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Pharmacological Assessment of *Glycyrrhiza glabra* Roots using Indomethacin-induced Enteropathy Rat Model: A Comparative Analysis of Petroleum Ether and Alcoholic Extracts

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KEYWORDS

Glycyrrhiza glabra, indomethacin, myeloperoxidase, prednisolone, enterocolitis, colon weight

ABSTRACT:

Glycyrrhiza glabra Linn, (G. glabra) commonly known as "Mulethi" in North India, has been investigated for its protective effects against indomethacin-induced enterocolitis in a rat model. The study also included toxicological profiling using the Up-and-Down Procedure (Test No. 425: Acute Oral Toxicity). Findings revealed the safety of GGPE and GGEE at 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 both plant extracts. In the disease model, GGPE at 400 mg/kg and GGEE at 200 and 400 mg/kg exhibited significant protective effects against indomethacin-induced enterocolitis. This was evident through improvements in macroscopic, biochemical, hematological, and histological data. Indomethacin administration (7.5 mg/kg, s.c. for two doses) induced alterations in various parameters, which were effectively mitigated by GGPE and GGEE. Specifically, these treatments significantly ($p<0.05$) reduced macroscopic lesion scores, enhanced antioxidant enzymes (SOD, CAT, GSH), liver function enzymes (SGOT, SGPT, ALP), and decreased bilirubin and LPO levels. Furthermore, GGPE and GGEE treatment considerably ($p<0.05$) elevated blood parameters (Hb, RBCs, WBCs, PLTs) and recovered body weight loss. GGEE at 400 mg/kg demonstrated effectiveness in restoring elevated levels of MPO and colon weight. Histopathological analysis indicated the amelioration of intestinal tissue, reducing the intensity of lesions. In conclusion, the study highlights that GGEE exhibits more pronounced protective effects against indomethacin-induced enterocolitis compared to GGPE, possibly due to the presence of polyphenolic phytoconstituents in GGEE.

INTRODUCTION

Glycyrrhiza glabra Linn (family: Leguminosae), commonly referred to as "Mulethi" in North India, is also known by the name's licorice and sweet wood. Indigenous to the Mediterranean and specific regions of Asia, this plant has a history of traditional use for various purposes. Traditional healers have ascribed its effectiveness as a diuretic, choleretic (stimulating bile secretion), and even as an insecticide. Moreover, it has been traditionally used to address issues such as coughs, colds, and painful swellings [1-3].

Inflammation is an intricate physiological process occurring in body tissues, orchestrated by a diverse range of signaling molecules, encompassing both pro-inflammatory and anti-inflammatory mediators. [4]. Common inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis lead to inflammation, resulting in severe gastrointestinal (GI) symptoms such as diarrhea, bleeding, abdominal pain,

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weight loss, anemia, edema, and hemorrhaging. Additionally, IBD is associated with ulceration of the mucosa and submucosa of the colon and rectum [5].

Current treatment of IBD includes anti-inflammatory drugs such as mesasalazine, sulfasalazine, corticosteroids, immunosuppressives (corticosteroids, azathioprine,), antibacterials (metronidazole, ornidazole, clarithromycin), biologics (infliximab, adalimumab, certolizumab pegol etc.) and probiotics (Saccharomyces boulardii, Lactobacillus sp, Bifidobacterium sp.) [6]. 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 [7]. Corticosteroids used in IBD treatments show severe side effects, such as diabetes mellitus, opportunistic infections, ocular effects, hypertension, venous thromboembolism, osteoporosis, etc. [8, 9]. 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 [10].

Globally, there is a growing demand for traditional medicine systems in the treatment of various diseases. In India, around 80% of the rural population depends on medicinal herbs or indigenous medicine systems. The Indian herbal industry employs nearly 960 plant species, generating a turnover exceeding 80 billion rupees [11, 12]. Although medicines derived from plants are generally expected to have low toxicity, specific medicinal plants used in traditional medicine have been reported to display toxic effects [13].

G. glabra extracts exhibit various pharmacological activities, including antitussive, antimicrobial, antioxidant, anti-inflammatory, antiulcer, anticancer, and more. These extracts contain pharmacologically active phytoconstituents such as triterpenes, saponins, flavonoids, alkaloids, glycyrrhizin, glycyrrhetic acid, glabridin, liquiritin, etc. [14]. Previous scientific reports have demonstrated their diverse pharmacological activities using various animal models. However, there is a current research gap in comparing extracts from solvents of different polarity to assess their protective effects against an indomethacin-induced enterocolitis rat model. The extraction of pharmacologically active phytonutrients can vary with solvents of different polarity, influencing overall biological activity. In this study, petroleum and ethanolic extracts were comparatively evaluated for their protective effects against an indomethacin-induced enterocolitis rat model. Additionally, toxicological profiling was conducted through an acute oral toxicity study using the Up-and-Down Procedure (Test No. 425) for acute oral toxicity.

MATERIALS AND METHODS

Chemicals and reagents

Tricholroacetic acid, ethyl acetate, acetic acid glacial, hydrochloric acid, anaesthetic ether, hydrogen peroxide, Ethyl diamine tetra acetic acid procured from Research Lab Fine Chem., Mumbai, India. Thiobarbituric acid was obtained from Loba Chemicals, Mumbai, India. Various other chemicals and reagents, including normal saline (sodium chloride injection IP 0.9% w/v), prednisolone (Wyselone®), indomethacin (Microcid®) tablets were sourced from the local market. Biochemical parameters kits were procured from Erba Diagnostic, India.

Collection and authentication of plant material

The *G. glabra* plant roots 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 23-18) was then deposited in the Herbarium of the Agarkar Research Institute for future reference.

