Protective Effect Of Spinacia Oleracea Leaves Against Indomethacin-Induced Enteropathy In Experimental Animals: A Comparative Investigation Of Petroleum Ether And Ethanolic Extracts

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ABSTRACT

Spinacia oleracea Linn., commonly known as spinach, has been traditionally used in ethnomedical therapy for various purposes, including the treatment of obesity, lung inflammation, lumbago, flatulence, and urinary calculi. In this study, we investigated the protective effects of petroleum ether (SOPE) and ethanolic (SOEE) extracts of Spinacia oleracea against indomethacin-induced enterocolitis in a rat model. Additionally, acute oral toxicity was evaluated using the Up-and-Down Procedure (Test No. 425: Acute Oral Toxicity). Results revealed the safety of both SOPE and SOEE at a dose of 2000 mg/kg, with normal observations in behavioral, body weight, organ weight, biochemical, and hematological parameters, except for slight alterations in respiration and salivation with SOPE and SOEE respectively. In the disease model, SOPE at 400 mg/kg and SOEE at 200 and 400 mg/kg exhibited significant protective effects against indomethacin-induced enterocolitis. These effects were evident through improvements in macroscopic, biochemical, hematological, and histological data. Overall, the findings suggest that SOEE exhibits more pronounced protective effects against indomethacin-induced enterocolitis compared to SOPE, possibly due to the presence of polyphenolic phytoconstituents in SOEE.

Keywords: Enterocolitis, Hematology, Indomethacin, Myeloperoxidase, Prednisolone, Spinacia oleracea,

INTRODUCTION

Spinacia oleracea Linn., commonly referred to as spinach and part of the Amaranthaceae family, is acknowledged for its diverse range of medicinal properties [1,2]. Ethnopharmacological studies have highlighted the therapeutic potential of Spinacia oleracea, revealing significant effects, including anti-epileptic, anti-Alzheimer, neuroprotective, anti-inflammatory, and antioxidant properties [3-5]. Moreover, several investigations have explored its effectiveness in addressing conditions such as osteoarthritis [6], antihyperlipidemia [7], antiglycation [8], appetite suppression [9], and diabetic wound healing [10]. Spinacia oleracea (S. oleracea) boasts a rich composition of chemical constituents, encompassing ascorbic acid, ferulic acid, luteolin, stigmasterol, apigenin, astragalin, para-coumaric acid, β-carotene, kaempferol, violaxanthin, methylenedioxy flavonol, rutin, quercetin, myricetin, caffeic acid, ortho-coumaric acid, protocatechuic acids, glycoglycerolipids, 20-hydroxyecdysone, spirasaponins, lutein, and various vitamins [11, 12]. This diverse array of constituents contributes to the wide spectrum of therapeutic effects attributed to S. oleracea, establishing it as a valuable resource in both traditional and modern medicine.

Inflammatory bowel disease, whether in its acute or chronic manifestation, significantly disrupts the quality of life and poses numerous health challenges for individuals across both developing and developed nations [13,14]. Key symptoms of inflammatory bowel disease including Crohn's disease and ulcerative colitis are notably rectal bleeding and diarrhea, contribute to a substantial burden, with approximately 1.7 billion reported cases annually. The colon and rectum are frequently affected areas in IBD, often characterized by ulceration in the mucosal and submucosal layers. Gastroenteritis stands as the second most prevalent cause of mortality globally, responsible for over one million deaths each year, with elevated mortality rates particularly evident in developing regions [15,16]. Conventional treatments for Inflammatory Bowel Disease (IBD) often come with various side effects, ranging from mild to more severe.

Current treatment of IBD includes anti-inflammatory drugs such as mesasalazine, sulfasalazine, corticosteroids, immunosuppressives, antibacterials, biologics and probiotics (17). Many of the drugs used in the treatment of IBD have severe side effects. Aminosalicylates used for IBD primarily consist of traditional sulfasalazine and other variants of 5-aminosalicylic acid medications. 5-aminosalicylic acid has many side effects such as abdominal, pain, nausea, flatulence, diarrhea, and headache, are generally mild. Nevertheless, sulfasalazine has more severe side effects, including hemolytic anemia, infertility, photosensitization, and granulocytosis, compared to 5-aminosalicylic acid [18]. Corticosteroids used in IBD treatments show severe side effects, such as diabetes mellitus, opportunistic infections, ocular effects, hypertension, venous thromboembolism, osteoporosis, etc. [19, 20]. Biologicals used in the treatments are associated with severe side effects, such as infections, immunogenicity and loss of response, malignancies, liver

dysfunction, abnormalities, heart failure, demyelination, and skin eruptions [21].

There is a rising global interest in traditional medicine systems for the treatment of diverse diseases. In India, approximately 80% of the rural population relies on medicinal herbs or indigenous medicine systems. The Indian herbal industry is actively engaged with nearly 960 plant species, contributing to a substantial turnover that exceeds 80 billion rupees [22, 23]. While plant-derived medicines are generally perceived to have low toxicity, it is noteworthy that certain medicinal plants employed in traditional medicine have been documented to exhibit toxic effects [24].

Phenolic compounds, encompassing flavonoids and phenolic acids, are recognized as potent bioactive substances with diverse health benefits, including antiulcer, antimicrobial, anticancer, antioxidant, cardioprotective, anti-inflammatory, hepatoprotective, and anti-diabetic properties [25, 26]. In a recent study by Mehmood A. et al., various polyphenolic compounds such as diosmetin-7-rutinoside, quercetin-3-caffeoylglucoside-6-malonylglucoside, isorhamnetin-3-caffeoyl-7-glucoside, p-hydroxybenzoic acid, quercetin-3-(p-coumaroyl-diglucoside)-7-glucoside, kaemferol-3-(p-coumaroyl-diglucoside)-7-glucoside, and vanillic acid hexoside were identified and quantified using the HPLC method [27]. Therefore, the present study aims to investigate the protective effects of Spinacia oleracea using an in vivo rat model. The extraction of pharmacologically active phytonutrients can vary based on solvents of different polarity, thereby influencing overall biological activity. Consequently, this research comparatively evaluates petroleum and ethanolic extracts for their protective effects against an indomethacin-induced enterocolitis rat model. Furthermore, a comprehensive toxicological profiling is conducted through an acute oral toxicity study using the Up-and-Down Procedure (Test No. 425) to assess acute oral toxicity levels.

MATERIALS AND METHODS

Chemicals and reagents

Ethyl acetate, glacial acetic acid, hydrochloric acid, trichloroacetic acid, anaesthetic ether, and hydrogen peroxide were procured from Research Lab Fine Chem in Mumbai, India. Ethylenediaminetetraacetic acid (EDTA) was also obtained from the same supplier. Thiobarbituric acid was sourced from Loba Chemicals in Mumbai, India. Additionally, various other chemicals and reagents, including normal saline (sodium chloride injection IP 0.9% w/v), prednisolone (Wyselone®), and indomethacin (Microcid®) capsule, were acquired from local markets. Biochemical parameter kits were obtained from Erba Diagnostic in India.

Collection and authentication of plant material

The S. oleracea plant leaves were sourced from the Pune, Maharashtra region. The identification process took place at the Agarkar Research Institute, an autonomous entity under the

Department of Science and Technology, Government of India, situated in Pune. A voucher specimen (AUTH 23-17) was then deposited in the Herbarium of the Agarkar Research Institute for future reference.

Preparation of plant extracts

The dried and powdered leaves of the S. oleracea plant underwent an extraction process, initially with petroleum ether (60–80°C), followed by successive extraction using a soxhlet extractor with 95% ethanol. The obtained extracts were concentrated through solvent recovery and carefully dried at 50°C in a hot air oven until complete dryness was achieved. Comprehensive extractive values were determined for all extracts, appropriately labeled, and then stored in sealed containers to ensure preservation.

