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Pharmacological evaluation of combinational treatment of herbal medicines with 5-fluorouracil for therapeutic enhancement in an animal model of colorectal cancer

Sagar Mahant[1], Krishna Patel[2], Arya Patel[2], Sanika Dongre[2], Dhvani Parmar[2], Rushi Shah[3], Diwyanshi Zinzuvadia[4], Alkeshkumar Patel[2]*

[1]CBCC Global Research, Ahmedabad, India.
[2]Ramanbhai Patel College of Pharmacy, CHARUSAT, Changa, India.
[3]Torrent Research Center, Ahmedabad, India.
[4]L J Institute of Pharmacy, Ahmedabad, India.

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ABSTRACT

Colorectal cancer (CRC) is the most frequent cancer in developing and developed countries. Chemotherapy acts on a few biomarkers, but CRC develops and progresses because of multiple genetic and epigenetic dysregulation. The animal study was conducted according to Committee for the Purpose of Control and Supervision of Experiments on Animals and Animal Research: Reporting of *In Vivo* Experiments guidelines. CRC was induced by 1,2-dimethylhydrazine at 35 mg/kg in all Sprague-Dawley male rats for 10 weeks except for normal control. Later, treatment is given for 5 weeks with standard 5-fluorouracil (5-FU) and test drug (Polyherbal mixture) alone and in combination to check synergism effects. Important parameters related to carcinogenesis, such as change in body weight, modified Bowen's score, colon length to weight ratio, liver and spleen index, complete blood count, estimation of uridine phosphorylase-1 enzyme, tumor necrosis factor-α, β-catenin by ELISA, and histopathology, were evaluated. Polyherbal mixture inhibited the UPP-1 enzyme, decreasing 5-FU toxicity on normal cells. Also, tumor necrosis factor-α and β-catenin levels were reduced significantly by combination compared to monotherapy alone. Combining chemotherapy with natural products may help reduce chemotherapy's dose and the cost of the treatment due to toxicities and hospitalization. Standard drug (5-FU) combined with a polyherbal mixture at a high dose produces a synergistic effect and reduces 5-FU toxicity.

INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer in both men and women globally. As per the GLOBOCAN 2020 report, it was estimated that the incidence of CRC cases worldwide is about 1,931,590, with the maximum number of cases prevalent in Asia [1]. Environmental, genetic factors, ion-transport mechanism [2], existing disease conditions, and diet [3] are major causes of CRC. Multiple hallmarks responsible for the development of CRC are high cell proliferation, rise in uridine phosphorylase enzyme-1 and β-catenin enzyme, proinflammatory cytokines, oxidative stress, presence of adenomatous polyposis coli (APC), mutation of the growth signal autonomy such as endothelial growth factor receptors, Kirsten rat sarcoma viral oncogene homolog (KRAS), and immune escape [4]. Genetic predisposition is the most critical risk factor in the development of colon cancer in certain populations, along with environmental exposures and abnormal lifestyle [5,6]. Environmental variables, such as sedentary life, overweight, processed foods, liquor, and meat consumption, are causes for the rise in CRC cases [7,8]. Uridine phosphorylase enzyme degrades uridine and aggravates toxicities of 5-fluorouracil (5-FU) in normal tissue. Due to a decrease in uridine level, its cytoprotective effect gets lost [9]. In CRC, the overexpression of β-catenin in the Wnt pathway leads to the upregulation of expression of urokinase plasminogen activator that causes progression of infiltration, metastasis as well as dormancy in human CRC [10]. Tumor necrosis factor-alpha (TNF-α), a proinflammatory

*Corresponding Author
Alkeshkumar Patel, Ramanbhai Patel College of Pharmacy, CHARUSAT, Changa, India. E-mail: alkeshpatel.ph @ charusat.ac.in

