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Scientific validation and mechanistic evaluation of Chuntaivatral chooranam in DMH-induced gastrointestinal cancer: An *in vivo* study

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Colorectal cancer is one of the leading causes of cancer-related morbidity and mortality worldwide, ranking third among males and second among females in industrialized countries. Its incidence is also rapidly increasing in developing nations, including India, largely due to changing dietary habits and lifestyle factors. 1,2-Dimethylhydrazine (DMH), a potent alkylating agent, is widely used to induce experimental colon carcinogensis. Chuntaivatral chooranam (CVC), a traditional Siddha polyherbal formulation, is commonly prescribed for gastrointestinal disorders such as piles, indigestion, and irritable bowel syndrome. However, its potential anticancer properties remain largely unexplored. The present study aimed to evaluate the chemopreventive efficacy of Chuntaivatral chooranam against DMH-induced colon cancer in Wistar rats. A total of 42 male Wistar rats were divided into nine groups, each consisting of six animals. Colon carcinogenesis was induced using DMH, and treatment groups received Chuntaivatral chooranam at different doses, either alone or in combination with 5-Fluorouracil. At the end of the experimental period, animals were sacrificed, and blood and tissue samples were collected for biochemical and molecular analyses. The results demonstrated that DMH administration significantly increased oxidative stress markers, tumor markers, and Phase I enzyme activities while decreasing antioxidant enzyme levels and membrane-bound ATPase activities. Treatment with Chuntaivatral chooranam, particularly in combination with 5-Fluorouracil, significantly reversed these alterations ($p<0.001$), reduced tumor incidence, and improved biochemical parameters. In conclusion, Chuntaivatral chooranam exhibited significant chemoprotective and antitumor activity against DMH-induced colon cancer, suggesting its potential as a complementary therapeutic agent in the management of colorectal cancer.

Keywords: Chemoprotection, Chuntai vatral chooranam, Colon cancer, Di methyl hydrazine, Oxidative stress markers, Phase I enzymes

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Colorectal cancer (CRC) is one of the biggest health issues globally with the occurrence rate amounting to 10% of all cancer-related deaths and 9.4% of cancer-related fatalities in 2020, second after lung cancer, which contributes to 18% of cancer-related mortality. It is estimated that by 2040 the total new cases of CRC in the world will have reached 3.2 million. This might be due to aging of populations, their increase, and the growing development of humans. This has been mainly contributed by the increased exposure to environmental risk factors, especially lifestyle and dietary modifications[1].

The colon and the rectal cancer incidence rates (AARs) per 100,000 in men are 4.4 and 4.1 respectively in India, and the colon cancer AAR in women is 3.9 per 100,000. In men, colon cancer is 8th and rectal cancer is 9th, but in women, colon cancer is also 9th, and rectal cancer is not in the top ten cancers[2]. It has also been noted that regional differences exist with AARs being higher in cities like Thiruvananthapuram, Bengaluru, and Mumbai in males and in Nagaland and Aizawl in females[3].

At the molecular level, research including the one carried out by The Cancer Genome Atlas Network has shown that colon and rectal cancers exhibit a common pattern of genomic changes, indicating that they should be classified together. Major mutations have been reported in such genes like APC, TP53, KRAS and PIK3CA as well as the newly identified genes like SOX9, ERBB2 and IGF2 that are linked

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to cellular growth and may serve as possible therapeutic agents.

Dimethylhydrazine (DMH) which is a potent DNA alkylating agent that has been shown to be found naturally in cycads is almost universally used to cause experimental colon carcinogenesis. DMH is an indirect carcinogen that metabolically activates to produce methylazoxymethanol (MAM) which reacts with DNA to form adducts, which eventually results into colon tumor formation.

An ancient traditional Siddha polyherbal preparation called Chuntaivatral chooranam (CVC) is a blend of seven herbs such as *Solanum torvum, Murraya koenigii, Mangifera indica, Trachyspermum ammi, Emblica officinalis, Punica granatum* and *Trigonella foenum-graecum*. Traditionally, CVC is employed for the treatment of gastrointestinal diseases like flatulence, indigestion, borborygmi, hemorrhoids and chronic diarrhea. Apart from application in treatment of gastrointestinal diseases, CVC exerts antibacterial activity against pathogens such as *Salmonella typhi*, Shigella flexneri and other common bacteria like *E. coli*[4]. CVC also possesses hepatoprotective, cardioprotective and nephroprotective properties. It also has anti-oxidant and anti-inflammatory properties due to the presence of bioactive compounds that possess hydroxyl radical scavenging property. It has also been used for the management of Type 2 Diabetes mellitus and due to the presence of tannins, saponins and flavonoids in it, it inhibits alpha-amylase and alpha-glucosidase enzymes[5].

