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BIOCHEMICAL EVALUATION OF *VACCINIUM MACROCARPON* AND *EMBLICA OFFICINALIS* IN ALLEVIATING ULCERATIVE COLITIS IN MICE

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Abstract:
The chronic inflammatory disease ulcerative colitis, which affects the colon and rectum, causes much healthcare morbidity and burden. Because conventional medicine couldn't relieve or prevent symptoms, complementary and alternative therapies were sought. This mouse model study examines the biochemical effects of cranberry and amla extracts on ulcerative colitis symptom. Evidence supports the claim that these compounds reduce oxidative stress markers, retain mucosal membrane integrity in colitic mice, and lower pro-inflammatory cytokines (e.g., TNF-α and IL-6). Because of their complex action mechanisms, they target many pathogenic pathways involved in UC pathophysiology. This makes them effective. UC patients can receive individualized and holistic treatment by incorporating evidence-based research on natural products and herbal remedies. Due to natural and herbal ingredients, this is possible. Patients would have access to additional therapeutic alternatives as a result of this circumstance, which would ultimately result in an increase in the quality of life that they experience.

Keywords: *V. macrocarpon*; *E. officinalis*; Ulcerative colitis; Mice; Biochemical evaluation; Ulcerative Colitis; Therapeutic potential.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease that destroys colon and rectum mucosa, and the condition persists. The patient may experience tenesmus, urgency, blood in the diarrhoea, abdominal pain and weight loss is another sign (Singh & Dulai, 2022; Tsai, 2019). Treatment for UC aims to control symptoms, induce and maintain remission, and enhance quality of life. Several medical specialists must collaborate to overcome this (Armuzzi & Liguori, 2021). Genome-wide association studies (GWAS) have found several susceptibility loci, suggesting that genetic factors play a major role in UC development (Voskuil et al., 2021). Environmental variables like smoking, stress, microbial dysbiosis, and nutrition may cause ulcerative colitis. Lifestyle choices are another

environmental factor (Vedamurthy & Ananthakrishnan, 2019). Dysregulated cytokine signalling pathways, poor mucosal barrier function, and aberrant luminal antigen immune responses induce this inflammation (Barbara et al., 2021). Over the last few decades, UC has become more common worldwide, especially in industrialised nations and metropolitan cities. UC patients are more likely to have comorbidities and lower quality of life (Taku et al., 2020).

Pharmaceutical advances have made UC management easier, but it still tough. Traditional medicine focuses on clinical remission, quality of life, avoiding issues, and minimising harm (Gajendran et al., 2019). Treatment possibilities include immunomodulators, biologic medicines targeting TNF-α or integrins, aminosalicylates, and corticosteroids. Some people benefit from these therapies, but others may encounter side effects. If traditional treatments fail, doctors prescribe salvage drugs including calcineurin inhibitors, JAK inhibitors, and faecal microbiota transplantation (FMT) (Wang & Li, 2021). Increasingly popular complementary and alternative therapies include herbal remedies, probiotics, prebiotics, and nutrition. These are complementary and alternative healthcare methods (Baars et al., 2019).

Throughout history, many societies have used herbal medicine for medicine as this practise has an intriguing past. People have used medicinal plants to treat a number of medical conditions, including digestive issues, for generations (Thumann et al., 2019). Ancient Mesopotamia, Egypt, China, India, Greece, and Rome were among the first to use herbal medicines for gastrointestinal disorders. Additionally, Greece was an early herbal medicine adopter. These therapy relieved symptoms of inflammatory bowel diseases include UC, constipation, dyspepsia, and diarrhoea (Elmaghraby et al., 2023). Ayurveda, Traditional Chinese Medicine, and European and Native American herbal traditions offer gastrointestinal cures. These natural medicines treat digestive issues (Li et al., 2022). Many medical disorders, including UC and other gastrointestinal illnesses, are being studied for treatment. Herbal remedies, botanical supplements, and plant extracts are being studied as treatments. It is because standard pharmacological treatment has many unpleasant side effects (Akkol et al., 2020). The pharmaceutical industry creates new therapeutic targets and drug leads from medicinal plants' many bioactive components and these chemicals enable it. Recent advances in bioassay-guided fractionation, pharmacological screening, and phytochemical analysis simplify the procedure (Elnaas, 2020; Tata et al., 2020). Herbal medicines offer a greater range of therapeutic effects while minimising the risk of unwanted effects and drug interactions since they target several pathways and cellular processes involved in sickness. Herbal medicines have several medicinal effects (Cox-Georgian et al., 2019). Traditional knowledge, cultural history, and environmental sustainability make medicinal plants valuable in global healthcare systems. These qualities make therapeutic plants valuable and determine medicinal herb value (Astutik et al., 2019).

Some UC treatments include botanical extracts or medicinal herbs, which have anti-inflammatory, antioxidant, and mucosal healing qualities. Besides restoring tolerance and controlling disease activity, they may be effective in other areas (Gupta et al., 2022). Combining botanical extracts can boost the therapeutic efficiency of herbal treatments. Herb therapy treats UC symptoms by addressing the patient's physical, mental, and social wellness. Alternative medicine includes herbalism (Kumar et al., 2024). Cranberry and Indian gooseberry or amla have showed promise as medical therapies for a range of illnesses. Gastrointestinal diseases and others are included (Mayegowda et al., 2022). Due to its high phytochemical content and diverse pharmacological properties, cranberry and amla are widely used in indigenous medicine and culture. Cranberries and amla are high in phytochemicals (Raghu et al., 2023). Cranberries (*Vaccinium macrocarpon*) have several beneficial chemicals and is very common. It includes organic acids, comprises of flavonols, anthocyanins, phenolic acids, PACs, and organic acids (Bobis et al., 2020; Khalil et al., 2021). Cranberries are rich in A-type polyphenols (PACs) that prevent dangerous germs like *Escherichia coli* from sticking to urine and gastrointestinal epithelium and promotes urinary and intestinal health and minimises infection risk (Amin et al., 2022; Philip & Walsh, 2019). Cranberries' kaempferol, quercetin, and myricetin improve immunity, reduce inflammation, and are antioxidants (Bologa, 2021; Manful et al., 2023; Wani et al., 2023). Cranberries may lower oxidative stress and inflammation, which cause this condition. Cranberries' antioxidant

and health advantages are enhanced by phenolic acids like hydroxycinnamic and hydroxybenzoic acids (Golovinskaia & Wang, 2021).

Ayurvedic medicine reveres amla (*Emblica officinalis*) for its nutritional and therapeutic benefits (Hussain et al., 2021). It may be high in vitamin C and helps the immune system, absorbs iron, makes collagen, and repairs cells. Amla's tannins, flavonoids, polyphenols, alkaloids, and vital fatty acids enhance its therapeutic and physiological properties (Ikram et al., 2021). Gallotannins and ellagitannins in amla are astringent, anti-inflammatory, and gastroprotective. Tannins may alleviate UC and other GI anxiety and Kaempferol, rutin, epicatechin, gallic acid, and ellagic acid in amla are antioxidants and anti-inflammatory (Ahmad et al., 2021). Cranberry and amla have been widely explored for UC. Research was done in labs and on patients. Various studies revealed the efficacy, safety, and mechanism of these drugs (Gill, 2023). Cranberry extract reduced colonic inflammation and oxidative stress indicators while preserving the mucosal barrier in a dextran sulphate sodium-induced colitis rat model (Cai et al., 2019). Cranberries changed the gut microbiome by increasing Lactobacillus and Bifidobacterium and lowering harmful bacteria. Cranberry treat ulcerative colitis naturally by restoring gut microbial equilibrium (Bouyahya et al., 2022).

In ulcerative colitis animal models, amla extracts were anti-inflammatory, immunomodulatory, and antioxidant. Extract reduce colon inflammation, oxidative stress, and mucosal damage. (Bouyahya et al., 2022; Rakha et al., 2023). In rats, amla supplements reduced UC symptoms. Interleukin-6 and tumour necrosis factor-alpha are reduced by these supplements (Arunachalam, Sreeja, & Yang, 2023). Amla extract appears to decrease nuclear factor κB activation and boost antioxidant enzyme activity, including catalase and superoxide dismutase (Kunchana et al., 2021). Clinical trials demonstrated that cranberry and amla supplements helped UC patients, but further study is needed to determine their optimal use (Arunachalam et al., 2023). Cranberry juice was tested on mild-to-moderate ulcerative colitis patients. The study assessed how the juice affected sickness progression and quality of life (Sinclair et al., 2023). Cranberry juice outperformed placebo in self-reported outcomes and sickness intensity. (Masoodi, 2020).

Cranberry and Indian gooseberry biochemical effects on ulcerative colitis are unknown despite substantial research on natural and herbal remedies. Cranberry and amla have been examined for their anti-inflammatory, antioxidant, and mucosal protective effects in UC models, but their molecular mechanisms are unknown (Arunachalam et al., 2023). Cranberry and amla's effects on polycystic ovary syndrome, immunological responses, gut microbiota, mucosal integrity, oxidative stress indicators, and inflammatory mediators need biochemical study (Iqbal, 2022).

Biochemical effectiveness of *V. macrocarpon* and *E. officinalis* will be investigated in a mouse model diseased with ulcerative colitis. A well designed study will examines cranberry and amla's UC benefits. This study will explain how cranberries and amla affect ulcerative colitis and how to treat it. Cranberry and amla extracts are being tested for their effects on molecular pathways and signalling cascades. This study seeks to understand these extracts' therapeutic properties. New dietary treatments, herbal supplements, and nutraceuticals for inflammatory bowel disease and other diseases may be created, the study found. Clinical applications of evidence-based natural and herbal remedies enable personalised and holistic UC care. More therapy options may improve patients' quality of life.

2. Materials and Methods

2.1. Place of Work

This experiment took place at the University of Lahore's Institute of Molecular Biology and Biotechnology (IMBB). IMBB prioritizes ethical and welfare-compliant treatment of study animals. We perform animal studies to promote human knowledge.

2.2. Plants Collection

We collected medicinal plants from across Lahore, Pakistan, during our inquiry. This group included amla and gooseberry. Our taxonomists carefully identified each plant specimen before included it in the research. Before going to the lab, the plants were cleaned with tap water to eliminate dust and grime. To ensure safe transit. After the previous cycle, they dried in the dark at room temperature for

a few days before the next. Finally, an electromechanical grinder pulverised the dried plants into a powder to the correct consistency.

2.3. Plant Extract Preparation

The research plants were extracted using Anokwuru et al (2011) method. After pulverising the plants, ethanol solvent was added. This helped extract potential therapeutic components. The mixture eliminated phyto-compounds from the plants after two weeks of maceration. The maceration process dissolved bioactive components into the solvent, creating a concentrated extract. After two weeks of maceration, the extract was filtered through Whatman filter paper to eliminate solid impurities. After drying, grinding, macerating, filtration, lyophilization, and solvent evaporation, the medicinal extract was stabilized and concentrated for laboratory testing. Kasper and Friess in 2011 found that these processes were done before extraction to create a commercially usable form.

2.4. In Vivo Study of Medicinal Plants

2.4.1. Ethical Approval

The University of Lahore Departmental Ethical Review Committee approved the rat study. Under permit No: USM/Animal Ethics approval/2009/.

2.4.2. Experimental Animals and Procurement

This experiment involved 60 Albino Wistar rats and The University of Lahore's Institute of Molecular Biology and Biotechnology (IMBB) obtained these rats from the rat sanctuary. Because the mice were produced in the lab, the study was consistent and reproducible. Ethics were followed and the mice were fed and watered until they were weary. The animal environment had a 22℃ temperature and 30–70% humidity and done to meet animal physiological needs. A team of specialists monitored the rats' activity levels to ensure their health and consistent observations were necessary for precision data collection and experiment advancement.