cytokine prevalent in the cancer microenvironment, is essential for controlling the body's immunological and inflammatory reactions. TNF is primarily recognized for its involvement in inducing inflammation, while it is also implicated in a number of physiological and pathological processes. TNF comes in two main varieties. The most well-known type of TNF is TNF-Alpha (TNF-), which is created by a number of immune cells, including macrophages, T cells, and natural killer cells. TNF- is a cytokine that stimulates inflammation and is classified as such. TNF has both inflammatory and apoptotic properties [11–13]. Apart from this, it also increases tumor-associated calcium signal transduction protein-2 expression via the extracellular signal-regulated kinase 1/2 signaling pathway, resulting in colorectal tumor progression [14]. 5-FU is a first-line treatment for CRC, but it has a limitation of cytotoxicity and resistance at advanced stages of CRC. The 1,2-dimethylhydrazine (DMH) is a carcinogen that causes CRC in rats. Preneoplastic abnormalities, such as many plaque lesions, aberrant crypt foci, and well-defined dysplasia, are seen after DMH therapy [15]. Considering the limitation of the 5-FU and multiple markers involved in the progression of CRC, we attempted to treat CRC with natural compounds. *In-silico* molecular docking was used to target the uridine phosphorylase and β-catenin, which govern cancer growth. Based on a literature review, herbal drugs were chosen for their anti-cancer potential and their capacity to lower other risk factors, such as oxidative stress, cell proliferation markers, and proinflammatory mediators. Herbal extracts include *Solanum nigrum, Nigella sativa, Garcinia indica,* and *Allium sativum,* which possess vital phytoconstituents that act on multiple markers of CRC. *Solanum nigrum* includes phytoconstituents, such as quercetin, Thymol, Naringenin, and others, and it is a traditional treatment with pharmacological properties such as preventing hepatotoxicity and cytotoxicity [16–20]. Thymoquinone, dithymoquinone, anthraquinonequercetin, thymol, and carvacrol are phytoconstituents found in *N. sativa* that function as anti-inflammatory and immunomodulatory agents [21]. *Garcinia indica* is beneficial in a number of ways, including as an antioxidant, anti-obesity, antibacterial, hepatoprotective, and cardioprotective substance. Coumaric acid, apigenin, and naringenin are mainly responsible for the action [22]. The phytoconstituents found in *A. sativum* include Allicin, Naringenin, Anthraquinone, and quercetin. It has been utilized as a medication since ancient times. Allicin is the main physiologically active ingredient in garlic, acting as a possible antioxidant agent that may aid in the treatment of CRC [23]. The mixture of extract of these four plants is hypothesized to treat CRC in the present study.

MATERIAL AND METHODS

Experimental animals

Fifty-four male Sprague-Dawley rats with body weights around 200–250 gm and ages 8–10 weeks were used. The animal experiment protocol (RPCP/IAEC/2021-22/R8) was approved by the Institute Animal Ethics Committee. The rats were kept in polypropylene cages with a hygienic corn cob bed with a 12-hour dark/12-hour light cycle. Temperature and relative humidity were maintained at 25°C ± 2°C and 50% ± 10%, respectively. The animal study was modified and prepared in alignment with Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (The ARRIVE Essential 10 and The Recommended Set) as well as CPCSEA regulation, INDIA. All animals were given free access to a pellet diet and regular drinking water [24].

Chemical procurement

DMH was purchased from Sigma Aldrich, while herbal extracts were purchased from Nutan Ayurvedic Research Centre, Gujarat. 5-FU manufactured by Celon Lab. Standard compounds such as thymoquinone, quercetin, and ellagic acid were purchased from Yucca Enterprise. TNF-α (RTA1021) and β-catenin (K11-0879) ELISA kits were purchased from Krishgen Biosystem, and the Uridine phosphorylase (MBS2605124) ELISA kit was purchased from My BioSource.

Experimental design

All the animals were divided into seven groups. DMH, a carcinogen, was administered to all groups except the normal control (NC) group. DMH was dissolved in normal saline containing 1.5% potassium EDTA as a vehicle. Late final pH was adjusted to 6.5–7 with 1 N sodium hydroxide solution and administered subcutaneously in each animal [25,26]. After 10 weeks, group 3 animals received 5-FU alone, whereas groups 4 and 5 received standard and test drug treatment at high and low doses. The remaining groups 6 and 7 were given test drug therapy alone at low and high doses respectively.

Group 1: NC; six animals; saline 10 ml/kg/day p.o.

Group 2: Disease control (DC); eight animals; DMH (35 mg/kg s.c.)—Once a week for 10 weeks [week 1 to 10].

Group 3: Standard control (STD); eight animals; DMH (35 mg/kg s.c.) [week 1 to 10] followed by 5-FU (10 mg/kg once a week for 5 weeks i.p.) [week 11 to 15].

Group 4: Standard + high test dose (STD + THP); eight animals; DMH (35 mg/kg s.c.) [week 1 to 10] followed by 5-FU (10 mg/kg i.p.) [week 11 to 15] + Polyherbal mixture (Low dose) (SN: 35 mg/kg; NS: 100 mg/kg; GI:75 mg/kg; AS: 30 mg/kg daily for 5 weeks p.o.) [week 11 to 15].

Group 5: Standard + low test dose (STD + TLP); eight animals; DMH (35 mg/kg s.c.) [week 1 to 10] followed by 5-FU (10 mg/kg i.p.) [week 11 to 15] + Polyherbal mixture (High dose) (SN: 140 mg/kg; NS: 400 mg/kg; GI:300 mg/kg; AS: 120 mg/kg daily for 5 weeks p.o.) [week 11 to 15].

Group 6: Test drug (TLP); eight animals; DMH (35 mg/kg s.c.) [week 1 to 10] followed by Polyherbal mixture (Low dose) (SN: 35 mg/kg; NS: 100 mg/kg; GI:75 mg/kg; AS: 30 mg/kg daily for 5 weeks p.o.) [week 11 to 15].

Group 7: Test drug (THP); eight animals; DMH (35 mg/kg s.c.) [week 1 to 10] followed by Polyherbal mixture (High dose) (SN: 140 mg/kg; NS: 400 mg/kg; GI:300 mg/kg; AS: 120 mg/kg daily for 5 weeks p.o.) [week 11 to 15].