CVC's effect on diarrhea and other gastrointestinal symptoms has been proved to be effective in previous clinical observations. However, its potential anti-cancer mechanisms remain unexplored which could open to new therapeutic possibilities.

Thus, this experiment will consider the anticancer efficiency of Chuntaivatral chooranam against the DMH-induced colon cancer model in the Wistar rats, thus filling one of the significant gaps in the existing literature and examining its potential as a new therapeutic agent.

Materials and Methods

Chemicals

1, 2-dimethylhydrazine (DMH) and 5-fluorouracil (5-FU) were acquired from Sigma-Aldrich (USA). All the other analytical grade chemicals and reagents were procured at SRL Pvt. Ltd., Chennai, Tamil Nadu and India.

Experimental animals

The study used 42 healthy adult male Wistar rats (weight 150-200 g). Animals were kept in normal laboratory conditions (temperature: 22±2°C; relative humidity: 50-60%; 12 h light dark cycle) and allowed to eat freely normal pellet diet and water ad libitum. Institutional ethical guidelines were adhered to in all the experimental procedures.

Experimental design

Rats were randomly divided into seven groups. Each group consists of 6 rats.

- Group I (Control): Received normal saline and normal diet.
- Group II (DMH Control): DMH was induced at a dose of 20 mg/kg body weight and 30 mg/kg body weight intraperitoneally every week during the first 10 weeks and second week respectively (induction period: 20 weeks).
- Group III (DMH + CC Dose 1): DMH-induced rat treated with Chuntaivatral chooranam at the dosage of 100 mg/kg body weight, orally by gavage injection twice for a period of 6 weeks.
- Group IV (DMH + CC Dose 2): Rats that were induced with DMH which received Chuntaivatral chooranam treatment (200 mg/kg body weight), orally through gavage at a dose of 200 mg/kg body weight, and which were put on alcohol every 6 weeks.
- Group V (DMH + Standard Drug): DMH induced rats were treated with 5-Fluorouracil at the dosage of 60 mg/kg body weight given once per week orally over 16 weeks.
- Groups VI (Combination Treatment): DMH-induced rats were treated with Chuntaivatral chooranam (200 mg/kg body weight, oral, twice a week) and 5-Fluorouracil (60 mg/kg body weight, oral, once a week) for 16 weeks.
- Group VII (Drug control): Control was normal rats treated with Chuntaivatral chooranam (200 mg/kg body weight) and 5-Fluorouracil (60 mg/kg body weight) for 16 weeks.

Promotion of colon carcinogenesis

DMH was just added to normal saline and injected intraperitoneally. It is metabolically activated to methylazoxymethanol (MAM), which is a colon carcinogenic inducer via the alkylation of DNA.

Drug preparation and administration

Chuntaivatral chooranam was made into a suspension in distilled water and was given orally

using a gavage. Each administration was prepared freshly to maintain stability and consistency in the dosage.

Sample collection

By the end of the experimental period, the animals were fasted overnight and cervical dislocated to death under mild anesthesia. The colon was removed, incised longitudinally, rinsed with ice-cold physiological saline, blotted and weighed.

The percentage of incidence of tumor was determined by the number of animals with tumors divided by the total number of animals in the group.

Histopathological analysis

The colon tissues were then fixed in the Bouin's fluid of 24-48 h, processed, embedded in paraffin, sectioned at 4-5 mm thickness and stained with hematoxylin and eosin (H&E) and observed under a microscope.

Biochemical assays

Oxidative stress markers

Lipid peroxidation (*LPO*)

Tissue homogenate was made in ice cold phosphate buffer. The sample was stirred with thiobarbituric acid (TBA), trichloroacetic acid (TCA) and hydrochloric acid. The mixture was boiled and then centrifuged to recover the mixture. The absorbance of the supernatant was measured at 532 nm to determine malondialdehyde (MDA) levels[6].

Catalase (*CAT*)

The homogenates were placed in a hydrogen peroxide substrate in phosphate buffer. The reaction was stopped after fixed incubation period with dichromate-acetic acid reagent. The mixture was subjected to boiling water bath, cooled and absorbance was measured at 570 nm. Hydrogen peroxide was used for catalase activity calculations[7].

Glutathione peroxidase (*GPx*)

The tissue homogenate, glutathione, sodium azide and hydrogen peroxide were added to the assay mixture in phosphate buffer. Trichloroacetic acid was added after the incubation period to halt the reaction. The mixture was centrifuged and the remaining glutathione in the supernatant was determined by Ellman's reagent. Absorbance at 412 nm was measured[8].