Preparation of plant extracts

The dried powdered roots of the *G. glabra* plant were subjected to extraction, first using petroleum ether (60–80°C), followed by successive extraction with 95% ethanol in a soxhlet extractor. The resulting extracts underwent concentration through solvent recovery and were thoroughly dried at 50°C in a hot air oven until complete dryness. Extractive values were meticulously

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determined for all extracts, accurately labeled, and subsequently stored in sealed containers for preservation.

Experimental animals

Male Sprague Dawley Rats and Swiss albino mice were obtained from Global Bioresearch Solution Pvt Ltd., Bhor, Pune. These animals were housed in polypropylene cages under controlled conditions, maintaining a room temperature of 22±1°C, a relative humidity ranging between 60% and 70%, and a 12:12-hour light and dark cycle. The accommodation was provided within an animal facility (615/PO/Re/S/2002/CPCSEA; dated on 11th June 2002). All procedures conducted in the study 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 approval from the Institutional Animal Ethical Committee (IAEC) at Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Dist. Pune – 412206, India, prior to the initiation of the experiment.

Phytochemical analysis

The analysis of diverse phytoconstituents in *G. glabra* roots was carried out through the utilization of petroleum ether, chloroform, and hydroalcoholic extracts. The methodology for this examination followed the outlined procedure by Khandelwal [15].

Acute Oral Toxicity

An assessment of acute oral toxicity was conducted using the Up-and-Down procedure, as described in Test No. 425 [16]. Healthy female albino mice, weighing 28-32 g, were chosen, and acclimatized for one week under standard conditions. The limit test was carried out at 2000 mg/kg p.o. as a single dose, with mice fasting overnight before dosing while having access to water *ad libitum*. A single mouse from each group received the dose of the vehicle or the respective test compound. Observations were closely monitored 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 observed for any toxic reactions, and their body weights were consistently monitored. After 14 days, blood was collected through the retro-orbital plexus method for hematological analysis, and blood serum was separated for biochemical evaluations. Vital organs were excised post-euthanasia via cervical dislocation, washed with normal saline, and weighed.

Experimental Design

The slightly modified method of Shanmugam S et al. [17] and Lin X-l et al [18] was followed for the experiment of indomethacin induced enterocolitis study. Male Wistar rats weighing between 200-230 g were selected in the study. Animals were divided into 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 mg/kg; (s.c.)	D-4 and D-5	-
G3	Prednisolone (2 mg/kg, p.o.,)	D-4 and D-5	D-4 to Day-11
G4	GGPE-100 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G5	GGPE-200 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G6	GGPE-400 mpk; p.o.	D-4 and D-5	D-1 to Day-11

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G7	GGEE-100 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G8	GGEE-200 mpk; p.o.	D-4 and D-5	D-1 to Day-11
G9	GGEE-400 mpk; p.o.	D-4 and D-5	D-1 to Day-11

In the experimental design, Group G1 served as the normal control and received subcutaneous injection of normal saline. Animals in groups G2 to G9 were administered indomethacin at a dose of 7.5 mg/kg subcutaneously for two consecutive days, D-4 and D-5. Group G3 was treated with standard prednisolone at a dose of 2 mg/kg orally for 8 days (D-4 to D-11), with both prednisolone and indomethacin treatments starting on the same day. Normal control rats were orally administered with 0.25% Na-CMC. Groups G4 to G6 were orally administered GGPE at dose levels of 100, 200, and 400 mg/kg, respectively. Similarly, ethanolic extracts of *G. glabra* (GGEE) were given 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 animal body weight was monitored throughout the experiment. On day 12, animals were humanely sacrificed, and the small intestine, caecum, and colon were isolated, cleaned with normal saline, and further processed for biochemical and histopathological analysis.

Assessment disease parameters

Determination animal body weight

All the animal body weight was monitored once in a day from day 0 to day 12 using animal weighing balance (Model- 440-21N, KERN Mettler, India.).

Examination macroscopic lesion score

Each rat intestinal tract was isolated, cleaned with normal saline, and macroscopically observed for any lesions. The lesion scoring, adapted from Morris et al [19] with minor modifications, was employed. 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 Saravanan Shanmugam et al [17].

Assessment of biological parameters

The blood samples were collected on day-12, and serum separation was achieved by centrifugation at 4000 rpm for 30 minutes using a Centrifuge 5810 R (Eppendorf, Hamburg, Germany). Subsequent analysis of the collected samples included assessing biochemical parameters such as 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 utilized 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

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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 were homogenized using phosphate buffer saline at pH 7.4 and subsequently analyzed for various antioxidant enzymes, including Superoxide dismutase (SOD), Catalase, and Reduced glutathione (GSH). Lipid peroxidation was assessed by estimating Thiobarbituric acid reactive substances in the tissue homogenate.

Determination Myeloperoxidase activity

The myeloperoxidase (MPO) activity was estimated in the intestinal tissue samples using the modified method of Krawisz JE et al [20]. The intestinal tissue was weighed and homogenized in ice-cold potassium phosphate buffer (pH 7.4; 1:10 ratio) using a Remi tissue homogenizer (RQ-127A, Remi Motors, India). Following 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 and 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). This mixture underwent one cycle of freezing and thawing, followed by 30 seconds sonication. The solution was further centrifuged, supernatant was collected and stored for further process. In the end, 0.1 mL of 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 was isolated, cleaned and length was measured in centimetre using ruler scale. The colon tissue was weighed on analytical weighing balance. Colon tissue weight ratio was calculated by the formula: Colon weight (gm) / Colon length (cm)

Histopathological analysis

The caecum tissue was dissected, preserved in 10% neutral buffered formalin, embedded in paraffin, and cut into solid sections with a thickness of 3-5 µm. Subsequently, these sections were stained with hematoxylin-eosin. To minimize bias, a blind examination of histological slides was carried out by a veterinary pathologist. The analysis of each tissue slide considered major lesions such as ulceration, hemorrhage, necrosis, and inflammatory cell infiltrations or any other observed lesions. The severity of observed lesions was scored as follows: 0= Not Present, 1= Minimal (<1%), 2= Mild (1-25%), 3= Moderate (26-50%), 4= Severe (51-100%) [21, 22]. The score for each animal was the sum of the observed lesion scores.

