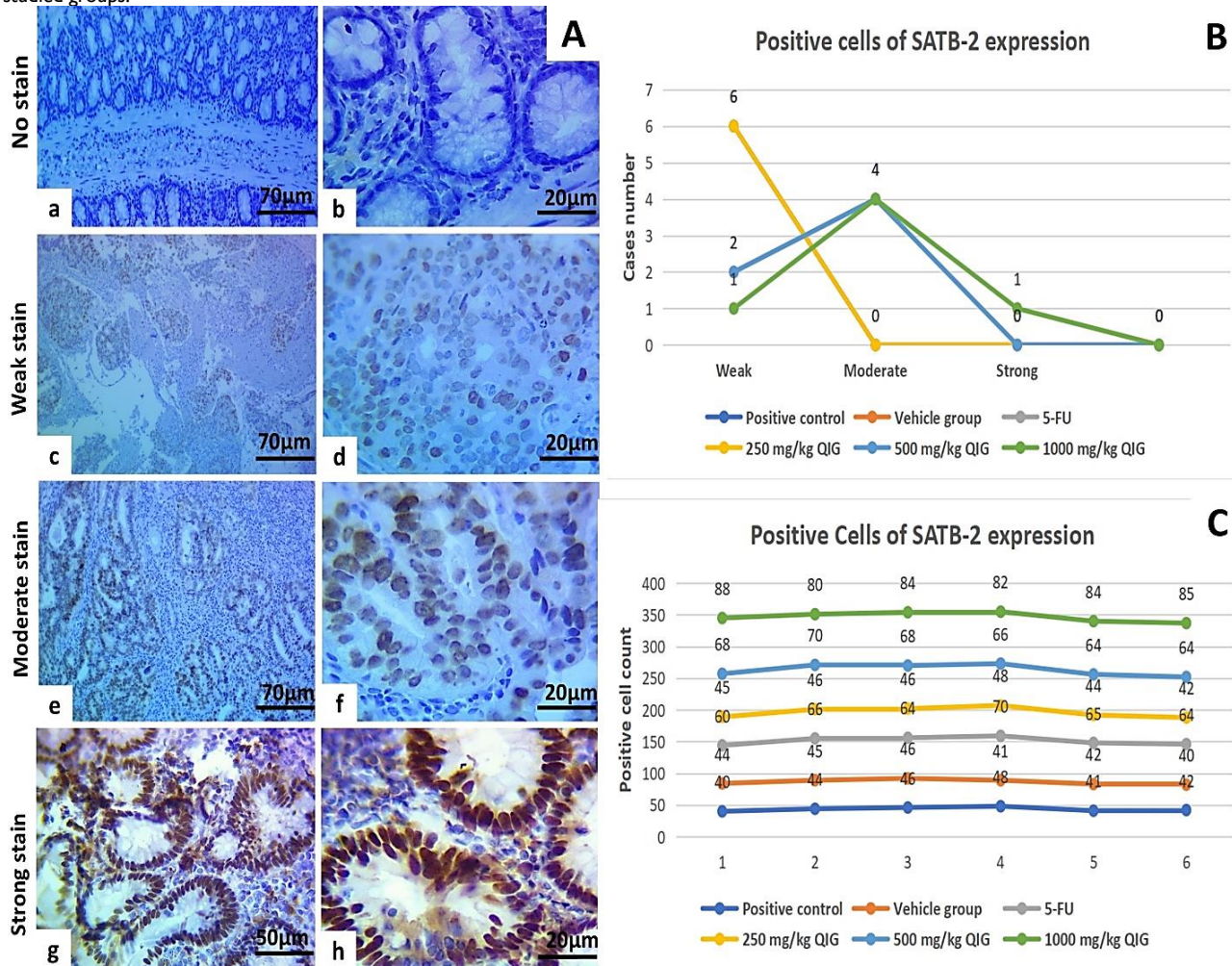


Fig. 9: A: Immunohistochemical sections of SATB-2 expression of CRC in rats showed: a and b: No positive cells (Score=0). c and d: Moderate-membranous expression (Score 8, 75%). e and f: Moderate-strong cytoplasmic expression (Score 12, 85%). g and h: Strong cytoplasmic-nuclear expression (Score 16, 80%). B: The percentages of different patterns of SATB-2 expression among the studied groups. C: The proportion of positive cells of SATB-2 expressions among the different groups.