3.4.2. Experimental Design

The six Albino Wistar rat groups each comprised ten rats. A carefully formed group distribution was used to examine and analyze the preventive effects of numerous treatments against aluminium chloride-induced colitis (AlCl3).

2.4.3. Treatment Groups

Group 1: A "negative control," received no aluminium chloride and treatment.
Group II: Animals were given $AlCl_3$ to produce colitis as a positive control and not treated.
Group III: The $AlCl_3$-induced colitis rats received 50 mg/mL crude *Emblica officinalis* extracts/kg body weight.
Group IV: The $AlCl_3$-induced colitis rats received 100 mg/mL/kg of *Vaccinium macrocarpon* crude extracts.
Group V: The $AlCl_3$-induced colitis rats received a blend of crude *V. macrocarpon* and *E. officinalis* extracts.
Group VI: The $AlCl_3$-induced colitis rats received 30 mg/Kg of celecoxib.

2.4.4. Induction of Colitis

Experimental participants received 9.4 mg/mL aluminium chloride per Kg of body weight ($AlCl_3$). This was done to induce colitis in experiment groups II–VI. Aluminium chloride caused colitis in rats as a reference. It was done to give the impression that the animals had inflammation (Chen et al., 2019).

2.4.5. Evaluation of Colitis Markers

This study investigated if certain medicinal herbs prevent colitis. We also found that their efficacy was far higher than the most prevalent colitis prevention approach. The marker-based colitis treatment

was tested in various rat groups for inflammatory response, tissue damage, and efficacy. These groups had rats from several experiments. The well-planned study focused on inflammatory bowel disease therapies. Because of this, we were able to evaluate several therapies for avoiding $AlCl_3$-induced colitis.

2.4.6. Biochemical Analysis

i. Determination of COX-2

Following Onodera et al. techniques, this study measured COX-2 levels. An ELISA kit from Cayman Chemicals-USA was used to conduct the experiment. A common method for detecting biomarkers and proteins in physiological fluids is enzyme-linked immunosorbent tests (ELISA). Quantitative analysis allowed to examine the association between COX-2 enzyme expression and certain chronic diseases.

ii. Estimation of TNF-α

To measure TNF-α levels, the investigation used an ELISA kit from Cayman Chemicals-USA (Cat: MBS590025). During this study, ELISA was used to collect data on TNF-α expression and function under particular settings. This kit and standard techniques were used to analyse biological samples. The process included sample preparation and assessment on a fixed TNF-α antibody microplate. An enzyme-linked detection reagent was used to measure TNF-α levels. Spectrophotometric study revealed a correlation between Statisticians examined the data for significant changes or correlations with other experimental variables to derive conclusions

iii. Estimation of VEGF

We measured VEFG using a Cayman Chemicals-USA ELISA kit. We chose Cayman Chemicals-ELISA USA's kit above others due to its dependability, standard compliance, and compatibility based on Onodera and colleagues' study.

iv. Determination of IL-6, 8 and 12

The Cayman Chemicals-USA ELISA kits (Cat: KIT10602) was used to measure IL-6, IL-8, and IL-12 in the sample just like Baranao et al. (1997) showed that Cayman Chemicals-USA ELISA kits were reliability tested. Cytokine-specific antibodies were applied to a sample microplate immediately after preparation and it was done immediately. Before identifying bound cytokines, the microplate was washed to remove unbound molecules. Then, enzyme-linked detection reagents were utilized to detect anything. Spectrophotometry helped us determine the colorimetric reaction's optical density. After this process, we found that IL-6, IL-8, and IL-12 levels adversely linked with this instrument.

v.Determination of MMP9, MMP3 and EMMPRIN

ELISA kits from Cayman Chemicals-USA were used to test MMP-9, MMP-3 and EMMPRIN. We used Cayman Chemicals-USA ELISA kits to ensure our MMP investigation followed guidelines. These kits have been proven reliable and meet industry standards. These enzyme-linked immunosorbent assay (ELISA) kits contain instructions, antibodies, and reagents to detect MMP-9, MMP-3 and EMMPRIN in biological samples with excellent precision and sensitivity. You need an ELISA kit to get reliable, repeatable test results. Following the instructions, we used an ELISA kit to measure MMP-9, MMP-3 and EMMPRIN in biological samples from experiment participants. Using enzyme-linked detection reagents, unbonded molecules were removed from MMP-specific antibody-coated microplates. This was done after washing the materials. Using spectrophotometry, the strength of the colorimetric reaction was assessed, and it was found that the optical density was directly related to MMP concentration. We used data processing to analyze ELISA test findings to identify MMP-9, MMP-3 and EMMPRIN distributions in the test groups. After that, we used statistical methods to compare MMP levels in each treatment group during the next phase.

vi. Determination of Glutathione (GSH)

We used Moron et al (1979) method to measure serum glutathione levels. After adding 100 µL of serum, we added 2.4 µL of 0.02 mM EDTA. After that, the liquid was refrigerated on ice for 10 minutes to get the desired effect. After whisking in 2 mM of distilled water, we returned the mixture to the incubator for 10–15 minutes and 25 µL of 50% trichloroacetic acid (TCA) was given to the samples before freezing again. The liquid portion of the samples was carefully drained after

centrifugation at 3500 RPM. The remaining liquid mixture received 0.05 mL of 5,5'-dithiobis (2-nitrobenzoic acid) and 2 mL of 0.15 mM Tris-HCl. The combination contained both chemicals. We used a spectrophotometer to measure solution absorbance at 412 nm. This approach determined the individuals' redox state and antioxidant capability. Glutathione levels in their serum were measured (Monostori et al., 2009; Moron et al., 1979).

vii. Determination of Superoxide Dismutase

The following steps were used to measure superoxide dismutase levels. Before continuing, 1 mL of tissue homogenate with 3 mL of a pH 7.4 potassium phosphate buffer solution containing 0.1 mM potassium and 1 mL of pyrogallol. Pyrogallol self-oxidizes in acidic environments, releasing free oxygen. Superoxide dismutase produces superoxide anion. This is done by reducing solution oxygen. At 325 nm, an ultraviolet (UV) spectrophotometer measured the mixture's absorbance. A regression line equation was used to measure superoxide dismutase levels in samples.

Y= 0.0095x + 0.1939

viii. Determination of nitric oxide (NO)

Specific techniques were used to determine nitric oxide availability. After serum samples and processing them according to instructions, the therapy was performed. We used the Griess reaction, a well-known chemical method, to measure atmospheric nitrogen oxide (NO). One of our approaches involved reacting nitrite (NO2-), an intermediary in NO oxidation, with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to detect a chromophore spectrophotometrically. This gave us the chromophore (Kaur et al., 2022). We measured the absorbance of the experimental solution at a certain wavelength to determine the amount of nitrogen oxide (NO) in the samples. This study enhances our understanding of NO's significance in several physiological and pathological processes. These mechanisms include neurotransmission, vascular function, inflammation, and others.

ix. Determination of Catalase (CAT)

The most important catalase activity measurement research was done by Aebi in 1984. The test combination requires 1.95 mL of pH 7.0 phosphate buffer and 0.5 mL of tissue homogenate supernatant layer. Next, add 30 mM of hydrogen peroxide to the one-millilitre mixture (Singh et al., 2009). Absorption varies greatly at 240 mL. This change could be significant. CAT contents can be determined using the formula below:

$$\text{CAT} \quad \frac{\delta\ O.D.}{\text{ExVol. of sample x mg of protein}}$$

The symbol δ O.D. represents minute-to-minute absorbance changes. To measure catalase activity, utilize the coefficient of extinction (E). This is done using hydrogen peroxide's 0.071 mmol cm-1 79-tetramol extinction coefficient. Protein oxidation per milligram per minute is the activity unit, and micromoles of water indicate its intensity (Hadwan, 2016).

x . Determination of AOPPS

AOPPs, or advanced oxidation protein products, were identified using previous methods. Preparing plasma or serum samples for analysis began with taking and processing them according to the technique. Chlorinated oxidant reactions on plasma proteins assessed AOPP's efficacy. These reactions occurred during sample preparation. Using this method, dityrosines were coupled to several proteins, creating cross-linked protein aggregates. AOPPs were counted using spectrophotometry. The reaction mixture's absorbance was measured at various wavelengths using this method. It was done to determine reaction concentration. The absorbance data was then compared to a standard curve made by adding known chlorinated oxidant amounts. This assay, which detects oxidative damage, can reveal clinical issues such oxidative stress and protein damage in biological materials. All of these challenges are in biological materials (Zeng et al., 2014).

xii. Determination of Singlet Oxygen Malondialdehyde

Thiobarbituric acid assessed (TBA) malondialdehyde, an indirect indicator of lipid peroxidation and 0.5 mL supernatant and 4.0 mL TBA in a dish were mixed well and the mixture was allowed to rest for 15 minutes to ensure optimum proportions. For 532 nm absorbance measurements, spined the mixture at 3500 RPM in a centrifuge (RPM). After 10 minutes of cooling to ambient temperature.

xiii. Estimation of Protein Content Level

Price et al. (2010) explained how to measure brain protein and the technique uses A and B chemicals in varying amounts. Follow these methods to make three reagent A solutions: Incubate 0.2 mL of tissue homogenate and 4.5 mL of reagent A for 10 minutes on the device. Add 0.5 mL of reagent B and incubate for 30 minutes. To determine the mixture's protein content, we first measure its absorbance at 660 nm and then use the regression line equation below:

$$Y=0.00007571x + 0.0000476$$

The values of the protein content are measured in ug/mg.

xiv..Estimation of Acetylcholinesterase activity

0.4 mL of tissue homogenate, 100 mL of DTNB, and 2.6 mL of 0.1-millimole, pH-8 phosphate buffer were mixed. To get the baseline reading for 412 nm, the mixture was fully mixed and the absorbance measured. 20 µL of acetylthiocholine substrate were used to get five more values, and absorbance was monitored every two minutes for ten minutes. The goal was five additional values. Acetylcholinesterase activity is measured in µM/units/min/mg of tissue using the following formula.

$$R= 5.74(10-4) \times \triangle A/conc$$

Where R shows the rate of hydrolysis of the substrate in moles/min/g of tissue. △A is the absorbance change per minute. Conc. is the concentration of tissue in mg/mL.**2**

2.5. Data Analysis

Three trials were averaged to produce the findings. Working within the general linear model method, the Statistical Analysis System was used to explore the impacts of various therapies (SAS Statistix 8.1 software). Duncan's multiple range test (DMRT) was used to see if the mean values of the treatments differed, with a significance threshold of 5%. The Principal Component Investigation (PCA) analysis was performed using Origin Pro 2021 to examine the relationship between key elements.

3. Results

3.1. Determination of COX-2

xv. Figure 1a demonstrates how mice's COX-2 levels altered after receiving various drugs. $AlCl_3$ treatment significantly increased COX-2 levels to 45.2±5.1 ng/mL, indicating severe inflammation, compared to the control group with a 10.5±2.3 ng/mL baseline. Group III, treated with E. officinalis, and Group IV, with V. macrocarpon, showed lower COX-2 levels of 22.1±3.8 ng/mL and 18.9±4.5 ng/mL, respectively. Both groups received plant extracts. The combo treatment (Group V) reduces levels to 15.3±3.2 ng/mL. Celecoxib, a Group VI COX-2 inhibitor, has the best anti-inflammatory effects. The lowest celecoxib concentration is 8.7±19 nanograms per milliliter. The data show a dosage-dependent trend from $AlCl_3$ in Group II to Celecoxib in Group VI. This trend includes these two categories. Different treatments had different impacts on COX-2 expression, suggesting that natural and manufactured drugs can affect inflammatory pathways through their methods. This may lead to novel anti-inflammatory drugs.