Reduced glutathione (*GSH*)

Trichloroacetic acid was added to the tissue homogenate and the mixture was centrifuged. The supernatant was mixed with phosphate buffer and Ellman's reagent (DTNB). The yellow-colored complex formed due to sulfhydryl groups was measured spectrophotometrically at 412 nm. A standard calibration curve of glutathione was used to calculate GSH concentration[9].

Glutathione-S-transferase (*GST*)

Phosphate buffer, reduced glutathione and 1-chloro-2, 4-dinitrobenzene (CDNB) were used as the reaction mixture and substrate, respectively. The reaction was started by adding tissue homogenate. The amount of glutathione-CDNB conjugate was measured spectrophotometrically using the increase in absorbance at 340 nm. Enzymes activities were expressed as mg-1 protein[10].

Membrane-bound enzymes

Na^+/K^+-ATPase

Under controlled conditions, the tissue homogenate was incubated with ATP substrate in sodium and potassium ions. Trichloroacetic acid was used to stop the reaction. The inorganic phosphate released on hydrolysis of ATP was measured colourimetrically. The absorbance was measured at 660 nm, and the enzyme activity was determined as μmol Pi released[11].

Mg^{2+}-ATPase

ATP, Magnesium chloride, buffer and tissue homogenate were added to the assay mixture and placed in the incubator at 37°C. The reaction was stopped using protein precipitating reagent. Inorganic phosphate released due to hydrolysis of ATP was measured colorimetrically. Absorbance was measured at 660 nm and the enzyme activity was determined[12].

Ca^{2+}-ATPase

Tissue homogenate was treated with ATP with the calcium ions. After incubation the reaction was stopped by trichloroacetic acid. The amount of inorganic phosphate that was released was determined by adding a suitable phosphate estimation reagent. Spectrophotometric absorbance values were measured at 660 nm and enzyme activity was determined[13].

Tumor marker enzymes

Carcinoembryonic antigen (*CEA*)

Immunoassay-based procedure was used for the analysis of serum samples and performed according to the manufacturer's instructions. The anti-CEA antibodies were added to the standards, controls, and

samples. After washing and adding the substrate color development was estimated spectrophotometrically. CEA concentration was determined using standard calibration curve and reported in ng/mL[14].

Gamma-glutamyltransferase (*GGT*)

Serum was placed in a buffer that contained glycylglycine and the γ-glutamyl-p-nitroanilide substrate. The reaction between GGT and p-nitroaniline resulted in the release of p-nitroaniline, which created a coloured product. Kinetically the colour formation was measured at 405 nm. The activity of enzymes was estimated by measuring the changes in absorbance and then reported in units per liter[15].

Lactate dehydrogenase (*LDH*)

Pyruvate substrate and NADH cofactor were added to the serum samples. LDH acted as catalyst to convert pyruvate to lactate and simultaneously oxidized NADH. Absorbance values were measured at 340 nm using a spectrophotometer to measure the consumption of NADH. The activity of enzymes was expressed in IU/L[16].

Xenobiotic enzymes

Cytochrome b5 reductase

Liver tissue was used to prepare microsomal fractions by differential centrifugation. The assay mixture comprised of microsomes, NADH and cytochrome b5 substrate. Spectrophotometric measurement of absorbance change at certain wavelengths was used to monitor reduction of cytochrome b5. The activity of enzymes was calculated and expressed as a function of microsomal protein in milligrams[17].

Cytochrome c reductase

Liver microsomal fractions were added to phosphate buffer to which was added NADPH and oxidized cytochrome c. Cytochrome c reduction was spectrophotometrically measured by the increase in absorbance at 550 nm. The enzyme activity was determined by its extinction coefficient and expressed as unit/mg protein[18].

Statistical analysis

Mean ± standard deviation (SD) was used to express all data of six animals in each group. One-way ANOVA and post hoc tests were used in statistical analysis. The mean difference that was taken to be statistically significant was a p-value below 0.05[19].

Results

Antitumor efficacy of Chuntaivatral chooranam on lipid peroxidation in control and experimental animals

Table 1 indicates that the concentration of lipid peroxidation in plasma and liver in control and experimental Wistar rats. Plasma and Liver LPO levels were significantly increased in group II colon cancer induced rats when compared with control animals. The LPO concentrations were significantly decreased in Combination of 5- Flurouraciland 200 mg/kg of body weight of Chuntaivatral chooranam treated Group VI rats when compared with 100 mg/kg of body weight of Chuntaivatral chooranam treated Group IV rats and 5-Fluro uracil treated rats. There were no changes in control animals treated with Combination of 5-Flurouracil and 200 mg/kg of body weight of Chuntaivatral chooranam compared with Group I control rats.