Statistical analysis

The data are expressed as mean ± standard deviation (SD; n=6, except n=5 for acute oral toxicity) for each group. Statistical analysis was carried out using GraphPad Prism software 5.0. One-way or Two-way analysis of variance (ANOVA) was performed, 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.

RESULTS

Phytochemical analysis

The yield of GGPE (Glycyrrhiza glabra petroleum ether extract) and GGEE (Glycyrrhiza glabra ethanolic extract) was determined to be 10.2% and 12.8%, respectively. The examination of phytoconstituents in *G. glabra* roots was carried out for both petroleum ether and ethanolic extracts. GGPE showed the presence of a mild level of tannins and a strong content of fixed oils and fats. Additionally, it exhibited the presence of a moderate level of phytosterols.

For GGEE, the examination showed the presence of alkaloids, carbohydrates, and saponins at moderate to strong levels. Additionally, glycosides, phytosterols, phenolic compounds, tannins, and flavonoids were observed at a strong level. Fixed oils, fats, proteins, and amino acids were found at a moderate level (Table 2).

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Table 2. Phytochemical analysis of petroleum ether and ethanolic extracts of *G. glabra*

Phytochemical tests	Pet. ether extract	Ethanolic extract
Alkaloids		
Dragendroff's test	-	++
Hager's test	-	+++
Wagner's test	-	++
Carbohydrates		
Molisch's test	-	+++
Barfoed's test	-	++
Benedicts 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 impact of Petroleum ether (GGPE) and alcoholic (GGEE) extracts of *G. glabra* roots at a dosage of 2000 mg/kg on behavioral patterns and body weight is presented in Tables 3 and 4. No instances of convulsions, tremors, or coma were observed in animals treated with GGPE and GGEE at the specified dose. 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 were marginally elevated during the initial 30 minutes in animals treated with GGPE and GGEE. No animal mortality was recorded in any group up to day 14. Throughout the experimental period, animals treated with GGPE and GGEE exhibited no itching behavior (Table 3). While GGPE and GGEE did not show a significant effect on % body weight change until day 14. The relative weight of liver heart and kidney were significantly ($p>0.05$) unaltered by the treatment of GGPE and GGEE (Table 4).

The influence of GGPE and GGEE on renal and liver function tests is detailed in Table 5. There were no significant ($p>0.05$) changes in serum creatinine and urea levels in the treatment groups compared to normal mice. Furthermore, liver function tests, including SGOT, SGPT, total proteins, total bilirubin, alkaline phosphatase, albumin, and globulin levels, were found to be within the normal range ($p>0.05$) in animals treated with GGPE and GGEE (Table 5). Additionally, the levels of total cholesterol, triglycerides, HDL-c, and LDL-c did not exhibit significant ($p>0.05$) alterations in mice treated with GGPE and GGEE compared to normal animals, as outlined in Table 6.

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Table 7 displays the impact of GGPE and GGEE on hematological parameters tested at 2000 mg/kg. Blood parameters, including hemoglobin, RBCs, WBCs, platelets, and differential WBCs such as monocytes, neutrophils, lymphocytes, eosinophils, and basophils, exhibited no significant ($p>0.05$) variations with the administration of the test plant extracts.

Table 3. Effect of petroleum ether and ethanolic extract of *G. glabra* on behavioural observations (acute oral toxicity study)

Parameters	**Observations**																	
	30 Min			**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
	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Itching	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
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	E	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	E	Nr	Nr	E	Nr	Nr	E
Salivation	Nr	E	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: GGPE; C: GGEE, Nr: Normal; Ab: Absent; NF: Not Found; NO: Not Observed, E: Elevated; L: Lowered

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

Groups; Dose (mg/kg)	**Body Weight Change (%)**			**Relative Organ Weight (per 10g)**		
	Day-1	**Day-7**	**Day-14**	**Heart**	**Kidney**	**Liver**
NC	3.55±3.17	4.56±3.32	7.04±3.64	0.072±0.00	0.166±0.02	0.651±0.03
GGPE-2000	2.74±1.98	5.32±2.83	7.65±2.12	0.076±0.00	0.163±0.01	0.686±0.02

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GGEE-2000	4.39±4.08	6.68±3.65	9.60±4.14	0.076±0.00	0.173±0.01	0.671±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. (A Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test)

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

Groups; Dose (mg/kg)	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	56.21±5.9	159.07±10.8	0.26±0.0	7.29±1.1	3.50±0.3	3.39±0.1
GGPE-2000	0.72±0.1	60.58±9.4	96.85±3.0	58.25±8.9	163.50±6.7	0.28±0.1	7.03±1.2	3.59±0.4	3.42±0.3
GGEE-2000	0.69±0.1	62.99±9.7	89.91±6.9	54.34±8.4	157.77±13.3	0.26±0.0	7.27±1.3	3.36±0.6	3.38±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 *G. glabra* on lipid profile (acute oral toxicity study)