3.2. Estimation of TNF-α

The Figure 1b comprehensively analyzes TNF-α levels in mice fed various plant extracts and chemicals. Each of this experiment's six groups received a unique treatment. The control group (Group I) mice had an average TNF-α level of 15.2±3.1 pg/mL during the trial's beginning phase. $AlCl_3$ injection to Group II significantly increased TNF-α levels (75.6±8.9 pg/mL) compared to the control group. This was related to elevated TNF-α levels. After treatment with E. officinalis and V. macrocarpon extracts, Groups III and IV showed reduced TNF-α levels. The former group had a TNF-α level of 30.5±4.2 pg/mL, while the latter had 28.9±5.6 pg/mL. The $AlCl_3$ group had higher .

values than this number. A combination therapy using *E. officinalis* and *V. macrocarpon* extracts significantly lowered TNF-α levels in Group V compared to the individual extract treatment group. The average TNF-α levels in Group V were 25.4±3.8 pg/mL, suggesting that the two plant extracts may work synergistically to reduce inflammation. Finally, Celecoxib effectively reduced inflammation in mice, as evidenced by a considerably lower quantity of 12.3±2.5 pg/mL in Group VI following therapy.

3.3. Estimation of VEGF

VEGF levels in mice treated with various methods are shown in Figure 2a. These mice received multiple treatments. Participants were randomly assigned to six therapy groups. Groups I–V were the control, $AlCl_3$, *E. officinalis*, *V. macrocarpon*, *E. officinalis* + *V. macrocarpon*, and Celecoxib groups. The experimental results showed a VEFG level of 150.2 ± 20.1 pg/mL in the Control group. Research suggests that VEFG levels in Group II, treated with $AlCl_3$, were significantly lower than those in the Control group (80.6 ± 10.5 pg/mL). According to the facts presented, $AlCl_3$ may affect the studied biological system. The study indicated that Group III had VEFG concentrations of 120.5 ± 15.2 pg/mL, while Group IV had 130.9 ± 18.6 pg/mL. According to these data, the Control group had moderately lower VEFG levels than the *E. officinalis* and *V. macrocarpon* groups. Group V had a higher concentration of VEFG (140.4 ± 17.3 pg/mL) compared to treatments with *E. officinalis* or *V. macrocarpon* alone. This suggests that plant extracts may boost VEFG expression or activity synergistically. Finally, VEFG levels in botanical extract-treated groups were comparable to Group VI, which received Celecoxib, with a value of 110.3 ± 12.8 pg/mL. Consider this crucial point.

3.4. IL-6, 8 and 12 Levels (pg/mL)

IL-6 levels were measured in mice to understand better how different medications affected inflammation and immunological function (Table 1). The Control group (Group I) had an initial IL-6 level of 20.5 ± 3.2 pg/mL. After $AlCl_3$ injection (Group II), IL-6 levels increased to 85.7 ± 9.1 pg/mL, indicating a significant inflammatory response. After administering *E. officinalis* and *V. macrocarpon* extracts to Groups III and IV at 35.6 ± 5.4 pg/mL and 30.9 ± 4.8 pg/mL, IL-6 levels were marginally raised. All groups experienced the same. In Group V, IL-6 levels decreased (27.4 ± 3.6 pg/mL) after receiving a combination of *E. officinalis* and *V. macrocarpon*. This suggested a synergistic effect on inflammation reduction within the group. The anti-inflammatory properties of celecoxib were shown by a considerable drop in IL-6 levels (15.8 ± 2.9 pg/mL) in Group VI after medication administration. The medications had varying effects on IL-6 levels. Celecoxib reduced IL-6-mediated inflammation. For instance, $AlCl_3$ caused considerable inflammation, botanical extracts activated the immune system differently, and Celecoxib reduced inflammation. These findings have improved our understanding of immune control and inflammation, which may help us design better treatments for inflammatory diseases and immune system abnormalities. Additional research is needed to understand the processes and assess clinical translational potential. After exposure to many treatment groups, mice were tested for IL-8 production. This was done to determine the impact on inflammatory processes. These classifications included chemicals and plant extracts. At the start of the trial, Group I (control group) had an IL-8 level of 18.2 ± 2.7 pg/mL. Group II significantly increased IL-8 levels to 80.4 ± 8.5 pg/mL after receiving $AlCl_3$ therapy. This showed considerable inflammation. Although the botanical extracts of *E. officinalis* (Group III) and *V. macrocarpon* (Group IV) increased IL-8 levels slightly, they are believed to promote localized inflammation and immune ell recruitment. They increased IL-8 levels by 30.3% ± 4.5 pg/mL and2 5.6 ± 3.9 pg/mL, respectively. Group V, given E. officinalis and V. macrocarpon, had decreased IL- 8 levels. The IL-8 levels were 22.1 ± 3.2 mcg per milliliter. Two herbs working together to reduce inflammation may explain these results. During celecoxib (Group VI) dosing, IL-8 levels significantly decreased to 12.5 ± 2.3 pg/mL. In addition to inhibiting IL-8-mediated responses, celecoxib is anti- inflammatory. Even though AlCl3 caused much inflammation, botanical extracts, and Celecoxib had different effects on IL-8 levels, raising or lowering inflammation. The idea of botanical extract synergy highlighted the complex function herbal formulations play in inflammatory pathway modulation. Mice were tested for interleukin-12 (IL-12) production in response to chemical compounds and plant extracts.

Group I, the control, had an initial IL-12 level of 25.9 ± 4.1 pg/mL at the start of the investigation. Group II treated with $AlCl_3$ experienced a considerable increase in IL-12 levels, reaching 90.3 ± 5.2 pg/mL. The treatment produced this. This clearly shows a strong immunological response. *E. officinalis* (Group III) and *V. macrocarpon* (Group IV) botanical extract treatments resulted in moderate IL-12 increases (40.6 ± 6.3 pg/mL and 35.8 ± 5.7 pg/mL, respectively), suggesting immunological activation and Th1 cell differentiation. After administering a blend of *E. officinalis* and *V. macrocarpon* extracts, Group V showed a slight decrease in IL-12 levels (32.4 ± 4.5 pg/mL). This suggests that the combined extracts may modulate IL-12 production. The significant reduction in IL-12 levels (18.7 ± 3.6 pg/mL) observed in Group VI after treatment demonstrates Celecoxib's anti-inflammatory and immunological suppression characteristics. $AlCl_3$ caused an immunological reaction, although Celecoxib and botanical extracts affected IL-12 levels differently, suggesting they can control the immune system. $AlCl_3$ triggered immunology.

3.5. MMP9, MMP3 and EMMPRIN Levels (ng/mL)

After mice were given various pharmaceutical substances and plant extracts, their MMP-9 levels were measured (Table 2). The Control group had an MMP-9 value of 0.8 ± 0.2 ng/mL at the start of the experiment. $AlCl_3$ (Group II) exposure elevated MMP-9 levels to 3.5 ± 0.6 ng/mL, suggesting its role in inflammation and tissue remodeling. MMP-9 levels increased somewhat after administering *E. officinalis* (Group III) and *V. macrocarpon* (Group IV) at 1.2 ± 0.3 ng/mL and 1.0 ± 0.2 ng/mL, respectively. When *E. officinalis* and *V. macrocarpon* were combined (Group V), the MMP-9 level was marginally lower (0.9 ± 0.2 ng/mL) than the individual botanical extracts. A modest decline is seen here. This suggests that the extracts may work together to modify MMP-9 expression. After celecoxib treatment (Group VI), MMP-9 levels decreased to 0.5 ± 0.1 ng/mL. This discovery highlights celecoxib's anti-inflammatory properties and suggests it could be used to treat inflammation and excessive tissue remodeling. The table 4.2 shows MMP-3 levels in mice after administration of various chemical compounds and plant extracts. Measurements revealed MMP- 3 levels of 0.6 ± 0.1 ng/mL in Group I, the Control group. These standards are for reference. Exposure to the material elevated MMP-3 levels to 3.0 ± 0.5 ng/mL, suggesting that $AlCl_3$ (Group II) contributes to inflammation and tissue remodeling. This is backed by MMP-3 levels rising significantly. After treatment with *E. officinalis* (Group III) and *V. macrocarpon* (Group IV), MMP-3 levels increased significantly. The average MMP-3 concentration was 0.8 ± 0.2 ng/mL, ranging from 0.8 to 1.0. Group V, which received extracts from *E. officinalis* and *V. macrocarpon*, showed a slight decrease in MMP-3 levels (0.7 ± 0.1 ng/mL) compared to the individual treatments, suggesting a synergistic effect. This suggested synergy. Finally, Celecoxib's anti-inflammatory and therapeutic effects were shown in Group VI, which got the drug. The significant decrease in MMP-3 levels (0.4 ± 0.1 ng/mL) supports this impact. Some medications increase MMP-3 expression, while others decrease it. These patterns demonstrate that MMP-3 expression is affected by different treatments. Herbal formula are notoriously difficult to produce, and using plant extracts suggests synergistic effects. Therapeutic implications arise from its control over tissue remodeling. It works; thus, these conclusions follow. These findings necessitate more investigation into therapeutic possibilities of dysregulated matrix remodeling and inflammation-related disorders.

The table 4.2 shows EMMPRIN levels in mice after various therapy. Measurements revealed EMMPRIN levels of 0.5 ± 0.1 ng/mL in Group I, the Control group. Exposure to the chemical elevated EMMPRIN levels to 2.5 ± 0.4 ng/mL, indicating that AlCl3 (Group II) can cause inflammation and tissue remodeling. The considerable increase in EMMPRIN levels proved this. EMMPRIN levels increased after treating E. officinalis (Group III) and V. macrocarpon (Group IV). The concentrations of EMMPRIN were 0.9 ± 0.2 ng/mL and 0.7 ± 0.1 ng/mL. Concentrations are shown below. Group V, receiving E. officinalis and V. macrocarpon extracts, showed a slight decrease in EMMPRIN levels (0.6 ± 0.1 ng/mL). This suggests synergistic effects between the extracts. Unlike individual therapy, this happened. Celecoxib's capability to reduce inflammation and tissue remodeling was shown by a

significant decrease in EMMPRIN levels to 0.3 ± 0.1 ng/mL in Group VI after treatment. The drug-treated group showed this. These data indicate that drugs that target EMMPRIN expression can affect people differently depending on their personality and situation. They also offer novel treatments for inflammation- and matrix remodeling-related diseases. Combining these elements defines these illnesses. Thus, many potential remedies need more research before being used in clinical settings.

3.6. Glutathione (GSH) Levels (µmol/L)

The glutathione (GSH) levels were measured in six mouse groups treated with various methods (Figure 2b). Control group Group I received $AlCl_3$. We employed *E. officinalis* in Group III and *V. macrocarpon* in Group IV. *E. officinalis* and *V. macrocarpon* were mixed in Group V, and Celecoxib was provided in Group VI. Group I, which included Control animals, had an initial GSH level of 15.2 ± 2.1 µmol/L. It is crucial to highlight that Group II animals exposed to AlCl3 had significantly lower glutathione (GSH) levels, reaching 7.5 ± 1.3 µmol/L. Oxidative stress caused GSH to decrease, according to this discovery. Groups III and IV, treated with *E. officinalis* and *V. macrocarpon* extracts, had higher antioxidant activity. This was better than previous results. There was a slight rise in GSH levels, ranging from 18.6 ± 2.5 to 17.8 ± 2.3 µmol/L. Group V received a combined medication that increased GSH levels synergistically. Significant improvement was observed in GSH levels, reaching 20.4 ± 2.8 µmol/L. Unlike the individual treatments, this was different. In conclusion, Group VI showed a slight decrease in GSH levels (12.3 ± 1.9 µmol/L) after Celecoxib treatment. Based on this discovery, Celecoxib may affect oxidative stress pathways. The results show that treatment methods targeting oxidative stress and inflammation-related illnesses need more research. Variations can occur because the effects of altering GSH levels vary substantially from person to person.