The dose of each test drug (*Solanum nigrum*-SN, *Nigella sativa*-NS, *Garcinia indica*-GI, *Allium sativum*-AS) was selected based on acute oral toxicity (OECD guideline 423) and published literature [27–32].

MOLECULAR DOCKING

The *in-silico* approach employs molecular docking software, which anticipates the interaction of specific enzymes, proteins, or genes with ligands. Some software names for molecular docking are AutoDock, FlexX, Autodock vina, and so

on [33–35]. The interaction of uridine phosphorylase, β-catenin, and phytoconstituents was studied using the *in vitro* molecular docking tool "Autodock Vina." Targets were identified using the protein data bank (PDB), and the structure of phytoconstituents for interaction was designed using ChemDraw. The standard drug for molecular docking against both enzymes is 5-FU. From UniProtKB Data Base (https://www.uniprot.org/), the sequence, structure, and functional information of β-Catenin and Uridine phosphorylase were retrieved with UniProt ID: P35222 (CTNB1_HUMAN), Q16831 (UPP1_HUMAN), respectively. β-Catenin and Uridine phosphorylase 3-D structures were downloaded from Research Collaboratory for Structural Bioinformatic PDB (https://www.rcsb.org/) with PDB IDs: 1JDH, 3NBQ, respectively, with resolutions of 1.90 Å and 2.30 Å. BIOVIA Discovery Studio 21.1 Visualizer was used to remove the co-crystallized ligands. Thymol, carvacrol, anthraquinone, naringenin, quercetin, thymoquinone, dithymoquinone, and allicin had their 3-D structures retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). A structural and active site investigation of all three proteins was carried out using the computed atlas of surface topography of proteins server (http://sts.bioe.uic.edu/castp). Molecular docking was performed using Autodock vina V.1.2.0.

EVALUATION PARAMETERS

Qualitative analysis-thin layer chromatography (TLC)

TLC was performed using toluene: ethyl acetate: glacial acetic acid as a mobile phase (4.5: 4: 0.5), and ALUGRAM® Xtra SIL G/UV_{254} precoated TLC sheets were used as a stationary phase.

Change in body weight

As an important marker of cachexia and decrease in food intake during CRC progression, change in body weight was measured by determining the difference between final body weight (at the end of 15th weeks) and initial body weight (before carcinogen induction) [36,37].

Modified Bowen's score scale

Based on the consistency of the stool, different scores were assigned to determine colitis and colon dysbiosis. 0 for regular stool, 1 for moist and soft stool (mild diarrhea), 2 for moist, and unformed stool (moderate diarrhea), 3 for watery stool (severe diarrhea), and 4 for occult blood stool [38–40].

Colon length-to-weight ratio

After sacrificing each animal, the colon was isolated. The length and weight of the colon were measured. The following formula was used to determine the ratio [41–43]:

Colon length to weight ratio = Colon length/Animal weight.

Liver index

Following the sacrifice of the animals, the liver was removed. Later, the weight of the liver was measured. The final ratio was calculated using the following formula [41–43]:

Liver index=Weight of liver/weight of the animal.

Spleen index

As the spleen can be considered the graveyard of blood cells, an increase in the spleen's weight can be considered as high cellular mortality. Following the sacrifice of the animals, the spleen was removed. The weight of each spleen was measured. The final ratio was calculated using the following formula [41–43]:

Spleen index = Weight of spleen/weight of animal.

Complete blood count (CBC)

Considering cellular turnover changes during carcinogenesis, CBC was estimated from each rat's blood (200 µl) using a Mindray BC-5130 analyzer. The percentage of lymphocyte, neutrophil, monocyte, and red blood cells (RBCs), and platelets were measured [44].

ELISA of TNF-α, uridine phosphorylase, and β-catenin

Proinflammatory cytokine, cell proliferation, and detoxifying protein levels are the main players in judging the success of the oncotherapy. A 100 µl plasma was used to estimate TNF-α and uridine phosphorylase, while 40 µl colon homogenate was used to estimate β-catenin by ELISA. At the end of the test, absorbances were measured at 450 nm using a microplate reader, and the results were interpreted.

Histopathological analysis

Based on palpation and morphological changes, the suspected colon part and liver were isolated and cleaned with normal saline before being placed in formalin for cell fixation. Tissues of the colon and liver were sent to a laboratory for histology in 10% formalin solution. Light microscopy was used to examine paraffin-embedded samples stained with hematoxylin and eosin (H and E). Slide images were captured using an inverted trinocular microscope (Carl Zeiss, Axio vert ALFL) [45].

Statistical analysis

All the values were expressed as mean ± SEM of six animals. Parameters were statistically analyzed with one-way ANOVA followed by Tukey's multiple comparison test and Kruskal Wallis test (for scoring) using graph pad prism software. $p < 0.05$ is considered a significant difference. Statistical analysis was done with GraphPad Prism 8.4.3 software [46].