Antitumor efficacy of Chuntaivatral chooranam on the activities of ATPases in liver of control and experimental animals

Figure 1 shows the activities of the membrane-bound enzymes Na^+/K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase in the liver of control and experimental Wistar rats. The activities of these enzymes were significantly altered in DMH-induced colon cancer rats compared with the control group. Liver ATPases activities were significantly decreased in colon cancer induced group II rats when compared with control animals. The ATPases activities were significantly increased in Combination of 5- Flurouracil and 200

Table 1 — Concentration of lipid peroxidation in plasma and liver of control and experimental groups of Wistar rats

Particulars	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Plasma	1.23±0.12	1.98±0.19a#	1.68±0.16b#	1.52±0.15b@	1.64±0.12b#	1.41±0.14 b#	1.23±0.12
Liver	1.26±0.14	3.29±0.31a#	2.95±0.25b#	1.89±0.22b#	1.95±0.22b#	1.36±0.14 b#	1.26±0.14

Each value is expressed as mean ± SD for six rats in each group.
Units: Plasma: nmoles of MDA liberated/mg protein; liver: nmoles of MDA liberated/mg protein
a - as compared with Group I
b - as compared with Group II
Statistical significance - #p<0.001, @p<0.01, *p<0.05, NS -Not significant

mg/kg of body weight of Chuntaivatral chooranam treated Group VI rats when compared with 100 mg/kg of body weight of Chuntaivatral chooranam treated Group IV rats and 5-Fluro uracil treated rats. There were no significantly changes in liver ATPases activities of Group VII control animals treated with Combination of 5- Flurouracil and 200 mg/kg of body weight of Chuntaivatral chooranam compared with Group I control rats.

Antitumor efficacy of Chuntaivatral chooranam on the level of CEA in colon of control and experimental animals

Figure 2 represents that the colon CEA concentration levels in control and experimental animals. CEA levels were significantly increased in colon cancer induced group II rats when compared with control animals. The colon CEA concentration levels were significantly decreased in Combination of 5- Flurouracil and 200 mg/kg of body weight of Chuntaivatral chooranam treated Group VI rats when compared with 100 mg/kg of body weight of Chuntaivatral chooranam treated Group IV rats and 5-Flurouracil treated rats. There were no significantly changes in colon CEA concentration of Group VII control animals treated with combination of 5- Flurouracil and 200 mg/kg of body weight of Chuntaivatral chooranam compared with Group I control rats.

Antitumor efficacy of Chuntaivatral chooranam on the level of Phase-1 enzyme activities of control and experimental animals in liver tissue

The (Table 2) represents the antitumor efficacy of Chuntaivatral chooranam on the activities of Phase I enzymes such as Cyt $P_{450,}$ Cyt b_5 and NADPH-Cyt C reductase in the liver of control and - animals. The phase I enzymes activities were found to be significantly ($p<0.001$) increased in colon cancer induced (Group-II) rats when compared with the control group (G-I) animals. Oral administration of Chuntaivatral chooranam (100 mg/kg of body weight) (G-III) and Chuntaivatral chooranam (200 mg/kg of body weight) (G-IV) individually caused a significant ($p<0.001$) decrease in these enzyme activities when compared with cancer bearing animals. Animals treated with 5- Flurouracil and 200 mg/kg of body weight of Chuntaivatral chooranam (G-VI) observed a very significant ($p<0.001$) decrease in these enzyme activities when compared with the colon cancer-induced group. However, there was no significant

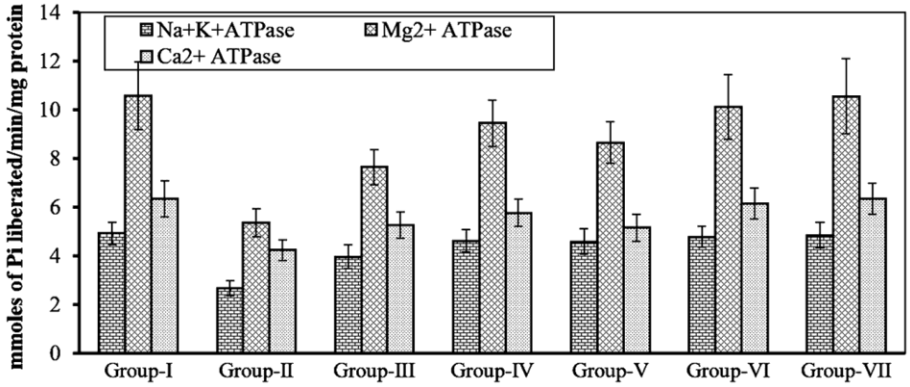

Fig. 1 — Antitumor efficacy of Chuntaivatral chooranam on the activities of ATPases in liver of control and experimental animals