Experimental animals

Male Sprague Dawley Rats and Swiss albino mice were sourced from Global Bioresearch Solution Pvt Ltd., Bhor, Pune. These animals were housed in polypropylene cages under controlled conditions, with a room temperature of 22±1°C, relative humidity between 60% and 70%, and a 12:12-hour light and dark cycle. The housing was provided within an animal facility (615/PO/Re/S/2002/CPCSEA; dated on 11th June 2002). All procedures conducted strictly adhered to the protocols established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The experimental protocols for animal studies (RDCOP/Pcol-13/IAEC/2022-23/13) received prior approval from the Institutional Animal Ethical Committee (IAEC) at Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Dist. Pune – 412206, India, before the commencement of the experiment.

Phytochemical analysis

The investigation into the diverse phytoconstituents in S. oleracea leaves encompassed the extraction using petroleum ether, and ethanolic solvents. The methodology for this analysis followed the procedure outlined by Khandelwal [28].

Acute oral toxicity

An evaluation of acute oral toxicity was conducted using the Up-and-Down procedure, following the guidelines outlined in Test No. 425 [29]. Healthy female albino mice, weighing 28-32 g, were selected, and acclimatized for one week under standard conditions. The limit test was performed at 2000 mg/kg p.o. as a single dose, with mice fasting overnight before dosing while having access to water ad libitum. Within each group, a single mouse was administered either the vehicle or the corresponding test compounds, namely, S. oleracea petroleum ether and ethanolic extracts. Close observations were made during the initial 0.5 hour, followed by continuous monitoring for 4 hours. Feed was provided 2 hours post-dosing. Once the treated mouse survived, the same dose was administered to all other

animals. A similar procedure was followed for a vehicle-treated control group (0.25% Na-CMC). The various animal groups were carefully observed for potential toxic effects within the initial 6 hours and subsequently at regular intervals throughout a 14-day period. Surviving mice were continuously monitored for any toxic reactions, and their body weights were consistently recorded. After 14 days, blood was collected through the retro-orbital plexus method for hematological analysis, and blood serum was separated for biochemical evaluations. Post-euthanasia via cervical dislocation, vital organs were excised, washed with normal saline, and weighed.

Experimental design

The experimental protocol for the study of indomethacin-induced enterocolitis followed a slightly modified method based on Shanmugam S. et al. [30] and Lin X.L. et al [31]. Male Wistar rats, with a weight range of 200-230 g, were chosen for the study, and they were divided into the following groups.

Table 1. Allocation of groups and drug treatment schedule

Groups	Treatment	Indomethacin Injection Schedule	Vehicle or Drug Treatment Schedule
G1	Normal saline (s.c.) + 0.25 % Na- CMC;	D-4 and D-5	D-1 to Day-11
G2	Indomethacin- 7.5 mpk; (s.c.)	D-4 and D-5	-
G3	Prednisolone (2 mpk, p.o.,)	D-4 and D-5	D-4 to Day-11
G4	SOPE-100 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G5	SOPE-200 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G6	SOPE-400 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G7	SOEE-100 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G8	SOEE-200 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G9	SOEE-400 mpk; p.o.	D-4 and D-5	D-1 to Day-11

In the experimental design, Group G1 functioned as the normal control, receiving a subcutaneous injection of normal saline. Groups G2 to G9 were subjected to subcutaneous administration of indomethacin at a dose of 7.5 mg/kg for two consecutive days, D-4 and D-5. Group G3 was treated with the standard prednisolone at a dose of 2 mg/kg orally for 8 days (D-4 to D-11), with both prednisolone and indomethacin treatments commencing on the same day. The normal control rats were orally administered with 0.25% Na-CMC. Groups G4 to G6 received oral administration of S. oleracea petroleum ether extract (SOPE) at dose levels of 100, 200, and 400 mg/kg, respectively. Similarly, ethanolic extracts of S. oleracea (SOEE) were administered to animals in groups G7 to G9 at dose levels of 100, 200, and 400, respectively. The dose volume for oral drug administration was 10 ml/kg, and the body weight of the animals was monitored throughout the experiment. On day 12, animals were euthanized, and the small intestine, caecum, and colon

were isolated, cleaned with normal saline, and further processed for biochemical and histopathological analysis.

Assessment of disease parameters

Animal body weight

The body weight of all animals was monitored once daily from day 0 to day 12 using an animal weighing balance (Model- 440-21N, KERN Mettler, India).

Macroscopic lesion score

The intestinal tract of each rat was isolated, cleaned with normal saline, and macroscopically examined for any lesions. A lesion scoring system, adapted from Morris G.P. et al [32] with minor modifications, was utilized. Lesion scores were assigned as follows:

Lesions	Score
no visible change	0
hyperemia at sites	1
lesions with a diameter of 1 mm or less	2
lesions with a diameter of 2 mm or less; number < 5	3
lesions with a diameter of 2 mm or less; number 5–10	4
lesions with a diameter of 2 mm or less; number > 10	5
lesions with a diameter more than 2 mm; number < 5	6
lesions with a diameter more than 2 mm; number 5–10	7
lesions with a diameter more than 2 mm; number > 10	8

Digital vernier caliper (Mitutoyo, Japan) was used measure lesion diameter. The percentage area affected in gastrointestinal tract parts was calculated as reported by Shanmugam S. et al [30].

Assessment of biological parameters

On day 12, blood samples were collected, and serum separation was accomplished by centrifugation at 4000 rpm for 30 minutes using a Centrifuge 5810 R (Eppendorf, Hamburg, Germany). The analysis of the collected samples involved assessing biochemical parameters, including plasma concentrations of alkaline phosphatase (ALP), serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and total bilirubin. Erba Diagnostics Limited's kit from India was employed for the analysis.

Determination of hematological parameters

Blood was collected from the rat in K2-EDTA coated tubes and processed for the analysis of hematology parameters. The parameters measured included hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs). The analysis was conducted using a Hematology analyzer (High Technology Incorporation: Microcc-20 Vet).

Antioxidant enzymes analysis

The intestinal tissues underwent homogenization using phosphate buffer saline at pH 7.4, and subsequent analysis included the evaluation of various antioxidant enzymes such as Superoxide dismutase (SOD), Catalase, and Reduced glutathione (GSH). The assessment of lipid peroxidation was conducted by estimating Thiobarbituric acid reactive substances in the tissue homogenate.

Determination of myeloperoxidase activity

The myeloperoxidase (MPO) activity in intestinal tissue samples was determined using a modified method based on Krawisz J.E. et al [33]. The procedure involved weighing the intestinal tissue, which was then homogenized in ice-cold potassium phosphate buffer (pH 7.4; 1:10 ratio) using a Remi tissue homogenizer (RQ-127A, Remi Motors, India). After homogenization, the samples underwent centrifugation at 4000 rpm for 20 min at 4 °C using a Centrifuge 5810R (Eppendorf, Hamburg, Germany). The pellet was removed, resuspended in 10 mL of ice-cold potassium phosphate buffer (50 mM, pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide and EDTA (10 mM). Following one cycle of freezing and thawing, along with 30 seconds of sonication, the solution was centrifuged, and the supernatant was collected and stored for further processing. In the final step, 0.1 mL of the supernatant was mixed with 1.9 mL of phosphate buffer, and 1000 µL of 1.5 mol/L 167 µg/mL O-dianisidine hydrochloride containing 0.0005% hydrogen peroxide. The changes in absorbance at 460 nm were monitored for 3 minutes using a UV-VIS spectrophotometer (V-530, JASCO instruments, Tokyo).