RESULTS

Molecular docking

The molecular docking was carried out between phytoconstituents and individually between the enzymes β-catenin and uridine phosphorylase enzyme-1, respectively (Table 1).

According to the molecular docking score, all of the phytoconstituents were closer to or had a high affinity for the β-catenin enzyme. Naringenin and quercetin obtained a higher docking score than other phytoconstituents and the standard medication. This indicates that both Naringenin as well as quercetin exhibited a greater affinity for the β-catenin enzyme. Dithymoquinone and anthracene obtained higher docking

scores than 5-FU. Thus, they have a stronger affinity for the β-catenin enzyme than 5-FU.

According to the molecular docking score, all of the phytoconstituents were closer to or had a high affinity for the uridine phosphorylase enzyme-1. Naringenin obtained a higher docking score than all other phytoconstituents and the standard medication. This indicates that Naringenin exhibited a greater affinity for the uridine phosphorylase enzyme-1. Following Naringenin, quercetin also displayed a higher docking score, indicating good binding affinity toward the uridine phosphorylase enzyme-1. Anthracene and dithymoquinone obtained higher docking scores than 5-FU. That is, they have a stronger affinity for the uridine phosphorylase enzyme-1 than 5-FU.

Table 1. Molecular docking score of phytoconstituents of herbal test drugs.

Sr.no.	Compound name	Docking score (kcal/mol)	
		β-catenin	Uridine phosphorylase
1.	5-FU	−4.80	−5.40
2.	Thymol	−5.00	−5.80
3.	Carvacrol	−5.10	−6.80
4.	Anthraquinone	−6.10	−7.20
5.	Naringenin	−6.40	−8.20
6.	Quercetin	−6.40	−8.10
7.	Thymoquinone	−5.20	−5.80
8.	Dithymoquinone	−6.30	−6.80
9.	Allicin	−3.30	−4.60

Overall, Naringenin and quercetin obtained a higher docking score than other phytoconstituents and the traditional medicines for both the enzymes uridine phosphorylase enzyme-1 and β-catenin (Fig. 1).

Qualitative analysis-TLC

The standard marker was placed on the first track, while the test herbal extract was placed on the second track. The presence of a specific phytoconstituent was confirmed by comparing it to a standard marker. After reaching the maximum height of the mobile phase, "Retention factor" (Rf value) was determined for test and standard. Rf sof the quercetin and ellagic acid were found to be 0.49 and 0.45, respectively.

Modified Bowen's score

NC group has shown semi-solid brown color stool and thus assigned a score "0" which indicates the absence of diarrhea. The DC group was assigned a score "4" because it displayed occult blood. The STD group was assigned a score "3" as it displayed moderate to severe diarrhea. The STD + TLP and STD + THP were assigned a score "1" because they showed mild diarrhea.

Change in body weight

The body weight of the DC group was significantly low ($p < 0.05$) due to cachexia and a decrease in food intake as compared to the NC and STD groups. The body weight of the THP and STD + THP groups was found to be comparable with the STD group. There is a significant decrease in body weight of TLP and STD + TLP as compared to the NC group and STD group, respectively (Fig. 2a).