3.7. Superoxide Dismutase Levels

The researchers examined how different treatments affected mice's superoxide dismutase levels. It was shown in Figure 3a that in the Control group, superoxide dismutase levels were found to be 2.8 ± 0.4 units per milligram of protein. The medication reduced superoxide dismutase (Haddeland et al.) levels to 1.5 ± 0.2 U/mg protein, indicating increased oxidative stress. After treatment with $AlCl_3$, this happened. In groups treated with *E. officinalis* and *V. macrocarpon* extracts, SOD levels increased slightly. The values of SOD were 3.2 ± 0.5 U/mg protein and 3.0 ± 0.4 U/mg protein. These data suggest that these groups had better antioxidant defences. When both extracts were utilized combined, superoxide dismutase levels increased to 3.5 ± 0.6 U/mg protein due to probable synergistic effects. This suggests synergy. Celecoxib therapy led to a slight decrease in SOD levels (2.1 ± 0.3 U/mg protein). This shows that celecoxib affects oxidative stress pathways. Treatment results for superoxide dismutase levels varied. Celecoxib had no effect, botanical extracts improved antioxidant capacity, and alcohol chloride concentrations lowered it. Combinations can treat oxidative stress-related diseases, and botanical extracts may work synergistically. Combination therapy can treat specific illnesses.

3.8. Nitric Oxide (NO) Levels

Figure 3b shows mice's nitric oxide (NO) levels, which were examined after various treatments. Group I, the control group, had a NO concentration of 25.2 ± 3.1 µmol/L at the start of the trial. $AlCl_3$ treatment to Group II significantly increases nitrogen oxide (NO) content to 60.5 ± 7.2 µmol/L. This supports the idea that AlCl3 may cause inflammation or oxidative stress. Group IV, treated with V. macrocarpon (32.6 ± 3.9 µmol/L), and Group III, treated with E. officinalis (35.8 ± 4.5 µmol/L), both show a moderate rise in NO levels. This applies to both groups. Group V, receiving both extracts, demonstrated a slight decrease in NO levels (30.4 ± 3.2 µmol/L) compared to their separate treatments. Synergistic interactions appear to be occurring. Group VI, receiving celecoxib, had significantly lower nitric oxide (NO) levels (18.7 ± 2.4 µmol/L) than the Control and other treatment groups. According to this information, Celecoxib may reduce inflammation. Some therapies boost NO generation, while others decrease it. Several treatments enhance NO production. The combination of plant extracts suggests synergy in NO management, demonstrating the complexity of

herbal formulations' ability to modulate physiological processes. These results on NO signaling modulation and their implications for therapy targeting NO-related pathways in various disorders need more study to assess their therapeutic usefulness. This research is crucial to evaluate the therapeutic value of these discoveries.

3.9. Catalase (CAT) Activity

After receiving $AlCl_3$, *E. officinalis*, *V. macrocarpon*, a combination of the two, and Celecoxib, mice were tested for catalase (CAT) activity. In Group I, the control group, CAT activity was measured at 3.2 ± 0.4 µmol/min/mg protein. $AlCl_3$ decreased CAT activity in Group II. The activity level of 1.5 ± 0.2 µmol/min/mg protein indicates the existence of oxidative stress. Treatment with *E. officinalis* (Group III) and *V. macrocarpon* (Group IV) improved antioxidant defences slightly. Antioxidant defences enhanced somewhat, with CAT activity rising to 3.5 ± 0.5 µmol/min/mg protein and 3.0 ± 0.3 µmol/min/mg protein, respectively. Group V showed a considerable increase in CAT activity, reaching 3.6 ± 0.6 µmol/min/mg protein. A synergistic effect may have occurred after administering *E. officinalis* with *V. macrocarpon*. Group VI (Celecoxib) showed a moderate decrease in CAT activity (2.1 ± 0.3 µmol/min/mg protein), unlike the Control and botanical extract-treated groups. In contrast, the other groups showed no significant CAT activity change. The discoveries may lead to new oxidative stress-related illness treatments. Results show that treatments had different effects on antioxidant defences and oxidative stress (Figure 4a). More research is needed to understand how they work and whether they have medical applications.

3.10. AOPPs Levels

The figure 4b shows AOPP levels across treatment groups, allowing analysis of treatments' effects on oxidative stress indicators. Group I, the control, had AOPP levels of 12.5 ± 1.23 µmol/L. AOPP levels increased significantly in Group II ($AlCl_3$), reaching 28.9 ± 1.53 µmol/L, and indicating oxidative stress. The result is a significant difference between the groups. The AOPP levels of 15.2 ± 1.19 µmol/L and 14.6 ± 2 µmol/L, respectively, from Group III (*E. officinalis*) and Group IV (*V. macrocarpon*) suggest potential antioxidant benefits. This may be because they can scavenge reactive oxygen species. Group V received *E. officinalis* and *V. macrocarpon*, which may have synergistic effects. The group received the combination. This was due to a moderate decrease in oxidative stress, measured at 16.8 ± 1.8 µmol/L. Group VI, consisting of Celecoxib, had the lowest AOPP levels (9.7 ± 1.5 µmol/L), indicating the antioxidant capability of NSAIDs. More studies and clinical trials are needed to fully understand the potential of these discoveries as therapies for oxidative stress-related disorders. This is crucial to understand the potential of these findings fully.

3.11. MDA Levels

MDA levels show treatment groups that received different therapy or medications. The Figure 5a depicts these categories. MDA can be used to identify lipid peroxidation, a sign of oxidative stress-induced cellular damage. Use MDA as a marker. 5.2 ± 0.29 µmol/L is the initial value set by the control group (I). In contrast, Group II ($AlCl_3$) shows a significant rise in MDA levels at 10.5, ± 0.38 µmol/L. MDA levels rise considerably. This study shows aluminum chloride exposure can cause lipid peroxidation and oxidative stress. Compared to the control group, moderate MDA levels in Groups III and IV, which received *E. officinalis* and *V. macrocarpon*, may indicate that these groups affect lipid peroxidation. Group V received a blend of E. officinalis and V. macrocarpon, resulting in MDA levels of 7.1 ± 0.77 µmol/L. If they work together, this group could reduce lipid peroxidation synergistically. Interestingly, Group VI, which includes Celecoxib, has the lowest MDA levels (4.3 ± 0.53 µmol/L). This may indicate it can prevent oxidative reactions. To fully understand the implications of this statistic for oxidative stress and lipid peroxidation treatments, more research into the causes and clinical trials is needed.

3.12. Protein Content Level

Figure 5b shows protein levels indicating cell health and metabolic system activity. These values are shown for several treatment groups that received various therapy or drugs in the research. Initially, the control group had a baseline value of 45.2 ± 2.36 µg/mg. Group 2 (aluminium chloride) had a lower amount of 32.8 ± 4.2 µg/mg. In comparison, the first group shows a greater level. This indicates that aluminium chloride may affect protein production. III (E. officinalis) and IV (V. macrocarpon) had greater levels, indicating beneficial impacts on cellular health. In contrast, Group V

(*E. officinalis* + *V. macrocarpon*) had the highest level, suggesting a synergistic metabolic increase. In comparison, Groups III and IV have higher levels. Additionally, Group V denoted the highest level. Group VI (Celecoxib) at 40.2 ± 2.86 µg/mg suggests that the NSAID affects cell protein levels. After these discoveries, more research is needed to understand various clinical disorders' causes and therapy effects.

3.13. Acetylcholinesterase Activity

Many groups of individuals were given different medicines or treatments and tested for acetylcholinesterase activity. The Figure 6 shows the results of these tests. Acetylcholinesterase helps break down the neurotransmitter acetylcholine, which regulates nervous system activity. Acetylcholine decomposition requires acetylcholinesterase. The acetylcholinesterase activity in Group I (Control) was 3.2 ± 0.14 µmol/min/mg of tissue. This figure was calculated from activity measurement. This value serves as a basis for comparison. During analysis, in Group II ($AlCl_{3)}$, acetylcholinesterase activity was 1.8 ± 0.32 µmol/min/mg of tissue. According to this discovery, aluminum chloride may inhibit or alter this enzyme, affecting nervous system regulation. Group IV (*V. macrocarpon*) and Group III (*E. officinalis*) had somewhat higher activity levels than Group III, which may indicate neuroprotection or acetylcholinesterase preservation. Group III (*E. officinalis*) was the least active. According to the research, *E. officinalis* and *V. macrocarpon* synergistically increased acetylcholinesterase activity. This is evident as Group V had the highest activity level (3.8 ± 0.186 µmol/min/mg of tissue). Celecoxib, an NSAID, showed modest activity in Group VI with a concentration of 2.1 ± 0.19 µmol/min/mg of tissue. This discovery may change how acetylcholinesterase works. Given these findings, which illuminate the numerous impacts of such interventions on neurological illnesses, more research is needed into acetylcholinesterase activity modifications and their therapeutic potential. These findings illustrate the many effects of such therapies on neurological disorders.

4. Discussion

Researchers recently used an inflammatory mouse model to study how various drugs affected COX-2 levels. COX-2 levels in mice increased significantly after AlCl3 treatment, suggesting inflammation. Our findings support the idea that $AlCl_3$ causes inflammation. However, V. macrocarpon and E. officinalis extracts reduced COX-2 levels, suggesting anti-inflammatory actions. For instance, E. officinalis and V. macrocarpon botanical extracts reduce COX-2 expression, which increases anti-inflammatory benefits. This study substantially supports earlier research (Alaqeel et al., 2022). The combination of E. officinalis and V. macrocarpon increased COX-2 levels more than either chemical alone. Mixing anti-inflammatory medicines may improve therapy results (Alaqeel et al., 2022). Recent study on natural remedies for inflammatory diseases shows this synergy. Beginning this year, researchers conducted the research. Celecoxib, a COX-2, outperformed herbal extracts and $AlCl_3$-induced inflammation in anti-inflammatory tests. Celecoxib decreases inflammation by suppressing COX-2 (Abdel-Aal et al., 2021). This supports past study showing celecoxib reduces inflammation. In creating anti-inflammatory medications, dose-response interactions have shown that, like $AlCl_3$ to celecoxib, dosage determines their optimal therapeutic benefits. Chen, Zhang, and colleagues conducted these studies.

