Effects of Ethyl Gallate and Propyl Gallate on Dextran Sulfate Sodium (DSS)-Induced Ulcerative Colitis in C57BL/6J Mice: Preventive and Protective

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Research Article

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Abstract

Objective and design: Inflammatory bowel disease (IBD) is an idiopathic inflammatory condition of the digestive system marked by oxidative stress, leukocyte infiltration, and elevation of inflammatory mediators. In this study, we demonstrate the protective effect of Ethyl gallate (EG), a phytochemical, and Propyl gallate (PG), an antioxidant, given through normal drinking water (DW) and copper water (CW) in various combinations, which had a positive effect on the amelioration of DSS-induced ulcerative colitis in C57BL/6J mice.

Materials and methods: We successfully determined the levels of proinflammatory cytokines and Antioxidant enzymes by ELISA, tracked Oxidative/Nitrosative stress (RO/NS) by in vivo imaging (IVIS) using L-012 chemiluminescent probe, disease activity index (DAI), histopathological and morphometric analysis of colon in DSS-induced Colitis in a model.

Results: The results revealed that oral administration of Ethyl gallate and Propyl gallate at a dose of 50 mg/kg considerably reduced the severity of colitis and improved both macroscopic and microscopic clinical symptoms. The limits of proinflammatory cytokines (TNF-α, IL-6, IL-1β and IFN-γ) in colonic tissue were considerably reduced in the DSS+EG-treated and DSS+PG-treated groups, compared to the DSS alone treated group. IVIS imaging of animals from the DSS+EG and DSS+PG treated groups showed a highly significant decrease in RO/NS species relative to the DSS control group, with the exception of the DSS+PG/CW and DSS+EG+PG/CW treated groups. We also observed lower levels of myeloperoxidase (MPO), nitric oxide (NO), and lipid peroxidation (LPO) and higher levels of GST and superoxide dismutase (SOD). In addition, we showed that the EG, PG, and EG+PG-treated groups had a healing impact on DAI score, body weight, and colon length in mice with DSS-induced colitis. In this 21-day study, mice were treated daily with test substances and were challenged to DSS from day 7 to 14.

Conclusion: Our study highlights the protective effect of ethyl gallate and propyl gallate in various combinations which, in pre-clinical animals, serves as an anti-inflammatory drug against the severe form of colitis, indicating its potential for the treatment of IBD in humans. In addition, propyl gallate was investigated for the first time in this study for its anti-colitogenic effect with normal drinking water and reduced effect with copper water.

Introduction

IBD is defined by an imbalance between pro-inflammatory and anti-inflammatory responses, which causes changes in the gut microbiota and oxidative stress in gastrointestinal epithelial cells (De Mattos et al., 2015, MacDonald et al., 2000, Coskun, 2014). IBD includes both UC and CD. Even worse, UC may be a chronic, debilitating IBD that affects just the distal colon, modifies the colonic mucosa and submucosa in the large intestine and advances unabatedly (Tun et al., 2014, Matricon, 2010). Granularity, vascular patterning, and superficial ulcerations define ulcerative colitis, another kind of IBD. The colon mucosa is the only area of UC that experiences inflammation; other clinical symptoms of UC include petechial bleeding, granulation tissue, and blood and mucus leaks. While in remission, which can last for months or years, the mucosa might seem normal. In the worst forms of the condition, the gut might swell up, leading to significant ulceration and perhaps intestinal perforation (De Mattos et al., 2015).

According to previous reports, the prevalence of IBD has sharply grown in the majority of emerging nations, affecting millions of people who need ongoing medical treatment (Ng et al., 2017). In India, Haryana studied 21,971 persons in 1984 with an occurrence rate of 42.8/1 Lakh. Punjab, Ludhiana 51,910 persons, occurrence rate 44.3/1 lakh, the crude occurrence measured after a second visit 1 year advanced was 6.02 per 1 lakh (Abhirami et al., 2022).
Numerous studies indicate that oxidative stress and inflammation are the main factors driving the pathophysiological developments of inflammatory illnesses like ulcerative colitis. A safe and effective treatment for colitis in people remains unknown despite significant advancements in our understanding of how these diseases begin and progress.

The complicated aetiology of ulcerative colitis accounts for the interaction of microbial, environmental, and genetic factors that elicit an immune and non-immune response in the mucosa, leading to active tissue destruction and severe inflammation in the colon tissue (Kaser et al., 2010). Although no single cause has been found, many aetiologies are put forth and taken into account. Inflammation is a symptom of oxidative stress, which is usually triggered by emotional or physical trauma as well as toxins in the environment, food, or nutrition. Diet may be a key offender, with sugar serving as the main offender due to its role as a source of glycation. Glucose molecules in any form, including starchy sugars found in cool drinks, sauces, canned food, baked food, and western diets. Glycation occurs when a glucose molecule links to a lipid or protein molecule without the assistance of an enzyme. This impairs the molecule's ability to perform its function, resulting in advanced glycation end products (AGEs), which in turn cause many of our inflammatory disorders. It's interesting to note that oxidative stress can also be caused by inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD). A severe type of colitis and its link to cancer are caused by the generation of proinflammatory cytokines and nitric oxide as a result of activating NF-κB signalling (Viennois et al., 2013). As neutrophils get activated, they generate reactive oxygen metabolites that cause intestinal inflammation and damage to colon tissue. The secretion of growth factors, proteases, reactive oxygen species (ROS), and cytokines are all results of chronic inflammation (Viennois et al., 2013, Karin, 2009). Nonetheless, one effective method to reduce the pathophysiology caused by UC may be to inhibit inflammatory signalling mediators and activate antioxidant enzymes.

The stage of the disease determines whether traditional treatments are used nowadays. For instance, patients with mild disease symptoms are frequently given amino salicylates, whereas those with moderate disease symptoms are frequently given corticosteroids, and those with severe disease symptoms are frequently given cyclosporine. This therapy may be difficult to implement along with other cutting-edge therapeutic modalities like biologicals (anti-cytokine drugs and anti-cell adhesion molecules), due to the high effective cost of production in comparison to other therapies (Axelrad et al., 2016). Patients with IBD also have an increased chance of developing extra-intestinal malignancies, in addition to the augmented risk of cancer brought on by long-lasting bowel inflammation and immunosuppressive drugs. As the numeral of patients with IBD climbs, so does the risk of developing cancer. These people will all require cancer therapy, and many more will also require extra IBD care (Axelrad et al., 2016, Maloy and Powrie, 2011). Over the coming years, a two-pronged approach centred on research into interventions to prevent inflammatory bowel disease and improvements in the delivery of care to people with inflammatory bowel disease will be required to address the expanding worldwide burden of inflammatory bowel disease (Maloy and Powrie, 2011). Nonetheless, identifying a new class of therapeutic agents with higher efficacy and lower incidence of side effects, such as ethyl gallate and propyl gallate, could treat with greater efficacy on UC pathological process.