Groups; Dose (mg/kg)	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
GGPE-2000	89.97±7.1	98.89±4.8	33.64±1.9	33.07±4.9
GGEE-2000	94.81±7.5	95.11±7.7	35.37±5.1	38.29±2.7

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 *G. glabra* on haematology parameters (acute oral toxicity study)

Groups; Dose (mg/kg)	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
TAPE-2000	15.3±0.9	5.6±0.3	5.8±0.2	789.0±34.4	1.2±0.4	31.0±4.8	63.2±6.0	1.6±0.5	0.2±0.4

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TAHE-2000	15.3±0.3	5.8±0.2	5.8±0.3	782.6±26.0	1.2±0.4	31.2±3.0	63.8±6.9	2.0±0.7	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 that the disease control animals exhibited a significant (p<0.001) decrease in animal body weight from day-6 to day-12 after the injection of indomethacin (D-4 and D-5) compared to normal control animals. Animals treated with GGPE at doses of 100 and 200 did not show any recovery in body weight loss. However, at a high dose of GGPE, 400 mg/kg, a considerable (p<0.05-0.001) increase in body weight change was observed from day-8 to day-11. Similarly, GGEE at 100 and 200 mg/kg treatment was unable to recover the body weight loss compared to disease animals, except at 200 mg/kg on day-10 (p<0.01). At a dose of 400 mg/kg, GGEE demonstrated a prominent increase in body weight from day-6 to day-12 (p<0.05 to p<0.001). Prednisolone, the standard drug, significantly (p<0.001) increased animal body weight from day-6 (p<0.05) to day-12.

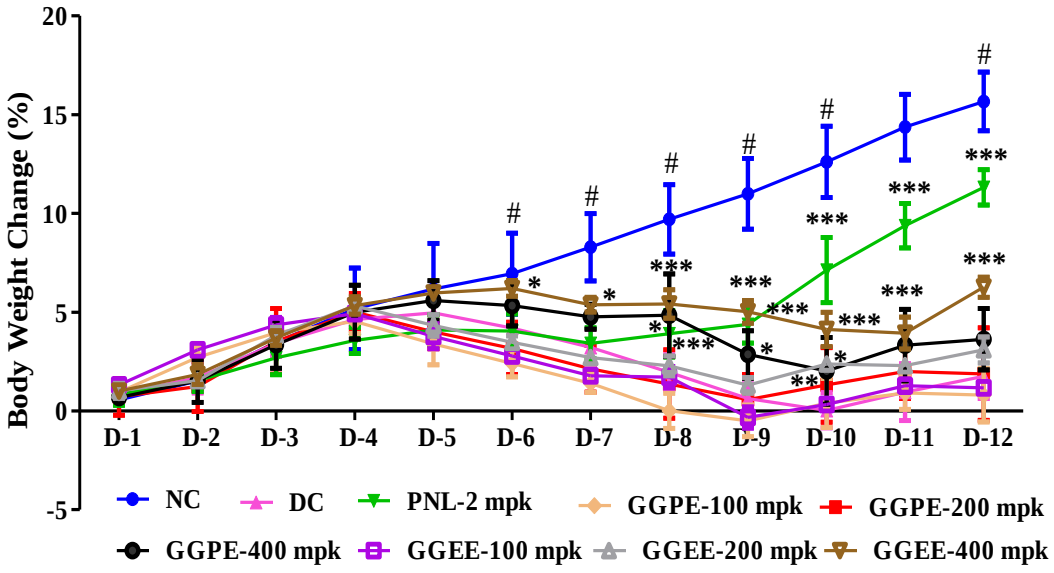

Figure 1: Effect of petroleum ether and ethanolic extract of *G. glabra* on animal body weight change. Values in the results are expressed as mean± SD, (n=6); Data is analyzed by Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly 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; *GGPE: Glycyrrhiza glabra* petroleum ether extract; GGEE: *Glycyrrhiza glabra* ethanolic extract

Macroscopic lesion score

The results presented in Table 8 indicate the effect of GGPE and GGEE on macroscopic lesion score and % affected area. The subcutaneous injection of indomethacin at the dose of 7.5 mg/kg resulted in significant lesions in the intestinal area of the disease control animals. Animals treated with GGPE at the dose levels of 100 and 200 mg/kg displayed a minimal effect (p>0.05) on macroscopic lesion score, % protection, and % affected area. However, the high dose of GGPE significantly reduced macroscopic lesion score and % affected area compared to diseased animals. It also exhibited a significant (p<0.01) protection (%) against lesions induced by indomethacin. On the other hand, GGEE treatment at 100 (p<0.01), 200 (p<0.001), and 400 mg/kg (p<0.001) demonstrated a significant effect

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against macroscopic lesions and % area affected. All three dose levels of GGEE significantly ($p<0.01$) protected the tissue against indomethacin-induced lesions. The standard drug, prednisolone, at the 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 *G. glabra* on macroscopic lesion score, % protection and % affected area

Groups; Dose (mg/kg)	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***
GGPE-100	7.50±0.5	5.32±5.8	7.50±5.5
GGPE-200	7.33±0.5	7.09±5.5	7.33±5.2
GGPE-400	6.33±0.8**	19.15±10.4**	6.33±8.2**
GGEE-100	6.33±0.8**	19.15±10.4**	63.33±8.2**
GGEE-200	5.50±0.8***	29.79±10.7***	55.00±8.4***
GGEE-400	4.83±0.8***	38.30±9.6***	48.33±7.5***

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 ($p<0.001$) elevation in serum GOT, GPT, alkaline phosphatase, and total bilirubin levels compared to normal. However, animals treated with GGPE at 400 mg/kg demonstrated a noteworthy reduction 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 ($p<0.05$), GPT ($p<0.05$, $p<0.01$), alkaline phosphatase ($p<0.05$, $p<0.01$), and total bilirubin ($p<0.01$, $p<0.05$) were significantly declined by the treatment of GGEE at 200 and 400 mg/kg. Prednisolone also demonstrated a significant ($p<0.001$) effect in restoring the altered biochemical parameters (Table 9).

