Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

Phytochemical Profiling and Anti-Colitis Potential of *Helianthus annuus* L. Seed Extract in Acetic Acid Induced Ulcerative Colitis in Wistar Rats

Indrajit Das[1], Moumita Ray[1], Ritu Khanra[1], Sakshar Saha[1]

[1]Department of Pharmaceutical Technology, JIS university, Agarpara, Kolkata- 700109, India

Correspondence:

Dr. Sakshar Saha, Assistant Professor, Department of Pharmaceutical Technology, JIS University, Kolkata, India

(Received: 05 January 2026 Revised: 15 February 2026 Accepted: 05 March 2026)

KEYWORDS

Ulcerative colitis, Helianthus annuus, Anti-inflammatory, Inflammation, TNF-α

ABSTRACT:

Introduction:

The current study was conducted to investigate the phytochemical composition and therapeutic potential of Helianthus annuus L. (sunflower) seed extract against ulcerative colitis (UC) in Wistar rats. UC, a chronic inflammatory bowel disease, was induced in rats to evaluate the anti-inflammatory and mucosal protective effects of the seed extract. Helianthus annuus L., commonly known as sunflower and belonging to the family Asteraceae, is one of the foremost sources of high-quality edible oil and is recognized for its substantial nutritional, culinary, and medicinal properties. In the in vivo analysis, ulcerative colitis was induced in Wistar rats using 7% acetic acid.

Objectives:

To evaluate the phytochemical constituents and assess the therapeutic efficacy of Helianthus annuus L. seed extract in acetic acid-induced ulcerative colitis in Wistar rats.

Methods:

Ulcerative colitis (UC) was induced in Wistar rats using 7% acetic acid. The treatment groups received 200 mg/kg and 400 mg/kg body weight of the seed extract, respectively, while a standard group received sulfasalazine. Phytochemical analysis was performed to identify bioactive constituents. Disease severity was evaluated through macroscopic scoring, colon weight/length ratio, stool consistency, and gastrointestinal pH. Nitric oxide (NO) and biochemical parameters including nuclear factor Kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) were measured. Histopathological examination of colon tissue was conducted to assess structural alterations.

Results:

Phytochemical analysis revealed the presence of biologically active compounds including saponins, tannins, flavonoids, alkaloids, and phenolic compounds, known for their antioxidant and anti-inflammatory properties. The results demonstrated that the sunflower seed extract reduced inflammation, oxidative stress, and mucosal damage in a dose-dependent manner. The higher dose (400 mg/kg BW) exhibited therapeutic outcomes comparable to the standard drug. Notably, NO, NF-κB, and TNF-α levels were markedly reduced in treated groups, and histological evaluation showed improved mucosal integrity.

Conclusions:

These findings suggest that Helianthus annuus seed extract holds promise as a natural therapeutic agent for managing ulcerative colitis (UC), possibly through its phytochemical constituents and anti-inflammatory action.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

1.Introduction

Ulcerative colitis or UC is a chronic bowel turbulence causing integrity to disrupt colon as identified by continuous colorectal incendiary and lesions. Event in various zone of the earth, UC has from 0.6 to 24.3 per 100,000 persons. Man, and women are uniformly altered. The goal of the recent study was to assess its impact *Helianthus annuus* on oxidative stress markers and inflammatory mediators for searching acetic acid-inducing ulcerative colitis or UC in rat models of its anti-ulcerative colitis activity [1]. Increasing the growing knowledge of the potential benefits of plant-based treatment resurrection of interest in herbal medicines. In the light of current health problems, a crowd botanical knowledge in the procedures of indigenous cure from conventional herbal remedies reviewed and re-examined. The need for a completely scientific investigation is increasing with interest in herbal therapy [2].

This renewal focus includes the practice of indigenous healing and an extension of ancient herbal traditions, which encouraged existing botanical knowledge to re - evaluate context of contemporary health challenges. The popularity of phyto medicines constant growth, there is a require for a systemic scientific investigation to prove its therapeutic claim. This synthesis of the traditional taid knowledge and this synthesis of modern scientific methods can be noticed to bridge the gap between melodious evidence and experiencedly valid intervention. Herbal therapy is being adopted more widely as successful supplement treatment so the main patient is as care method and in some cases [3]. A plant in the Asteraceae family, *Helianthus annuus* or sunflower, is one of the notable plants receiving increased attention as a result of this comeback. Reaching up to 4 meters in height, this annual herbaceous plant is distinguished by its large, oval leaves that are alternately lined with a stiff, hairy stem [4]. The sunflower inflorescence, a compound flower head known scientifically as a capitulum, is its distinctive feature. Within this capitulum are hundreds or perhaps thousands of individual flowers arranged in an intricate spiral pattern. Yellow, petal-like ray florets adorn the outer edges, while a dark-coloured, cylindrical disk occupies the centre [5]. Sunflower seeds and oil have been used for a long time due to their antibacterial, anti-inflammatory, anti-diabetic and anti-cardiovascular properties. According to research, the seeds contain powerful antioxidants namely Fig.1 represent quercetin, caffeic acid, tocopherol and chlorogenic acid that can help fight free radicals and reduce oxidative stress, which is key elements in the pathophysiology of ulcerative colitis. The anti-inflammatory properties of sunflower seed extract may also help regulate cytokine levels and reduce mucosal damage associated with colitis [6]

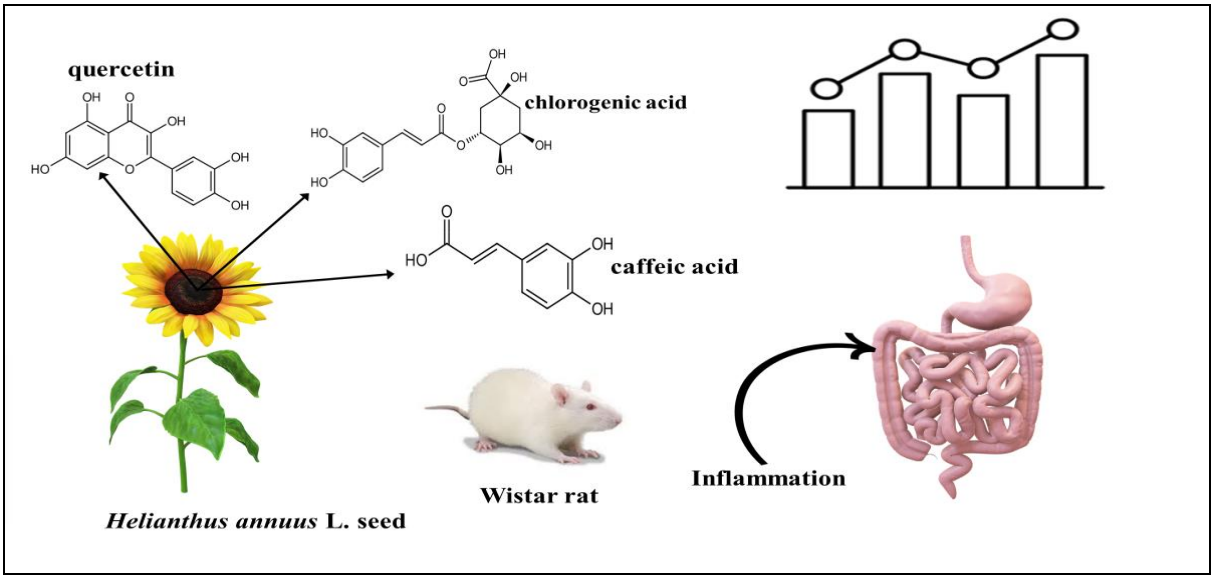


Figure 1: Investigating the Anti-Inflammatory Potential of *Helianthus annuus* Seed Extracts in a Rat Model of Colitis

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

Sunflower seeds or *Helianthus annuus* L, cultivated and consumed worldwide, serve as a rich source of numerous nutrients, including protein, unsaturated fats, dietary fibre, as well as a various types of vitamins, especially vitamin E, and essential minerals namely copper,zinc, selenium, folate, and iron. Sunflower seeds have a wide range of culinary uses; they are used as cooking oil, fried or eaten as a savoury dish, used in desserts, and are widely used in animal and pet food as a valuable source of sulfur-containing amino acids [7]. Furthermore, sunflower seeds and sprouts have significant health advantage, including anti-inflammatory, anti-hypertensive, antibacterial, and antioxidant properties, and the potential to support wound healing and heart health[8]. These benefits are mostly related to the natural phenolic compounds, polyunsaturated fatty acids, flavonoids and several vitamins of the sunflower plant. This collection of data emphasizes how important it is to conduct thorough scientific research on the therapeutic potential of herbal medicines, especially in light of the numerous health benefits associated with *Helianthus annuus* [11][12].By systematically evaluating the in vitro anti-inflammatory and anti-inflammatory characteristics of *Helianthus annuus* seeds, this effort aims to fill the current knowledge gap [13]. The study also attempts to determine whether the plant has the potential to be a natural source of bioactive molecules that could be used as building blocks for drugs targeting chronic degenerative diseases in the future. The findings of this study will help provide a strong scientific basis for its pharmacological use and guide future research on its broader therapeutic potential [14][15].

2.Methods

2.1. Compiling and verification of the herb

The whole fresh herb of Sunflower was collected in the month of August 2023 from the village of Murshidabad district of West Bengal. The air-dried seeds were collected from UPL Limited, C/O Bharathi Bhramha Seeds Dist- Rangareddy, Telangana. The plant was determined and authenticated by the survey of Botanical Survey of India, Howrah with the authentication number JISU/2023/HA/001.