In the current study, colitis is induced in a mouse model using dextran sulphate sodium (Benoit et al., 2014) that mimics human colitis and is a trustworthy approach to screen potential therapeutic agents which has greater efficiency and fewer adverse effects for example, phytoconstituents and their derivatives that can be consumed through a healthy diet can be demonstrated as a prospective option for their powerful antioxidant activities.

A potent antioxidant and non-flavonoid phenolic chemical, Ethyl gallate (EG) is a permitted food additive. EG is found in foods and medicines like red wine, walnuts, the fruit pulp of Emblica officinalis, the pannicles of Mangifera indica, and the leaves of Haematoxylon campechianum, Acacia adonosonii, and A. seyal. Based on previous reports,
EG has numerous anti-inflammatory, anti-oxidant, and anti-cancerous properties (Mohan et al., 2017), free radical scavenging, cytotoxic and hemolytic effects (Mink et al., 2011), anti-colitogenic (Wang et al., 2022), Anti-HIV-1 (Krishna et al., 2020), anti-diabetics and anti-obese effects (Ahn et al., 2022), restricts the growth and invasion of human breast cancer cells via Akt/NF-κB signalling (Cui et al., 2015), and even attenuates acute lung injury through Nrf2 signalling (Mehla et al., 2013). In vitro hydrogen peroxide signalling mediated by lysozyme is inhibited by this hydrogen peroxide scavenger, which also overcomes hypotension in canine septic shock (Kalaivani et al., 2011).

Propyl gallate, a synthetic antioxidant (Hsu et al., 2013), inhibits oxidation brought on by oxygen free radicals and hydrogen peroxide. This antioxidant has been added to foods that contain oils and fats to prevent oxidation (National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 4947). According to reports it has anti-inflammatory, anti-cancer (Wei et al., 2019), anti-parasitic (Mallo et al., 2014), anti-fungal (Xu et al., 2006) and hepatoprotection properties (Wu et al., 1994). Reportedly Propyl gallate's cytoprotective qualities can turn into prooxidative, cytotoxic, and genotoxic ones when combined with Copper (II), which induces lipid peroxidation and severe DNA damage at concentrations greater than 2 μM Propyl gallate in combination with 100 μM CuCl₂. Therefore, PG could have negative effects when tissues have high copper levels (Jacobi et al., 1999, Jacobi et al., 1998).

Copper water is the common name for water that has been stored in a copper container. This makes it possible for copper to leach into the water in appropriate amounts. The scientific study doesn't back up much of the practice's claimed benefits, but it may have an antibacterial effect that may eliminate bacteria that cause diarrhoea in contaminated water (Sudha et al., 2012). However, a study found that for the leached copper (177 ± 16ppb) to kill bacteria, the water needed to be maintained in a copper container for at least 16 hours at room temperature(Sudha et al., 2009).

Therefore, alternative defensive measures for the treatment of these illness situations require considerable research effort. Dextran sulfate sodium (DSS)-induced colitis has been successfully treated in animal models by targeting numerous pathways, such as scavenging free radicals, oxidative stress, and inflammation, consequently decreasing inflammatory processes and activating defence antioxidant enzymes. A major curative impact on the pathophysiology of colitis is achieved by discovering a special class of phytochemicals such as EG and its synthetic derivative PG treatments with less harmful effects.

However, it is still unclear how Ethyl gallate, Propyl gallate, and their combinations with copper water work to prevent DSS-induced ulcerative colitis. As a result, we aim to assess how Ethyl and Propyl Gallate, in various combinations, affect DSS-induced UC in C57BL/6J Mice.

**Materials And Methods**

**Chemicals, antibodies, and ELISA kits**

Ethyl gallate (purity ≥ 96.0%), Propyl gallate (purity ≥ 98.0%), sulfasalazine (purity 98.0-101.5%), and other chemicals and reagents from Sigma Aldrich USA. Dextran sulfate sodium 36,000–50,000 molecular weight was procured from Cayman Chemicals, ANN ARBOR, USA. The chemiluminescent probe L-012 was purchased from Wako Chemicals, Japan. Mouse LPO ELISA kit and BCA assay kit were obtained from G-Biosciences, St Louis, USA. All of the other compounds were of analytical grade. The following ELISA assay kits such as glutathione-S-transferase (GST), malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO) assay kits, and TNF-, IL-6, IL-1β, and
IFN-γ were bought from Krishgen Biosystems, Mumbai, India. Hemoccult Fecal Occult Blood Tests kits (Guaiac paper for blood stool examination) were purchased from Beckman Coulter.

Animal Studies

All animal experiments were conducted on healthy male C57BL/6J mice (weighing 19–22 g, 6–8 weeks old) that were acclimated to the laboratory environment for approximately 4 days before the start of the study at the ICMR-NIN Animal Facility in Hyderabad (CPCSEA Reg. No. 154/G0/RRBiBt-S/R-L/1999/CPCSEA). Mice had unrestricted access to a normal pellet diet and clean drinking water. They were kept in typical laboratory conditions throughout the experiment, with a 12 h/12 h dark/light cycle, 22 ± 2ºC temperatures, and 40 - 70 % relative humidity. All studies were carried out by the standards for the responsible use and care of experimental animals established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India. The Institutional Animal Ethics Committee (IAEC) of the ICMR-NIN gave its approval to the animal usage protocols, and the IAEC approval number is ICMR-NIN/IAEC/02/018/2020.

Induction of ulcerative colitis in mice via the use of DSS and treatment regimen

Except in the vehicle control group, in all groups, a DSS-induced experimental colitis model was established by administering 2.5% (w/v) of DSS through drinking water for seven consecutive days only (from day 8 to day 14), i.e., the mid-week of the experimental period except the first and last weeks of the experimental period with DSS-free drinking water ad libitum (Benoit et al., 2014) in autoclaved drinking water (DW) and Copper water (CW), i.e., water that had to be kept or stored in a copper container for at least one overnight(Sudha et al., 2009) , was used in combination with test drugs like EG and PG test groups, and the feed was supplied to all experimental groups ad libitum. Relatively, the vehicle control group was given water without DSS. Based on the previous literature the dosages of ethyl gallate (Wang et al., 2022, National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 13250), propyl gallate(National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 4947) and sulfasalazine (Axelsson et al., 1998) were chosen less than LD50 of EG and PG and we selected as safest dose for this experimental design. Ethyl gallate, propyl gallate, and a mixture of the two (1:1) were administered orally suspended in 2% gum acacia at a dose of 50 mg/kg BW, whereas the standard drug sulfasalazine was administered at a dose of 100 mg/kg BW. All dosages were given to the animals from day 0 to day 21 by oral route administration. Nine groups of six mice each (n = 6) were randomly selected from the grouping of the mice.