Colon tissue weight ratio

The colon tissue underwent isolation, cleaning, and length measurement in centimetres using a ruler scale. Following this, the tissue was weighed on an analytical balance. The colon tissue weight ratio was then calculated using the formula: Colon weight (gm) / Colon length (cm).

Histopathological analysis

The caecum tissue was carefully dissected, preserved in 10% neutral buffered formalin, embedded in paraffin, and sectioned into solid slices with a thickness ranging from 3 to 5 µm. These sections were subsequently stained using hematoxylin-eosin. To minimize bias, histological slides were blindly examined by a veterinary pathologist. The evaluation of each tissue slide took into consideration significant lesions such as ulceration, hemorrhage, necrosis, inflammatory cell infiltrations, or any other observed abnormalities. Lesion severity was scored as follows: 0 = Not Present, 1 = Minimal (<1%), 2 = Mild (1-25%), 3 = Moderate (26-50%), 4 = Severe (51-100%) [34, 35]. The score for each animal was determined by summing the observed lesion scores.

Statistical analysis

The data are presented as mean ± standard deviation (SD; n=6, except n=5 for acute oral toxicity) for each group. Statistical analysis was conducted using GraphPad Prism software 5.0. One-way or Two-way analysis of variance (ANOVA) was employed, followed by Dunnett's multiple comparison t-test or Bonferroni's multiple comparison t-test, respectively, to determine statistical differences. Significance levels were indicated as *p<0.05, **p<0.01, ***p<0.001 compared to the disease control group, and #p<0.05 compared to the normal control.

RESULT & DISCUSSION

Phytochemical analysis

The yield of S. oleracea petroleum ether extract (SOPE) and S. oleracea ethanolic extract (SOEE) was determined to be 10.2% and 13.6%, respectively. A thorough analysis of phytoconstituents in S. oleracea leaves was conducted for both petroleum ether and ethanolic extracts. SOPE exhibited the presence of a mild level of carbohydrates, tannins, saponins, phenolic compounds, flavonoids, and a substantial content of fixed oils and fats. Furthermore, it demonstrated a moderate level of phytosterols. In the case of SOEE (S. oleracea ethanolic extract), the analysis revealed the presence of alkaloids, carbohydrates, glycosides, and saponins at moderate to strong levels. Furthermore, phytosterols, phenolic compounds, proteins, amino acids, tannins, and flavonoids were observed at strong levels. Fixed oils and fats were found at a mild level (Table 2).

Table 2. Phytochemical analysis of petroleum ether, and ethanolic extract of S. oleracea

Phytochemical tests	Pet. ether extract	Ethanolic extract
Alkaloids		
Dragendorff's test	-	++
Hager's test	-	++
Wagner's test	-	+
Carbohydrates		
Molisch's test	+	+++
Barfoed's test	-	++
Benedict's test	+	++
Glycosides		
Molisch's test after hydrolysis	-	+++
Phytosterols		
Liebermann's Burchard's test	++	+++
Fixed oils and fats		

Spot test	+++	+
Saponification test	+++	+
Saponins		
Foam test	+	+++
Haemolysis test	+	++
Phenolic compounds and tannins		
Ferric chloride test	+	+++
Lead acetate test	+	+++
Proteins and amino acids		
Biuret test	-	+++
Ninhydrin test	-	+++
Flavonoids		
Shinoda test	+	+++

Mild: +; Moderate: ++; Strong: +++; - Absent

Acute oral toxicity

The influence of Petroleum ether (SOPE) and ethanolic (SOEE) extracts of S. oleracea leaves at a dose of 2000 mg/kg on behavioral patterns and body weight is presented in Tables 3 and 4. No occurrences of convulsions, tremors, or coma were noted in animals treated with SOPE and SOEE at the specified dosage. All parameters, including eyes, fur and skin condition, mucous membrane, salivation, sleep, and urination (color), were found to be normal. The respiration rate and salivation showed slight increases during the initial 30 minutes in animals treated with SOPE and SOEE, respectively. No instances of animal mortality were recorded in any group up to day 14. Throughout the experimental period, animals treated with SOPE and SOEE displayed no itching behavior (Table 3). While SOPE and SOEE did not exhibit a significant impact on % body weight change until day 14, the relative weight of the liver, heart, and kidneys remained significantly unaltered ($p>0.05$) due to the treatment of SOPE and SOEE (Table 4).

The impact of SOPE and SOEE on renal and liver function tests is elaborated in Table 5. No significant ($p>0.05$) changes were observed in serum creatinine and urea levels in the treatment groups compared to normal mice. Moreover, liver function tests, including SGOT, SGPT, total proteins, total bilirubin, alkaline phosphatase, albumin, and globulin levels, were within the normal range ($p>0.05$) in animals treated with SOPE and SOEE (Table 5). Additionally, the levels of total cholesterol, triglycerides, HDL-c, and LDL-c did not show significant ($p>0.05$) alterations in mice treated with SOPE and SOEE compared to normal animals, as detailed in Table 6.

Table 7 presents the effects of SOPE and SOEE on hematological parameters tested at a dosage of 2000 mg/kg. Blood parameters, encompassing hemoglobin, RBCs, WBCs, platelets, as well as differential WBCs including monocytes, neutrophils, lymphocytes, eosinophils, and basophils, did not demonstrate significant ($p>0.05$) variations following the administration of the test plant extracts.

Table 3. Effect of petroleum ether and ethanolic extract of S. oleracea on behavioural observations (acute oral toxicity study)

Parameters	Observations																	
	0.5 h			4 h			24 h			48 h			Day-7			Day-14		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	**A**	**B**	C
Convulsions & tremors	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Coma	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Faeces consistency	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Eye	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Fur & Skin	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Itching	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Mucous membrane	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Respiration	Nr	E	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	E	Nr	Nr	E	Nr	Nr	E
Salivation	Nr	Nr	E	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	E	Nr	Nr	E	Nr	Nr	E
Somatomotor activity & behaviour pattern	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Sleep	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Urination (colour)	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr

A: Normal control; B: SOPE; C: SOEE, Nr: Normal; Ab: Absent; NF: Not Found; NO: Not Observed, E: Elevated; L: Lowered

Table 4. Effect of petroleum ether and ethanolic extract of S. oleracea on animal body weight change and relative organ weight (acute oral toxicity study)

Groups	Body Weight Change (%)			Relative Organ Weight (g per 10g of Body Weight)		
	Day-1	Day-7	Day-14	Heart	Kidney	Liver
NC	3.38±2.86	4.84±2.26	13.79±8.06	0.068±0.00	0.157±0.02	0.611±0.02
SOPE-2000	2.49±1.67	5.44±2.50	17.44±2.86	0.069±0.00	0.150±0.01	0.619±0.02
SOEE-2000	4.19±3.64	6.48±3.18	18.19±2.50	0.069±0.00	0.160±0.01	0.621±0.02

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to normal control at respective time points. (Body weight change- A Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test; Relative organ weight- A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 5. Effect of petroleum ether and ethanolic extract of S. oleracea on renal and liver function tests (acute oral toxicity study)

Groups	Renal Function Test		Liver Function Test						
	Creatinine (mg/dL)	Urea (mg/dL)	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphatase (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
NC	0.70±0.0	57.67±6.4	88.72±10.8	56.2±5.9	159.0±10.8	0.26±0.0	7.29±1.1	3.50±0.3	3.39±0.1
SOPE-2000	0.72±0.1	61.39±9.0	97.71±3.1	59.0±9.3	164.0±7.1	0.29±0.1	7.11±0.7	3.66±0.3	3.41±0.3
SOEE-2000	0.69±0.1	62.80±9.6	90.80±6.7	55.7±8.4	159.0±13.3	0.26±0.0	7.13±1.1	3.51±0.4	3.49±0.3