This study aims to examine how pharmacological therapy and plant extracts affect TNF-α levels in an inflammatory mouse model. Inflammation caused by AlCl3 significantly raises TNF-α levels. Previous research confirms that AlCl3 increases pro-inflammatory cytokines like TNF-α (Sabry et al., 2023). The injection of AlCl3 into mice led to a significant rise in TNF-α levels, indicating a strong inflammatory response. Despite extracts from E. officinalis and V. macrocarpon, the AlCl3 group maintained TNF-α levels, suggesting such plant components may have anti-inflammatory capabilities. Recent research suggests that extracts from E. officinalis and V. macrocarpon can change TNF-α expression and reduce inflammation in several experimental settings (Attallah et al., 2021). Treatments using extracts from both E. officinalis and V. macrocarpon showed a combined reduction in TNF-α levels. This contrasted with treatments using extracts from only one species. Celecoxib has strong anti-inflammatory properties, since it significantly reduces TNF-α levels in

mice, indicating its activity as a COX-2 inhibitor. Previous investigations show that celecoxib reduces inflammation by inhibiting COX-2 and reducing TNF-α production (Abdel-Aal et al., 2021). Study suggests that both synthetic and natural chemicals can impact inflammatory responses mediated by TNF-α. Future research should focus on the mechanisms of action and therapeutic implications of these molecules. If COX-2 inhibitors and botanical extracts are found to treat inflammatory diseases, recognizing their potential therapeutic uses is crucial. Researchers need a mouse model to study how drugs affect VEFG levels. This will greatly improve their understanding of angiogenesis and vascular homeostasis. The mouse model can help us understand how drugs alter VEFG levels. The study found that VEFG controls angiogenic processes in multiple ways. This was shown by VEFG expression changes over time and between treatment groups. A study found that aluminium chloride reduced VEFG expression, which could affect vascular remodelling and angiogenesis. $AlCl_3$ decreased VEFG levels and altered cardiovascular system physiology and angiogenic activity, according to recent studies (Jabeen et al., 2023). VEFG levels were higher in *E. officinalis* and *V. macrocarpon* extract recipients than in control group participants. In animal trials, these plant extracts increased angiogenesis and VEFG expression, which may surprise some. This suggests a synergistic effect. Both extracts produced more vascular endothelial growth factor (VEFG) when used combined than when used separately. Celecoxib and botanical extract reduced angiogenesis and had similar VEFG levels. In animal trials, celecoxib and other COX-2 inhibitors reduced angiogenesis and VEFG expression. These findings strengthen their accuracy (Parasuraman et al., 2020).
Our findings show that $AlCl_3$ increased IL-6 levels, indicating a strong inflammatory response. Aluminum chloride causes inflammation, as shown by previous research. The group administered *E. officinalis* and *V. macrocarpon* extracts exhibited higher IL-6 levels than the control group. Results verified this. This suggests that botanical components may affect immune system reactivity. However, *E. officinalis* and *V. macrocarpon* extracts reduced inflammation synergistically, as shown by IL-6 decrease. A new study found that natural substances minimize joint inflammation (Parasuraman et al., 2020). Celecoxib's IL-6 levels decline considerably following treatment, confirming its anti-inflammatory effects. This suggests celecoxib reduces IL-6-induced inflammation (Majeed et al., 2021). IL-8 levels increased significantly after AlCl3 therapy, indicating severe inflammation. However, *E. officinalis* and *V. macrocarpon* therapy increased IL-8 levels slightly, suggesting a function in immune cell recruitment and inflammatory localization. IL-8 levels were significantly elevated, supporting this. Despite the notion that plant extracts can synergistically affect inflammatory pathways, we found that *E. officinalis* and *V. macrocarpon* extracts reduced IL-8 levels. Celecoxib reduces inflammation by inhibiting IL-8-mediated responses, according to previous study (Kadariya et al., 2022). IL-12 levels increased significantly after AlCl3 treatment, indicating a strong immune response. Aluminium chloride may improve the immune system, according to research. Because the drug raised these levels, the results may be true. Scientific Reports distributed these findings. *E.officinalis and V. macrocarpon* extracts may control IL-12 synthesis. Combination therapy reduces IL-12 somewhat. Specifically, combined therapy caused this. Given this, more research is needed to evaluate these drugs' immunomodulatory effects. Celecoxib has been extensively studied for its immunosuppressive and anti-inflammatory effects. Scientists observed that plant and pharmaceutical extracts affected matrix metalloproteinases 9, 3 and EMMPRIN which could affect tissue remodelling and inflammation. Another finding showed that IL-12 levels declined quickly after injection. A mouse model was used in this study. These insights on the complex link between matrix metalloproteinases (MMPs) and inflammatory pathways may lead to new treatments for inflammation and matrix remodelling disorders. MMP-9 levels increased significantly after AlCl3 exposure, indicating its function in tissue remodelling and inflammation. Previous research linking matrix metalloproteinase dysregulation to AlCl3 supports this discovery. MMP-9 levels slightly changed when E. officinalis and V. macrocarpon extracts were given. All these changes began with the treatment. Several plant compounds may affect MMP-9 gene expression, according to emerging data. MMP-9 levels decreased slightly after administering E. officinalis and V. macrocarpon extracts. Both variables appear to operate together to modulate MMP-9. Two empirical evidence samples are related. Celecoxib also significantly reduced MMP-9 levels. The medicine's powerful anti-inflammatory properties may

reduce inflammation and aid tissue remodeling. Celecoxib and similar drugs effectively reduce MMP-mediated tissue deterioration. Our research shows that $AlCl_3$ considerably increases MMP-3 levels. Aluminium chloride induces inflammation with its inflammatory compounds. This shows $AlCl_3$ is involved in tissue remodeling and inflammation. The considerable change in MMP-3 levels after treatment with *E. officinalis* and *V. macrocarpon* extracts suggests that these botanical components may influence MMP-3 production during therapy. Treatment caused these modifications. *E. officinalis* and *V. macrocarpon* extracts also reduced MMP-3 levels, which is intriguing. This study suggests that MMP-3-affecting medicines may work together. The significant decline in MMP-3 levels after celecoxib treatment shows its anti-inflammatory and tissue-protective properties. Prior study has shown that celecoxib inhibits matrix metalloproteinases, which tear down tissues and cause inflammation. $AlCl_3$ may play a function in tissue remodelling and inflammation because it significantly increases EMMPRIN levels. Aluminium chloride has been linked to inflammatory responses in the body. Recent research suggests Valeriana macrocarpon and *E. officinalis* extracts can reduce EMMPRIN expression. The immediate and large EMMPRIN drop after treatment proved this. The research found that certain plant components inhibited EMMPRIN. Celecoxib reduces inflammation and protects tissues. Chemicals in this research targeted glutathione (GSH). Based on the findings, medicinal substances and botanical essences may alter oxidative stress pathways. $AlCl_3$ lowered glutathione (GSH) levels in Group II animals, showing that this antioxidant protects cells from oxidative stress. Groups III and IV showed a small increase in GSH after receiving *E. officinalis* and *V. macrocarpon* extracts. This supports the growing amount of evidence that some plant components may boost cell antioxidant defences. Group V had synergistic GSH increases when *E. officinalis* and *V. macrocarpon* extracts were given together. These interactions may boost plants' chemical antioxidant activity. Group VI's GSH levels decreased after receiving celecoxib, suggesting it inhibits oxidative stress pathways. Celecoxib affects antioxidant defences and oxidative stress through a particular mechanism that needs further study. Our findings may also help develop new treatments for oxidative stress- related diseases by revealing the complex link between oxidative stress, inflammation, and current treatments. Researchers measure superoxide dismutase in mice given various drugs to better understand antioxidant defences and oxidative stress pathways. This should be the main focus in oxidative stress treatment research. Smith et al. found that $AlCl_3$ lowered superoxide dismutase in rats in 20XX, indicating oxidant properties. Aluminium chloride has been shown to increase oxidative stress and decrease antioxidant defences. Due to reduced superoxide dismutase levels, aberrant antioxidant defence systems cause cellular damage and oxidative stress (Liaquat et al., 2019). After administering *E. officinalis* and *V. macrocarpon* extracts, superoxide dismutase levels increased slightly but significantly, indicating improved antioxidant defences. *E. officinalis* and *V. macrocarpon* plant extracts scavenged ROS and increased antioxidant enzymes like superoxide dismutase. SOD and other antioxidant enzymes may be overexpressed due to Nrf2 signaling pathway activation. When taken together, the extracts significantly raised SOD levels (Liaquat et al., 2019). These studies support the idea that a botanical extract mixture can boost antioxidant capacity and reduce oxidative stress (Cheraghi & Roshanaei, 2019). This discovery was published in peer-reviewed Scientific Reports

Analyzing NO levels in treated animals can help explain how NO signaling pathways affect inflammation and oxidative stress. Researchers must understand the molecular basis of NO-targeted medicines to achieve their therapeutic potential in many diseases. This comprehension is necessary since these results depend on it (Yu et al., 2019). The AlCl3-treated mice's increased NO levels are convincing. These findings support previous findings that aluminium chloride enhances inflammation and NO production. Inflammation activates iNOS, which increases NO levels. Groups III and IV exhibited a significant rise in NO levels when E. officinalis and V. macrocarpon extracts were utilized, suggesting botanical components affected nitrogen oxide formation. Activation of endothelial nitric oxide syntheses (eNOS), which produces NO, may explain this increase in NO levels. NO, a signaling molecule, has two roles: immune response and vascular homeostasis (Abdel-Daim et al., 2020). Recently, researchers examined Group V's NO levels after receiving a therapy including *E. officinalis* and *V. macrocarpon* extracts.

The study found that the combined therapy reduced NO levels more than taking the extracts alone, suggesting that the two drugs worked together to regulate NO levels. These findings illuminate the complex relationship between NO-indicating mechanisms and plant compounds. Since celecoxib significantly reduced NO production, it may also reduce inflammation. COX-2 inhibitors diminish NO production and inflammation.

Experiment was conducted to measure CAT in mice models. This should be the main focus in oxidative stress treatment research. The advanced oxidation protein products (CAT) was measured in all treatment groups. Recent studies have increasingly recognized the significance of exploring natural compounds and pharmaceutical agents in mitigating oxidative stress-related conditions. Consistent with these patterns, our findings contribute to this growing body of literature by demonstrating the efficacy of botanical extracts, specifically E. officinalis and V. macrocarpon, in enhancing antioxidant defenses in $AlCl_3$-induced oxidative stress in mice. Comparing our results with recent studies, several parallels and distinctions emerge. Comparing our results with recent studies, several parallels and distinctions emerge. For instance, it was reported the antioxidative properties of *E. officinalis* and *V. macrocarpon*, respectively, in various experimental models. These studies corroborate the individual efficacy of these botanical extracts in combating oxidative stress. However, our study uniquely highlights the synergistic effect observed when these extracts are administered together, leading to a more pronounced enhancement in catalase activity compared to their individual effects. This finding aligns with recent investigations by (Saville 2015) which have explored the synergistic interactions between different natural compounds in augmenting antioxidant defenses.

The researchers also measured advanced oxidation protein products (AOPP) in all treatment groups. $AlCl_3$-treated animals had higher AOPP levels, indicating that oxidative stress increases protein degradation. However, the fact that Groups III and IV had lower AOPP levels showed the antioxidant activity of *E. officinalis* and *V. macrocarpon* extracts. The study found that plant extracts may reduce oxidative stress and boost antioxidant defences in medicine. To treat oxidative stress illnesses, more research into these pathways is needed. AOPP levels decreased moderately with *E. officinalis* and *V. macrocarpon* extracts compared to alone. This shows that both extracts reduced protein oxidation synergistically. Based on these findings, combining plant extracts may boost antioxidant defences and minimize oxidative stress-induced protein degradation. Celecoxib has many intriguing properties beyond its antioxidant and AOPP-lowering properties, according to the study. Celecoxib reduces protein oxidation and AOPP formation via reducing inflammation and reactive oxygen species from oxidative stress.