2.2. Extraction

Fig 2. shows that the dried seeds are then crushed and grinded to make a powder form.The fat was removed from the sunflower seed powder by dissolving it in hexene. powdered *Helianthus Annus* L. seeds were subjected to water extraction through maceration, which was conducted over a period of five days. After 3-4 days, filtration was performed to obtain the extract. The filtrate was also collected and allowed to evaporate on water bath in 60°C until a satisfactory amount of solvent was lost. The resulting semi-solid extract was then collected and kept in a freezer for following examination.

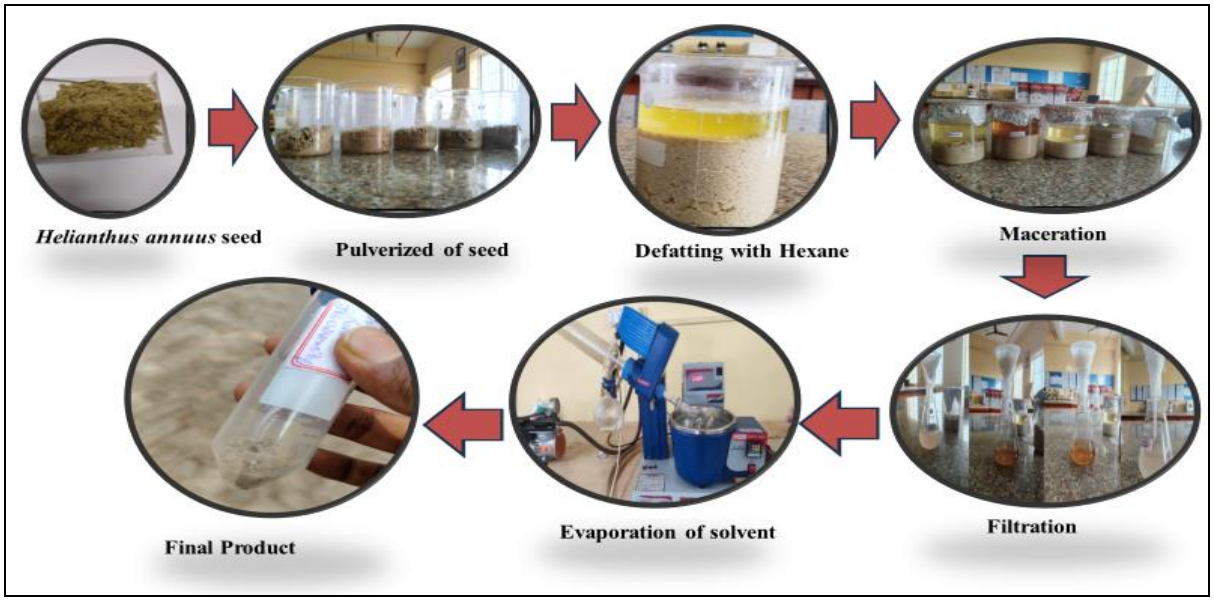


Figure 2: Various stages of the Extraction process

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

2.3. Phytochemical screening

The phytochemical analysis of the seed and its extract involves a qualitative assay to identify various phytochemicals in the plant material. Phytochemical screening: Qualitative study of secondary metabolites and the qualitative phytochemical analysis [16] of the *Helianthus annuus* L. seeds extract was carried according to Kokate *et al*, 2005. Analysis was conducted to detect the existence of various phytonutrients including carbohydrates, alkaloids, glycosides, tannins, flavonoids, phenols, oils and fats.

2.4. Preparation of Test Sample:

For conducting the following phytochemical tests, a 1mg/ml test solution was prepared. (The amount of 10mg crude extract was measured in a Falcon tube and 10 ml of distilled water was added to it).

2.5. Total Flavonoid Content

The total flavonoid amount of the crude extract was ascertained with some modifications as depicted by Liu. The TFC was determined from the linear equivalence y = 0.0086x + 0.071 of a standard curve prepared with quercetin and the total flavonoid amount of the extract was convey in milligrams per gram of extract [17].

2.6. Total Phenolic Content

The total phenolic amount of the crude extract was ascertain as depicted by Javanmardi *et al*. The TPC was determined from the linear equivalence y = 0.0087x + 0.0645 of a standard curve pre with gallic acid and the amount of total phenolic amount in the extract was convey in milligrams of extract per gram of extract [18].

3.*In vitro* antioxidant activity

3.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH)radical scavenging activity

The method depicted by Sanchez-Moreno *et al*. was used to evaluate the scavenging activity of DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals. A 0.002% w/v DPPH solution in methanol was prepared [19]. For the test, 150 µl of each and every concentration of extract was blend with 150 µl of DPPH solution, and control solutions were adapted without DPPH for color correction. The reaction combination was hatched in the dark for 30 min at ambient temperature, and the UV-VIS spectrophotometer (Shimazu UV -1800) was used to observed absorbance at 517 nm. The radical scavenging activity [20] was analysis by this equation:

Percentage inhibition = [{Absorbance of control - (Absorbance of test sample- Color factor)}/

(Absorbance of control)] *100

3.2. Reducing power assay

The reducing power of the samples was evaluated using a technique developed by Oyaizu in 1986. Extract samples (1.0 ml) of different concentrations were incorporated with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2M phosphate buffer (pH 6.6) [21]. This mixture was incubated in hot air oven at 50°C for 20 min. Afterwards incubation, 2.5 ml of 10% trichloroacetic acid was addition and the blend was centrifuged at 650 rpm for 10 min. Then, 2.5 ml of solution withdrawn from mixture and addition 2.5 ml of distilled water and 0.5 ml of 0.1% iron (III) chloride. The color change at 700 nm was observed by a spectrophotometer. Ahmadi F *et al*. showed that ascorbic acid was adapted to differentiate the reducing power. The enhanced the absorbance, superior the reducing power of the sample [22]. The reducing power activity was determined by the formula:

Percentage inhibition = [{Absorbance of control- (Absorbance of test sample- Color factor)}/

(Absorbance of control)] *100

4.*In vitro* anti-inflammatory study

4.1. Protein denaturation study using egg albumin

The reaction amalgam included 2.8 ml of phosphate-buffer saline (pH),0.2 ml of egg albumin (from a fresh hen's egg) and 2 ml of test extract at different concentrations (20µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100µg/mL). An uniform capacity of double-purified water was used as a control. The combination was incubated in a biological oxygen demand incubator at 37°C ± 2°C for 15 min and then heated at 70°C for 5 min. After cooling, the absorbance was calculated at 660 nm using the vehicle as a blank. Diclofenac sodium was used as standard and traditional/herbal drugs were treated in the same way to determine absorbance. The test extracts were selected to be as close as feasible to the benchmark therapeutic regimen. The percentage

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

obstacle of protein denaturation was determined using the following equation [23]: -

Percentage inhibition = [{Absorbance of control-(Absorbance of test sample- Color factor)}/

(Absorbance of control)] *100

4.2.RBC membrane stabilization assay

The goat blood film stabilization techniques have been used to evaluate in vitro anti-inflammatory properties. Blood was assembled from a goat and mixed with a uniform volume of Alsever solution, which contains 0.8% sodium citrate,0.5% citric acid, 2% dextrose, and 0.42% Nacl. Blood samples were kept at 4°C for 24 hours until use. The samples were centrifuged at 2500 rpm for 5 minutes, and the supernatant was discarded. The cell suspension was then cleaned with a 0.9% w/v sterile saline solution (NaCl) and again centrifuged at 2500 rpm for 5 minutes. This washing process was frequent three times until the supernatant became transparent and colorless. The filled cell capacity was determined and the cellular material was renovated into a 40% suspension (v/v) with phosphate-buffer saline (10 mM, pH 7.4) for use in the experiment [24].

4.3 Heat Induced Haemolysis

A mixture of 0.05 ml of hydromethanolic seed extract and 0.05 ml of goat blood cell suspension was mixed with 2.95 ml of phosphate buffer (pH 7.4). This mixture was incubated in a shaking water bath at 54°C for 20 min. Afterwards incubation, the mixture was centrifuged at 2500 rpm for 3 min and the absorbance of the supernatant was calculated at 540 nm using a UV/VIS spectrometer. The phosphate buffer solution served as a control for the analysis [25].

Percentage inhibition = [{Absorbance of control-(Absorbance of test sample- Color factor)}/

(Absorbance of control)] *100

5.Quantitative Analysis - Liquid Chromatography–Mass Spectrometry (LC-MS)

Liquid Chromatography–Mass Spectrometry (LC-MS) was employed to identify and characterize the bioactive compounds present in the water extract of *Helianthus annuus* L. seed. The analysis was conducted at the Sophisticated Analytical Instrument Facility, Panjab University, using a Waters SYNAPT-XS HDMS system (Model: DBA064) operated with MassLynx Version 4.2 software. Before analysis, the extract was centrifuged at 12,000 rpm for 10 minutes to remove any particulates. Chromatographic separation was achieved using a UPLC ACQUITY H CLASS Series system equipped with a Waters Acquity BEH C18 column (2.1 × 100 mm, 1.7 µm particle size).