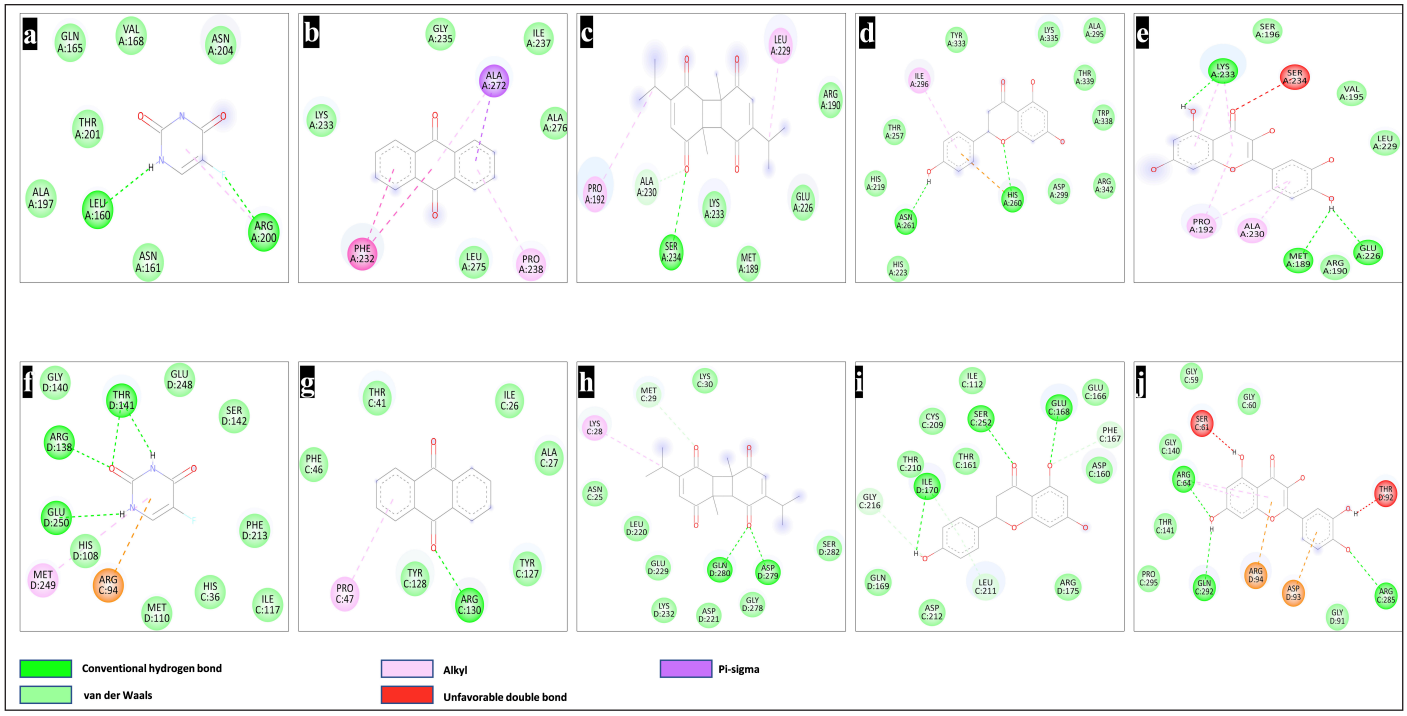

Figure 1. 2-D interaction of phytoconstituents with β-catenin (1JDH) and UPP-1 (3NBQ). Interaction of β-catenin with 5-FU (a), Anthracene (b), Dithymoquinone (c), Naringenin (d), quercetin (e), Interaction of UPP-1 with 5-FU (f), Anthracene (g), Dithymoquinone (h), Naringenin (i), and quercetin (j).

Colon length/weight ratio

The colon length-to-weight ratio of the DC group was significantly lower as compared to all other groups. Amongst all test groups, STD + THP gave improved results when compared with the STD group. All other test groups gave comparable results with the STD group (Fig. 2b).

Liver index

The liver weight was increased in the DC group due to dysplasia, and the animal weight decreased due to cachexia, from which it was concluded that this group has a high liver index as compared to all other groups. Only the THP group showed a significant decrease ($p < 0.05$) in liver weight, which is comparable with the NC group. All other test groups gave comparable results to that of the STD group (Fig. 2c).

Spleen index

There was an increase in spleen weight due to high blood cell turnover, whereas there was a decrease in the body weight of animals in the DC group, from which it was concluded that the spleen index of the DC group was higher as compared to all other groups. The spleen index of the THP group was significantly ($p < 0.05$) lower as compared to the NC, DC, and STD groups. The spleen index of STD + THP and STD + TLP were also comparable with the NC group (Fig. 2d).

Complete blood count

Percentage of lymphocytes

DC group displayed a significant decrease ($p < 0.05$) in the lymphocyte count as compared to all other groups, which can be associated with failure of antitumor immunity. The TLP and THP groups displayed an increase in the number of lymphocytes as compared with the STD group. The STD + TLP and STD + THP groups had similar results when compared with the STD group, which can be an indication of STD causing a decrease in overall number of lymphocytes (Fig. 3a).

Percentage of neutrophils

The DC group showed a significantly higher ($p < 0.05$) neutrophil count as compared to all groups, which is an indication of poor prognosis. The THP, STD + THP, and STD + TLP groups displayed a similar number of neutrophils when compared with the STD group. The TLP group showed a significant decrease in the percentage of neutrophils as comparable to NC, DC, and STD groups (Fig. 3b).

Percentage of monocytes

The monocyte count was higher in the DC group as compared to all other groups, indicating poor prognosis. The results of all test groups were found to have comparable results with the NC group and STD group (Fig. 3c).

RBC

Only the THP group was found to have comparable results with the STD group. The STD + THP, TLP, and STD + TLP groups were found to have decreased RBC count as compared to the STD group, possibly due to lower values found in a few animals (Fig. 3d). When CRC reaches the advanced stage, there are chances of the development of anemia that were not found in our study.

Enzyme-linked immunosorbent assay

TNF-α

The TNF- α levels were found to be low in the NC group, whereas they were highest during diseased conditions. The results of THP and TLP were comparable with STD. The TNF- α levels of STD + THP and STD + TLP were significantly lower ($p < 0.05$) as compared to STD, which can be an indication of the synergistic effect of STD and polyherbal formulation (Fig. 4b).

β-Catenin

The amount of β-catenin in the NC group was found to be low but too high in the DC group. The STD + THP group displayed a low amount of β-catenin as compared to STD and all other test groups; it gave comparable results with the NC group (helps in the reduction of tumor cell proliferation). The THP, TLP, and STD + TLP groups have comparable results with the STD group (Fig. 4c).