Table 9: Effect of petroleum ether and ethanolic extract of *G. glabra* on liver function test and haematology parameters

Parameters	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#

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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***
GGPE-100	64.0±6.9	88.5±4.2	96.7±7.3	1.0±0.1	11.9±1.8	5.1±1.0	7.3±0.8	447.2±50.6
GGPE-200	71.0±5.7	93.3±9.8	100.1±5.7	1.0±0.1	12.2±1.1	5.4±0.7	7.8±0.7	495.2±29.6
GGPE-400	75.3±12.7*	96.9±8.0*	112.7±12.6**	0.9±0.1*	12.1±1.8	6.2±0.5	8.2±0.4	541.5±44.1*
GGEE-100	67.9±7.2	91.6±4.5	98.5±6.7	0.9±0.1	12.4±1.9	5.7±0.5	7.7±0.4	472.5±76.5
GGEE-200	77.1±8.6*	96.9±8.0*	110.0±6.5*	0.9±0.1**	14.1±1.0*	6.5±0.5*	8.4±0.7	550.8±43.2*
GGEE-400	78.8±12.1*	99.9±6.9**	115.3±12.7**	0.8±0.1***	14.6±0.9**	8.3±1.4***	8.6±0.6*	603.5±19.3***

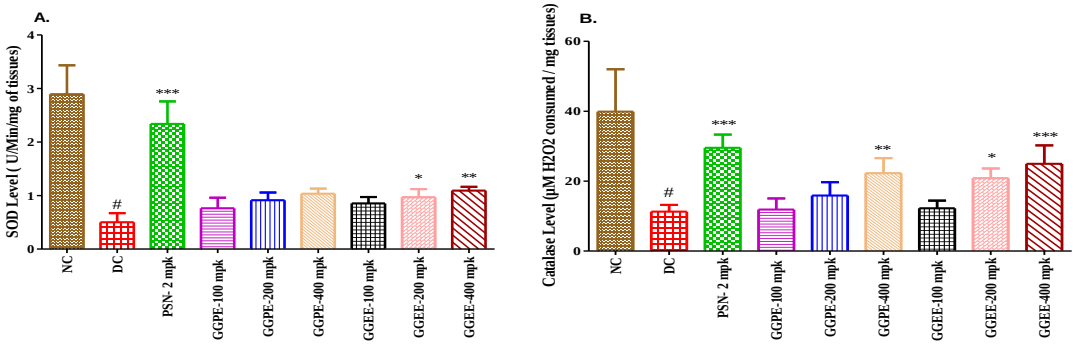
Values in the results are expressed as mean ± SD (n=6). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to disease control. #p<0.05, significantly different in comparison to normal control. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Hematology parameters

Table 9 illustrates the effect of GGPE and GGEE on haematological parameters. Rats of 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). However, treatment with GGPE at doses of 100, 200, and 400 mg/kg displayed minimal (p>0.05) effect on restoring haematological parameters, except for PLTs level, which was significantly elevated at the 400 mg/kg dose. Additionally, GGEE significantly elevated Hb, RBCs, and PLTs levels at 200 and 400 mg/kg compared to disease rats. WBCs level was considerably elevated only at the high dose of 400/kg of GGEE. The standard drug, prednisolone, exhibited a significant (p<0.001) effect in elevating all investigated blood parameters.

Antioxidant enzymes analysis

The levels of antioxidant enzymes, including SOD, catalase, and reduced glutathione, were significantly reduced in diseased rats compared to normal rats (Figure 2 A-C). After treatment with GGPE at 400 mg/kg, there was a notable increase only in catalase (p<0.01) and reduced glutathione (p<0.05). The doses of 200 and 400 mg/kg of GGPE were found to be ineffective (p>0.05) in restoring the altered SOD, catalase, and reduced glutathione levels. In contrast, GGEE at 200 and 400 mg/kg effectively elevated SOD, catalase, and reduced glutathione levels (p<0.05-0.01). Prednisolone significantly (p<0.01-0.001) increased the levels of the investigated antioxidant components. Similarly, elevated lipid peroxidation was restored significantly by GGPE (200 mg/kg; p<0.05) and GGEE (200 and 400 mg/kg; p<0.01-001) and prednisolone (p<0.001) (Figure 2D).