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to normal control. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 6. Effect of petroleum ether and ethanolic extract of S. oleracea on lipid profile (acute oral toxicity study)

Groups	Lipid Profile			
	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
NC	91.98±5.9	95.81±6.7	36.89±5.5	35.51±5.7
SOPE-2000	91.21±7.5	99.50±3.9	34.79±2.2	34.20±4.9
SOEE-2000	93.78±7.5	94.58±8.2	36.06±4.4	37.21±4.6

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to normal control. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 7. Effect of petroleum ether and ethanolic extract of S. oleracea on haematology parameters (acute oral toxicity study)

Groups	Haematology Parameters								
	Hb (g/dL)	RBCs (10^6/µL)	WBCs (10^3/µL)	Platelets (10^3/µL)	Monocytes (%)	Neutrophiles (%)	Lymphocytes (%)	Eosinophiles (%)	Basophiles (%)
NC	15.4±1.0	6.0±0.2	5.6±0.5	768.2±30.6	1.8±0.4	32.6±5.0	60.2±11.0	2.0±1.0	0.4±0.5

SOPE-2000	15.4±0.6	5.6±0.3	5.7±0.3	805.8±51.2	1.4±0.5	31.2±4.0	63.8±7.2	1.6±0.9	0.2±0.4
SOEE-2000	15.3±0.6	5.7±0.2	5.9±0.5	787.0±25.3	1.2±0.4	32.0±2.5	65.2±6.5	1.6±0.5	0.2±0.4

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to normal control. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Animal body weight

Figure 1 illustrates a notable decline in animal body weight from day-6 to day-12 in disease control animals after the indomethacin injection (D-4 and D-5), demonstrating a significant (p<0.001) difference compared to normal control animals. Animals treated with SOPE at doses of 100 and 200 did not show any recovery in body weight loss. However, at a high dose of SOPE, 400 mg/kg, there was a substantial (p<0.05-0.001) increase in body weight observed from day-7 to day-12. Similarly, SOEE at 100 treatment was ineffective in recovering body weight loss compared to disease animals. However, SOEE at 200 mg/kg was effective in regaining body weight from day-9 to day-10 (p<0.05-0.001). At a dose of 400 mg/kg, SOEE displayed a significant increase in body weight from day-7 to day-12 (p<0.05-0.001). Prednisolone, the standard drug, significantly (p<0.001) increased animal body weight from day-8 (p<0.05) to day-12.

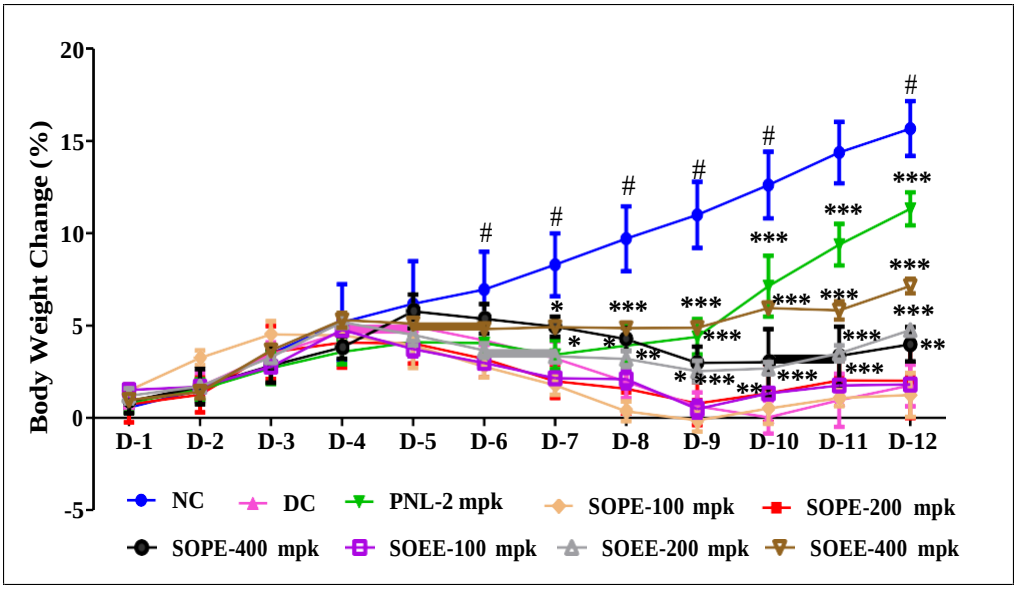


Figure 1. Effect of petroleum ether and ethanolic extract of S. oleracea on animal body weight change. Values in the results are expressed as mean± SD, (n=6); Data is analyzed by Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to DC. #p<0.05 significantly different in comparison to NC**. Abbreviations:** NC: Normal control; DC: Disease control; PSN-2: Prednisolone at 2 mg/kg; SOPE: Spinacia oleracea petroleum ether extract; SOEE: Spinacia oleracea ethanolic extract

Macroscopic lesion score

The findings presented in Table 8 delineate the impact of SOPE and SOEE on macroscopic lesion score and % affected area. Subcutaneous injection of indomethacin (7.5 mg/kg) resulted in notable lesions in the intestinal area of the disease control animals. Animals treated with SOPE at the dose levels of 100 and 200 mg/kg exhibited minimal effects ($p>0.05$) on macroscopic lesion score, % protection, and % affected area. However, the high dose of SOPE, 400 mg/kg, significantly ($p<0.01$) reduced macroscopic lesion score and % affected area compared to diseased animals. It also demonstrated significant ($p<0.01$) protection (%) against lesions induced by indomethacin. Conversely, SOEE treatment at 200 ($p<0.01$) and 400 mg/kg ($p<0.001$) showcased a significant effect against macroscopic lesions and % area affected. Mid and high doses of SOEE (200 and 400 mg/kg) significantly ($p<0.01$) protected the tissue against indomethacin-induced lesions. The standard drug, prednisolone, at a dose of 2 mg/kg, exhibited a significant ($p<0.001$) positive impact on lesion score, % protection, and % affected area.

Table 8. Effect of petroleum ether and ethanolic extract of S. oleracea on macroscopic lesion score, % protection and % affected area

Groups	Macroscopic Lesion Score	% Protection	% Area Affected
NC	0.00±0.0	0.00±0.0	0.00±0.0
DC- INDO-7.5; s.c.	7.83±0.4#	0.00±0.0	7.83±4.1#
Prednisolone-2	1.17±0.8***	85.11±9.6***	1.17±7.5***
SOPE-100	7.67±0.5	3.55±5.5	76.67±5.2
SOPE-200	7.50±0.5	5.32±5.8	75.00±5.5
SOPE-400	6.50±0.8**	17.02±10.7**	65.00±8.4**
SOEE-100	7.17±0.4	8.87±4.3	71.67±4.1
SOEE-200	6.33±0.8**	19.15±10.4**	63.33±8.2**
SOEE-400	6.00±1.1***	23.40±14.0***	60.00±11.0***

Values in the results are expressed as mean ± SD (n=6). *$p<0.05$, **$p<0.01$, ***$p<0.001$, significantly different in comparison to disease control. #$p<0.05$, significantly different in comparison to normal control. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Biochemical parameters

In the biochemical analysis, disease control animals exhibited a significant ($p<0.001$) increase in serum levels of GOT, GPT, alkaline phosphatase, and total bilirubin compared to normal. However, animals treated with SOPE at 400 mg/kg showed a notable decrease

in SGOT ($p<0.05$), SGPT ($p<0.05$), alkaline phosphatase ($p<0.01$), and total bilirubin ($p<0.05$) levels. Similarly, elevated levels of serum GOT ($p<0.05$-0.01), GPT ($p<0.05$-0.01), alkaline phosphatase ($p<0.01$) were significantly reduced by treatment with SOEE at 200 and 400 mg/kg. The total bilirubin level was considerably reduced by 100 ($p<0.05$), 200 ($p<0.01$), and 400 mg/kg ($p<0.001$) of SOEE. Prednisolone also exhibited a significant ($p<0.001$) effect in restoring the altered biochemical parameters (Table 9).