DISCUSSION

Colitis-associated cancer is one of the most common forms of colorectal cancer (CAC) (Zhu *et al.*, 2021). Colon cancer is associated with colitis induced by AOM-DSS (Wu *et al.*, 2025). To determine its potential therapeutic significance in the management of colorectal cancer, Assessing the impact of *Quercus infectoria* gall extract on colon's adenocarcinoma is the goal of the current investigation. QIG provide considerable health advantages and have particular anti-inflammatory effects against colitis (Ding *et al.*, 2025). The key components of oak, according to evaluations, are flavonoids like quercetin and polyphenolic components like tannins and gallic acid (Hashim *et al.*, 2013; Thakur *et al.*, 2024), these results align with our HPLC findings (Fig. 1). In the current study, the group that received the cancer-induction chemicals (AOM-DSS) experienced a decrease in body weight that is in accordance with subsequent investigations (Mai *et al.*, 2019), and the rats exhibit severe diarrhea and fourth score in DAI, as a part of inflammatory process and colon tumorigenesis. This aligns with the findings of a recent study that highlighted the effect of AOM/DSS on CRC and

causing diarrhea (Sun *et al.*, 2023). According to the findings of this study, the rats receiving high dose QIGE treatment demonstrated the correction in the altered hematologic indices, there is no direct study specifically reporting the impact of QIG extract on correction of blood parameters in colon adenocarcinoma, however, a study done by (Castejón *et al.*, 2019) in rats with ulcerative colitis, revealed that the *Quercus* extract decreased neutrophil infiltration and inflammatory cytokines. The hypothesis is that *Q. infectoria* extract could have a beneficial impact on blood parameters by limiting tumor growth and lowering systemic inflammation. The present investigation focused on inflammatory cells such neutrophils, lymphocytes, and monocytes, which together are important components of the tumor microenvironment of colon adenocarcinoma. These cells control tumor growth, metastasis, and immune responses by interacting with cancer cells and stromal constituents (Li *et al.*, 2025). In this study, the elevated platelet count observed in rats with colorectal adenocarcinoma is mainly due to tumor-driven thrombocytosis, a common paraneoplastic response in colorectal cancer. The tumor and its microenvironment produce cytokines and growth factors such as interleukin-6

(IL-6) that stimulate increased platelet production by megakaryocytes in the bone marrow. Increased platelet counts correlate with cancer progression, angiogenesis, metastatic spread and reflecting a poor prognosis (Braun *et al.*, 2021). Platelet evaluation in the treatment of colorectal cancer with *Quercus infectoria* gall extract (QIGE) in rats is important because platelets serve as indicators of systemic inflammation, immune status, and potential toxicity. Improvements or normalization in platelet counts or indices with QIGE treatment would suggest reduced tumor burden and inflammation, correlating with the reported antitumor effects. Additionally, platelet evaluation provides insight into the safety and tolerability of QIGE. The role of M1 and M2 macrophage important as a potential future direction for further immunotherapy confirming. Considering the side effects of chemotherapy and based on complete blood count results, anemia, neutropenia, leukopenia, and thrombocytopenia recorded in the rats of the 5-FU-treated group, which is in accordance with earlier researches that highlighted myelosuppression as one of the most prominent 5-FU adverse effects (Ishibashi *et al.*, 2021; Salama *et al.*, 2022; Khan *et al.*, 2023). Additionally, the rats in the same group exhibited diarrhea and DAI score two mostly as a result of chemotherapy-induced intestinal mucosal damage (Das *et al.*, 2016) and a reduction in body weight which corresponds to a prior study (Yoneda *et al.*, 2021).