The mobile phase consisted of:

- **Solvent A**: 0.1% formic acid in LC-MS grade water
- **Solvent B**: 0.1% formic acid in acetonitrile

The flow rate was maintained at **0.2 mL/min**, and the injection volume was **5 µL**. A gradient elution was applied as follows:

Time (min)	%A	%B	Curve
0.00	95	5	6
5.00	95	5	6
30.00	10	90	6
35.00	10	90	6
36.00	95	5	6
45.00	95	5	6

Mass spectrometric detection was performed using electrospray ionization (ESI) in positive mode. Data were acquired in MRM (Multiple Reaction Monitoring) mode with unit resolution. The MS parameters were set as follows:

- **Desolvation gas flow**: 950 L/hr
- **Cone gas flow**: 50 L/hr
- **Desolvation temperature**: 550 °C
- **Source temperature**: 120 °C
- **Capillary voltage**: 3.22 kV
- **Cone voltage**: 50 V
- **Collision energy**: 4 eV

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

- **Source offset**: 80 V

The total run time for each analysis was 45 minutes. The system operated in data-dependent mode, allowing automatic switching between MS and MS/MS acquisition for comprehensive profiling of metabolites.

6.In vivo ulcerative colitis study

6.1. Acute oral toxicity study

Organization for Economic Co-operation and Development (OECD) guideline 425 for chemical testing was superseded for operating an acute oral toxicity study of seeds of *Helianthus annus* in male Wistar rats [26].

6.2. Procurement and acclimatization of animals

The research was carried out at Jadavpur University 188, Raja Subodh Chandra Mallick Road, Jadavpur, Kolkata, West Bengal 700032, where healthy adult male albino Wistar rats weighing 150±20 g were used. The rats were accommodating in polypropylene cages lined with husk, maintained under standard environmental conditions: temperature 25 ± 2° Celsius, relative humidity 55 ± 10% and 12hrs light-dark cycle. They were fed a high-fat diet and had free access to water. They were given a week to acclimatize before carrying out any experiments. All experimental protocol the animal ethics committee of Jadavpur University was approved the study and it was conducted in granting with the relevant regulations of CCSEA (Committee for the control and Supervision of Experiments on Animals) and institutional guidelines (Reference Number: JU/IAEC-25/106)

6.3. Acetic Acid Induced Ulcerative Colitis in Wistar Rats Model

A whole of 25 healthy wistar rats were split into 5 groups (n=5 in each group) [Fig. 3] to evaluate the therapeutic efficacy of *Helianthus annuus* L. seed extract against acetic acid-induced ulcerative colitis**.** Firstly, Days 1-7of the study groups 3, 4, and 5 received daily oral administration of the respective treatments [27]. On Day 8 of the study, after overnight fasting, ulcerative colitis was induced in anesthetized Wistar rats by the rectal administration of 1 mL of 7% acetic acid using a lubricated polyethylene catheter inserted almost 6–8 cm into the rectum. The rats were held with their heads down for approximately 30 sec to stop leakage and ensure even distribution of the solution within the colon. Following induction, treatment was continued for an additional three days (Day 9–11). On Day 12, all animals were sacrificed under deep anesthesia. The entire colony was carefully excised, washed with cold saline, and assessed for macroscopic injury, colon length and weight. Tissue samples were then processed for biochemical assays (NO), inflammatory marker analysis (e.g., TNF-α, NFkβ), and histopathological examination to evaluate structural and cellular changes associated with inflammation and healing [28][29].

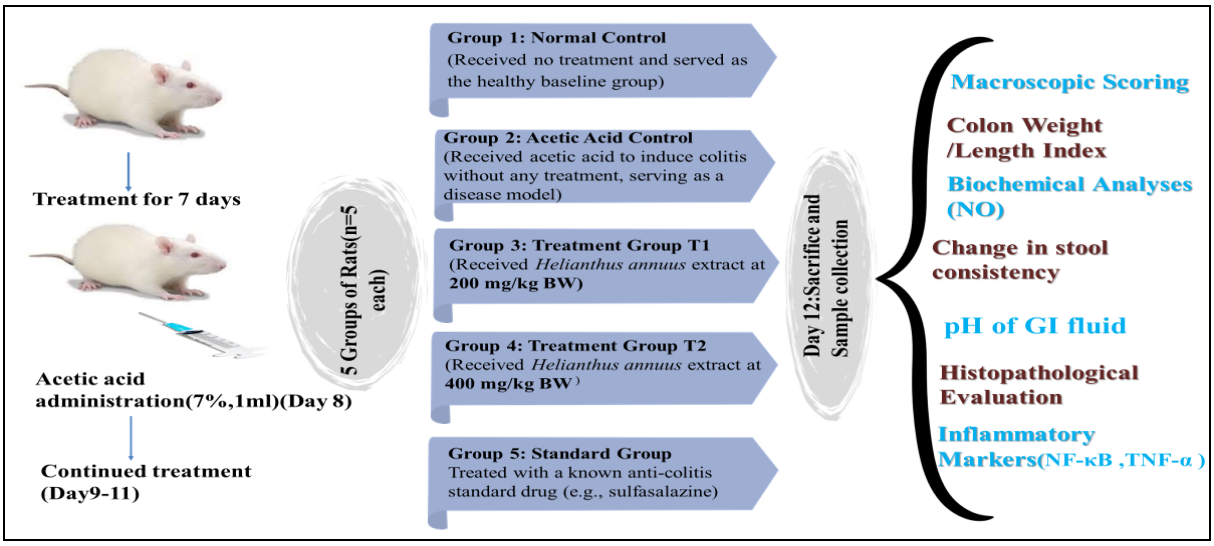


Figure 3: Group division and treatment schedule of Acetic Acid induced ulcerative colitis study of the *Helianthus annus* L. seed extract

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

6.4. Assessment of colon damage by macroscopic scoring

Colonic ulceration and inflammation were assessed using the scoring manner described by Wallace *et al*. [30]. A colonic segment approximately 8 cm long was dissected from the proximal end to the anus. A longitudinal incision was then created each individual colonic tissue. Colonic tissue lesions were scoring using the scale in Table 1.

Table 1: Scoring decision making factor for ulcers and inflammation

Score	Appearance
0	No visible damage
1	Localized hyperemia without erosions or ulcers
2	Presence of erosions or ulcers without notable inflammation
3	Erosions or ulcers with localized inflammation
4	Two or multiple spot ulceration/inflammation
5	Multiple major ulcerations or widespread lesions extending>1 cm

6.5. Colon Weight/Length Index

The colon was weighed and its length was determined to calculate the weight/length ratio, which serves as an indicator of inflammation-induced edema and tissue thickening, both hallmark signs of colitis.

6.6. Change in stool consistency

In experimental models of colitis, stool consistency is a straightforward but important measure of inflammation and gastrointestinal health. With the aim of estimate the degree of colonic damage and the therapeutic impact of test substances, stool consistency was visually observed across various treatment groups in this study.

6.7. Estimation of pH of GI fluid

Excessive inflammation in colon may cause to rise the gastric pH towards acidic. Estimation of gastric pH among various groups may give an indication of healing and degree of inflammation.

6.8. Estimation of tissue antioxidant

Colon tissue was homogenized in appropriate buffers and centrifuged to obtain supernatants for biochemical assays:

- **NO (Nitric Oxide):**

NO is a signaling molecule with both harmful and beneficial effects, depending on its concentration and the context. At high concentrations, NO can be pro-inflammatory and contribute to tissue damage. Excessive NO can react with superoxide to form peroxynitrite (ONOO-), a highly reactive molecule that can cause oxidative stress and inflammation.

6.9. Estimation of Inflammatory Markers

The concentration of tumor necrosis factor-alpha (TNF-α) in colon tissue homogenate was measured using a marketable ELISA or enzyme linked immunosorbent assay kit,as directed by the manufacturer Krishgen Biosystem (Mumbai, India) specific for rat/mouse TNF-α, consequents the manufacturer's rules. Absorbance was calculated at 450 nm using a microplate reader, and the concentrations were stated as pg/mg of protein. A standard curve was get ready using known concentrations of TNF-α to calculate sample values [31].

Levels of nuclear factor kappa B (NF-κB) p65 subunit was calculated using a specific enzyme linked immunosorbent assay (ELISA) were conducted to quantify pro-inflammatory marker such as NFkβ in colon homogenates, which reflect the degree of immune response and inflammation. The assay was carried out as per the instructions provided by the manufacturer.Optical density was read at 450 nm, and values were determined from a standard curve [32].Results were stated as pg/mg of protein.

6.10. Histopathological Evaluation

Elhefnawy EA *et al*. [33] showed that sections of colon tissue were fixed in 10% formalin, inserted in paraffin, cut into 5 µm thick sections. They were stained using hematoxylin and eosin (H&E) and examined under a light microscope for histopathological changes such as epithelial erosion, crypt architecture distortion, inflammatory cell infiltration, and mucosal ulceration.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

6.11. Statistical Analysis

The experimental value was obtained as mean ± standard deviation (SD). Statistical investigation was executed using IBM SPSS software (version 20). The significant difference was set at ($p < 0.001$) and one-way ANOVA (Analysis of Variance) Post-hoc analysis(Tukey test) was performed.

6. Results

6.1. Phytochemical screening: Qualitative study of secondary metabolites

The preliminary phytonutrients study of the extract of *H. Annus* disclose the existence or absence of various secondary metabolites. The study has shown the existence of flavonoids, Phenol, Tannin, alkaloids in the water extract of *H.Annus* as shown in Table 2.The extract did not show any positive result for carbohydrates, glycoside and terpenoid.