Table 9. Effect of petroleum ether and ethanolic extract of S. oleracea on liver function test and haematology parameters

Groups	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphatase (U/L)	Total Bilirubin (mg/dL)	Hb (g/dL)	RBCs (10^6/µL)	WBCs (10^3/µL)	PLTs (10^3/µL)
NC	87.3±8.9	114.8±9.5	158.7±9.7	0.3±0.0	15.6±1.1	9.3±0.7	9.7±1.0	647.8±68.6
DC- INDO-7.5; s.c.	61.9±7.8#	84.0±5.6#	92.3±7.2#	1.1±0.1#	11.8±1.6#	4.9±0.8#	7.3±0.8#	437.3±65.7#
Prednisolone; 2	84.1±8.0***	108.9±6.5***	152.2±13.4***	0.5±0.1***	14.8±1.0**	9.1±1.3***	9.4±0.4***	657.0±76.6***
SOPE-100	64.5±7.3	89.3±3.9	97.0±7.8	1.0±0.1	12.54±1.8	4.93±0.7	7.20±0.7	458.50±49.7
SOPE-200	71.8±5.9	94.3±9.7	100.9±5.9	1.0±0.1	12.05±1.0	5.41±0.9	7.66±0.8	491.33±34.2
SOPE-400	76.2±12.7*	97.9±7.8*	113.5±12.7**	0.9±0.1*	13.90±1.4*	6.25±0.5*	8.24±0.4	544.50±48.7*
SOEE-100	69.0±4.7	90.1±4.2	98.5±7.9	0.9±0.1*	13.1±1.5	5.8±0.5	7.7±0.2	473.2±72.1
SOEE-200	72.5±4.2*	97.4±6.9*	102.6±5.7	0.9±0.1**	13.8±1.6*	6.7±0.9**	8.3±0.4*	558.7±40.7**
SOEE-400	78.0±5.7**	101.2±8.7**	114.3±11.6**	0.8±0.1***	14.9±0.9**	8.7±1.1***	8.6±0.7*	609.5±15.2***

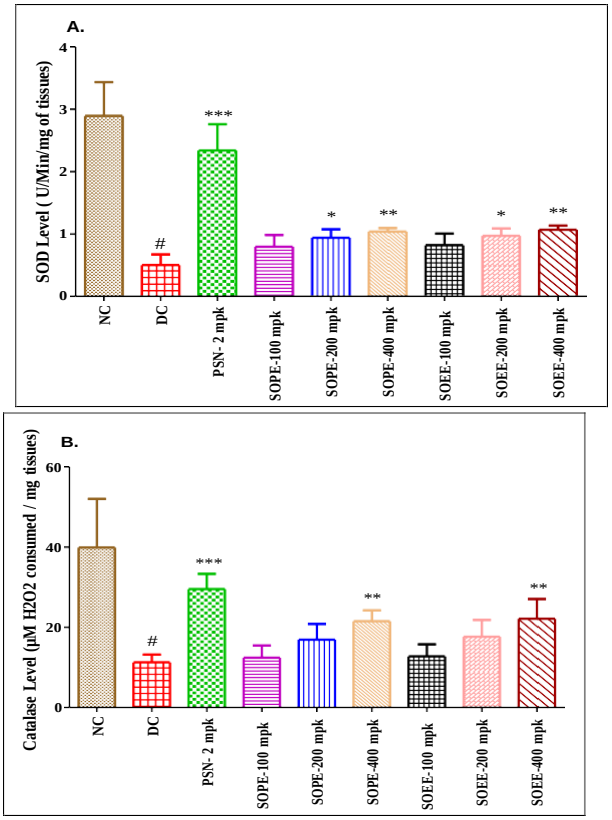
Values in the results are expressed as mean ± SD (n=6). *$p<0.05$, **$p<0.01$, ***$p<0.001$, significantly different in comparison to disease control. #$p<0.05$, significantly different in comparison to normal control. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Hematology parameters

Table 9 depicts the impact of SOPE and SOEE on haematological parameters. Animals in the disease control group exhibited a significant ($p<0.001$) decrease in haemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). Treatment with SOPE only at doses of 100 and 200 showed minimal ($p>0.05$) effect on restoring haematological parameters; however, at 400 mg/kg, all blood parameters were significantly improved ($p<0.05$) except for WBCs. Additionally, SOEE significantly elevated Hb ($p<0.05$-0.01), RBCs ($p<0.01$-0.001), WBCs ($p<0.05$), and PLTs levels ($p<0.01$-0.001) at 200 and 400 mg/kg compared to disease rats. The standard drug, prednisolone, exhibited a significant ($p<0.001$) effect in increasing all examined blood parameters.

Antioxidant enzymes analysis

The levels of antioxidant enzymes, namely SOD, catalase, and reduced glutathione, showed a significant decrease in diseased rats compared to the normal group (Figure 2 A-C). Following treatment with SOPE and SOEE at doses of 200 ($p<0.05$) and 400 mg/kg ($p<0.01$), there was a notable increase in SOD levels. Similarly, the catalase level was significantly raised ($p<0.01$) with SOPE and SOEE treatment at 400 mg/kg. While SOEE at 400 mg/kg effectively restored reduced glutathione levels, SOPE exhibited no significant effect at any dosage. Moreover, both SOPE and SOEE at 400 mg/kg demonstrated a significant ($p<0.05$) decrease in lipid peroxidation. Prednisolone significantly ($p<0.01$-0.001) restored the altered levels of the investigated antioxidant components, including lipid peroxidation (Figure 2D).