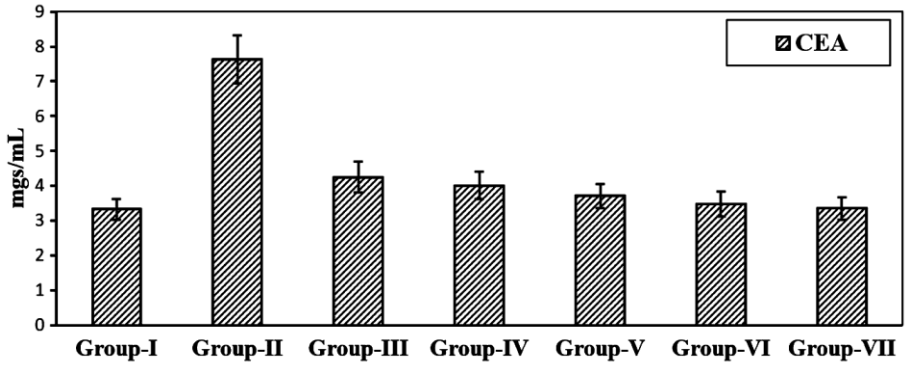

Fig. 2 — Antitumor efficacy of Chuntaivatral chooranam on the CEA level in colon of control and experimental animals

Table 2 — Antitumor efficacy of Chuntaivatral chooranam on the activities of Phase I enzymes in the liver of control and experimental group animals

Particulars	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII
Cyt-p450	0.66	0.87 a#	0.79 b#	0.75 b#	0.73 b#	0.68 b#	0.65
Cyt b5	0.48	0.71a#	0.64 b#	0.56 b#	0.52 b#	0.48 b#	0.45
NADPH Cyt-C	93.23	145.4a#	130.65 b#	109.08 b#	95.85 b#	94.62 b#	91.68

Each value is expressed as mean ± SD for six rats in each group.
Units: Plasma: IU/L; Liver: IU/L
a - as compared with Group I
b - as compared with Group II
Statistical significance - $^{\#}p<0.001$, $^{@}p<0.01$, *p<0.05, NS -Not significant.

changes in the activities of these phase I enzymes between the control and the control animals treated with the combination of 5- Flurouracil and 200 mg/kg of body weight of Chuntaivatral chooranam (G-VII).

Discussion

Cancer is a considerable health problem characterized by the proliferation and spread of abnormal cells. While the term "cancer" typically refers to malignant neoplasms. The development of cancer is a multistep chemical process involving mainly three stages: initiation, promotion, and progression or development.

Both external and internal agentsinvolved to the onset of cancer. External agents include such as tobacco use, exposure to harmful chemicals, radiation, and certain infectious agents. Internal agents involve inherited genetic mutations, hormonal imbalances, and immune system dysfunctions. These agents may act independently or in combination to trigger or accelerate the process of carcinogenesis. Treatment methods for cancer include surgery, radiation therapy, chemotherapy, hormone therapy, immunotherapy, and targeted therapy, depending on the type, size and stage of the disease.

Lipid peroxidation

Oxidative stress, particularly through the process of lipid peroxidation, plays a notable role in carcinogenesis. Lipid peroxidation (LPO) is a chain reaction initiated by free radicals that oxidize polyunsaturated fatty acids within cellular membranes, serving as a key marker of oxidative cellular damage. During this process, free radicals combined with lipids, generating reactive species byproducts such as malondialdehyde (MDA), hydroperoxides, and hydroxyl radicals[20].

Among these, MDA is of main concern as it has been shown to induce mutagenesis in different tissues by DNA adducts formation. The damage induced by lipid peroxidation can also expand to proteins, causing to oxidative modifications. In addition, MDA can react with cellular DNA to form DNA-MDA adducts, which may involve to carcinogenic mutations[21].

Plasma concentration of MDA is widely used as a biochemical indicator of lipid peroxidation. Increased concentration of these peroxidation products have been implicated in the early stages of cancer development. The incidental of LPO can considerably disrupt the structural and functional integrity of cell membranes, leading to deceased membrane fluidity, raised permeability, inhibiting of membrane-bound enzymes, and reduced essential fatty acids.