Table 2: Identification of various secondary metabolites

Sl. No.	Secondary Metabolites	Result
1	Carbohydrates	-
2	Phenol	+
3	Glycoside	-
4	Tannin	+
5	Alkaloids	+
6	Flavonoid	+
7	Terpenoid	-

6.2. Total Phenolic Content

The phenolic content was evaluated following to the linear calibration curves of standard compounds Gallic acids standard curve. The total phenolic content was differentiate with the gallic acid standard and estimated the aqueous extract at 761.66 mg/g of crude extract using the formula $y = 0.0087x + 0.0645$ where R^2 value is 0.9812.

6.3. Total Flavonoid Content

The total flavonoid content (mg/gm) concentration was determined using the identical standard curve formula for quercetin. $Y=0.0105X – 0.3686$

Total Flavonoids phenolic content in terms of quercetin is 424.68 mg/gm.

6.4. *In vitro* antioxidant analysis

6.4.1. Reducing power assay:

Reducing power assay results clearly show that *Helianthus annuus* seed extract has a substantial, concentration-dependent antioxidant capacity. The Fig. 4 show that % inhibition increased significantly from 45.8% at 20 µg/mL to 79.4% at 100 µg/mL in comparison to the baseline of 23.6% ($p < 0.001$). Interestingly, a significant rise in reducing power was noted above 60 µg/mL, indicating a threshold above which the extract's ability to donate electrons becomes noticeably more prominent. The strong redox activity of the phytochemical components is shown by the statistically significant enhancement observed at all tested concentrations (20,40,60,80,100 µg/ml).

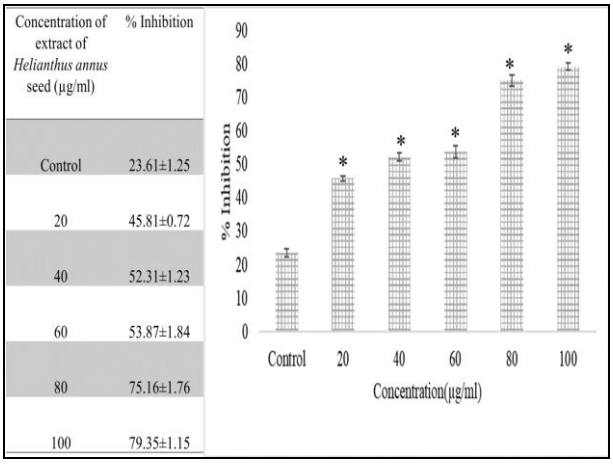
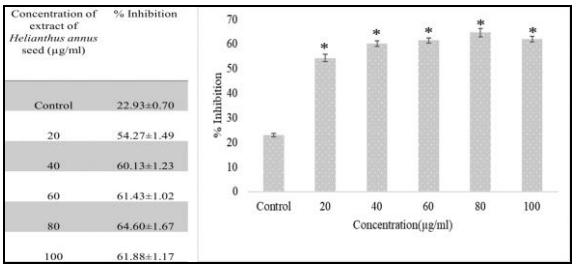


Figure 4: Estimation of Reducing power assay of extract of *Helianthus annus* seed extract using various concentration(µg/ml). Data are stated as mean ± standard deviation (n=3) ∗ indicates a notable change relative to control (P<0.001)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

6.4.2. DPPH radical scavenging activity:

The bar graph presents the results of the DPPH test, a common method used to evaluate the antioxidant activity of extracts prepared with different concentration. All concentration extracts demonstrated significantly higher antioxidant activity compared to the control group ($p < 0.001$). Fig.5 shows that even the lowest measured extract concentration 20 µg/mL considerably increased free radical neutralization to 54.3%, whereas the untreated control showed only a slight inhibition 22.9%. A plateau in scavenging ability was suggested by the peak inhibition 64.6% at 80 µg/mL and the minor fall to 61.9% at 100 µg/mL. Strong hydrogen-donating potential, a defining characteristic of powerful antioxidant chemicals, is demonstrated by the violet DPPH radical's quick decrease to pale yellow.

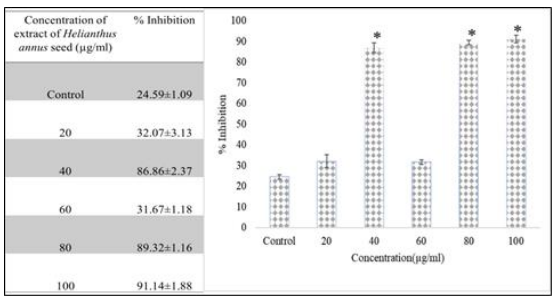


Concentration of extract of *Helianthus annus* seed (µg/ml)	% Inhibition
Control	22.93±0.70
20	54.27±1.49
40	60.13±1.23
60	61.43±1.02
80	64.60±1.67
100	61.88±1.17

Figure 5: Estimation of DPPH radical scavenging of extract of *Helianthus annus* seed extract using various concentration(µg/ml) . Data are stated as Mean± Standard (n=3) ∗ indicates a notable variation relative to the control(P<0.001)

6.5. *In vitro anti-inflammatory analysis*

6.5.1. Egg albumin denaturation assay:



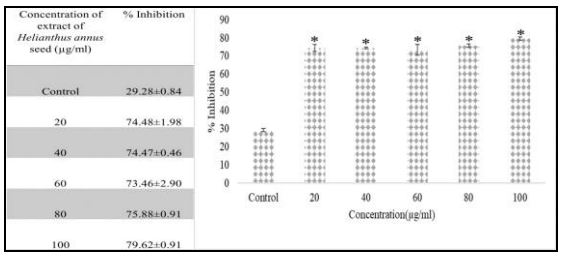
Concentration of extract of *Helianthus annus* seed (µg/ml)	% Inhibition
Control	24.59±1.09
20	32.07±3.13
40	86.86±2.37
60	31.67±1.18
80	89.32±1.16
100	91.14±1.88

Figure 6: Estimation of egg albumin assay of extract of *Helianthus annus* seed extract using various concentration(µg/ml). Data are stated as Mean ± Standard(n=3) ∗ indicates a notable variation relative to the control(P<0.001)

The anti-inflammatory activity of *Helianthus annuus* seed extract was evaluated using the egg albumin denaturation method, and the results revealed a concentration-dependent effect. Fig.6 as shown in the control group showed a baseline inhibition of 24.59 %. At 20 µg/ml, the inhibition slightly increased to 32.07 %, while at 40 µg/ml, a marked elevation to 86.86 % was observed, indicating a significant protective effect against protein denaturation ($p < 0.001$). Interestingly, the inhibition at 60 µg/ml dropped to 31.67%, suggesting a non-linear trend. However, further increase in concentration to 80 and 100 µg/ml resulted in robust inhibition of 89.32 % and 91.14 %, respectively, both statistically significant. These findings suggest potent anti-inflammatory activity of the extract at higher concentrations, with maximum efficacy observed at 100 µg/ml.

6.5.2. HRBC Membrane stabilization

The *Helianthus annuus* L.seed extract demonstrated significant membrane stabilizing activity in a concentration-dependent manner, indicative of its anti-inflammatory potential. Different concentration of the extract (20,40,60,80,100 µg/ml) were used to evaluated the Fig.7 as shown in % inhibition of haemolysis. The control group exhibited a baseline inhibition of 29.28 %. Treatment with the extract at 20 µg/ml resulted in a substantial increase in inhibition to 74.48 %, which remained consistent at 40 µg/ml (74.47%) and 60 µg/ml (73.46%). A slight increase was observed at 80 µg/ml (75.88 %), with the maximum inhibition recorded at 100 µg/ml (79.62%). All treated groups showed statistically notable differences compared to the control ($p < 0.001$).



Concentration of extract of *Helianthus annus* seed (µg/ml)	% Inhibition
Control	29.28±0.84
20	74.48±1.98
40	74.47±0.46
60	73.46±2.90
80	75.88±0.91
100	79.62±0.91

Figure 7: Estimation of HRBC membrane stability assay of extract of *Helianthus annus* seed extract using various concentration(µg/ml). Data are stated

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

as Mean ± Standard(n=3) ∗ indicates a notable variation relative to the control(P<0.001)

7.Quantitative Analysis (LC-MS)

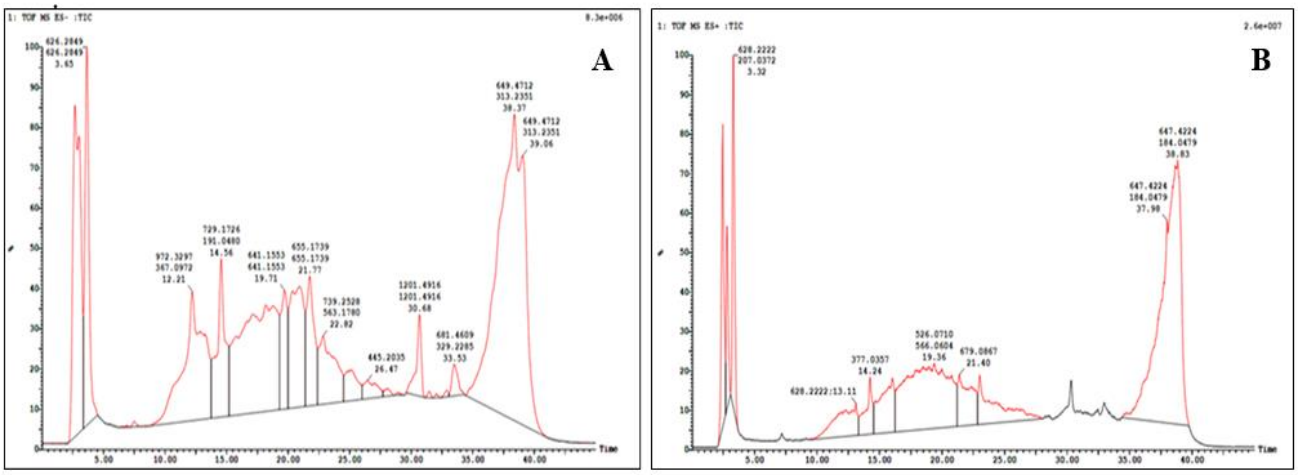


Figure 8: LC-MS chromatograms of the water extract of *Helianthus annuus* L. seed recorded in (A) positive and (B) negative ionization modes.