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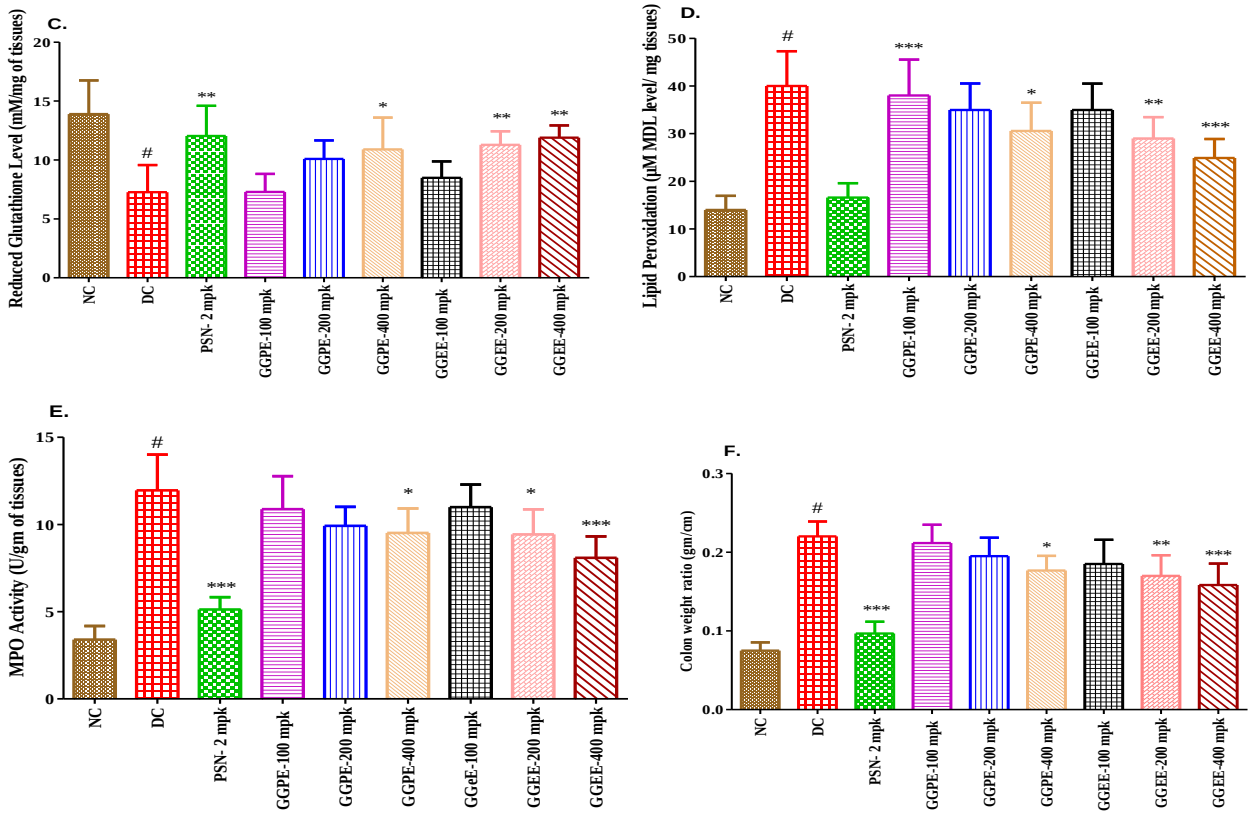

Figure 2: Effect of petroleum ether and ethanolic extract of *G. glabra* on antioxidant enzyme levels, MPO activity and colon weight ratio in rats A) SOD B) Catalase C) Reduced glutathione D) Lipid peroxidation E) MPO activity F) Colon weight ratio. Values in the results are expressed as mean± SD, (n=6); 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; *GGPE: Glycyrrhiza glabra* petroleum ether extract; GGEE: *Glycyrrhiza glabra* ethanolic extract

Myeloperoxidase activity

Figure 2-E illustrates the myeloperoxidase activity in intestinal tissue samples. The disease control animals displayed a significant ($p<0.001$) elevation in MPO activity compared to normal. Animals treated with GGPE at a higher dose, 400 mg/kg, considerably decreased ($p<0.05$) the MPO level. Lower doses of GGPE, at 100 and 200 mg/kg, had a marginal ($p>0.05$) effect on MPO activity. However, GGEE at mid and high doses, 200 ($p<0.05$) and 400 mg/kg ($p<0.001$), significantly decreased MPO activity, and the results were found to be dose dependent. Prednisolone, the standard drug, exhibited a significant ($p<0.001$) decline in MPO activity.

Colon tissue weight ratio

The colon tissue weight ratio was significantly ($p<0.001$) elevated in diseased rats compared to normal. Treatment with GGPE at a high dose of 400 mg/kg was found to be effective in significantly ($p<0.05$) decreasing the colon tissue weight ratio. However, GGEE at 200 ($p<0.01$) and 400 mg/kg ($p<0.001$) displayed a prominent inhibition in colon weight ration. The dose-dependency pattern was observed with GGEE treatment. Prednisolone also significantly ($p<0.001$) inhibited colon weight ratio at the dose of 2 mg/kg (Figure- 2G).

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Histopathological analysis

The caecum tissue in diseased animals 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 13.83±0.98. Treatment with GGPE at 100 and 200 mg/kg showed no significant effect ($p>0.05$) in minimizing the lesions. However, at the 400 mg/kg dose, moderate lesions were observed in the tissues, and the lesion score was significantly ($p<0.01$) reduced to 11.13±0.52. Similarly, animals treated with GGEE at 200 ($p<0.01$) and 400 mg/kg ($p<0.001$) doses displayed a significant decrease in the lesion score (12.00±1.55; 9.00±1.41, 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).