Uridine phosphorylase 1

Inhibition of uridine phosphorylase-1 is responsible for cytoprotection and lowering chemo-induced toxicities in vital organs. The uridine phosphorylase-1 enzyme concentration was lowest in the NC group and highest in the

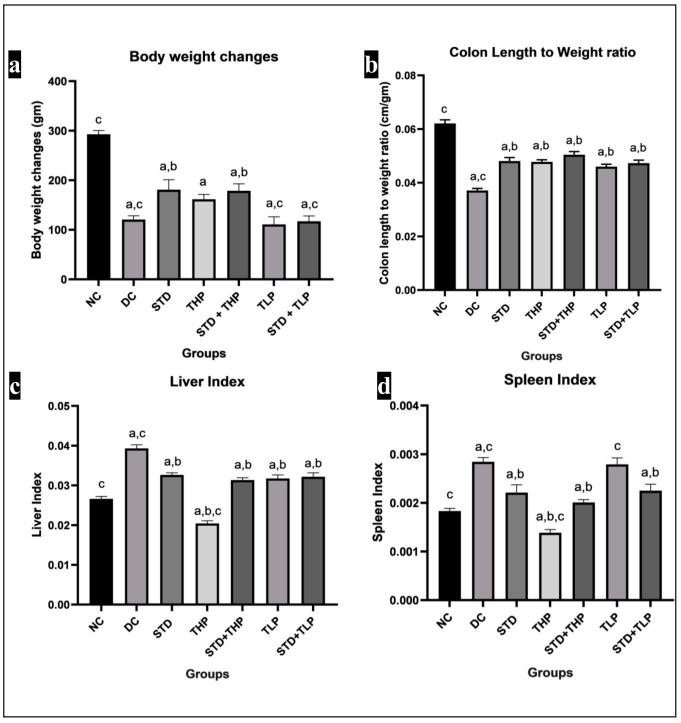

Figure 2. Morphological changes after CRC treatment. Body weight changes (a), colon length to weight ratio (b), liver index (c), and spleen index (d), respectively. [a, b, c] significantly different from NC, DC, and Standard (Std), respectively, at $p < 0.05$ and $n = 6$.

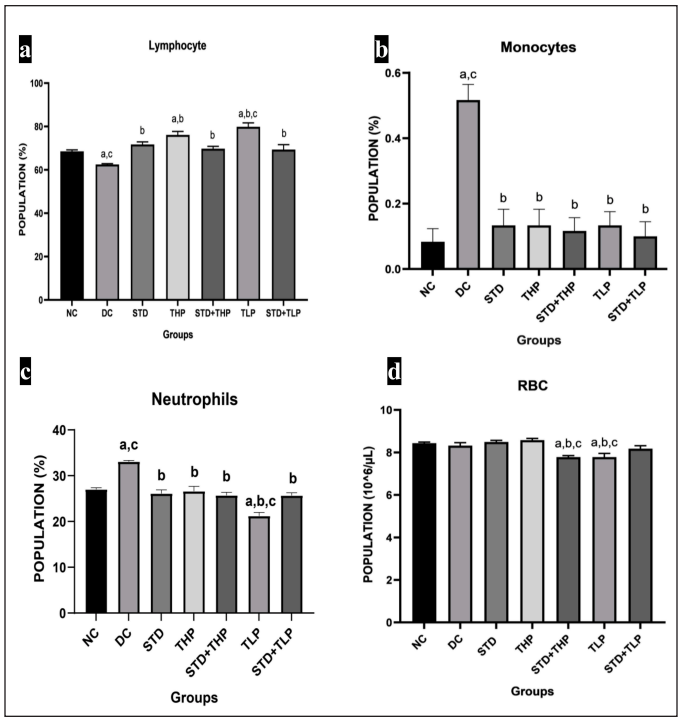

Figure 3. Hematological changes after CRC treatment for lymphocyte population (%) in different groups (a), Monocytes population (%) in different groups (b), Neutrophils population (%) in different groups (c), RBC population (106/µl) in different groups. a, b, and c are significantly different from NC, DC, and Standard (Std), respectively, at $p < 0.05$ and $n = 6$. RBC = Red blood cells.