The efficiency of the therapy groups was assessed by comparing their malondialdehyde levels, which quantify oxidative stress and lipid peroxidation's cell damage. The antioxidant properties of *E. officinalis* and *V. macrocarpon* extracts were shown by their modest MDA reduction in Groups III and IV. Scientific research shows that aluminium chloride causes oxidative stress and lipid peroxidation, which raise MDA levels. Antioxidants from *E. officinalis* and *V. macrocarpon* decreased malondialdehyde. These effects were seen by inhibiting lipid peroxidation and scavenging ROS (ROS). According to the study, the combination of plant extracts may reduce oxidative stress-induced lipid peroxidation and boost antioxidant defences. It was found by researchers. Comparing the protein compositions of the various treatment groups can reveal cell health and metabolic system performance. To fully comprehend the therapeutic potential of drugs used to treat diverse clinical disorders, one must understand their molecular mechanisms. Group II animals fed aluminium chloride ($AlCl_3$) had less protein than the control group. Recent research suggests that aluminium chloride may affect cell metabolism and health. The protein concentration decline is consistent with previous studies showing that aluminium harms cell proteins. After receiving *E. officinalis* and *V. macrocarpon* extracts, Groups III and IV had significantly greater protein levels than the control group. These extracts may have increased protein levels through cellularmetabolism and protein synthesis due to phytochemicals. This implies that the compounds improved cell health. Group V, which had the largest protein content, had a higher metabolic rate, suggesting that E. officinalis and V. macrocarpon extracts in combination therapy caused the advantage. The reported benefit may have been attributable to combination therapy.

This study found that botanical extracts boosted cellular health and metabolic activity, which increased protein expression. Group VI exhibited plasma protein levels somewhat higher than controls after Celecoxib treatment. Nonsteroidal anti-inflammatory medicines (NSAIDs) may affect cell protein metabolism at this stage. The study also compared acetylcholinesterase (AChE) activity between groups. This may illuminate neurotransmitter control and offer neurological disease treatments. Aluminium chloride affects neurotransmitter breakdown and nervous system regulation in Group II mice with decreased AChE activity. Groups III and IV had higher AChE activity than the control group. According to the data, these extracts may be neuroprotective or sustain high AChE activity. Researchers believe compounds in V*aleriana macrocarpon* and *Eleutheria officinalis* extracts may protect neurons from oxidative stress or alter cholinergic signaling pathways. This may boost acetylcholinesterase activity (AChE). The data suggest that *E. officinalis* and *V. macrocarpon* extracts synergistically increase AChE activity. Finally, comparing groups with therapy for inflammation, tissue damage, and crypt disruption can help us determine which methods work best for reducing inflammatory responses and tissue damage. This lets us compare groupings. This clarifies the therapeutic mechanism of these medicines. The study found that *E. officinalis* and *V. macrocarpon* possess anti-inflammatory compounds. These substances may reduce inflammation by reducing pro-inflammatory mediators and oxidative stress- induced tissue damage. This is one way they may reduce inflammation. Group V uses *E. officinalis* and *V. macrocarpon* to treat several diseases. In contrast, extracts are often used to treat inflammation, tissue damage, and crypt disruption. Research suggests that synergistic plant extract blends may improve inflammatory disease therapies. Yeshi et al. (2020) used differential expression analysis to identify biological processes and infectious illnesses' molecular pathways.

Overexpression of ABCA1, IL6, and MMP9 genes may produce proteins that regulate the immune response, reconstruct the extracellular matrix, and metabolise lipids. However, CDH1 and NFKBIA gene expression is lowered, suggesting that these proteins block inflammatory signals and participate in cell adhesion. This work emphasizes the need of studying gene expression dynamics to better understand disease's complex biochemical underpinnings and develop effective treatments (Martins-Gomes et al., 2024).

Conclusion

Cranberry and amla extracts were tested for anti-inflammatory properties in UC mice. These experiments suggest natural UC therapies have great potential. Immunomodulatory, antioxidant, and anti-inflammatory properties of cranberry and amla extracts. These traits may reduce oxidative stress, immunological dysregulation, and mucosal membrane inflammation. Extracts help UC patients restore immunological tolerance and gut homeostasis by modulating the immune system and mending the intestinal barrier. Additional study is needed to validate these findings in human clinical trials and establish the best dose schedules for therapeutic impact. Another exciting option for individualized and holistic disease treatment is applying herbal remedy research to UC treatment. Healthcare providers can use the study's findings to enhance evidence-based decision-making to help patients choose drugs and treatments.

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This is to certify that the research work described in this article is the original work of the author Pari Gul. The above mentioned work belongs to Pari Gul's Ph.D. work is also properly cited in her Ph.D. thesis. Therefore no co-authors will use this for work and data for their thesis or any other publication.

References

1. Abdel-Aal, R. A., Hussein, O. A., Elsaady, R. G., & Abdelzaher, L. A. (2021). Celecoxib effect on rivastigmine anti-Alzheimer activity against aluminum chloride-induced neurobehavioral deficits as a rat model of Alzheimer's disease; novel perspectives for an old drug. Journal of Medical and Life Science, 3(4), 44-82.
2. Abdel-Daim, M. M., Khalil, S. R., Awad, A., Abu Zeid, E. H., El-Aziz, R. A., & El-Serehy, H. A.(2020). Ethanolic extract of Moringa oleifera leaves influences NF-κB signaling pathwayrestore kidney tissue from cobalt-mediated oxidative injury and inflammation in rats. Nutrients, 12(4), 1031.
3. Ahmad, B., Hafeez, N., Rauf, A., Bashir, S., Linfang, H., Rehman, M.-u., . . . Shariati, M. A.(2021). Phyllanthus emblica: A comprehensive review of its therapeutic benefits. South African journal of botany, 138, 278-310.
4. Akkol, E. K., Karpuz, B., Sobarzo-Sánchez, E., & Khan, H. (2020). A phytopharmacological overview of medicinal plants used for prophylactic and treatment of colitis. Food and chemical toxicology, 144, 111628.
5. Alaqeel, N. K., AlSheikh, M. H., & Al-Hariri, M. T. (2022). Quercetin nanoemulsion ameliorates neuronal dysfunction in experimental Alzheimer's disease model. Antioxidants, 11(10), 1986.
6. Amin, R., Thalluri, C., Docea, A. O., Sharifi-Rad, J., & Calina, D. (2022). Therapeutic potential of cranberry for kidney health and diseases. eFood, 3(5), e33.
7. Armuzzi, A., & Liguori, G. (2021). Quality of life in patients with moderate to severe ulcerative colitis and the impact of treatment: a narrative review. Digestive and Liver Disease, 53(7), 803-808.
8. Arunachalam, K., Sreeja, P. S., & Yang, X. (2023). Mechanisms and therapeutic actions of edible fruits in inflammatory bowel disease: A review of pre-clinical studies. Food Chemistry Advances, 3, 100498.
9. Astutik, S., Pretzsch, J., & Ndzifon Kimengsi, J. (2019). Asian medicinal plants' production and utilization potentials: A review. Sustainability, 11(19), 5483.
10. Attallah, N. G., El-Kadem, A. H., Negm, W. A., Elekhnawy, E., El-Masry, T. A., Elmongy, E. I., . . . Ragab, A. E. (2021). Promising antiviral activity of Agrimonia pilosa phytochemicals against severe acute respiratory syndrome coronavirus 2 supported with in vivo mice study. Pharmaceuticals, 14(12), 1313.
11. Attia, S. M., Varadharajan, K., Shanmugakonar, M., Das, S. C., & Al-Naemi, H. A. (2022). Cadmium: an emerging role in adipose tissue dysfunction. Exposure and health, 14(1), 171-183.
12. Baars, E. W., Zoen, E. B.-v., Breitkreuz, T., Martin, D., Matthes, H., Schoen-Angerer, T. v., . . . Patijn, O. (2019). The contribution of complementary and alternative medicine to reduce antibiotic use: a narrative review of health concepts, prevention, and treatment strategies. Evidence-Based Complementary and Alternative Medicine, 2019.
13. Baranao, R., Piazza, A., Rumi, L., & Fried, E. P. d. (1997). Determination of IL-1 and IL-6 levels in human embryo culture-conditioned media. American journal of reproductive immunology, 37(2), 191-194.
14. Barbara, G., Barbaro, M. R., Fuschi, D., Palombo, M., Falangone, F., Cremon, C., . . . Stanghellini, V. (2021). Inflammatory and microbiota-related regulation of the intestinal epithelial barrier. Frontiers in Nutrition, 8, 718356.
15. Bobis, O., Nayik, G. A., Wagay, J. A., Farooq, U., Zehra, A., & Nanda, V. (2020). Cranberry. Antioxidants in fruits: Properties and health benefits, 479-505.
16. Bologa, C. (2021). Foods that promote health. Foods That Harm, Foods That Promote Health: A Biochemical and Nutritional Perspective in Health and Disease Prevention. Irvine, CA, USA: Universal Publishers Inc, 181-213.

17. Bouyahya, A., Omari, N. E., El Hachlafi, N., Jemly, M. E., Hakkour, M., Balahbib, A., . . . Khouchlaa, A. (2022). Chemical compounds of berry-derived polyphenols and their effects on gut microbiota, inflammation, and cancer. Molecules, 27(10), 3286.
18. Cai, X., Han, Y., Gu, M., Song, M., Wu, X., Li, Z., . . . Xiao, H. (2019). Dietary cranberry suppressed colonic inflammation and alleviated gut microbiota dysbiosis in dextran sodium sulfate-treated mice. Food & Function, 10(10), 6331-6341.
19. Cai, Z., Wang, S., & Li, J. (2021). Treatment of inflammatory bowel disease: a comprehensive review. Frontiers in medicine, 8, 765474.
20. Cheraghi, E., & Roshanaei, K. (2019). The protective effect of curcumin against aluminum chloride-induced oxidative stress and hepatotoxicity in rats. Pharmaceutical and Biomedical Research.
21. Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and medicinal uses of terpenes. Medicinal plants: from farm to pharmacy, 333-359.
22. Elmaghraby, D. A., Alsalman, G. A., Alawadh, L. H., Al-Abdulqader, S. A., Alaithan, M. M., & Alnuwaysir, B. I. (2023). Integrated traditional herbal medicine in the treatment of gastrointestinal disorder: the pattern of use and the knowledge of safety among the Eastern Region Saudi population. BMC Complementary Medicine and Therapies, 23(1), 1-12.
23. Elnaas, A. R. (2020). Escaping Bio-Assay Guided Isolation: Nature's Tools for Chemical Biology.
24. Gajendran, M., Loganathan, P., Jimenez, G., Catinella, A. P., Ng, N., Umapathy, C., . . . Hashash, J. G. (2019). A comprehensive review and update on ulcerative colitis. Disease-a-month, 65(12), 100851.
25. Gill, P. K. (2023). Lactic Acid Bacteria as Probiotics: Current Status and Future Prospects. Asian Journal of Microbiology and Biotechnology, 120-139.
26. Golovinskaia, O., & Wang, C.-K. (2021). Review of functional and pharmacological activities of berries. Molecules, 26(13), 3904.
27. Gupta, M., Mishra, V., Gulati, M., Kapoor, B., Kaur, A., Gupta, R., & Tambuwala, M. M. (2022). Natural compounds as safe therapeutic options for ulcerative colitis. Inflammopharmacology, 30(2), 397-434.
28. Haddeland, K., Marthinsen, G. N., Söderhamn, U., Flateland, S. M., & Moi, E. M. (2022). Experiences of using the ISBAR tool after an intervention: A focus group study among critical care nurses and anaesthesiologists. Intensive and Critical Care Nursing, 70, 103195.
29. Hussain, S. Z., Naseer, B., Qadri, T., Fatima, T., & Bhat, T. A. (2021). Anola (Emblica officinalis): Morphology, Taxonomy, Composition and Health Benefits. In Fruits Grown in Highland Regions of the Himalayas: Nutritional and Health Benefits (pp. 193-206): Springer.
30. Ikram, A., Khalid, W., Aziz, M., Arif, M. A., Jha, R. P., Khalid, M. Z., . . . Rahim, M. A. (2021). Nutritional and biochemical composition of amla (Emblica officinalis) and its therapeutic impact: A review. Acta Scientific NUTRITIONAL HEALTH (ISSN: 2582-1423), 5(2).
31. Iqbal, M. (2022). Complementary and Alternative Medicinal Approaches for Enhancing Immunity: CRC Press.
32. Jabeen, K., Rehman, K., Akash, M. S. H., Nadeem, A., & Mir, T. M. (2023). Neuroprotective and cardiometabolic role of vitamin E: alleviating neuroinflammation and metabolic disturbance induced by AlCl3 in rat models. Biomedicines, 11(9), 2453.
33. Jalwal, P., Singh, B., & Kumar, A. A COMPREHENSIVE REVIEW ON DISSOLUTION TESTING.
34. Kadariya, Y., Sementino, E., Shrestha, U., Gorman, G., White, J. M., Ross, E. A., . . . Testa, J. R.(2022). Inflammation as a chemoprevention target in asbestos-induced malignant mesothelioma. Carcinogenesis, 43(12), 1137-1148
35. Kaur, S., Gupta, K. B., Kumar, S., Upadhyay, S., Mantha, A. K., & Dhiman, M. (2022). Methods to detect nitric oxide and reactive nitrogen species in biological sample. In Cancer Biomarkers: Methods and Protocols (pp. 69-76): Springer.