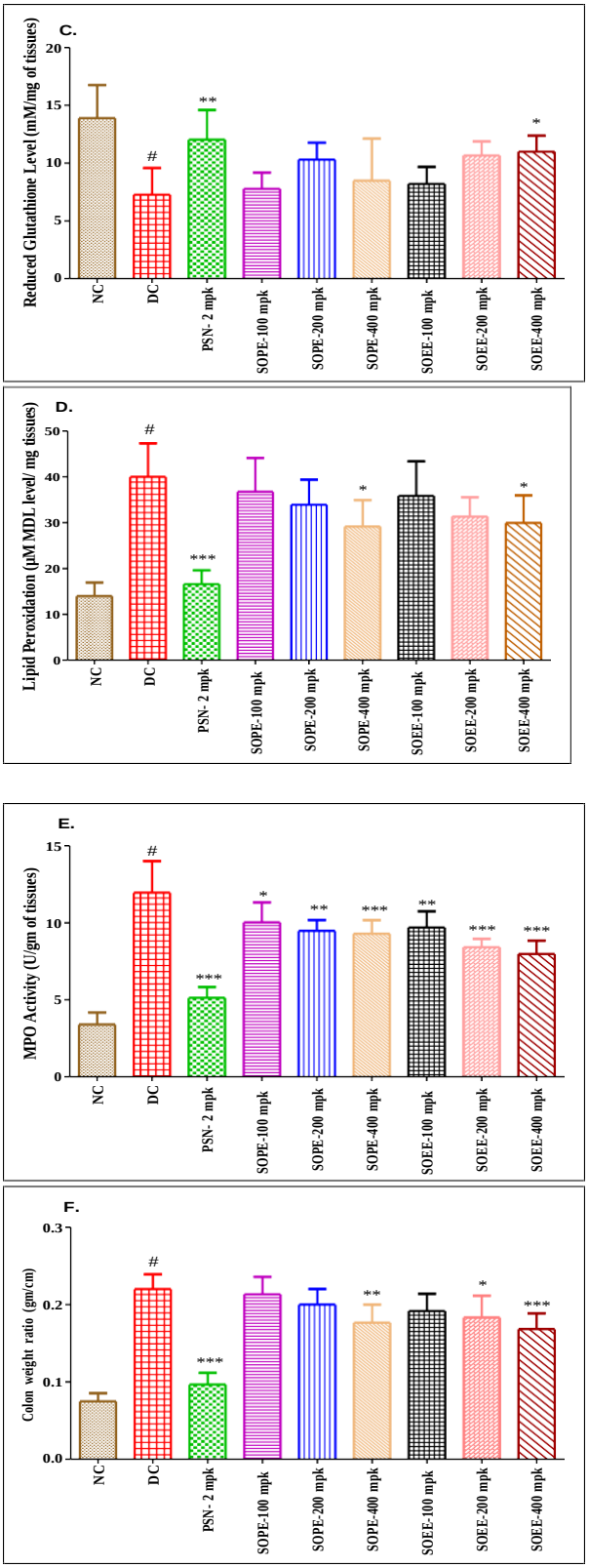


Figure 2. Effect of petroleum ether and ethanolic extract of S. oleracea on antioxidant enzyme levels, MPO activity and colon weight ratio in rats A) SOD B) Catalase C) Reduced glutathione D) Lipid Peroxidation E) MPO activity F) Colon weight ratio. Values in the results are expressed as mean± SD, (n=6); Data is analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's multiple

comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to DC. #p<0.05 significantly different in comparison to NC. **Abbreviations:** NC: Normal control; DC: Disease control; PSN: Prednisolone; SOPE: Spinacia oleracea petroleum ether extract; SOEE: Spinacia oleracea ethanolic extract

Myeloperoxidase activity

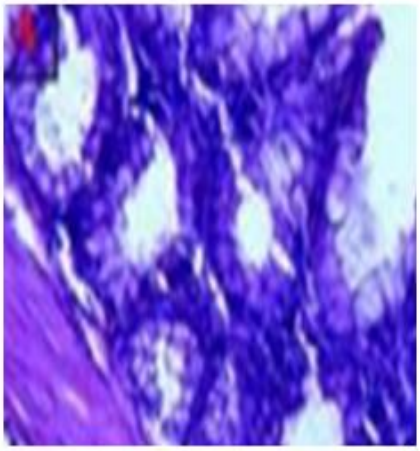
Figure 2-E depicts the myeloperoxidase activity in intestinal tissue samples. The disease control animals exhibited a significant (p<0.001) increase in MPO activity compared to the normal group. Animals treated with SOPE at 100 (p<0.05), 200 (p<0.01), and 400 mg/kg (p<0.001) showed a notable decline in MPO levels in a dose-dependent manner. Similarly, SOEE at 100, 200, and 400 mg/kg significantly (p<0.01-0.001) reduced MPO activity, with results showing dose-dependency. Prednisolone, the standard drug, demonstrated a significant (p<0.001) decrease in MPO activity.

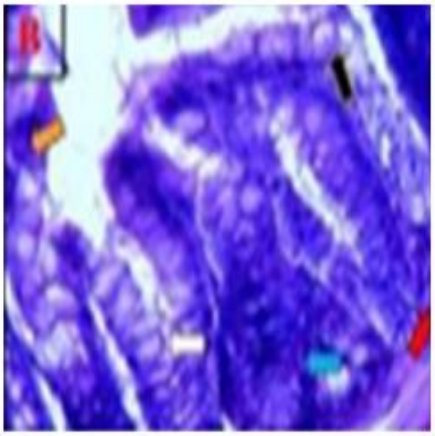
Colon tissue weight ratio

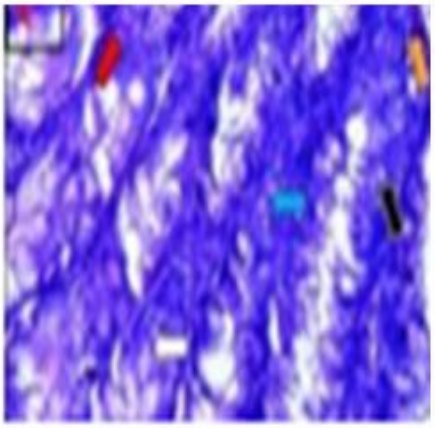
The colon tissue weight ratio exhibited a significant (p<0.001) increase in diseased rats compared to the normal group. Treatment with SOPE at a high dose of 400 mg/kg demonstrated effectiveness in significantly (p<0.01) reducing the colon tissue weight ratio. Conversely, SOEE at doses of 200 (p<0.05) and 400 mg/kg (p<0.001) displayed notable inhibition in the colon weight ratio, with a clear dose-dependent pattern observed with SOEE treatment. Prednisolone also significantly (p<0.001) inhibited the colon weight ratio at a dose of 2 mg/kg (Figure- 2F).

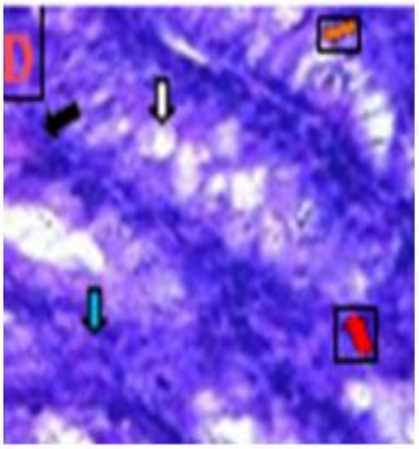
Histopathological analysis

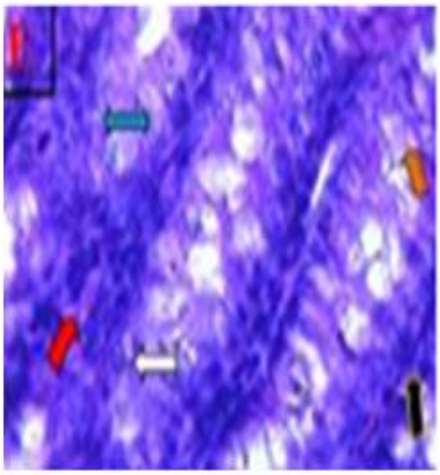
In diseased animals, the caecum tissue exhibited significant (p<0.001) severe lesions, including ulceration, hemorrhage, necrosis, and leukocytic infiltrations compared to normal (Figure 3 A-I). The lesion score of the disease control rats was determined to be 14.33±0.82. Treatment with SOPE at a dose of 100 showed no significant effect (p>0.05) in minimizing the lesions. However, at doses of 200 (p<0.05) and 400 mg/kg (p<0.001), moderate lesions were observed in the tissues, and the lesion score was significantly (p<0.01) reduced to 12.50±1.52 and 10.67±0.52, respectively. Similarly, animals treated with SOEE at doses of 100 (p<0.01), 200 (p<0.001), and 400 mg/kg (p<0.001) displayed a significant reduction in the lesion score (12.00±0.63; 11.50±1.22, and 10.17±1.17, respectively). All the lesions were found to be at a mild level (Figure 4). The administration of the standard drug, Prednisolone, resulted in a significant (p<0.001) improvement in elevated lesions and lesion score (Figure 4).