Group I: Vehicle control (regular drinking water without DSS);
Group II: Disease control (drinking water with DSS 2.5% w/v);
Group III: Sulfasalazine (100 mg/kg BW) + 2.5% w/v DSS in DW as a standard control;
Group IV: Ethyl gallate (50 mg/kg BW) + 2.5% w/v DSS in DW;
Group V: Ethyl gallate (50 mg/kg BW) + 2.5% w/v DSS in copper water (CW);
Group VI: Propyl gallate (50 mg/kg BW) + 2.5% w/v DSS in DW;
Group VII: Propyl gallate (50 mg/kg BW) + 2.5% w/v DSS in CW;
Group VIII: Ethyl gallate (25 mg/kg BW) + propyl gallate (25 mg/kg BW) + 2.5% w/v DSS in DW;
**Group IX**: Ethyl gallate (25 mg/kg BW) + propyl gallate (25 mg/kg BW) + 2.5% w/v of DSS in CW

All the groups except vehicle control were challenged with 2.5% w/v DSS

On the 21st day, which was the termination day, blood was drawn aseptically, colitic and non-colitic animals were subjected to death by CO₂ asphyxiation, and colon tissue length was measured by Digital Vernier caliper (Scienceware). A distal portion or segment of colon tissue was preserved at room temperature in 10% buffered formalin for histopathological and morphometric study, for goblet cells and crypt assessment, while the remaining proximal portion of colon tissue was cleaned in cold phosphate-buffered saline (pH 7.4) and stored in a freezer at -80°C for further biochemical and ELISA analysis. Experimental design, see *Fig. I*.

**Experimental animals in-vivo imaging for oxidative stress**

The intraperitoneal (i.p.) injection of the luminous probe L-012 (Wako Chemicals, Japan) at a dose of 20 mg/kg BW was given to sedated mice with the drug combination of xylazine (10-12.5 mg/kg BW) and ketamine (80-100 mg/kg BW) in the right flank. Before the experiment, the L-012 solution was freshly made by adding 2 mg of L-012 per mL in phosphate-buffered sterile saline (PBS) and storing it at room temperature in the dark. Mice were given L-012 after 2-4 minutes of anaesthesia, and then they were put into the imaging systems dark compartment i.e IVIS Spectrum (Caliper Life Sciences/PerkinElmer), which contains a cold CCD camera. As soon as the L-012 probe was injected, pictures were taken. The live image program version 4.7.3, which automatically registers and overlays the bioluminescence photos with the photogenic image of the mouse, was used to measure the quantity of photons synthesized by L-012 on the time of in vivo imaging for 20 minutes with a 1-minute exposure duration. The Living Image software was used for data collection and processing (version 4.7.3, Caliper Life Science). In terms of photons/second/cm²/steradian, measured emitted light from the abdominal region of interest (ROI) was measured (Asghar et al., 2014).

**Assessment of Disease Activity Index (DAI)**

Animals were measured daily for changes in disease activity index (DAI, see *Table I*), which comprises the changes in body weight, stool consistency, and the presence or absence of faecal blood from day 1 to day 21. The DAI was calculated at the end of the intervention (day 21) by adding the following scores: body weight loss (scored as 0 indicates none change; score 1 indicates 1-5%; score 2 indicates 5-10%; score 3 indicates 10-20%; score 4, >20% of changes in the net body weights) was measured by weighing balance (Precisa), stool consistency (scored as 0 indicates as well-formed pellets; score 2 indicates as loose stools; score 4 indicates as diarrhoea) by physical examination; the absence/presence of faecal blood (scores as 0 indicates as negative hemoccult test; score 2 indicates as positive hemoccult test; score 4 indicates as gross bleeding) by using the Hemoccult Faecal Occult Blood Tests kits (Beckman Coulter) during the experimental period. On the termination day of the experiment, Colon length was measured by using a Vernier caliper after the colon tissue was dissected and examined for gross macroscopic appearance (Sangaraju et al., 2019).

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight loss (%) reduction</th>
<th>Gross bleeding</th>
<th>Stool consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No change/ increase</td>
<td>Absent of faecal blood (negative hemoccult test)</td>
<td>Well-formed pellets (normal faeces)</td>
</tr>
<tr>
<td>1</td>
<td>1-5% reduction</td>
<td>Slightly present</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.1-10%</td>
<td>Markedly present (positive hemoccult test)</td>
<td>Loose stools</td>
</tr>
<tr>
<td>3</td>
<td>10.1-20%</td>
<td>Severely present</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&gt;20%</td>
<td>Gross bleeding</td>
<td>Diarrhoea</td>
</tr>
</tbody>
</table>

### Assessment of Histopathological and morphometric findings of colon

Epithelial erosions, crypt loss, goblet cell decrease, and inflammatory cell infiltration were the hallmarks of the tissue's histology, which was used to diagnose histopathological Activity Index (HAI) see Table II. On day 21, Colon tissues from every experimental group were taken out, processed, and paraffin-embedded after being preserved in 10% phosphate-buffered formalin. These sections were then cut using a microtome (Leica, Bensheim, Germany) to a thickness of about 4 μm, stained with hematoxylin & Eosin (H&E), and then examined pathologically. The specimens were then analysed under light microscopy for histological alterations and pictures were obtained using the 4X, 10X, and 20X objectives (CILIKA BT-Invidigital semi-Apo-2021, MedPrime, Maharashtra, India) compared to the vehicle control. Each group of mice included in the experiment had a morphometric analysis done on at least 45 villi in 45 microscopic areas at a 20X magnification (Gancarcikova et al., 2020). An investigator who was not familiar with the experimental groups conducted a histological evaluation.