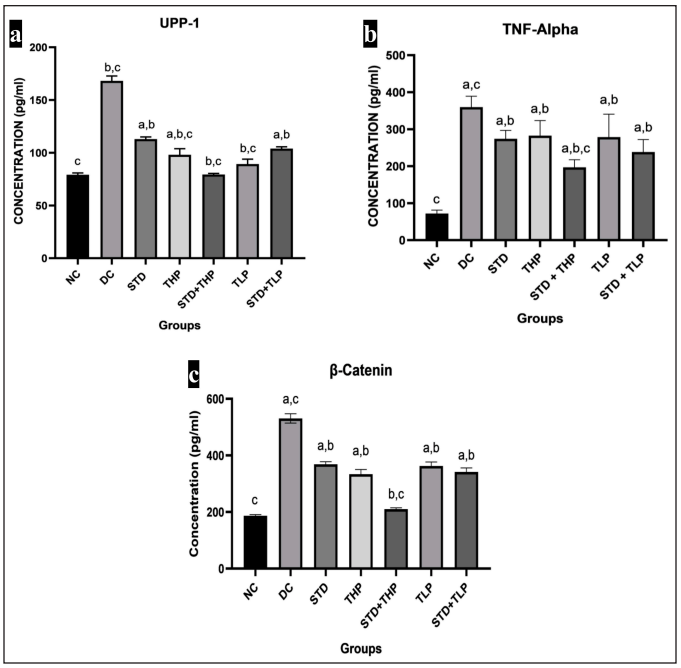

Figure 4. Cytokine and driver proteins change after CRC treatment. ELISA for UPP-1 concentration (pg/ml) in different groups (a), TNF-α concentration (pg/ml) in different groups (b), and β-catenin concentration (pg/ml) in different groups (c). a, b, and c are significantly different from NC, DC, and Standard (Std), respectively, at $p < 0.05$ and $n = 6$. CRC = Colorectal cancer; ELISA = Enzyme link immunosorbent assay; TNF-α = Tumour necrosis factor–α; UPP-1 = Uridine phosphorylase-1.

DC group as compared to all other groups, respectively. The uridine phosphorylase-1 levels were significantly lower ($p < 0.05$) in the THP group, STD + THP group, and TLP group as compared to the STD group. The uridine phosphorylase-1 levels in the STD + TLP group were comparable with the STD group (Fig. 4a).

Histopathological study

NC group animals have all normal layers of mucosa, submucosa, muscularis, and serosa with normal cell proliferation with a proper nucleocytoplasmic ratio in the colon and absence of liver metastasis. The DC animals showed moderately differentiated adenocarcinoma infiltrated to lamina propria in colon and liver dysplasia. The STD and STD + THP groups showed mild dysplasia in the colon with absence of liver metastasis. The TLP group, STD + TLP, and THP groups displayed adenocarcinoma in the colon. Mild liver dysplasia was observed in the TLP and STD + TLP groups, whereas no liver metastasis was observed in the case of the THP group (Fig. 5).

DISCUSSION

Uridine phosphorylase degrades uridine to uracil by the pyrimidine salvage pathway and increases 5-FU toxicities in normal tissues. It gets overexpressed in many gastric cancer cells. Uridine is a biochemical modulator that lowers 5-FU host toxicity and maintains the drug's antitumor efficacy [47]. The β-catenin protein regulates cell division. By boosting transcriptional factors, intranuclear beta-catenin promotes cell proliferation and malignancy. Wnt/catenin signaling is necessary for intestinal homeostasis and APC gene mutations. APC works as a tumor suppressor gene, preventing cells from dividing and developing too swiftly or uncontrolled. TNF-α accelerated cell proliferation and metastasis as well as induced chemotherapy resistance [48]. DMH metabolized in the liver, releasing metabolic intermediates such as azoxymethane and methyl azoxy methanol that are subsequently transformed into active electrophilic methyl diazonium glucuronide in the liver and get discharged into the intestinal lumen by organotropism. In mucosal cells, bacterial glucuronidases hydrolyze glucuronides to form active carbonium ions, which methylate nucleic acids and proteins, producing oxidative stress and cancer. In addition to colon selectivity, DMH alkylates hepatocellular DNA and acts as a hepatic necrogenic agent [49]. Adenocarcinoma, on the other hand, is a cancer that originates from the epithelial cells of glands or glandular-like structures [50]. Successful treatment necessitates anti-cancer activity on tumor cells and cytoprotection to normal cells. Single herbal medicine does not have all the necessary properties to work at multiple hallmarks of the CRC, so the best four herbal extracts were chosen based on molecular docking and available literature. The multiple therapeutic effects were reported by polyherbal mixture like activation of the caspase 2/3/9 for apoptosis, [51] anti-inflammatory, [51,52] and antioxidant effect [51–53]. Diarrhea is an indication of colitis, loss of aquaporin channels from the tissue, or side effects of the test drug due to gastrointestinal damage [54]. Polyherbal mixture lowered the modified Bowen's