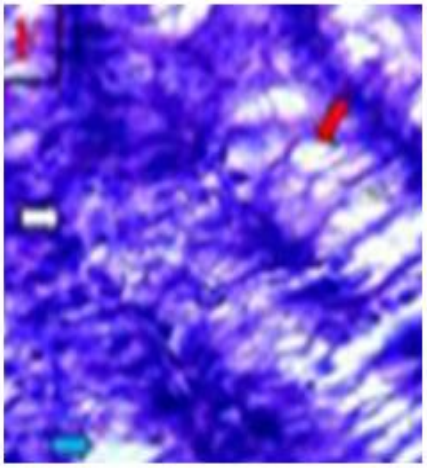












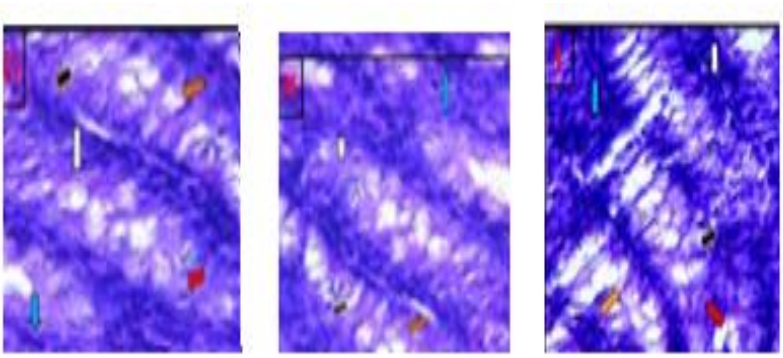


Figure 3. Effect of petroleum ether and ethanolic extract of S. oleracea on histopathological analysis (H & E stain, 400X) of caecum tissue. A) NC B) DC C) PSN at 2 mg/kg D) SOPE-100 mg/kg E) SOPE-200 mg/kg F) SOPE-400 mg/kg G) SOEE-100 mg/kg H) SOEE-200 mg/kg I) SOPE-400 mg/kg. Histopathological images of H&E-stained caecum was tissues taken at high magnification (400X) displays, ulceration (orange arrow), hyperemia (red arrow), cellular infiltration (blue arrow), goblet cell hyperplasia (white arrow) and necrosis (black arrow) **Abbreviations:** NC: Normal control; DC: Disease control; PSN: Prednisolone; SOPE: Spinacia oleracea petroleum ether extract; SOEE: Spinacia oleracea ethanolic extract.

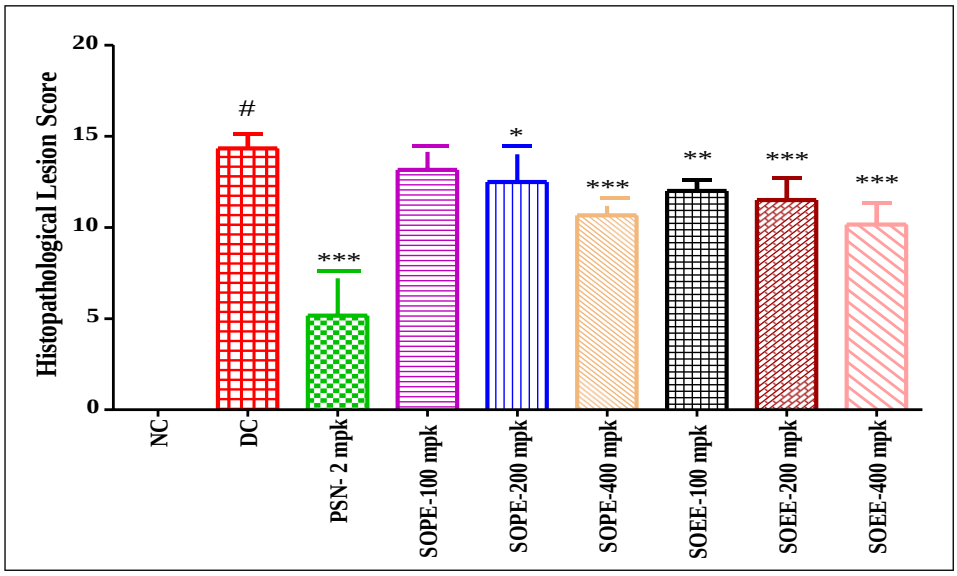


Figure 4. Effect of petroleum ether and ethanolic extract of S. oleracea on histopathological lesion scores of caecum tissue. Values in the results are expressed as mean± SD, (n=6); Data is analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to DC. #p<0.05 significantly different in comparison to NC. **Abbreviations:** NC: Normal control; DC: Disease control; PSN: Prednisolone; SOPE: Spinacia oleracea petroleum ether extract; SOEE: Spinacia oleracea ethanolic extract

DISCUSSION

In this study, we conducted a comparative analysis to assess the protective effects of petroleum and ethanolic extracts of S. oleracea against indomethacin-induced enterocolitis, alongside an examination of acute oral toxicity in experimental animals. Performing an initial toxicological evaluation is essential to ensure the safety of herbal medicines, as these natural products may contain bioactive compounds with potential unintended adverse effects on human health. Clinical signs and symptoms serve as primary indicators among various toxicity markers, providing valuable insights into the potential adverse effects of drugs on vital organs [36, 37]. The acute oral toxicity of SOPE and SOEE was investigated in Swiss albino mice following OECD guideline-425. Our findings revealed that various behavioral observations, including eyes, fur and skin condition, mucous membrane, salivation, sleep, and urination (color), all exhibited normal patterns in animals treated with SOPE and SOEE. Although there was a slight increase in respiration rate and salivation in the SOPE and SOEE-treated animals, overall somatomotor activity and behavior patterns remained normal. Importantly, no instances of animal mortality were recorded in any group during the 14-day observation period. Furthermore, renal and liver function tests, as well as hematological parameters, showed no significant alterations. Additionally, there were no changes in organ weights of the heart,

kidney, and liver. Our study provides valuable insights into the acute oral toxicity of SOPE and SOEE, suggesting an overall favorable safety profile due to the absence of animal mortality and the observation of normal behavioral and physiological parameters. However, the observed changes in respiration rate and salivation emphasize the importance of conducting further investigations to better understand the underlying physiological responses. The interpretation of these findings is crucial within the context of the intended use and dosage of herbal medicines, and ongoing monitoring is essential for a comprehensive understanding of their safety profile.