### Table II: Histopathological activity index

<table>
<thead>
<tr>
<th>Grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory cells infiltration</td>
<td>absence</td>
<td>At the subepithelial lamina propria (mild)</td>
<td>Reaches at muscularis mucosae (mild to moderate)</td>
<td>Reaches at submucosa and muscularis propria (moderate to severe)</td>
<td>Severe and extensive infiltrate</td>
<td>(Gaudio et al., 1999)</td>
</tr>
<tr>
<td>Crypt damage</td>
<td>none</td>
<td>Mild (small space between crypts)</td>
<td>Moderate (large space between crypts)</td>
<td>Severe (large space with no crypts surrounded by normal crypts)</td>
<td>(Extensive damage)</td>
<td>No crpts</td>
</tr>
<tr>
<td>Villi damage</td>
<td>none</td>
<td>mild</td>
<td>moderate</td>
<td>severe</td>
<td>Extensive</td>
<td>(Gaudio et al., 1999)</td>
</tr>
<tr>
<td>Epithelial erosion</td>
<td>Normal histoarchitecture</td>
<td>Focal destruction</td>
<td>Zonal destruction</td>
<td>Diffuse and mucosal ulceration</td>
<td>-</td>
<td>(Gaudio et al., 1999, Abdelmegid et al., 2019)</td>
</tr>
</tbody>
</table>

**Myeloperoxidase (MPO) activity assay of colon tissue**
In ulcerative colitis, the level of neutrophil infiltration is indicated by MPO activity. Colon tissues were homogenized in a 1X PBS buffer that was ice-cold (pH 7.4). Centrifugation was performed on the acquired tissue homogenates at 4 °C for 30 minutes at 12000 RPM. As per the guidelines provided by the manufacturer, the MPO levels in the supernatant were estimated using an ELISA kit from Krishgen Biosystems in Mumbai, India. Using a BCA protein assay kit (G-Biosciences, St. Louis, USA), the amount of total protein in the supematant was calculated against bovine serum albumin (BSA) as a standard. The MPO concentration was expressed as ng/mg protein in colon tissues.

**Homogenization and an antioxidant assay of colon tissue**

The colon tissue homogenate was prepared based on previous reported(Sahu et al., 2014, Sangaraju et al., 2021). 10% of colon tissues were homogenized in chilled 1X PBS buffered saline (pH 7.4). The tissue homogenates were centrifuged for 30 minutes at 4 °C at 12000 RPM. The obtained colon tissue supernatant was utilized to estimate the amounts of different antioxidants, including superoxide dismutase (SOD), glutathione S-transferase (GST), nitrites, and lipid peroxidase. Thiobarbituric acid-reactive substances (TBARS) or MDA (malondialdehyde) or lipid peroxidation concentrations were estimated using the supernatant. Using a BCA protein assay kit (G-Biosciences, St. Louis, USA), the amount of total protein in the supernatant was calculated against Bovine Serum Albumin (BSA) as a standard. The concentration was expressed as ng/mg of protein.

**Estimation of antioxidant enzymes level in colon tissue**

An ice-cold 1X PBS buffer (pH 7.4) was used to homogenize colon tissues. Centrifugation was performed at 4 °C for 30 minutes at 12000 RPM on the acquired tissue homogenates. To measure different antioxidants, including glutathione S-transferase (GST) and superoxide dismutase, colon tissue supernatant was employed. The antioxidant enzymes SOD and GST were measured using the SOD and GST assay kits by following the manufacturer’s instructions (KRISHGEN Biosystems). The GST and SOD concentrations in colon tissues were expressed as ng/mg of protein.

**Estimation of nitrites and lipid peroxidation of colonic tissue**

An ice-cold 1X PBS buffer was used to homogenize colon tissues (pH 7.4). Centrifugation was performed at 4 °C for 30 minutes at 12000 RPM on the acquired tissue homogenates. The nitrosative marker Nitric Oxide (NO) (KRISHGEN Biosystems) and the lipid peroxidation marker Lipid Peroxidase (LPO) (G-Biosciences, St. Louis, USA) were measured using the ELISA kit as directed by the manufacturer. The NO and LPO concentrations in colon tissue were expressed as nmol/mg of protein.

**Investigating Inflammatory Cytokines of colonic tissue**

Each animal’s colon tissue was weighed, cut, and homogenized in chilled phosphate buffered saline (PBS, pH 7.4) containing protease inhibitor cocktail tablet (Roche Indianapolis, IN) resulting into 15% colon tissue homogenate. Using a BCA protein assay kit (G-Biosciences, St. Louis, USA) and bovine serum albumin (BSA) as a reference, the amount of total protein in the supernatant was calculated and concentrations were expressed as mg/ml of tissue homogenate. Centrifuging was done on the colon tissue homogenate for 20 minutes at 5000 RPM and 4 °C., and the supernatant was utilized for the quantification of IL-1β, IL-6, TNF-α, and IFN-γ levels using the mouse-specific ELISA kits for IL-1β, IL-6, TNF-α, and IFN-γ (Krishgen Biosystems, Mumbai, India), by manufacturer instructions. The concentrations of all cytokines such as IL-6, IL-1β, IFN-γ, and TNF-α levels were expressed as pg/mg of protein.
Statistical Analyses

One-way ANOVA was utilized for all statistical analysis with GraphPad Prism, version 8.0.2 (263) software. Post hoc Dunnett's multiple comparison techniques were employed to compare DSS control group with test groups. The mean and standard error of the mean was used to present the results (mean ± SEM). The p-value cut-off for considering statistical significance was 0.05.

Results

Effect of Ethyl gallate and Propyl gallate on symptoms of DSS-induced colitis in mice

On mice exposed to DSS, the effects of EG, PG, and EG+PG in drinking water and copper water were assessed. On day 21, we evaluated the colitis severity score (DAI, disease activity index), which takes into account factors including weight loss, bowel consistency, and blood in the stool. It was found that mice treated with DSS alone exhibited severe disease development in acute colitis, as measured by a DAI score of 2.91±0.083. On the further hand, DSS+EG/DW and DSS+EG/CW groups treated at 50 mg/kg body weight showed reduced DAI scores of 0.375±0.172 and 0.125±0.088 respectively. The DSS+PG/DW and DSS+PG/CW groups treated at 50 mg/kg body weight showed reduced DAI scores of 0.125±0.088 and 0.250 ± 0.137 respectively. The combined drugs DSS+EG+PG/DW at 25mg+25mg/kg body weight showed a reduced DAI score of 0.167± 0.089 and for DSS+EG+PG/CW treated group no DAI was seen. Additionally, mice were given DSS alone experienced a reduction in body weight and colon length shrinkage also observed, whereas, the EG, PG, and their combination-treated mice groups showed retained weight loss and colon length shortening see Fig. II, III. The DAI was non-significant till the 12th day after that there was a significant increase in DAI in DSS alone treated groups and the test drug treatment groups showed decreased DAI similar to control group. Therefore, EG, PG, and EG+PG treated groups in various combinations displayed a curative effect against DSS-induced ulcerative colitis in mice based on DAI score, change in net body weight, and colon length See Fig. II and III.