Table 3: Identified phytoconstituents from the water extract of *Helianthus annuus* L. seed as determined by LC-MS analysis

Sl. No	m/z Value	RT (min)	Ionization Mode	Compound Name	Molecular Formula	Area %	Reference
1	262.0954	2.79	Positive	Dietziamide A	$C_{13}H_{14}N_2O_4$	2.79%	Natural Products Atlas
2	262.0954	2.79	Negative	L-cis-Cyclo(aspartylphenylalanyl)	$C_{13}H_{14}N_2O_4$	5.11%	HMDB
3	207.0372	3.32	Positive	3-Hydroxybenzoic acid	$C_7H_6O_3$	5.92%	ChemSpider
4	377.0357	14.24	Positive	Quercetin-3-O-glucoside (Isoquercitrin)	$C_{21}H_{20}O_{12}$	2.83%	PubChem
5	191.048	16.55	Negative	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	5.11%	PubChem
6	526.071	19.36	Positive	Digoxin	$C_{41}H_{64}O_{14}$	21.87%	PubChem
7	503.1612	2.7	Negative	Dihydromyricetin	$C_{15}H_{12}O_8$	9.85%	PubChem
8	729.1726	14.56	Negative	Rutin	$C_{27}H_{30}O_{16}$	4.95%	PubChem

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

9	649.4712	38.37	Negative	Triacylglycerol (C36:2)	$C_{39}H_{72}O_6$	31.28%	LipidMaps
10	972.3297, 367.0972, 641.2088	12.21	Polyphenolic compound	Proanthocyanidin dimer	$C_{30}H_{26}O_{12}$	9.98%	PubChem

Fig.8 as shown in LC-MS analysis of the water extract of *Helianthus annuus* L. seed was performed in both positive and negative ionization modes, revealing a complex phytochemical profile composed of several bioactive constituents. A total of ten major compounds were tentatively identified based on accurate m/z values, retention times (RT), molecular formulas, and relative abundances, many of which have documented pharmacological activities. Table 3 represent in the positive ionization mode, Digoxin (m/z 526.071, RT: 19.36 min) was the most prominent compound, comprising 21.87% of the total chromatographic area. Digoxin is a well-established cardiac glycoside with potent positive inotropic activity, acting via Na^+/K^+-ATPase inhibition, and is commonly used in the management of congestive heart failure and atrial fibrillation. 3-Hydroxybenzoic acid (m/z 207.0372, RT: 3.32 min, 5.92%) was also identified and is known for its antioxidant and anti-inflammatory properties, having demonstrated radical scavenging and anti-proliferative effects in vitro. Another phenolic compound, quercetin-3-O-glucoside (isoquercitrin) (m/z 377.0357, RT: 14.24 min, 2.83%), Chen *et al*.,has been widely studied for its neuroprotective, anti-inflammatory, and anti-osteoporotic effects, including enhancement of bone formation and protection against oxidative stress in neuronal cells and osteoblasts [34,35]. Dietziamide A, a diketopiperazine detected at m/z 262.0954 (RT: 2.79 min, 2.79%), is reported to possess antimicrobial activity, although its pharmacological characterization remains limited and warrants further exploration [36]. In the negative mode, triacylglycerol (C36:2) (m/z 649.4712, RT: 38.37 min) was the most abundant compound with a relative area of 31.28%, representing a major class of neutral lipids that serve as metabolic energy reservoirs but may also reflect lipid metabolic activity in plant tissues [37]. Dihydromyricetin (m/z 503.1612, RT: 2.70 min, 9.85%) is a flavanonol with diverse bioactivities, including hepatoprotective, neuroprotective, antioxidant, and anti-alcohol intoxication effects, supported by evidence of modulation of GABAergic signaling and inhibition of lipid peroxidation [38]. Guo *et al*., and Zhang *et al*., investigate that notably, recent studies have shown that dihydromyricetin also possesses anti-osteoporotic potential by suppressing osteoclast differentiation and activity, and improving bone microarchitecture in ovariectomized models [39,40]. Proanthocyanidin dimers (m/z 972.3297, RT: 12.21 min, 9.98%) were also detected and are known for their strong antioxidant, anti-inflammatory, and cardioprotective properties, mediated through the modulation of oxidative stress and endothelial function [41]. The flavonol glycosides rutin (m/z 729.1726, RT: 14.56 min, 4.95%) and kaempferol-3-O-rutinoside (m/z 191.0480, RT: 16.55 min, 5.11%) were identified, both of which exhibit antioxidant, vasoprotective, and anti-inflammatory activities. Importantly, rutin has demonstrated anti-osteoporotic effects through its ability to enhance bone mineral density, inhibit osteoclastogenesis, and stimulate osteoblast differentiation in both in vitro and in vivo models [42,43,44]. Additionally, L-cis-Cyclo(aspartylphenylalanyl) (m/z 262.0954, RT: 2.79 min, 5.11%), a diketopiperazine derivative, has been reported to possess antitumor and antiviral activities by disrupting protein synthesis and interfering with cellular proliferation [45].

Together, these results highlight the presence of a diverse group of phytochemicals with significant therapeutic potential, including cardiovascular, neuroprotective, anti-inflammatory, antioxidant, and bone-protective (anti-osteoporotic) effects. The identification of these compounds supports the biological relevance of the sample and provides a strong foundation for further pharmacological evaluation.

8.*In-vivo* study

8.1. Acute oral toxicity study

The acute oral toxicity of the extract was evaluated following to the 2008 OECD 425 guideline. No signs of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

toxicity were observed after application of *Helianthus annus* L. seeds at doses up to 2000 mg/kg b.w.Generally 1/5 to 1/10 of the lethal dose is chosen for calculating the effective dose. Therefore, doses of 200 and 400 mg/kg b.w. were chosen in the study.

8.2. Estimation of Colon-Weight/Length Index

Groups of study	Colon weight (g)/length ratio (cm)
Normal	7.6 ± 0.66
Disease Control	11.64 ± 0.79
Test 1	9.31 ± 0.41
Test 2	7.09 ± 0.45
Standard	5.04 ±0.72

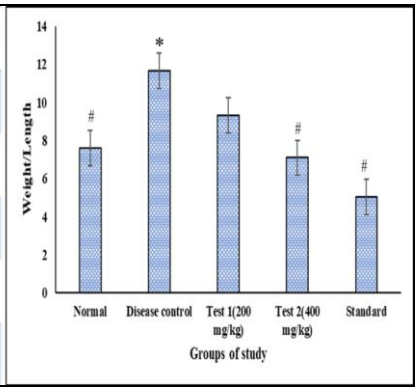


Figure 9: Estimation of Weight/Length ratio of descending colon of various groups of rats(n=5). Data are stated as mean ± standard deviation. ∗ indicates a notable variation relative to the normal control and # indicates the notable variation when compared with disease control (p <0.001).

As shown in the Fig.9 with a colon weight/length ratio of 7.6 cm, the Normal group (Group 1) showed signs of a healthy, non-inflammatory colon. With a markedly higher ratio of 11.64 cm, the Disease Control group (Group 2), which was given acetic acid without any treatment, demonstrated severe tissue damage, edema, and colonic inflammation brought on by the induction of ulcerative colitis. With a colon index value of 9.31cm, Test Group 1 (T1, 200 mg/kg BW) showed a moderate decrease, indicating partial therapeutic or protective benefits. In comparison to the lower dose, Test Group 2 (T2, 400 mg/kg BW) had an enhanced anti-inflammatory effect, as evidenced by a further drop in the ratio to 7.09cm. With the lowest ratio of 5.04 cm, the Standard group (Group 5), which was given a well-known anti-inflammatory drug, was showing strong therapeutic efficacy and was getting close to normal values.