Supporting report ofthose patients with colon cancer exhibited significantly higher MDA levels compared to healthy controls. In the present study, increased lipid peroxidation levels were observed in animals bearing colon cancer. However, treatment with Chuntaivatral chooranam and 5- Flurouracil resulted in a marked reduction in these levels, indicating a protective effect against oxidative damage[22].

Membrane bound ATPases

ATPases are membrane-bound enzymes that play an important role in maintaining ionic gradients between the intra and extracellular environments. These enzymes, including Na^+/K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase, control the active transport of sodium, potassium, magnesium, and calcium ions across cell membranes using energy derived from ATP hydrolysis. For instance, Na^+/K^+-ATPase actively transports three sodium ions out of the cell and brings in two potassium ions, thereby maintaining essential ion gradients and membrane potential.

These ATPases are tightly integrated into the plasma membrane and are involved in critical cellular processes such as ion homeostasis, signal transduction, and membrane integrity. Their activity is regulated by hormones, proteins, and secondary messengers. However, in cancerous conditions, the

function of these enzymes is often compromised due to oxidative stress, particularly lipid peroxidation, which damages membrane phospholipids[23].

In the present study, a significantly decreased in the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase was showed in animals bearing colon cancer. This decrease may result from increased susceptibility of cancer cell membranes to free radical-induced lipid peroxidation. Such oxidative damage causes membrane integrity and impairs the function of membrane-bound enzymes, ultimately damaging cellular homeostasis.

Glutathione (GSH) is a tripeptide and plays a protective role against oxidative damage by neutralizing free radicals and thereby preventing lipid peroxidation, which otherwise affects membrane stability and osmotic balance in cells. Ca^{2+}-ATPase and Mg^{2+}-ATPase are especially critical in regulating intracellular calcium and magnesium levels, keeping them at submicromolar concentrations. In cancer, inhibition of Ca^{2+}-ATPase may result in excessive accumulation of calcium ions, causing to membrane disruption and cell death[24].

The observed alterations in ATPase enzyme activity in both erythrocyte membranes and colon tissues in carcinoma-bearing animals align with findings reported in other cancer studies. The decrease in Na^+/K^+-ATPase and Mg^{2+}-ATPase activity likely stems from increased free radical production, which damages cell membrane phospholipids and protein components of the ATPase complex. In this damage disrupts ion transport, impairs membrane permeability, and affects essential cell functions including metabolism, signaling, and overall membrane fluidity.

In the present study, cancer-bearing animals indicated decreased concentration of Na^+/K^+-ATPase and Mg^{2+}-ATPase activity, while Ca^{2+}-ATPase activity was increased due to compensatory calcium influx under stress conditions. Treatment with Chuntaivatral chooranam and 5- Flurouracil, both individually and in combination, significantly changed the activities of these ATPases in erythrocyte membranes and colon portion. Notably, the combination therapy helped restore ATPase activities toward normal levels, suggesting improved membrane stability and function.

This restoration may be attributed to increased levels of glutathione and decreased lipid peroxidation due to the antioxidant properties of Chuntaivatral chooranam and 5- Flurouracil. Chuntaivatral chooranam can increase ATPase activity. Therefore, these findings suggest that the combined treatment Chuntaivatral chooranam and 5- Flurouracilof helps preserve membrane asymmetry and integrity by counteracting oxidative stress and maintaining ion homeostasis[25.]

Carcino embryonic antigen

Carcinoembryonic antigen (CEA) is a type of cell surface glycoprotein that is typically expressed during fetal development but becomes transcriptionally inactive in normal adult tissues. However, it is frequently reactive and over expressed in various human carcinomas, including those of the lung, breast and colon. The increased concentration of CEA noted in these cancers suggests its development in both cancer progression and notably, there is no genetic difference between tumor-associated CEA and its normal counterpart.

CEA serves as a dependable tumor marker, particularly in breast and colon cancer, where it is secreted into the bloodstream at increased levels. Several studies have shown that CEA levels are significantly increased in patients with colon cancer. The progression of tumor growth has been found to correlate with increased serum CEA levels, as also noted in colon cancer-bearing animals (Group II) in the present study.

In summary, the present observation suggest that the increased CEA levels correlated with cancer progression can be effectively decreased through treatment with Chuntaivatral chooranam and 5-Flurouracil, highlighting their potential role in colon cancer management.

Phase I enzymes

The body's primary defense mechanisms against colon cancer include Phase I and Phase II enzyme reaction systems. These two (phase I and II) families of enzymes are essential for detoxifying and prevent the body from various carcinogens encountered through diet and environmental exposure.