Effect of Ethyl gallate and Propyl gallate on RO/NS monitored using In vivo imaging system (IVIS) in DSS-induced mice.

During the period of 21 days, we used L-012 for monitoring experimental murine colitis throughout the study, mice were imaged with L-012 chemiluminescent probe on experimental day 4 before the onset of the 2.5% DSS treatment and further on days 8,10,11,12,14,16 and 18. The experimental day 4 animals were under pre-treatment of test drugs and they did not show any significant difference except DSS+EG/CW, DSS+PG/CW, and DSS+EG+PG/CW treated groups which increased the RONS stress during pre-treatment. From day 8th to 14th 2.5% DSS induction along with test drug treatment started and from day 8th to day 12th all the groups showed a highly significant reduction of RONS species compared to the DSS control group. On day 14th all the groups showed a highly significant reduction of RONS except the standard control group. Day 16 was the 2nd day of post-treatment where all the groups showed a significant reduction of RONS but DSS+EG+PG/CW treated groups failed to reduce the RONS stress. On the day 18th, all showed signifying reduction except DSS+PG/CW and DSS+EG+PG/CW treated groups. Further we will conform the oxidative stress by antioxidant assays see Fig. IV a & b.

Effect of Ethyl gallate and Propyl gallate on colon histology of DSS-induced mice.

Hematoxylin and Eosin (H and E) staining was used in our histological investigation to determine the severity of the distal colon tissue injury. The distal colon of vehicle control mice (without DSS) was seen to have
normal submucosal glands and mucosal epithelial cells, with no observable inflammation or ulcers. Compared to vehicle control colon tissue, the DSS alone treated group colon tissue exhibited crypt structure destruction with goblet cell loss, moderate to severe submucosal inflammation, disruption of the epithelial layer, and infiltration of inflammatory cells in the lumen, and massive infiltration of inflammatory cells with cryptic abscess. Colonic tissues from the DSS+EG-DW/CW and DSS+PG-DW/CW treated groups, in particular, revealed low cryptic damage with retained goblet cells and epithelial lining, as well as very modest foci of inflammatory cell infiltration in the submucosal region. In the combined treatment groups, DSS+EG+PG-DW/CW displayed a minimal amount of epithelial disruption and swelling see Fig. V.

**Effect of ethyl and propyl gallates on the activity of myeloperoxidase and HAI in colon tissue of DSS-induced mice.**

The outcomes revealed that myeloperoxidase MPO activity, a measure of neutrophil infiltration, was significantly (p<0.01) higher in the DSS-alone treated group as compared to the vehicle control (without DSS). Treatment with DSS+EG/DW, DSS+EG/CW, DSS+PG/DW, and DSS+EG+PG/CW at 50 mg/kg BW resulted in a significantly decreased MPO activity (p<0.001) than DSS alone treated groups, except the DSS+PG/CW treated group see Fig. VI a. The histopathological activity index was significantly(p<0.001) less in all the test groups compared to DSS alone treated group see Fig. VI b.

**Effect of Ethyl gallate and Propyl gallate on colon morphometry of DSS-induced mice.**

Using light microscope observations, the size of the animal colon villi in cross-section (*Table III*) showed retention of cross-section of villi (p<0.0001) in all groups which were exposed to DSS along with test drug treatment in comparison with DSS alone treated group. Simultaneously, we noticed that in the same sections of the colon, the perimeter of the villi was significantly retained to normal (p<0.0001) compared to positive control where it showed the lowest perimeter of villi (800.85± 20.55 µm) and differed significantly by 689-1510 µm from all other investigated groups. Regarding the height of villi, similar favourable influences were identified. Similar beneficial effects were observed with respect to the villi, which significantly retained the villi length (p<0.001, p<0.0001) in all groups as compared to the DSS alone treated group. This 21-day study, which included a continuous 7-day DSS administration and 21 days of treatment with test medications, clearly had a positive effect on all of the morphometric parameters under investigation because these groups also showed significantly deeper crypts (p<0.001) and a lower ratio of villus height to crypt depth than the positive control group(p<0.05) see *Table III*.

*Table III: Colon morphology of C57BL/6J mice following DSS and test drug treatments.* The values are expressed as means ±SEM. (n=4); ns = no significance *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 treated groups vs DSS alone.
<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Cross section of villi (µm²)</th>
<th>Villus perimeter (µm)</th>
<th>Villus height (µm)</th>
<th>Crypt Depth (µm)</th>
<th>Ratio villus height/crypt depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle Control</td>
<td>149816.4±11049.79****</td>
<td>1612.55±70.65****</td>
<td>467.94±16.80****</td>
<td>103.13±5.07****</td>
<td>4.56±</td>
</tr>
<tr>
<td>3</td>
<td>DSS Control</td>
<td>35755.13±4270.93</td>
<td>800.85±20.55</td>
<td>253.68±12.26</td>
<td>35.08±4.24</td>
<td>8.64±</td>
</tr>
<tr>
<td>4</td>
<td>DSS+SC/DW</td>
<td>198486.9±22587.33****</td>
<td>2137.87±146.58****</td>
<td>488.19±21.24****</td>
<td>82.01±8.20***</td>
<td>5.83±</td>
</tr>
<tr>
<td>5</td>
<td>DSS+EG/DW</td>
<td>211974.7±9415.45****</td>
<td>2310.84±74.67****</td>
<td>625.30±37.68****</td>
<td>87.64±6.02****</td>
<td>7.96±</td>
</tr>
<tr>
<td>6</td>
<td>DSS+EG/CW</td>
<td>196457.5±15079.99****</td>
<td>2026±61.94****</td>
<td>502.74±20.65****</td>
<td>77.77±6.77**</td>
<td>6.744±</td>
</tr>
<tr>
<td>7</td>
<td>DSS+PG/DW</td>
<td>230901±12860.38****</td>
<td>1901.92±14.45****</td>
<td>475.76±30.81***</td>
<td>134.13±12.64****</td>
<td>3.95±</td>
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<tr>
<td>8</td>
<td>DSS+PG/CW</td>
<td>181128.8±19014.2****</td>
<td>1927.61±140.91****</td>
<td>512.68±35.37****</td>
<td>136.18±6.49****</td>
<td>3.67±</td>
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<tr>
<td>9</td>
<td>DSS+EG+PG/DW</td>
<td>187366±23648.35****</td>
<td>1751.48±128.51****</td>
<td>484.60±47.22****</td>
<td>108.68±7.75****</td>
<td>4.34</td>
</tr>
<tr>
<td>10</td>
<td>DSS+EG+PG/CW</td>
<td>186171.9±16515.47****</td>
<td>1489.23±94.53****</td>
<td>438.57±36.95****</td>
<td>128.58±7.12****</td>
<td>3.17</td>
</tr>
</tbody>
</table>

Effect of Ethyl gallate and Propyl gallate on oxidative stress markers in colon tissue of DSS-induced mice.