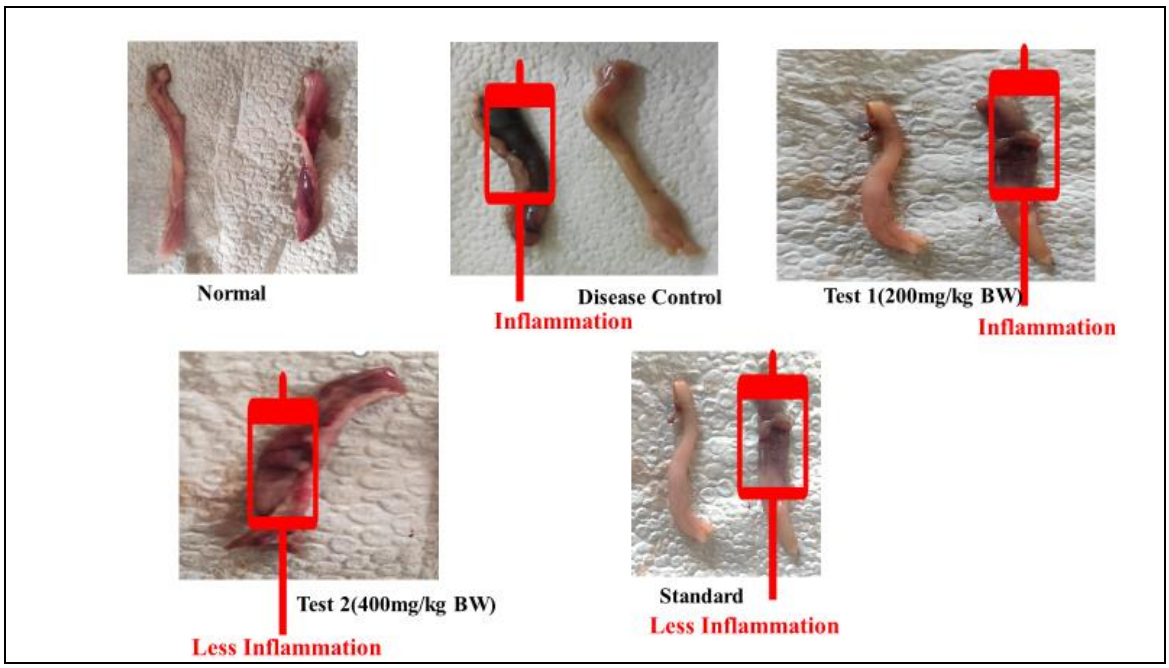


Figure 10: Macroscopic view of colonic inflammation of various groups of rats

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

8.3. Macroscopic Evaluation

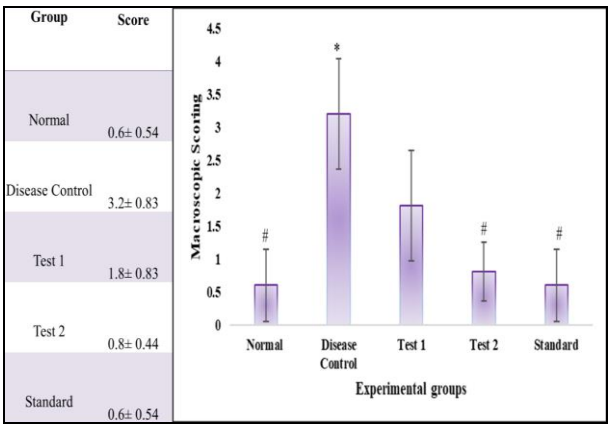


Figure 11: Estimation of macroscopic scoring of descending colon of various groups of rats(n=5). Data are stated as mean ± standard deviation. ∗ indicates a notable variation relative to the normal control and # indicates the notable variation when compared with disease control (p <0.001).

Extract from *Helianthus annuus* seeds as shown in Fig.10 the following was discovered by the descending colon's macroscopic scoring: With an average score near 0.5, the Normal group (Group 1) displayed little to no damage, suggesting a healthy, intact colonic mucosa. As shown in Fig.11 with a notably high mean score of roughly 3.3, the Disease Control group (Group 2), which was given acetic acid without treatment, showed clear inflammation and ulceration along with severe mucosal damage. In comparison to the disease control group, Test Group 1 (T1, 200 mg/kg BW) showed a moderate improvement with fewer apparent lesions, as evidenced by a lower macroscopic score of about 1.8. The macroscopic score of Test Group 2 (T2, 400 mg/kg BW) further decreased to about 1.1, indicating a greater protective effect against colonic damage at the higher dose. The Standard group displayed the lowest score among the treated groups, approximately 0.7, closely resembling the Normal group and confirming its potent anti-inflammatory and mucosal protective properties.

These findings confirm that acetic acid-induced colonic damage was considerably reduced by both test regimens, especially the higher dose (T2). With a nearly normal restoration in the group receiving standard therapy, the macroscopic scoring amply illustrates the test compounds' dose-dependent protective effect.

8.4. Change in stool consistency

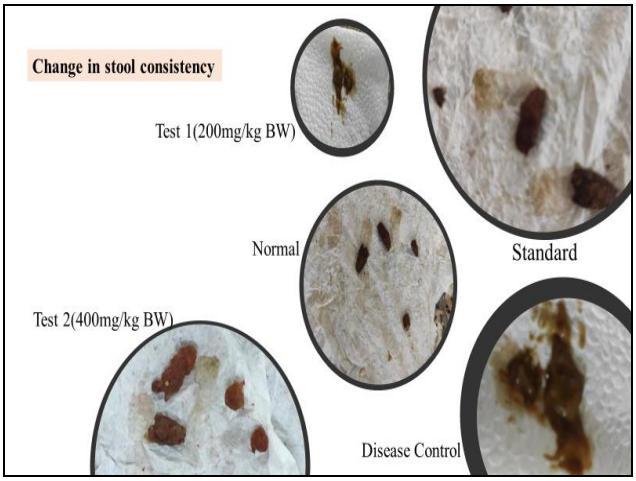


Figure 12: Image of different groups of rat stool consistency

Fig.12 represent Normal Group: The rats in this group had solid, well-formed pellets that were indicative of normal mucosal integrity and intestinal physiology. Disease Control Group: Untreated rats given 7% acetic acid induction had loose, watery, and mucus-filled feces, which are a sign of ulcerative colitis's severe diarrhea, mucosal inflammation, and colonic dysfunction. Rats in Test Group 1 (T1, 200 mg/kg BW) had softer, semi-formed feces with traces of mucus. This suggested a modest therapeutic benefit of the test chemical at this dosage, as evidenced by the partial improvement in inflammation and gastrointestinal function. Test Group 2 (T2, 400 mg/kg BW): The animals in this group showed mainly well-formed feces that were better looking and had less mucus, which suggests that the higher dose had a stronger anti-inflammatory and healing effect**.** Standard Group: This group exhibited firm, dark, pellet-like stools similar to the normal group. This consistency indicates restoration of normal bowel function and confirms the effectiveness of the standard drug in counteracting acetic acid-induced colonic damage.

These visual observations correlate with the biochemical and macroscopic results, supporting the efficacy of the test compounds particularly at higher doses in mitigating colonic inflammation and restoring normal stool consistency in ulcerative colitis.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

8.5. Estimation of pH of GI fluid

Table 4: Estimation of pH of GI fluid

Group	GI Fluid Volume	Estimated Gastric pH
Normal Control	1.2 mL	1.8
Disease Control	2.9 mL	5.8
Standard	1.4 mL	2.8
Test I (200mg/kg BW)	1.6 mL	3.4
Test II (400mg/kg BW)	1.9 mL	2.9

The gastrointestinal (GI) fluid analysis reveals significant alterations in both volume and pH among the different groups, indicating the effects of disease and treatment. As shown in Table 4 the disease control group shows a marked increase in GI fluid volume (2.9 mL) and a notable drop in pH (5.8), suggesting enhanced secretion and acidity associated with ulcerative colitis pathology. In contrast, the Normal Control maintains a balanced fluid volume (1.2 mL) and near-neutral pH (1.8), reflecting physiological homeostasis. The Standard treatment group shows partial normalization with a fluid volume of 1.4 mL and pH of 2.8, indicating therapeutic efficacy. Test I (200 mg/kg BW) and Test II (400 mg/kg BW) demonstrate dose-dependent improvements: Test I slightly reduces fluid volume (1.6 mL) and raises pH (3.4), while Test II achieves better normalization (1.9 mL, pH 2.9). These findings suggest that both test treatments mitigate disease-induced changes in GI fluid characteristics, with higher doses showing greater effectiveness in restoring near-normal physiological conditions.

8.6. Histopathological Study

The histopathological examination of colon tissue, as presented in Fig. 13, reveals distinct characteristics across different groups. The histopathological study of colon tissues shows that effect of Test 1(200 mg/kg BW), Test II(400 mg/kg BW), Normal, Disease Control, and standard oral administration of AA for 8 days in colon samples stained with hematoxylin and eosin (H&E) protocol. Histopathological study of colon samples from normal controls showed normal appearing mucosal lining, well-defined tissue architecture, well-preserved mucosal crypts and no hypertrophy of goblet cells without any visible signs of inflammation or necro-inflammatory injury. In contrast, the disease control group exhibited extensive potential ulcers, multiple mucosal erosions, hemorrhages in the mucosa and submucosa and severe inflammatory cell infiltration and loss of normal crypt structure. The standard (sulfasalazine) group showed slightly dilated but overall healthy crypts showing goblet cell hyperplasia and mucus and almost preserved mucosa with insignificant inflammation. A mild polymorphic infiltrate is also seen in otherwise healthy submucosa and mucosa and reduced inflammation compared to disease control. Test I(200 mg/kg BW) group exhibits signs of moderately preserved architecture, better mucosal integrity than disease control and some goblet cells and villi preserved. Test treatment shows improvement ,possibly effective in reducing inflammation and restoring normal histology. Test II (400 mg/kg BW) group displays an signs of similar to Test I , possibly slightly more better preserved compared than Test I and less edema and distortion, relatively better crypt integrity. Test I and Test II both demonstrate positive histological outcomes, suggesting that the treatments being tested are effective, with Test 2 possibly showing slightly superior effect for better preservation.

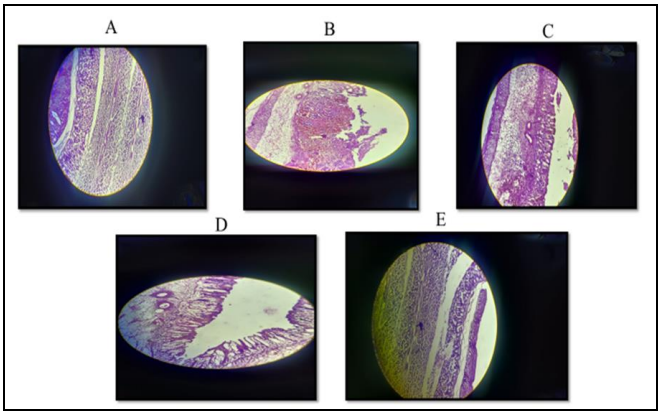


Figure 13: Histological sections (5x X 20x) of colon tissue of experimental rats, differentiated in normal(A), Disease control (B), Test 1(C), Test II(D), Standard Drug-Sulfasalazine (E).