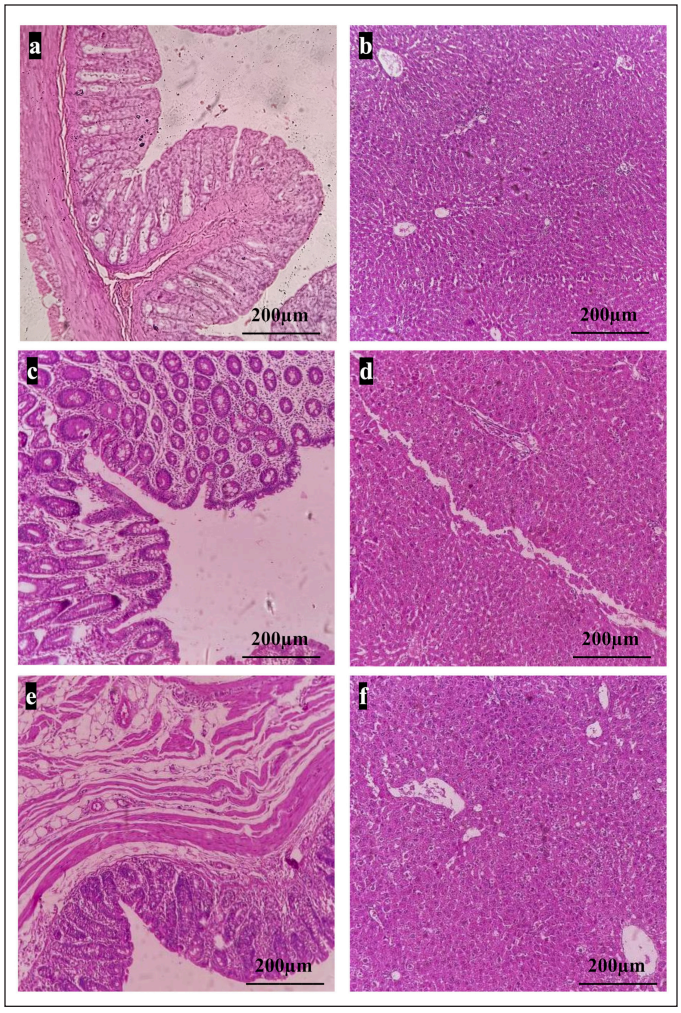

Figure 5. Polyherbal mixture ameliorated DMH-induced CRC. H and E staining of rat colon and liver. A NC group of colon (a) and liver (b) shows normal histoarchitecture; DC group of colon (c) shows infiltration up to the lamina and moderately to poorly differentiated adenocarcinoma, while liver (d) shows mild to moderate dysplasia, colon of STD + THP (e) shows mild dysplasia but no malignancy, whereas in liver (f) metastasis was not observed (Scale = 200 µm). CRC = Colorectal cancer, DMH = 1,2-dimethylhydrazine.

score compared to the DC and STD groups. Compared to the STD alone, the THP with 5-FU normalizes the colon length-to-weight ratio. TNF-α was reduced in both the standard and polyherbal test mixtures at high and lower doses compared to the STD alone. Compared to the STD, the β-catenin level in the STD + THP group was lower, indicating that the polyherbal combination reduced the β-catenin level. Polyherbal mixture raises the uridine level and accelerates the cytoprotection against 5-FU by decreasing UPP-1 in STD + THP compared to the STD, implying that polyherbal combination inhibits UPP-1 enzyme and increases the uridine level. STD + THP group animals showed dysplasia and a normal liver in histopathology. As per the result, the polyherbal mixture showed a synergistic effect with the 5-FU when given at a higher dose. The polyherbal combination improved the 5-FU antitumor efficacy while lowering its toxicity. Histopathological analysis of the colon from DMH administered to a different group of rats has shown mild dysplasia, tumor cell infiltration up to the lamina propria, moderately differentiated adenocarcinoma, and liver metastasis. Mild dysplasia was observed in the STD and STD + THP groups. Therefore, it can be deduced that STD + THP can prevent cancerous tumor growth in the tissue. In addition, the high dose of polyherbal mixture prevents the spread of cancer cells to the liver, thereby avoiding invasive adenocarcinoma.

CONCLUSION

The uridine phosphorylase-1 enzyme is responsible for reducing uridine levels in host tissue. The rise in uridine level reduces 5-FU toxicity in normal cells. By inhibiting β-catenin, tumorigenesis can be halted at the dysplasia level. TNF-α inhibition by polyherbal mixture reduces oxidative stress and inflammatory changes. The positive modulation by a combination of herbal medicine with 5-FU is supported by improvement in prognosis measured by changes in body weight, blood cell counts, and diarrhea score. The results indicated less toxicities and more anti-cancer efficacy in the treatment of CRC. The combination of standard and test drugs (high dosage) yielded the most notable results when compared to the test group alone. Histological evaluation revealed that the polyherbal combination was effective in reducing DMH-induced inflammation and dysplasia. According to the findings of the current investigation, the polyherbal combination can help to minimize DMH-induced dysplasia and prevent CRC progression. A rare research study is available that aims to evaluate herbals with first-line chemotherapy for CRC. Further investigation of Caspase2/3/9, inflammatory indicators, such as interleukins, KRAS, MutL protein homolog 1 and carcinoembryonic antigen, can assist in determining the molecular mechanism of polyherbal combination. In this study, we evaluated the effects of herbal medicines and 5-FU for the initial stages of CRC, but its evaluation in advanced stages of CRC and associated comorbidities need to be evaluated in appropriate animal models.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The animal experiment protocol (RPCP/IAEC/2021-22/R8) was approved by the Institutional Animal Ethics Committee. The study was conducted as per the CPCSEA & ARRIVE guidelines.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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