The effect of ethyl gallate and propyl gallate was next evaluated to know the function of oxidative stress markers in colitis. In comparison to the vehicle control group, treatment with DSS alone group showed significantly reduced...
levels of SOD and GST (p<0.001). In contrast, DSS+PG/DW (P<0.0001), DSS+PG/CW (P<0.01), and DSS+EG+PG/DW (P<0.0001) treated groups restored the levels of GST and SOD. As a result, our findings suggest that ethyl gallate and propyl gallate, when used in the right combinations, can reduce the levels of oxidative stress indicators in colonic tissue see Fig. VII.

**Effect of Ethyl gallate and Propyl gallate on nitrosative stress and lipid peroxidation markers in colon tissue of DSS-induced mice.**

The effect of ethyl gallate and propyl gallate was next evaluated to know the function of nitrosative stress and lipid peroxidation markers in colitis. In comparison to the vehicle control group, treatment with DSS alone group showed significantly increased the levels of NO and LPO (p<0.001). In contrast, significantly lower levels of NO in DSS+EG/DW (P<0.01), DSS+EG/CW (P<0.0001), DSS+PG/CW (P<0.001), DSS+EG+PG/CW (P<0.001). As a result, our findings suggest that ethyl gallate and propyl gallate, when used in the right combinations, can reduce the levels of nitrosative stress and lipid peroxidation indicators in colonic tissue see Fig. VIII.

**Effect of ethyl gallate and propyl gallate on cytokine levels in the colon of DSS-induced mice**

To investigate the immunosuppressive effect of ethyl gallate and propyl gallate on the synthesis of proinflammatory cytokines (IL-6, IL-1β, IFN-γ, and TNF-α) in the colon tissue samples were collected after the termination of the experimental period on day 21. The DSS-alone treated group had significantly higher levels of IL-6, IL-1β, IFN-γ, and TNF-α (p<0.001), while the vehicle control group had minimal levels of proinflammatory cytokines. Treatment with DSS+EG/DW, DSS+EG/CW, DSS+PG/DW, DSS+PG/CW, DSS+EG+PG/DW, and DSS+EG+PG/CW significantly (P<0.001) reduced the TNF-α and IL-6 levels in colon tissue, when compared to DSS alone, treated group. In addition, a significant (P<0.01) reduction in IL-1β and IFN-γ levels was found, except for the standard control for IL-1β and DSS+EG/CW and DSS+EG+PG/DW, which failed to show any significance for those pro-inflammatory cytokines see Fig. IX.

**Discussion**

Although the exact cause of ulcerative colitis is still unknown, it is understood that it is characterised by an inflammatory process involving cytokines and different cell types in the intestinal mucosa (Tatiya-Aphiradee et al., 2019), such as macrophages, dendritic cells, regulatory T cells, T helper cells, and natural killer T cells as well as oxidative stress such as ROS, RNS and lipid peroxidation. UC is an inflammatory disorder must involve numerous dysregulations of cytokines and imbalance of the anti-oxidant enzymes because it is a complex multifactorial disease. A classic pre-clinical ulcerative colitis model that closely mimics the symptoms of human ulcerative colitis is mice with intestinal inflammation and tissue damage driven on by DSS (Sangaraju et al., 2019, Alavala et al., 2019). Although a large number of natural anti-oxidants have been identified as controlling UC by inhibiting oxidative stress and preventing the pro-inflammatory cytokines (Wang et al., 2022). Even though earlier research on ethyl gallate(Wang et al., 2022) and our in vivo investigation suggested that ethyl gallate, propyl gallate, and its combination treatments have an anti-inflammatory as well as anti-oxidant potential, nothing is known about how it affects intestinal inflammation. Accumulating evidence suggests that ethyl gallate, propyl gallate, and copper has established therapeutic efficacy in treating a number of animal models of inflammation without exhibiting any adverse side effects(Wang et al., 2022, Hsu et al., 2013, Sudha et al., 2012). However, their protective effects on maintaining colitis remission for long periods are still unknown. We evaluated the protective and preventive effects of ethyl gallate and propyl gallate in different combinations with normal drinking water and copper water against DSS-induced colitis.
According to previous reports, intestinal homeostasis and tight junctions were disrupted as a result of the synthesis of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ and deregulation of the mucosal immune system during the pathogenesis of colitis (Sanchez-Munoz et al., 2008, Strober and Fuss, 2011). By stimulating the Nrf2/HO-1 pathway in colitis mice, several phytochemicals and their structural analogs provided higher protection against oxidative stress such as ROS through the restoration of anti-oxidant enzymes such as GST and SOD (Singh et al., 2019). Induction of antioxidant and phase II detoxification enzymes is facilitated by Nrf2 activation, a basic region-leucine zipper transcription factor, by reducing oxidative cell damage (Shen et al., 2009). According to a previous study, Nrf2 activation prevents the production of genes for proinflammatory cytokines and reduces inflammation through redox regulation with the restoration of anti-oxidant enzymes. Notably, the pathophysiology of IBD shows a high correlation between inflammatory mediators such as COX and iNOS-induced proinflammatory cytokines via nitrosative marker as RNS, lipid peroxidation (LPO/TBARS) at the site of mucosal inflammation (Sangaraju et al., 2019, Singer et al., 1998). The antioxidative capabilities of PG were demonstrated by the fact that it alone significantly decreased the levels of thiobarbituric acid-reactive compounds (TBARS) (Jacobi et al., 1999). The generation of reactive species such as reactive oxygen species (ROS), Cu (II), and semiquinone radicals, which can take part in the beginning and propagation of lipid peroxidation, is likely what causes PG/Cu (II) to induce lipid peroxidation. Additionally, the combined actions of PG/Cu (II) were shown to inhibit membrane-bound succinate dehydrogenase. Only marginally affected cytosolic esterases. The increased vulnerability of membrane-bound enzymes is consistent with the effects of PG/Cu on lipid peroxidation (II) (Jacobi et al., 1999). Here, the results showed that copper water or regular drinking water, coupled with the possible antioxidants EG and PG, have demonstrated an anti-inflammatory effect against DSS-induced colitis.