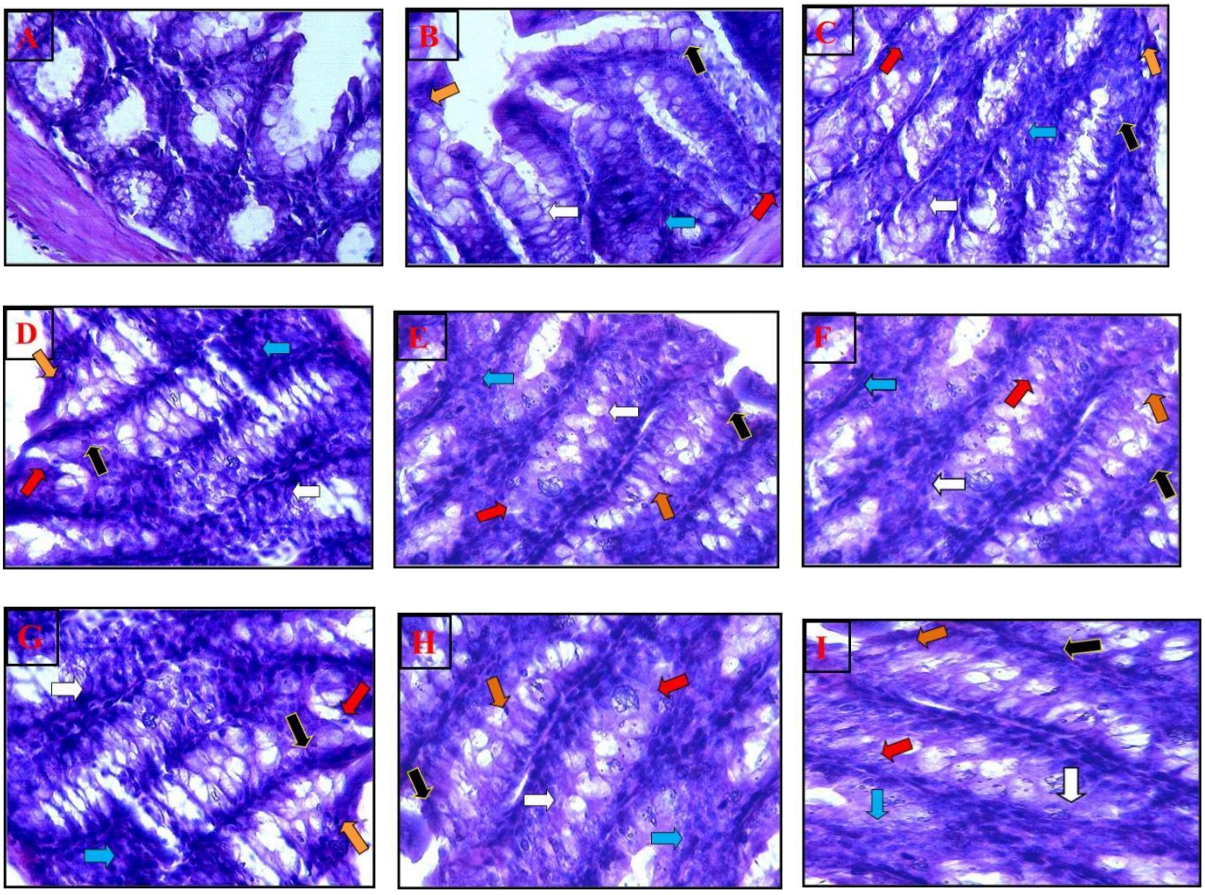

Figure 3: Effect of petroleum ether and ethanolic extract of *G. glabra* on histopathological analysis (H & E stain, 400X) of caecum tissue. A) NC B) DC C) PSN- 2 mg/kg D) GGPE-100 mg/kg E) GGPE-200 mg/kg F) GGPE-400 mg/kg G) GGEE-100 mg/kg H) GGEE-200 mg/kg I) GGPE-400 mg/kg. Histopathological images of H&E-stained caecum 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; GGPE: *Glycyrrhiza glabra* petroleum ether extract; GGEE: *Glycyrrhiza glabra* ethanolic extract

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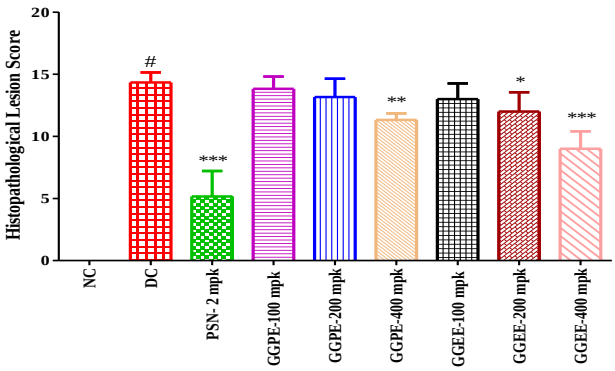

Figure 4: Effect of petroleum ether and ethanolic extract of *G. glabra* on histopathological lesion scores of caecum tissue. Values in the results are expressed as mean± SD, (n=6); Data is analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test; *p<0.05, **p<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; *GGPE: Glycyrrhiza glabra* petroleum ether extract; GGEE: *Glycyrrhiza glabra* ethanolic extract

DISCUSSION

In this investigation, we conducted a comparative assessment of the protective effects of petroleum and ethanolic extracts of *G. glabra* against indomethacin-induced enterocolitis, along with an evaluation of acute oral toxicity in experimental animals. Undertaking a preliminary toxicological assessment is crucial to ensuring the safety of herbal medicines, given that 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 insights into the potential adverse effects of drugs on vital organs [23, 24]. The acute oral toxicity of GGPE and GGEE was investigated in Swiss albino mice following OECD guideline-425. The findings revealed that various behavioral observations, including eyes, fur and skin condition, mucous membrane, salivation, sleep, and urination (color), all displayed normal patterns in animals treated with GGPE and GGEE. There was a slight increase in respiration and salivation in both treated groups, and somatomotor activity and behavior patterns were generally normal. Importantly, no instances of animal mortality were recorded in any group during the 14-day observation period. Additionally, renal, liver function tests, and hematological parameters showed no significant alterations. Furthermore, there were no changes in organ weights of the heart, kidney, and liver. This study provides valuable insights into the acute oral toxicity of GGPE and GGEE, 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 highlight the need for additional 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.