Shanmugam S. et al. [30] and Vemu B. et al. [40] have previously reported a significant decrease in body weight following indomethacin administration. In line with these findings, our study also observed notable body weight loss after two doses of subcutaneous indomethacin injection at 7.5 mg/kg. However, treatment with SOPE at a high dose of 400 mg/kg and SOEE at mid (200 mg/kg) and high doses (400 mg/kg) showed substantial recovery in body weight loss. NSAIDs have been extensively employed to attain analgesic, antinociceptive, and anti-inflammatory effects; nevertheless, a significant drawback associated with their usage is linked to gastrointestinal (GI) side effects. There is a rising prevalence of enteropathy attributed to NSAID consumption, whereas the occurrence of upper gastroduodenal damage is on the decline [38]. The principal pathological alterations induced by NSAIDs encompass endothelial physical barrier impairment resulting from NSAID-induced disruptions in prostaglandin E synthesis, oxidative stress, inflammatory stress, immune dysregulation, and dysbiosis of intestinal microorganisms [39]. The administration of indomethacin led to intestinal ulceration, which can be distinguished into topical and resolution phases. In the topical phase, there was observed sloughing of mucus and epithelial layers, accompanied by a decrease in the protective effects of phosphatidylcholine [41, 42]. During the resolution phase, the inhibition of cyclooxygenase- 1 (COX-1) enzymes resulted in a decline in the synthesis of anti-inflammatory mediators. Studies in humans have indicated that COX-1 expression is maximum in the small intestine, and injury from non-selective NSAIDs is primarily observed in the upper gastrointestinal tract, highlighting the role of COX-1 in the resolution phase for anti-inflammatory prostaglandin synthesis. In rats, NSAID-induced injury is mainly limited to the lower gastrointestinal tract, possibly due to variations in COX-1 enzyme distribution [43, 40]. In our current investigation, indomethacin administration resulted in hyperemia, bleeding, mucosal destruction, and hemorrhagic lesions in the GI tract, contributing to an increased macroscopic lesion score. However, treatment with SOPE and SOEE significantly mitigated these pathophysiological changes, as evidenced by a notable decrease in the macroscopic lesion score. Interestingly, SOEE demonstrated superior effectiveness in ameliorating the pathological changes induced by indomethacin in the intestine compared to SOPE.

Numerous scientific studies have reported that the administration of indomethacin for two consecutive days induces chronic inflammation in the gastrointestinal (GI) tissues of rats [44, 45]. In this study, we aimed to investigate the protective effects of petroleum and ethanolic extracts of S. oleracea leaves using an indomethacin-induced enterocolitis rat model. Various endpoint parameters, including animal body weight, macroscopic scoring, biochemical analysis, antioxidant enzymes, myeloperoxidase activity, hematology, and histopathological parameters, were evaluated to confirm the protective effects of the plant extracts.

The analysis of serum biochemicals, such as SGPT, SGOT, ALP, and bilirubin enzymes, serves as a valuable quantitative method for evaluating gastrointestinal damage, as these enzymes are typically found at elevated concentrations in the cytoplasm. Normally, these enzymes are not present in the serum; however, during tissue damage, their leakage into the serum can provide crucial diagnostic information regarding the nature and consequences of the damage [46]. In our study, indomethacin administration led to a decline in SGPT, SGOT, ALP levels, and a significant elevation in bilirubin enzymes. However, treatment with SOPE (400 mg/kg) and SOEE (200 and 400 mg/kg) effectively ameliorated these altered enzyme levels. Similarly, subcutaneous administration of indomethacin at 2 mg/kg resulted in a significant reduction in hematology parameters, including Hb, RBCs, WBCs, and PLTs. The reduced levels of these blood components were attenuated by the ethanolic extract of S. oleracea. SOPE effectively restored the altered changes in Hb, RBCs, and PLTs only at a dose of 400 mg/kg. These findings are consistent with earlier reports by Shanmugam S. et al [30].

The excessive production of free radicals in gastrointestinal tissues can have deleterious effects on proteins and nucleic acids [47, 48]. Enzymatic antioxidants like SOD, CAT, GSH, as well as non-enzymatic antioxidant compounds, play crucial roles in counteracting the excessive free radicals generated during diseased conditions [49]. In our study, the activities of enzymatic antioxidants, including SOD, CAT, and GSH, in intestinal tissues were notably decreased, while lipid peroxidation was increased following indomethacin administration, indicating a clear association of oxidative stress with intestinal tissue damage. However, both SOPE and SOEE significantly elevated the levels of antioxidant enzymes and reduced lipid peroxidation, except for SOPE, which did not alter GSH levels.

Neutrophils are equipped with the enzyme Myeloperoxidase (MPO), which contributes to the generation of hypochlorous acid (HOCl), known for its antibacterial properties. This enzyme plays a pivotal role in the breakdown of gastrointestinal mucin and the peroxidation of lipids and proteins, leading to the release of hydroxyl and chloride radicals, ultimately resulting in oxidative stress [50]. The data from our study revealed that indomethacin administration led to an increase in MPO activity, while treatment with SOPE and SOEE resulted in a significant decrease in MPO activity, indicating their potent inhibitory effects on MPO.

Moreover, the administration of indomethacin resulted in an increased colon weight in diseased animals compared to the normal group, thus confirming the clinical manifestation of the disease. However, treatment with SOPE at a high dose and SOEE at mid and high doses significantly decreased colon weight. The observed overproduction of pro-inflammatory mediators such as MPO, IL-1β, and TNF-α suggests their involvement in colon inflammation [47]. The protective effect of SOPE at a high dose of 400 mg/kg and SOEE at mid and high doses (200 and 400 mg/kg) resulted in a significant reduction in colon weight. The more pronounced effect of SOEE compared to SOPE could be attributed to the presence of various polyphenolic compounds known to possess anti-inflammatory and antioxidant activities.

Histopathological examination of the caecum tissues revealed significant abnormal changes in the caecum tissues of diseased rats, including ulceration, hyperemia, cellular infiltration, goblet cell hyperplasia, and necrosis. Treatment with SOPE and SOEE at doses of 200 and 400 mg/kg demonstrated protective effects by attenuating the severity of these lesions to mild levels and reducing their intensity. The results obtained with SOEE were particularly noteworthy and found to be more effective compared to SOPE.

The current study confirms that treatment with SOPE and SOEE effectively mitigates indomethacin-induced enterocolitis, as demonstrated by macroscopic, histological, and biochemical analyses. Moreover, SOEE exhibits more robust protective effects against indomethacin-induced enterocolitis compared to SOPE. S. oleracea leaves contain numerous polyphenolic components, including diosmetin-7-rutinoside, quercetin-3-caffeoylglucoside-6-malonylglucoside, isorhamnetin-3-caffeoyl-7-glucoside, p-hydroxybenzoic acid, quercetin-3-(p-coumaroyl-diglucoside)-7-glucoside, kaemferol-3-(p-coumaroyl-diglucoside)-7-glucoside, and vanillic acid hexoside. Additionally, S. oleracea leaves have been previously demonstrated to possess antioxidant, anti-inflammatory, and anti-ulcer activities [51,52]. Phytochemical analysis reveals a high quantity of polyphenolic compounds in SOEE. Therefore, the robust protective effects observed against indomethacin-induced enterocolitis by SOEE are likely attributed to these phytoconstituents.

CONCLUSION

In conclusion, this study investigated the protective effects of petroleum ether (SOPE) and ethanolic (SOEE) extracts of Spinacia oleracea leaves against indomethacin-induced enterocolitis in rats. The findings suggest that both SOPE and SOEE are safe at a single oral dose of 2000 mg/kg. However, SOEE exhibited superior efficacy in mitigating various parameters associated with enterocolitis compared to SOPE, likely due to its higher concentration of potent bioactive compounds such as phenolic compounds and flavonoids. These compounds are known for their antioxidant, anti-inflammatory, and antiulcer properties. SOEE demonstrated significant antioxidant

activity by restoring levels of SOD, catalase, reduced glutathione, and lipid peroxidation. Additionally, it exhibited anti-inflammatory effects by inhibiting myeloperoxidase activity, indicating its potential therapeutic benefits in reducing oxidative stress and inflammation. The presence of phytosterols in SOPE also contributed to its protective effects. Overall, the study highlights the potential of Spinacia oleracea extracts, particularly SOEE, as a natural remedy for mitigating indomethacin-induced enterocolitis. However, further research is needed to fully understand the mechanisms underlying these therapeutic effects and to explore their potential clinical applications in treating gastrointestinal disorders.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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