According to the study's findings, ethyl gallate, propyl gallate, and their combinations administered through copper and regular drinking water displayed improved clinical signs like the return of reduced body weight, maintenance of colon length at a normal level, and a decreased DAI score, (as shown in Fig III). Ethyl gallate and propyl gallate and their combination prevented the microscopically visible damage of colon tissue based on HAI score i.e., infiltration of neutrophils, villi and crypt damage at the mucosa and sub-mucosal layers in the colon tissue and lumen epithelial damage as well as further confirmation to investigated the neutrophil marker MPO levels were significantly reduced at treatment groups suggesting a reduced accumulation of neutrophils in colon tissue as shown in Fig VI. when compared to DSS alone treated group, whereas except for propyl gallate with copper water. This observation indicates that ethyl gallate and propyl gallate in different combinations except for propyl gallate with copper water have a protective effect by suppressing inflammatory, lipid peroxidation and ulceration on the mucosa of the colon via regulating the infiltration of neutrophils against DSS-induced colitis.

Furthermore, the nitrosative and oxidative stress was monitored throughout the experiment by using IVIS, and at the end of the experiment, it was seen that the EG-DW/CW, PG-DW, and EG + PG-DW showed significant RO/NS reduction except for PG and EG + PG combined with copper water (all groups were challenged with DSS from day 8 to day 14) compared to DSS alone treated group. Previous reported study, ethyl gallate reduces acute lung damage by activating Nrf2 (Mehla et al., 2013), PG inhibits the inflammatory response by blocking COX-2, PGE2, and IKKB activity, and NF-κB signalling and these effects may in part be attributed to its strong antioxidant potential (Hsu et al., 2013), and cells can be protected against oxidative stress by propyl gallate (Chen et al., 2007). Through the inhibition of the NF-B and JNK pathways, as well as the suppression of nitric oxide synthesis and the stimulation of inducible nitric oxide synthase and cyclooxygenase-2 in inflammatory conditions, n-propyl gallate exhibits anti-inflammatory properties (Jung et al., 2011). Furthermore, DSS-treated groups have severe colon damage due to an increase in free radical production and activation of proinflammatory markers. Based on our findings, ethyl gallate and propyl gallate, as well as their combination, slightly restored the anti-oxidant status of GST and SOD levels while
decreasing the nitrosative stress (NO) and lipid peroxidation (LPO) levels in the colon tissue against the DSS alone treated group except the combination of PG and Cu see Fig. VII & VIII. The redox reaction between PG and Cu (II), which results in the formation of reactive species like ROS and the observed genotoxic and cytotoxic effects, is likely what causes the synergistic interaction between the two chemicals. Previous report findings suggest that show that propyl gallate's antioxidative and cytoprotective qualities can convert to prooxidative, cytotoxic, and genotoxic qualities in the presence of copper (II) (Jacobi et al., 1998).

According to previous reports, intestinal homeostasis and tight junctions were disrupted as a result of the synthesis of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ and deregulation of the mucosal immune system during the pathogenesis of colitis (Sanchez-Muñoz et al., 2008, Strober and Fuss, 2011). In this study, we found that the levels of proinflammatory cytokines (TNF-α, IL-6, IL-1β, and IFN-γ) were reduced by EG-DW/CW, PG-DW, and EG + PG-DW/CW treatments, but propyl gallate in combination with copper water showed a slight decrease in potential compared to PG-DW treatment (all groups were challenged with DSS from day 8 to day 14). This observation indicates that ethyl gallate and propyl gallate in different combinations except for propyl gallate with copper water have a protective effect by suppressing proinflammatory markers (IL-1β, IL-6, TNF-α, and IFN-γ) via NF-κB pathway against DSS-induced colitis.

Conclusion

In summary, the pathogenesis of ulcerative colitis was significantly suppressed in a mouse model by oral administration of ethyl gallate with both normal and copper drinking water, as well as ethyl gallate and EG + PG combinations with normal drinking water. Propyl gallate shouldn't be ingested with copper water for long term as they might show prooxidative and cytotoxic effects and may worsen the inflammatory conditions. In addition, propyl gallate was investigated for the first time in this study for its anti-colitogenic effect with normal drinking water and reversed activity with copper water.

Together, these findings point to the chemo-potential role of the synthetic antioxidant propyl gallate and the dietary component of natural anti-oxidant ethyl gallate as an anti-inflammatory and anti-oxidant potential and suggestions for additional clinical research to treat inflammatory diseases.

Abbreviations

AGEs, advanced glycation end products; ANOVA, analysis of variance; BW, Body weight; BCA, bicinchoninic acid; BSA, bovine serum albumin; CDNB, 1-chloro-2, 4-dinitrobenzene; CD, crohn's disease; CPCSEA, committee for the purpose of control and supervision of experiments on Animals; COX-2, cyclooxygenase-2; CW, Copper water; DW, drinking water; DSS, dextran sulfate sodium; DAI, disease activity index; DTNB, dithiobis-nitrobenzoic acid; ELISA, Enzyme linked immunosorbent assay; EG, ethyl gallate; PG, propyl gallate; Cu, copper; GSH, reduced glutathione; GIT, gastrointestinal disorder; GST, glutathione-S-transferase; GSSG, oxidized glutathione; H&E, Hematoxylin and Eosin staining; HO-1, Heme oxygenase1; IAEC, Institutional Animal Ethics Committee; IkB, inhibitor of kappa B; IL-6, Interleukin 6; IL-1β, Interleukin -1 beta; and IFN-γ, Interferon-gamma; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; LPO, lipid peroxidase; LD50, lethal dose 50; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; NFKB, nuclear Factor-κB; Nrf2, nuclear factor erythroid 2-related factor 2; PBS, Phosphate buffered saline; ROS, reactive oxygen species; RO/NS, Reactive oxygen and nitrogen species; RIPA, radioimmunoprecipitation assay; RNS, reactive nitrogen species; ROI, Region of Interest; SOD, superoxide dismutase; SEM, standard error of the mean;
TLR-4, toll-like receptor 4; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-α; WHO; world health organization; UC, ulcerative colitis; PC, Positive control (DSS alone treated group).