In the normal control group (A), the yellow arrow indicates an intact mucosal epithelium, while the red

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

arrow highlights well-organized crypt architecture and a healthy submucosa. In the disease control group (B), the yellow arrow shows disrupted epithelial lining, the red arrow marks areas of ulceration and crypt loss, and the blue arrow indicates dense inflammatory cell infiltration. In Test Group I (C), the green arrow points to partially preserved crypts with mild mucosal disruption. In Test Group II (D), the blue arrow demonstrates significant restoration of mucosal structure and reduced inflammation. In the standard drug-treated group (E), the red arrow shows intact crypt architecture, the green arrow denotes well-preserved mucosal lining, and the blue arrow highlights minimal inflammatory infiltration, resembling the normal histology.

8.7. Estimation of tissue antioxidant

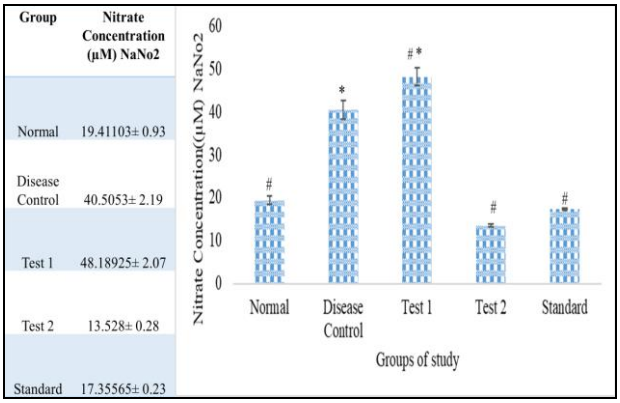


Figure 14: Estimation of NO in colon tissues. Data are stated as Mean ± Standard Deviation (n=5). ∗ indicates a notable variation relative to the normal control and # indicates the notable variation when compared with disease control (p <0.001).

The estimation of Tissue anti-oxidant (NO) was carried out using colon tissue homogenates. Estimation of tissue anti-oxidant has exhibited that the water extract of seeds of *Helianthus annus* has notable effect on acetic acid (AA)induced ulcerative colitis. The nitrate concentration (measured as $NaNO_2$, µM) in colon tissue homogenates is presented in Fig.14. In the disease control group, a notable increase in nitrate levels was observed (~40 µM) compared to the normal control group (~20 µM), indicating enhanced nitric oxide (NO) production and oxidative stress associated with ulcerative colitis ($p < 0.001$). Administration of the standard treatment effectively normalized nitrate levels to approximately 20 µM**,** comparable to the normal group. Among the test groups, Test I (200 mg/kg) exhibited a further increase in nitrate concentration (~48 µM), significantly higher than both the disease and normal controls ($\#p < 0.001$), suggesting a potential pro-oxidant effect at this dose.

In contrast, Test II (400 mg/kg**)** markedly reduced nitrate concentration to approximately 18 µM**,** showing a notable decrease compared to the disease control and even slightly lower than the normal control (#p < 0.001). This indicates a dose-dependent antioxidant effect**,** likely through suppression of iNOS or inducible nitric oxide synthase activity or reactive nitrogen species.

8.8. Estimation of Inflammatory Markers - TNF-α

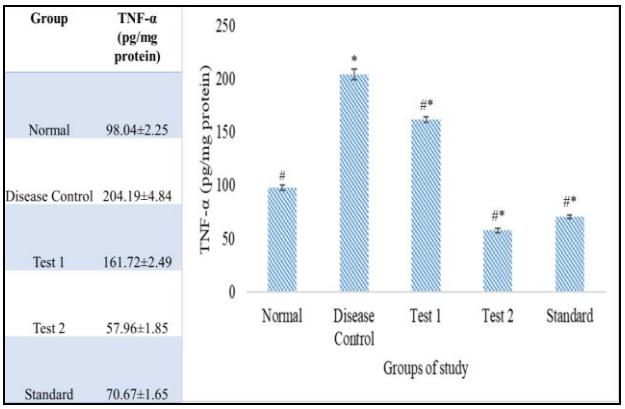


Figure 15: Estimation of TNF-α (pg/mL) in colon tissues. Data are stated as Mean ± Standard Deviation(n=5). ∗ indicates a notable variation relative to the normal control and # indicates the notable variation when compared with disease control (p <0.001).

The estimation of protein (TNF-α) was carried out using colon tissue homogenates. Estimation of protein has exhibited that the water extract of seeds of *Helianthus annus* has notable effect on acetic acid induced ulcerative colitis. Fig.15 illustrates the levels of TNF-α (pg/mg protein) across different groups. A significant elevation in TNF-α was noticed in the disease control group (~200 pg/mg), differentiate to the normal control group (~110 pg/mg), confirming active inflammation in the colonic tissues ($p < 0.001$).Treatment with the standard drug markedly reduced TNF-α levels to approximately 100 pg/mg. Similarly, the test groups exhibited a dose-dependent reduction in TNF-α levels:

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

Test I (200 mg/kg) showed a moderate decrease (~170 pg/mg), while Test II (400 mg/kg) demonstrated a substantial decrease (~90 pg/mg), both showing statistical significance when differentiate to the disease control group (#$p < 0.001$).

8.9. Estimation of Inflammatory Markers - NF-κB

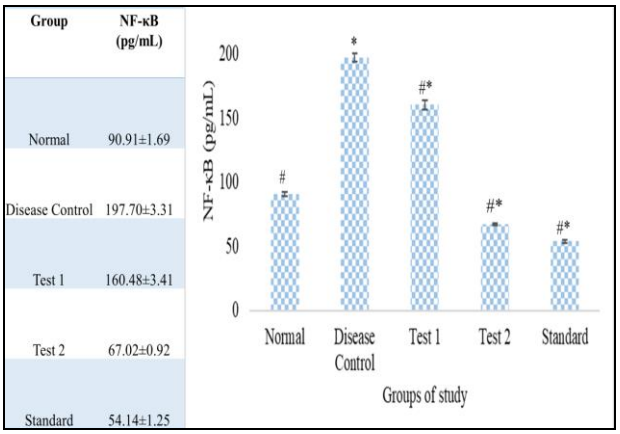


Figure 16: Estimation of NF-κB (pg/mL) in colon tissues. Data are stated as Mean ± Standard Deviation(n=5). ∗ indicates a notable variation relative to the normal control and # indicates the notable variation when compared with disease control (p <0.001).

The stated of nuclear factor kappa B (NF-κB) in colon tissue is depicted in Fig. 16. An increase in NF-κB levels was noticed in the disease control (~190 pg/mL) differentiate to the normal control (~100 pg/mL), indicating pronounced inflammatory activation in ulcerative colitis ($p < 0.001$). Treatment with the standard drug markedly reduced NF-κB levels to approximately 60 pg/mL, showing significant improvement relative to the disease control group(#$p <$ 0.001). A dose-dependent reduction in NF-κB levels was also evident in the test groups, Test I (200 mg/kg) reduced levels to ~160 pg/mL (#$p < 0.001$ vs disease control) and Test II (400 mg/kg) brought NF-κB down to ~70 pg/mL, closely aligning with the standard group.

9.Discussion and Conclusion:

In conclusion, the study provides compelling evidence that *Helianthus annuus* L. seed extract exerts significant therapeutic effects in mitigating the severity of UC or ulcerative colitis in a rat model. The observed benefits are likely due to the synergistic outcome of its diverse phytochemical components, which exhibit antioxidant, anti-inflammatory, and mucosal-protective actions. These results not only support the long-standing usage of sunflower seeds to treat inflammatory diseases, but they also clear the way for the creation of plant-based treatments for ulcerative colitis. To transform these preclinical findings into effective, safe, and efficacious treatments for UC patients in humans, further research,invloving mechanistic studies and clinical trials is needed. In an experimentally produced model of UC or ulcerative colitis in Wistar rats, this learning thoroughly evaluated the phytochemical constituents and therapeutic efficacy of *Helianthus annuus* L. (sunflower) seed extract. Due to the limited efficacy and extensive adverse effects of current pharmaceutical therapies, ulcerative colitis (UC), a chronic and relapsing inflammatory bowel disease, significantly reduces the quality of life and continues to be a therapeutic challenge. In this context, natural plant-based therapies with anti-inflammatory and antioxidant properties are increasingly being explored as complementary or alternative options. Phytochemical profiling of the seeds of *Helianthus annuus* L., a plant that has long been used for various medicinal purposes, has shown the presence of a wide range of bioactive chemicals. Ulcerative colitis symptoms were significantly reduced after treatment with *Helianthus annuus* L.seed extract at doses of 200 mg/kg and 400 mg/kg BW. The extract had a protective outcome on the colon, normalizing the weight/length ratio of the colon, improving stool consistency, and significantly reducing the macroscopic damage score. In vivo, induction of colitis using acetic acid triggers significant pathological changes including increased macroscopic scores, mucosal ulceration, and proinflammatory biomarkers. Treatment with *H. annuus* extract significantly improves these parameters. Important in UC development, TNF-α and NF-κB expression mediate leukocyte recruitment and inflammatory cascades.TNF-α and NF-κB levels were significantly lower in the extract-treated groups, indicating that inflammatory pathways were suppressed. Furthermore, the treated groups had a potent antioxidative effect as evidenced by a significant reduction in nitric oxide (NO) levels, which is a contributing factor to oxidative stress and mucosal injury in colitis . Histopathological evaluation supported these results, revealing that *Helianthus annuus* seed extract preserved the integrity of the colonic mucosa, reduced inflammatory cell

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

infiltration, and reduced ulceration differentiate to the disease control group. The high-dose extract (400 mg/kg BW) demonstrated effects comparable to, or in some parameter's superior to, the standard treatment group, further emphasizing the therapeutic potential of the extract.