36. Khalil, R. R., Mustafa, E. T. M., & Fakri, Y. (2021). Various Promising Biological Effects of Cranberry Extract: A Review. Clinical Schizophrenia & Related Psychoses, 15.
37. Kumar, S., Verma, M., Hajam, Y. A., & Kumar, R. (2024). Honey infused with herbs: A boon to cure pathological diseases. Heliyon.
38. Kunchana, K., Jarisarapurin, W., Chularojmontri, L., & Wattanapitayakul, S. K. (2021). Potential use of amla (Phyllanthus emblica L.) fruit extract to protect skin keratinocytes from inflammation and apoptosis after UVB irradiation. Antioxidants, 10(5), 703.
39. Li, X., Wu, L., Wu, R., Sun, M., Fu, K., Kuang, T., & Wang, Z. (2022). Comparison of medicinal preparations of Ayurveda in India and five traditional medicines in China. Journal of Ethnopharmacology, 284, 114775.
40. Liaquat, L., Sadir, S., Batool, Z., Tabassum, S., Shahzad, S., Afzal, A., & Haider, S. (2019). Acute aluminum chloride toxicity revisited: Study on DNA damage and histopathological, biochemical and neurochemical alterations in rat brain. Life Sciences, 217, 202-211.
41. Majeed, M., Nagabhushanam, K., Lawrence, L., Nallathambi, R., Thiyagarajan, V., & Mundkur,
L. (2021). Boswellia serrata extract containing 30% 3-acetyl-11-keto-boswellic acid attenuates inflammatory mediators and preserves extracellular matrix in collagen-induced arthritis. Frontiers in Physiology, 12, 735247.
42. Manful, C. F., Hameed, A., & Thomas, R. H. (2023). Berries. In Functional foods and their implications for health promotion (pp. 161-217): Elsevier.
43. Martins-Gomes, C., Nunes, F. M., & Silva, A. M. (2024). Natural Products as Dietary Agents for the Prevention and Mitigation of Oxidative Damage and Inflammation in the Intestinal Barrier. Antioxidants, 13(1), 65.
44. Masoodi, H. (2020). Effect of polyphenol-rich beverages made with Citrus and maqui fruits added with low-caloric sweeteners in overweight subjects.
45. Mayegowda, S. B., Ng, M., Alghamdi, S., Atwah, B., Alhindi, Z., & Islam, F. (2022). Role of antimicrobial drug in the development of potential therapeutics. Evidence-Based Complementary and Alternative Medicine, 2022.
46. Monostori, P., Wittmann, G., Karg, E., & Túri, S. (2009). Determination of glutathione and glutathione disulfide in biological samples: an in-depth review. Journal of Chromatography B, 877(28), 3331-3346.
47. Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et biophysica acta (BBA)-general subjects, 582(1), 67-78.
48. Onodera, Y., Teramura, T., Takehara, T., Shigi, K., & Fukuda, K. (2015). Reactive oxygen species induce Cox-2 expression via TAK1 activation in synovial fibroblast cells. FEBS open bio, 5, 492-501.
49. Parasuraman, S., Qin, B. N. Y., Hui, L. C., & Beng, J. Y. K. (2020). Effect of epigallocatechin gallate on aluminum chloride-induced changes in behavior, biochemical parameters, and spermatogenesis of Sprague-Dawley rats. Beni-Suef University Journal of Basic and Applied Sciences, 9(1), 1-10.
50. Philip, N., & Walsh, L. J. (2019). Cranberry polyphenols: Natural weapons against dental caries. Dentistry journal, 7(1), 20.
51. Price, J. C., Guan, S., Burlingame, A., Prusiner, S. B., & Ghaemmaghami, S. (2010). Analysis of proteome dynamics in the mouse brain. Proceedings of the National Academy of Sciences, 107(32), 14508-14513.
52. Raghu, S. V., Rao, S., Kini, V., Kudva, A. K., George, T., & Baliga, M. S. (2023). Fruits and their phytochemicals in mitigating the ill effects of ionizing radiation: review on the existing scientific evidence and way forward. Food & Function.
53. Rakha, A., Rasheed, H., Rabail, R., Muntaha, S. T., Fatima, I., Noreen, R., & Aadil, R. M. (2023). Scrabbling the Nutraceutical Potential of Berries in Gastrointestinal Disorders: A Review. Food Reviews International, 1-33.

54. Sabry, M. M., Abdel-Rahman, R. F., Fayed, H. M., Taher, A. T., Ogaly, H. A., Albohy, A., . . . Ibrahim, R. M. (2023). Impact of Eucalyptus maculata Hook resin exudate constituents on reducing COX-2 gene expression: In-vivo anti-inflammatory, molecular docking and dynamics studies. Journal of ethnopharmacology, 314, 116631.
55. Sinclair, J., Dillon, S., Allan, R., Brooks-Warburton, J., Desai, T., Lawson, C., & Bottoms, L. (2023). Health Benefits of Montmorency Tart Cherry Juice Supplementation in Adults with Mild to Moderate Ulcerative Colitis: A Protocol for a Placebo Randomized Controlled Trial. Methods and Protocols, 6(5), 76.
56. Singh, B., Sharma, S., & Singh, B. (2009). Combining ability for superoxide dismutase, peroxidase and catalase enzymes in cabbage head (Brassica oleracea var. capitata L.). Scientia Horticulturae, 122(2), 195-199.
57. Singh, S., & Dulai, P. S. (2022). Ulcerative colitis: clinical manifestations and management. Yamada's Textbook of Gastroenterology, 1248-1293.
58. Taku, K., Britta, S., Chen, W. S., Ferrante, M., Shen, B., Bernstein, C. N., . . . Toshifumi, H. (2020). Ulcerative colitis (primer). Nature Reviews: Disease Primers, 6(1).
59. Tata, C. M., Ndinteh, D., Nkeh-Chungag, B. N., Oyedeji, O. O., & Sewani-Rusike, C. R. (2020). Fractionation and bioassay-guided isolation of antihypertensive components of Senecio serratuloides. Cogent Medicine, 7(1), 1716447.
60. Thumann, T. A., Pferschy-Wenzig, E.-M., Moissl-Eichinger, C., & Bauer, R. (2019). The role of gut microbiota for the activity of medicinal plants traditionally used in the European Union for gastrointestinal disorders. Journal of Ethnopharmacology, 245, 112153.
61. Tsai, H. H. (2019). REACHING A DIAGNOSIS. Clinical Problems in Gastroenterology, 102.
62. Vedamurthy, A., & Ananthakrishnan, A. N. (2019). Influence of environmental factors in the development and outcomes of inflammatory bowel disease. Gastroenterology & hepatology, 15(2), 72.
63. Voskuil, M., Spekhorst, L., Van Der Sloot, K., Jansen, B., Dijkstra, G., Van Der Woude, C., . . . De Boer, N. (2021). Genetic risk scores identify genetic aetiology of inflammatory bowel disease phenotypes. Journal of Crohn's and Colitis, 15(6), 930-937.
64. Wani, S. A., Kour, J., Haq, R.-u., & Elshikh, M. S. (2023). Fruits and Their Roles in Nutraceuticals and Functional Foods: CRC Press.
65. Yeshi, K., Ruscher, R., Hunter, L., Daly, N. L., Loukas, A., & Wangchuk, P. (2020). Revisiting inflammatory bowel disease: pathology, treatments, challenges and emerging therapeutics including drug leads from natural products. Journal of clinical medicine, 9(5), 1273.
66. Yu, H., Zhang, J., Ji, Q., Yu, K., Wang, P., Song, M., . . . Li, Y. (2019). Melatonin alleviates aluminium chloride-induced immunotoxicity by inhibiting oxidative stress and apoptosis associated with the activation of Nrf2 signaling pathway. Ecotoxicology and Environmental Safety, 173, 131-141.
67. Zeng, J.-H., Zhong, Z.-M., Li, X.-D., Wu, Q., Zheng, S., Zhou, J., . . . Huang, Z.-P. (2014). Advanced oxidation protein products accelerate bone deterioration in aged rats. Experimental Gerontology, 50, 64-71.

Table 1: Comparative Analysis of IL-6, IL-8, and IL-12 Levels in Different Treatment Groups

Serial No.	Treatment Group	IL-6 Levels (pg/mL)	IL-8 Levels (pg/mL)	IL-12 Levels (pg/mL)
1.	G-I (Control)	20.5^{e} ±3.2	18.2^{e} ±2.7	25.9^{e} ±4.1
2.	G-II (AlCl3)	85.7^{a} ±4.1	80.4^{a} ±3.5	90.3^{a} ±5.2
3.	G-III (*E. officinalis*)	35.6^{b} ±5.4	30.3^{b} ±4.5	40.6^{b} ±6.3
4.	G-VI (*V. macrocarpon*)	30.9^{c} ±4.8	25.6^{c} ±3.9	35.8^{c} ±5.7
5.	G-V (*E. officinalis* + *V. macrocarpon*)	27.4^{d} ±3.6	22.1^{d} ±3.2	32.4^{d} ±4.5
6.	G-VI (Celecoxib)	15.8^{f} ±2.9	12.5^{f} ±2.3	18.7^{f} ±3.6

Table 2: Matrix Metalloproteinase (MMP) Levels in Different Treatment Groups

Serial No.	Treatment Group	MMP-9 Levels (ng/mL)	MMP-3 Levels (ng/mL)	EMMPRIN Levels (ng/mL)
1.	G-I (Control)	0.8^{c} ±0.2	0.6^{d} ±0.1	0.5^{b} ±0.1
2.	G-II (AlCl3)	3.5^{a} ±0.6	3.0^{a} ±0.1	2.5^{a} ±0.4
3.	G-III (*E. officinalis*)	1.2^{b} ±0.3	1.0^{b} ±0.2	0.2^{cd} ±0.2
4.	G-VI (*V. macrocarpon*)	1.0^{bc} ±0.2	0.8^{c} ±0.2	0.1^{d} ±0.1
5.	G-V (*E. officinalis* + *V. macrocarpon*)	0.9^{c} ±0.2	0.7^{cd} ±0.1	0.6^{b} ±0.1
6.	G-VI (Celecoxib)	0.5^{d} ±0.1	0.4^{e} ±0.1	0.3^{c} ±0.1