Combining CEA with CA 19-9 tumor markers enhances prognostic data and diagnostic precision, especially when monitoring the progression and recurrence of CRC (Stikma *et al.*, 2014). In the present investigation and according to the tumor markers, in rats with adenocarcinoma, tumor marker levels were significantly elevated. Notably, treatment with either 5-FU or a high dose of QIGE effectively restored tumor marker levels back within the normal range. The histopathology results exhibited that QIG extract at high dose regresses the tumor invasion towards well-differentiated adenocarcinoma and the invasion only seen at the mucosa layer. This comes from the phenolic compounds of QIGE that have a wide range of therapeutic effects, including antioxidant (Kamarudin *et al.*, 2021) antimicrobial, anti-inflammatory, and anti-tumor actions (Azarakhsh *et al.*, 2025). An Experiment revealed that natural products could potentially use for preventing cancer cell proliferation, arrest of cell cycle and death of cancer cells (Liang *et al.*, 2022). Tannic acid also has the ability to decrease tumor volume while increasing intra-tumoral necrosis (Bona *et al.*, 2020). Ellagic acid had chemo-preventive features, making it a potential natural anticancer component for cancer treatment (Čižmaríková *et al.*, 2023). An Iraqi study revealed that ellagic acid helpful in preventing intestinal mucositis caused by 5-FU (Al-hoshary and Zalzal, 2023). Quercetin and rutin exhibit anticancer properties in colon cancer (Suman *et al.*, 2025).

The key effector in the canonical Wnt signaling pathway, which is essential to tissue regeneration and repair, is beta-catenin. When beta-catenin receives Wnt signals, it avoids destruction, enters the nucleus, and activates genes that promote cell growth and tissue repair. (Wu *et al.*, 2025). Wnt/beta-catenin signaling can be enhanced or modified by SATB2, a potent regulator that can affect the progression of cancer. They collaborate as an

essential molecular pair for directs cell fate during tissue repair and regeneration (Yu *et al.*, 2017). The current study's immunohistochemistry results proved that a high cytoplasmic- nuclear ratio with strong staining and peak scores of β -catenin is observed in grade III CRC in the CP, and low dose QIGE groups significantly. While the most common pattern of expression was cytoplasmic in grade II CRC in the median dose QIGE and 5-FU groups. The normal membranous pattern was only detected in high dose QIGE with grade I and (score= 8-12) that improved the regression and differentiation considerably. This result is relevant since colon cancer's invasiveness, poor differentiation, and tumor growth are all associated with abnormal activation of the Wnt/ β -catenin signaling system (Zhao *et al.*, 2024). SATB2 is a colorectal differentiation diagnostic marker (Schmitt *et al.*, 2021). In the current data, the SATB2 was weakly expressed in the poorly invasive CRC (grade III) in the CP and low dose QIGE, whereas in median dose QIGE and 5-FU groups the intensity and score increased significantly. Nevertheless, at 1000 mg/kg QIGE extract, the proportion and intensity of SATB2 expression are reported to be significantly ($P \geq 0.5$) increased, showing strong expression. SATB2 expression tends to be higher in well-differentiated tumors and is linked with less aggressive tumor behavior, whereas its absence is linked to tumor growth and poor differentiation (Liu *et al.*, 2019; Shaban Al-Omar *et al.*, 2024). Thus, a significant increase in SATB2 expression by high-dose QIGE suggests a positive effect on promoting a shift from a poorly differentiated state to a well-differentiated one and may indicate regression towards a less invasive, more organized adenocarcinoma phenotype (Cheng *et al.*, 2025). This aligns well with our previous observations of tumor regression and improved differentiation markers (β -catenin) under high-dose QIGE treatment. The effects of QIGE extract on SATB2 and β -catenin expression are currently not well supported by direct empirical data in the relevant research. As a future direction, in order to evaluate proteins and genes linked to colorectal cancer, Western blotting and qPCR are becoming essential molecular techniques and they could have significant potential for allowing earlier detection of colorectal cancer (Li *et al.*, 2025).

Conclusions: Phytochemicals derived from plants have gained considerable research attention for cancer chemoprevention due to their wide accessibility, cost-effectiveness, and reduced side effects in cancer control and management. The current study proved that oral administration of QIGE at 1000 mg/kg in rats with colonic adenocarcinoma effectively restored body weight and fecal consistency, improved hematological parameters and tumor biomarkers, and significantly regressed the tumor progression in the colon tissue, indicated by appropriate expression of neoplastic signaling pathways.

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Author's contribution: SMAH and SHI, supervised, and implemented the original idea, HSHR Conducted the practical work, gathered the samples, and authored the paper, SMAH performed the histopathological and immunohistochemical (IHC) analyses.

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