Ethics Approval and Consent to Participate

All animal experiments were conducted in compliance with CCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC), Jadavpur University, Kolkata, India (Approval No.: JU/IAEC-25/106). All procedures involving animals adhered to the ethical standards for the care and use of laboratory animals.

Consent to participate is not applicable as the study did not involve human participants.

Consent for Publication

Not applicable. This study does not involve any individual person's data (human photographs, personal details, or clinical information) requiring consent.

Availability of Data and Materials

All datasets generated and analysed during the current study are included in this manuscript and its supplementary materials. Additional raw data (LC–MS output files, histology images, and biochemical assay data) are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests related to this work.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors gratefully acknowledge the Department of Pharmaceutical Technology, JIS University, for providing laboratory facilities. The authors also thank the Sophisticated Analytical Instrument Facility (SAIF), Panjab University, for providing LC–MS analytical support. Technical assistance received from the staff of the animal house facility at Jadavpur University is sincerely appreciated.

List of Abbreviations

SS	*Helianthus Annus* (Sunflower Seed)
HAE	*Helianthus Annus* extract
DPPH	2,2-diphenyl-1-picrylhydrazyl
UV	Ultra Violet
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
%	Percentage
BW	Body Weight
µg	Microgram
µl	Microliter
PBS	Phosphate Buffer Solution
NaOH	Sodium Hydroxide
KOH	Potassium Hydroxide
^{o}C	Degree celsius
gm	Grams
w/v	Weight / Volume
TPC	Total Phenolic content
TFC	Total flavonoids content
rpm	Revolution per minute
LPO	Lipid Peroxidation
NO	Nitric Oxide

References:

1. Harborne JB (2006) *Phytochemical techniques*. 1st ed. New Delhi: New India Publishing Agency.
2. Liu MXQ, Weber C, Lee CY, Brown J, Liu RH (2002) Antioxidant and antiproliferative activities of raspberries. *J Agric Food Chem* 50(10):2926–2930.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 526-544 | ISSN:2251-6727

3. Javanmardi J, Stushnoff C, Locke E, Vivanco JM (2003) Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem* 83(4):547–550.

4. Olugbami JO, Gbadegesin MA, Odunola OA (2015) In vitro free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*. *Pharmacogn Res* 7(1):49.

5. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F (1998) A procedure to measure the antiradical efficiency of polyphenols. *J Sci Food Agric* 76:270–276.

6. Oyaizu M (1986) Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr Diet* 44(6):307–315.

7. Ahmadi F, Kadivar M, Shahedi M (2007) Antioxidant activity of *Kelussia odoratissima* Mozaff. in model and food systems. *Food Chem* 105(1):57–64.

8. Banerjee S, Chanda A, Adhikari A, Das A, Biswas S (2014) Evaluation of phytochemical screening and anti-inflammatory activity of leaves and stem of *Mikania scandens* (L.) Willd. *Ann Med Health Sci Res* 4(4):532–536. https://doi.org/10.4103/2141-9248.139302

9. Rajagopala PLR, Sreejitha KR, Kiron SS, Jose R, Saritha M (2013) Anti-inflammatory (in vitro) activity of the leaves of *Ficus gibbosa* Blume by HRBC membrane stabilisation. *IOSR J Pharm Biol Sci* 7(4):55–57.

10. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV (2018) In vitro anti-inflammatory properties of selected green leafy vegetables. *Biomedicines* 6(4):107. https://doi.org/10.3390/biomedicines6040107

11. Ahmad F, Tabassum N (2013) Preliminary phytochemical, acute oral toxicity and antihepatotoxic study of roots of *Paeonia officinalis* Linn. *Asian Pac J Trop Biomed* 3(1):64–68. https://doi.org/10.1016/S2221-1691(13)60025-8

12. Cellat M, Tekeli İO, Türk E, Aydin T, Uyar A, İşler CT, Gökçek İ, Etyemez M, Güvenç M (2023) *Inula viscosa* ameliorates acetic acid-induced ulcerative colitis in rats. *Biotech Histochem* 98(4):255–266. https://doi.org/10.1080/10520295.2023.2176923

13. Abdelsameea AA, Alsemeh AE, Alabassery N, Samy W, Fawzy A, Abbas NAT (2023) Icosapent ethyl alleviates acetic acid-induced ulcerative colitis via modulation of SIRT1 signaling pathway in rats. *Int Immunopharmacol* 115:109621. https://doi.org/10.1016/j.intimp.2022.109621

14. El Mahdy RN, Nader MA, Helal MG, Abu-Risha SE, Abdelmageed ME (2023) Eicosapentaenoic acid mitigates acetic acid-induced ulcerative colitis through modulation of NF-κB and TGF-β/EGFR signaling pathways. *Life Sci* 327:121820. https://doi.org/10.1016/j.lfs.2023.121820

15. Wallace JL, Keenan CM (1990) An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. *Am J Physiol* 258(4 Pt 1):G527–G534.

16. Rashidian A, Rashki A, Abdollahi A, Haddadi NS, Chamanara M, Mumtaz F, Dehpour AR (2019) Dapsone reduced acetic acid-induced inflammatory response in rat colon tissue through inhibition of NF-κB signaling pathway. *Immunopharmacol Immunotoxicol* 41(6):607–613. https://doi.org/10.1080/08923973.2019.1678635

17. Chamanara M, Abdollahi A, Rezayat SM, Ghazi-Khansari M, Dehpour A, Nassireslami E, Rashidian A (2019) Thymol reduces acetic acid-induced inflammatory response through inhibition of NF-κB signaling pathway in rat colon tissue. *Inflammopharmacology* 27(6):1275–1283. https://doi.org/10.1007/s10787-019-00583-8

18. Elhefnawy EA, Zaki HF, El Maraghy NN, Ahmed KA, Abd El-Haleim EA (2023) Genistein and/or sulfasalazine ameliorate acetic acid-induced ulcerative colitis in rats via modulation of multiple inflammatory signaling pathways. *Biochem Pharmacol* 214:115673. https://doi.org/10.1016/j.bcp.2023.115673

19. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. (2018) Isoquercitrin promotes osteogenic

differentiation and bone formation via the BMP signaling pathway. *J Cell Physiol* 233(3):2230–2241.

20. Du L, Zheng R, Zhang D, Li Q, Lv X, Wang Y (2022) Neuroprotective effects of isoquercitrin in models of oxidative stress. *Neurochem Int* 152:105204.
21. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, et al. (2009) Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res* 50(Suppl):S9–S14.
22. Li H, Song F, Duan LR, Sheng JJ, Xie YH, Yang Q (2017) Dihydromyricetin prevents alcohol-induced liver injury by enhancing antioxidative defense in mice. *Front Pharmacol* 8:104.
23. Guo Y, Li S, Li J, Zhang Y, Zhu L (2017) Dihydromyricetin prevents bone loss by suppressing osteoclast activity in ovariectomized mice. *Mol Med Rep* 15(5):3031–3038.
24. Zhang Y, Liu X, Wang H, Guo Y, Zhang L, Tang Z, et al. (2024) Dihydromyricetin alleviates bone loss via gut microbiota modulation in ovariectomized mice. *Front Pharmacol* 15:1452921.
25. Cos P, De Bruyne T, Hermans N, Apers S, Van den Berghe D, Vlietinck AJ (2004) Proanthocyanidins in health care: current and new trends. *Planta Med* 70(8):717–729.
26. Ganeshpurkar A, Saluja AK (2017) The pharmacological potential of rutin. *Saudi Pharm J* 25(2):149–164.
27. Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M (2011) A review on the dietary flavonoid kaempferol. *Mini Rev Med Chem* 11(4):298–344.
28. Park H, Kim JY, Jung JY, Kim DH, Park SJ, Kim SH, et al. (2014) Rutin promotes osteoblast differentiation by enhancing Runx2 expression. *Biochem Biophys Res Commun* 473(4):1277–1281.
29. Zhai Y, Guo Y, Du X, Bai Y, Zhu L (2014) Rutin suppresses osteoclast formation via modulation of the RANKL signaling pathway. *Molecules* 19(1):253–265.
30. Lee YS, Cha BY, Saito K, Yamaguchi K, Yamada Y, Kaneko K, et al. (2016) Nobiletin improves hyperglycemia and bone loss in ovariectomized mice. *J Bone Miner Metab* 34(5):510–521.
31. Roy RN, Laskar S, Sen SK, Ghosh AK (2015) Diketopiperazines: new class of anticancer agents. *Curr Med Chem* 22(2):237–253.
32. Neurath MF (2014) Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 14(5):329–342.
33. Keshavarzian A, et al. (2003) Increased colonic nitric oxide production and inducible nitric oxide synthase expression in patients with ulcerative colitis. *Gut* 52(5):731–739.