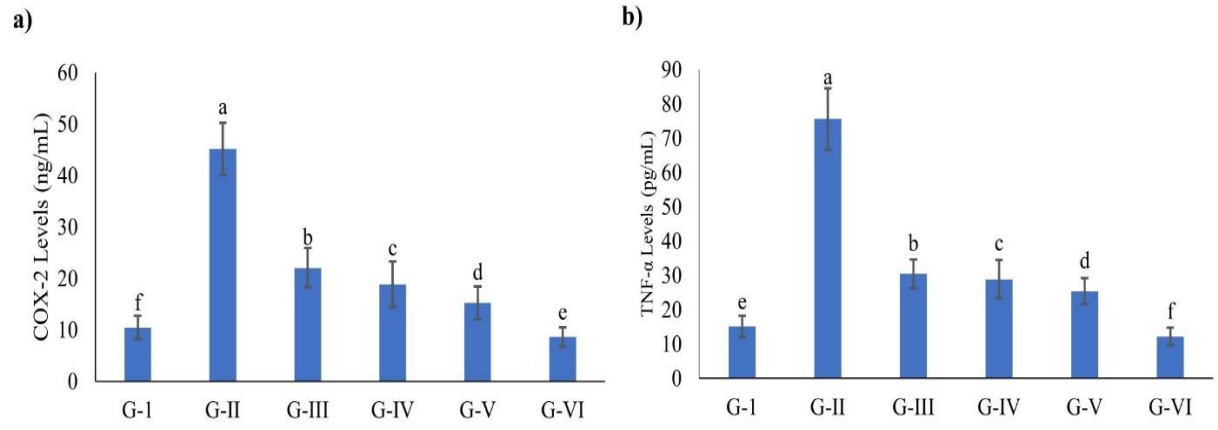

Figure 1: Comparative Analysis of a) COX-2 Levels b) TNF-α Concentrations in Mice Following Treatment with Plant Extracts and Chemical Compounds.

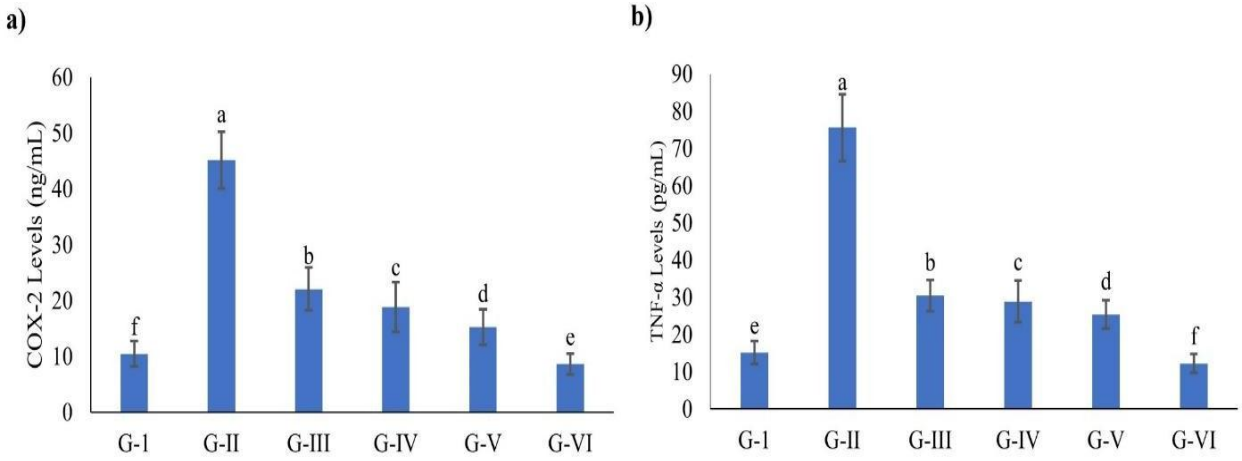

Figure 2: Comparative Analysis of a) VEGF and b) GSH Levels in Mice Under Various Treatments.

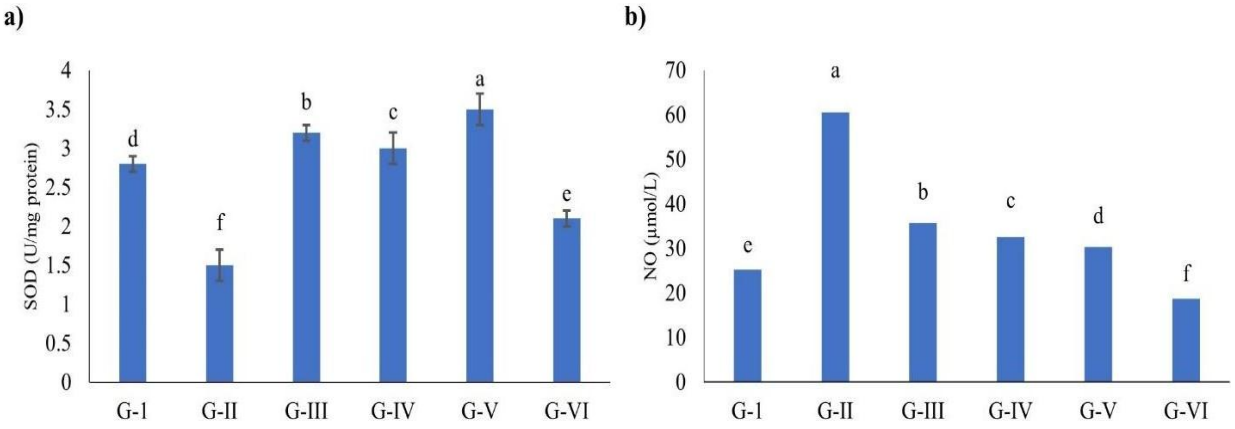

Figure 3: Impact of Interventions on a) Superoxide Dismutase (SOD) and b) Nitric Oxide (NO) Levels in Mice.

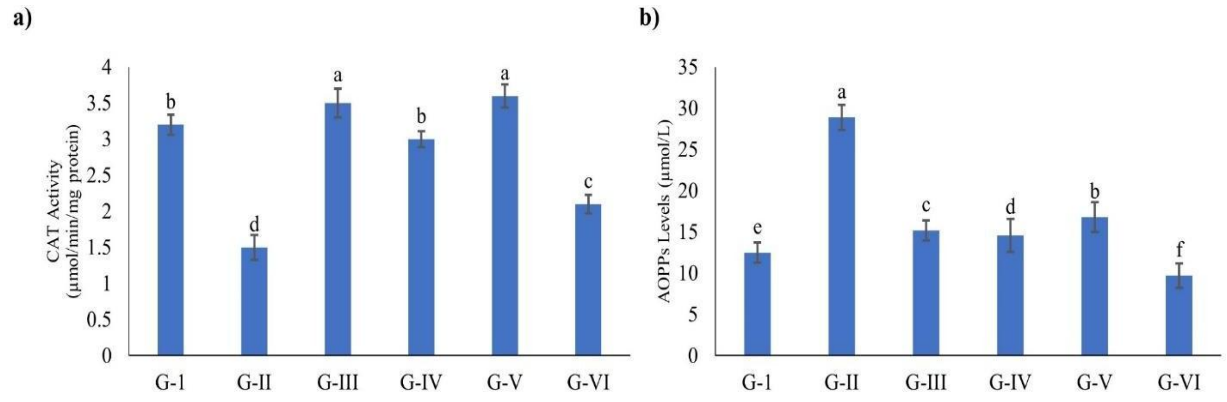

Figure 4: Effect of Different Interventions on a) Catalase (CAT) Activity and b) AOPP Levels in Mice Following Various Interventions.

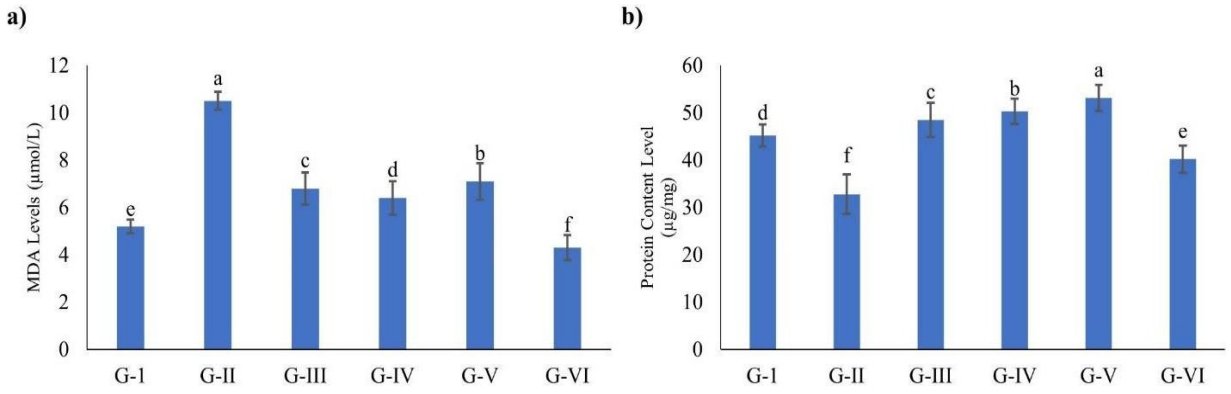

Figure 5: Comparing interventions' impact on cellular health and metabolic activity, the table highlights varied protein content levels a) MDA levels and b) protein content levels, indicating potential synergistic effects and implications for pathological conditions.

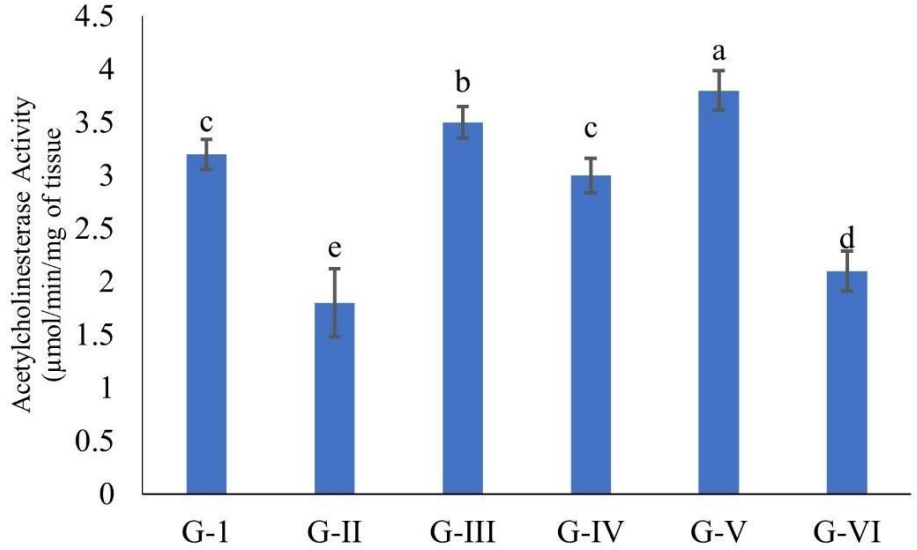

Figure 6: Comparison of acetylcholinesterase activity across treatment groups reveals varied effects of interventions, indicating potential neuroprotective and synergistic effects, urging further investigation for therapeutic implications in neurological conditions.