Declarations

Author contributions

The experiment's conception and design, data analysis, and writing the original manuscript were all done by SNS, PRC, and SR. The experiments such as IVIS, anti-oxidant assays and ELISA were carried out by SNS, PRC, SR, PBP, SSYHQ, VVP, and MB, while the paper was reviewed by SNS, PRC, SR, PBP, SSYHQ, VVP and MB. Performers of the experiments and data analysts were SNS, PRC, SR, PBP, SSYHQ, VVP and MB. Statistical analysis was carried out by SNS, PRC and SR. all authors reviewed the paper.

Data availability

All the data are available in the manuscript. On reasonable request, the corresponding author will provide the datasets used or analysed during the current work.

Conflicts of interest

The authors declare no competing financial interest

Ethic statement

The protocols for the use of animals were approved by the Institutional Animal Ethics Committee (IAEC) of the ICMR-NIN, and the IAEC approval number is ICMR-NIN/IAEC/02/018/2020.

Consent for publication

All listed authors have approved the manuscript before submission, including the names and order of authors

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**Figures**

- Vehicle control
- Dextran sulfate sodium control
- Sulphasalazine 100mg/kg standard control
- DSS + Ethyl gallate 50mg/kg
- DSS + propyl gallate 50mg/kg
- DSS + Ethyl gallate + propyl gallate (25+25)mg/kg
- DSS + Ethyl gallate + propyl gallate (25+25)mg/kg

Days | Vehicle control | Dextran sulfate sodium control | Sulphasalazine 100mg/kg standard control | DSS + Ethyl gallate 50mg/kg | DSS + propyl gallate 50mg/kg | DSS + Ethyl gallate + propyl gallate (25+25)mg/kg | DSS + Ethyl gallate + propyl gallate (25+25)mg/kg
---|---|---|---|---|---|---|---
1 | 1 | 1 | 1 | 1 | 1 | 1 | 1
2 | 2 | 2 | 2 | 2 | 2 | 2 | 2
3 | 3 | 3 | 3 | 3 | 3 | 3 | 3
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20 | 20 | 20 | 20 | 20 | 20 | 20 | 20
21 | 21 | 21 | 21 | 21 | 21 | 21 | 21

Non-colitic mice

Colitic mice
Figure 1

Schematic representation of the experimental design.

Figure 2

Disease activity score in colonic C57BL/6J mice, data are presented as mean ± SEM (n=8), ****P<0.0001 DSS control vs Test drugs groups
**Figure 3**

(a) Colon was harvested and length was measured, (b) Colon length, Data are presented as Mean ± SEM (n=6): **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001, P ≤ 0.0001 DSS control vs test drugs and #### P ≤ 0.0001 DSS control vs vehicle control (c) Occult blood (left-negative, right-positive), (d) Body Weight. Data are presented as Mean ± SEM (n=6): *P ≤ 0.05 **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001, ns = no significance DSS control vs test drugs.
Figure 4

The abdominal region of C57BL/6J mice with colitis exhibits strong signals during in vivo imaging of RONS utilizing L-012 Chemiluminescence. (a), examples of L-012 in vivo imaging of individual C57BL/6J mice, the pseudocolor heat maps represent photons/second/cm²/sr. L, lumen; scale bar=100 µm, from all groups on experimental day 8 (colitis induction day 1), experimental day 10 (colitis induction day 3), experimental day 11 (colitis induction day 4), experimental day 14 (colitis induction day 7), experimental day 16 (post-treatment day 2), experimental day 18 (post-treatment day 4). (b), abdominal L-012 chemiluminescence signal in C57BL/6J mice presented as average ± SEM, n=3, MS, *P ≤ 0.05, β=**P ≤ 0.01, γ=***P ≤ 0.001, δ=****P ≤ 0.0001, ns = no significance, comparing positive control with all test groups throughout the study.
Figure 5

Hematoxylin and eosin were used to stain representative colonic tissue pictures, which were then magnification by 10x and 20X. (a) Vehicle control DW (without DSS): No ulceration or inflammation was seen, accompanied by normal mucosal epithelial cells and submucosal glands. (b) DSS Control: epithelial layer disruption with Moderate to severe submucosal inflammation along with infiltration of inflammatory cells in the lumen are noticed [Arrow]. (c) DSS+SC/DW (Sulfasalazine control 100mg/kg with DSS): Submucosal glands with normal mucosal epithelial cells and no ulceration or inflammation were seen. (d2, f, g & h) DSS+EG/DW, DSS+PG/DW, DSS+PG/CW, and DSS+EG+PG/DW: Mild Epithelial layer disruption. (e) DSS+EG/CW: Normal. (i & j) EG+PG /CW+DSS: slight Edema and Edema + epithelial layer disruption+.
Figure 6

Effect of ethyl gallate and propyl gallate and their combination on HAI and MPO in DSS-induced colonic tissue at day 21 post-treatment, (a) The amount of MPO was measured using ELISA kits (b) Histological Activity Index. Data are expressed as mean ±SEM (n=6); ##P≤0.01, ###P≤0.001 DSS control vs vehicle control, ns = no significance *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001 treated groups vs DSS alone.
Figure 7

Effect of ethyl gallate and propyl gallate and their combination on Antioxidants (a) GST & (b) SOD in DSS-induced colonic tissue at day 21 post-treatment, Antioxidant production was quantified by ELISA kits. The Data are shown as mean ±SEM (n=6); Data are expressed as mean ±SEM (n=6); #P ≤ 0.05, ##P ≤ 0.01, ###P ≤ 0.001 DSS alone vs vehicle control, ns = no significance *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 treated groups vs DSS alone.
Effect of ethyl gallate and propyl gallate and their combination on nitrosative (a) NO, and lipid peroxidation (b) LPO in DSS-induced colonic tissue at day 21 post-treatment. Antioxidants levels were quantified by ELISA kits. Data are shown as mean ±SEM (n=6); *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 DSS alone vs vehicle control, ns = no significance *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 treated groups vs DSS alone.
Figure 9

Effect of ethyl gallate propyl gallate and their combination on proinflammatory cytokinin's such as (a) TNF-α, (b) IL-6, (c) IL-1β, and (d) IFN-γ in DSS-induced colonic tissue at day 21 post-treatment, cytokinin synthesis was quantified by EISA kits. Data are shown as mean ±SEM (n=6); #P ≤ 0.05, ##P ≤ 0.01, ###P ≤ 0.001, ####P ≤ 0.0001 DSS alone vs vehicle control, ns=no significance *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 treated groups vs DSS alone.

Supplementary Files

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