Phase I and Phase II enzymes are mainly involved in the metabolic processing of xenobiotics. Foreign substances such as environmental carcinogens by converting nontoxic into more water-soluble and excretable forms. However, during this process, some compounds can be bioactivated into reactive metabolites capable of damaging DNA. Phase I enzymes, especially those in the cytochrome P450 family, along with cytochrome b5 and NADPH

cytochrome c reductase, are primarily responsible for the initial activation of these carcinogens.

For example, during the biotransformation of DMH (1, 12 - Dimethyl hydrazine) in the microsomal mixed-function oxidase system, reducing equivalents from NADPH are shifted via cytochrome P450, with cytochrome b5 acting as a serving electron donor in this process[26].

The microsomal cytochrome P450 system is crucial in the oxidative activation and detoxification of xenobiotics. It not only promotes the excretion of these compounds but also influences the duration and intensity of their toxic effects. The cytochrome P450 monooxygenases plays a pivotal role in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs), contributing to an increased risk of cancer[27].

In the present study, there was a marked increase in the activity of Phase I enzymes including CYP450, cytochrome b5, and NADPH cytochrome c reductase in the colon portion of cancer-bearing animals. All the phase I detoxification enzyme activities were significantly decreased in Chuntaivatral chooranam and 5- Flurouracil treated animals. This upregulation may be attributed to membrane lipid damage induced by free radicals, as previously described by[27]. However, lipid peroxidation (LPO) levels observed in the colon of cancer-bearing rats suggest a potential link between oxidative stress and enhanced Phase I enzyme activity, contributing to further membrane damage and carcinogenesis.

Broader scientific and clinical implications of Chuntaivatral chooranam

CVC exhibits potent synergistic effects due to the presence of numerous bioactive compounds. The presence of synergistic effect offers a platform for development of new drug leads for mitigation of chronic and drug-resistant infectious diseases. Since CVC has been traditionally used for the treatment of gastrointestinal disorders, it can be administered along with curd for the enhancement of gut microbiota in humans. CVC also has been effective in the management of non-communicable diseases such as Diabetes and Cardiovascular diseases; hence it can be employed as a plant-based alternative to treat these conditions especially in low- and middle-income countries[5].

Conclusion

Based on the biochemical findings, we conclude that the combination of Chuntaivatral chooranam and 5- Flurouracil exhibits significant chemotherapeutic potential against DMH-induced colon cancer. This effect is likely mediated through enhanced immune modulation and a reduction in the adverse effects commonly associated with 5-Flurouracil treatment. Ongoing studies aim to further elucidate the specific anticancer mechanisms of Chuntaivatral chooranam.

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Author Contributions

MN supervised the experimental procedures and interpreted the findings. MN and VM carried out the literature review, manuscript preparation, and statistical evaluation. BCW and PS conducted the laboratory investigations. AS and PG assisted with data collection, data analysis, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Ethics Approval

According to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the Institutional Regulatory Authority's Ethical Committee authorized the procedure with register number-765/Po/Re/S/03/ CPCSEA, New Delhi, India.

Use of Generative AI in Scientific Writing

No generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, editing, or compilation of this manuscript.

Data Availability

Provided in the tables within the text.

Reference

1 Keum N, Lee D H, Greenwood D C, Manson J E & Giovannucci E, Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized

controlled trials, *Ann Oncol*, 30 (5) (2019) 733-743. doi:10.1093/annonc/mdz059.

2 Bray F, Colombet M, Aitken J F, *et al*., *Cancer incidence in five continents*, Vol XII. Lyon: International Agency for Research on Cancer; 2023. https://ci5.iarc.who.int. Accessed March 25, 2024.

3 Bopanna S, Kedia S, Das P, *et al*., Long-term follow-up reveals high incidence of colorectal cancer in Indian patients with inflammatory bowel disease, *United European Gastroenterol J*, 5 (5) (2017) 708-714. doi:10.1177/2050640616680552.

4 Shree Devi M, Sathiyarajeswaran P, Kannan M, Mohanasrinivasan V, Subathradevi C, *et al*., GC-MS, FT-IR analysis and antibacterial study of bioactive compounds of Chundaivattral Chooranam-A Siddha polyherbal formulation, *Int J Pharm Tech Res*, 8 (10) (2015) 204-209.

5 Shenbagaraj S, Ramamoorthy V, Balasubramanian S, Priya Ramachandran S S, Parameswaran S, *et al*., A comprehensive drug review on cuntaivarralcūranam for its anti-diabetic potential on matumēkam (type II diabetes mellitus), *J Nat Remed*, 24 (2024) 2341-2353.

6 Devasagayam T P A & Tarachand U, Decreased lipid peroxidation in the rat kidney during gestation, *Biochem Biophys Res Commun*, 145 (1) (1987) 134-138.

7 Sinha A K, Colorimetric assay of catalase, *Anal Biochem*, 47 (2) (1972) 389-394.

8 Rotruck J T, Pope A L, Ganther H E, Swanson A B, Hafeman D G, *et al*., Selenium: biochemical role as a component of glutathione peroxidase, *Science*, 179 (4073) (1973) 588-590.

9 Sedlak J & Lindsay R H, Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent, *Anal Biochem*, 25 (1968) 192-205.

10 Habig W H, Pabst M J & Jakoby W B, Glutathione S-transferases: the first enzymatic step in mercapturic acid formation, *J Biol Chem*, 249 (22) (1974) 7130-7139.

11 Bonting S L, Sodium-potassium activated adenosine triphosphatase and cation transport, In: Bittar EE, editor, *Membranes and Ion Transport*, London: Wiley-Interscience, (1970) p. 257-363.

12 Ohnishi T, Suzuki Y, Suzuki Y & Ozawa K, A comparative study of plasma membrane Mg^{2+}-ATPase activities in normal, regenerating and malignant cells, *Biochim Biophys Acta*, 684 (1) (1982) 67-74.

13 Hjertén S & Pan H, Purification and characterization of two forms of a low-affinity Ca^{2+}-ATPase from erythrocyte membranes, *Biochim Biophys Acta*, 728 (2) (1983) 281-288.

14 Gold P & Freedman S O, Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques, *J Exp Med*, 121 (3) (1965) 439-462.

15 Rosalki S B & Rao M L, Serum gamma-glutamyltranspeptidase activity in alcoholism, *Clin Chim Acta*, 39 (1) (1972) 41-47.

16 King J, The dehydrogenases or oxidoreductases—lactate dehydrogenase, In: Van D, editor, *Practical Clinical Enzymology*, London: D Van Nostrand Company Ltd; (1965) p. 83-93.

17 Omura T & Sato R, The carbon monoxide-binding pigment of liver microsomes, I. Evidence for its hemoprotein nature, *J Biol Chem*, 239 (7) (1964) 2370-2378.

18 Phillips A H & Langdon R G, Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization and kinetic studies, *J Biol Chem*, 237 (8) (1962) 2652-2660.

19 Zar J H, *Biostatistical Analysis*, 5th ed. Upper Saddle River (NJ): Prentice Hall; 2010.

20 Yang G, Milne G L, Nogueira M S, Yi H, Lan Q, *et al*., Lipid peroxidation and colorectal cancer risk: a time-varying relationship [preprint]. medRxiv, (2025). doi:10.1101/2025.02.16.25322362.

21 Rice-Evans C & Burdon R, Free radical-lipid interactions and their pathological consequences, *Prog Lipid Res*, 32 (1) (1993) 71-110. doi:10.1016/0163-7827(93)90006-I.

22 Ayala A, Muñoz M F & Argüelles S, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, *Oxid Med Cell Longev*, 2014 (2014) 360438. doi:10.1155/2014/360438.

23 Guerra E, Relli V, Ceci M, Tripaldi R, Simeone P, *et al*., Trop-2, Na+/K+-ATPase, CD9, PKCα, cofilin assemble a membrane signaling super-complex that drives colorectal cancer growth and invasion, *Oncogene*, 41 (12) (2022) 1795-1808. doi:10.1038/s41388-022-02220-1.

24 Chen D, Song M, Mohamad O & Yu S P, Inhibition of Na+/K+-ATPase induces hybrid cell death and enhanced sensitivity to chemotherapy in human glioblastoma cells, *BMC Cancer*, 14 (2014) 716. doi:10.1186/1471-2407-14-716.

25 Kumudha V & Mohamed Musthafa M, *In vitro* anticancer activity of Siddha herbal formulation Kodiveli Chooranam against colorectal cancer, *World J Pharm Res*, 7 (18) (2018) 964-969.

26 Aparicio J, Esposito F, Serrano S, Falco E, Escudero P, *et al*., Metastatic colorectal cancer: first-line therapy for unresectable disease, *J Clin Med*, 9 (12) (2020) 3889. doi:10.3390/jcm9123889.

27 Ozawa S, Inter-individual differences in efficacy and toxicity induced by therapeutic drugs and xenobiotics in relation to genetic polymorphisms in xenobiotic metabolizing enzymes, Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku, (116) (1998) 69-81.