NSAIDs have been widely used to achieve analgesic, antinociceptive, and anti-inflammatory effects; however, a major limitation of NSAID usage has been related to GI side effects. The prevalence of enteropathy due to NSAID consumption is growing, while the rate of upper gastroduodenal damage is decreasing [25]. The main pathological changes induced by NSAIDs are the damage of endothelial physical barrier due to NSAIDs caused disorders of prostaglandin E synthesis, oxidative stress, inflammatory stress, immune dysregulation and intestinal microorganism dysbiosis [26].

Bhaskar Vemu et al. [27] and Saravanan Shanmugam et al. [17] reported a significant decrease in body weight following indomethacin administration. Consistent with these findings, our study demonstrated notable body weight loss after two doses of subcutaneous indomethacin injection at 7.5 mg/kg. Treatment with GGPE at a high dose and GGEE at mid and high doses showed substantial recovery in body weight loss. The administration of indomethacin resulted in intestinal ulceration, distinguishable into topical and resolution phases. In the topical phase, there was observed sloughing of mucus and epithelial layers, coupled with a reduction in the protective effects of phosphatidylcholine [28, 29]. During the resolution phase, the inhibition of COX-1 enzymes led to a decline in the synthesis of anti-inflammatory mediators. Human studies indicated that

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COX-1 expression is highest in the small intestine, and injury from non-selective NSAIDs is confined to the upper gastrointestinal tract, underscoring the role of COX-1 in the resolution phase for anti-inflammatory prostaglandin synthesis. In rats, NSAID-induced injury is limited to the lower gastrointestinal tract, potentially due to variations in COX-1 enzyme distribution [30, 27]. In the current investigation, indomethacin administration resulted in hyperemia, bleeding, mucosal destruction, and hemorrhagic lesions in GI tract, contributing to an increased macroscopic lesion score. Treatment with GGPE and GGEE significantly mitigated these pathophysiological changes, evidenced by a notable decrease in the macroscopic lesion score. GGEE demonstrated superior effectiveness in ameliorating the pathological changes induced by indomethacin in the intestine compared to GGPE.

Several scientific reports have documented that the administration of indomethacin for two consecutive days induces chronic inflammation in the GI tissues of rats [31, 32]. In the present investigation, we studied the protective effects of petroleum and ethanolic extracts of *G. glabra* seeds using indomethacin induced enterocolitis rat model. Different end point parameters viz. animal body, macroscopic scoring, biochemical analysis, antioxidant enzymes, myeloperoxidase activity, hematology and histopathological parameters were studied to confirm the protective effects of plants extracts.

The analysis of serum biochemicals, including SGPT, SGOT, ALP, and bilirubin enzymes, proves to be a valuable quantitative method for assessing gastrointestinal (GIT) damage, as these enzymes are present at elevated concentrations in the cytoplasm. Under typical circumstances, these enzymes are not present in the serum; however, during instances of tissue damage, there may be leakage, leading to their presence in the serum. The examination of these serum enzymes is crucial for clinical diagnosis, providing information on the nature and consequences of tissue damage [33]. In the present investigation, SGPT, SGOT, ALP, level was declined, and bilirubin enzymes significantly declined by the indomethacin. However, GGPE and GGEE found effective in ameliorating the altered these enzymes levels. Similalrly, subcutaneous indomethacin at the 2 mg/kg led to significant decrease in hematology parameters including Hb, RBCs, WBCs and PLTs. The reduced level of these blood components were attenuated particularly by ethanolic extract of *G. glabra*. GGPE was found ineffective in restoring the altered changes in the blood parameters. The results were found in line with earlier reports of Saravanan Shanmugam et al 2020.

The excessive generation of free radicals in GIT tissues has detrimental effects on proteins and nucleic acids [34, 35]. Enzymatic antioxidants, such as SOD, CAT, GSH, and lipid peroxidation, along with non-enzymatic antioxidant groups, are crucial in safeguarding against the surplus free radicals produced during disease conditions [36]. In this study, the activities of enzymatic antioxidants including SOD, CAT and GSH, in the intestinal tissue were noticeably reduced and lipid peroxidation was increased by indomethacin administration, indicating a clear association of oxidative stress with intestinal tissue damage. However high dose of GGPE and GGEE at mid and high doses significantly elevated the antioxidant enzymes and reduced lipid peroxidation.

Neutrophils contain Myeloperoxidase enzyme, contributing to the generation of hypochlorous acid (HOCl), which possesses antibacterial properties. This enzyme plays a role in the depolymerization of gastrointestinal mucin and the peroxidation of lipids and proteins, resulting in the release of hydroxyl and chloride radicals, ultimately causing oxidative stress [37]. The data of the current study displayed that the indomethacin elevated MPO activity, and treatment with GGPE at a high dose and GGEE at mid and high doses demonstrated a considerable decline in MPO activity, confirming their strong inhibitory action against MPO.

Furthermore, indomethacin increased colon weight in the diseased animals compared to normal, confirming the clinical manifestation. Treatment with GGPE at a high dose and GGEE at mid and high doses significantly declined colon weight. The overproduction of pro-inflammatory mediators, including MPO, IL-1β, and TNF-α, suggests their involvement in the inflammation of the colon [34]. The protective effect of GGPE and GGEE could be attributed to the presence of various polyphenolic compounds, which are proven to have anti-inflammatory and antioxidant activity.

Histopathological analysis of caecum tissues displayed prominent abnormal changes in the diseased rat's caecum tissues such as ulceration, hyperemia, cellular infiltration, goblet cell hyperplasia and necrosis.

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Treatment of GGPE (400 mg/kg) and GGEE at 200 and 400 mg/kg displayed protective effects by restoring altered lesions to mild levels and reduced the intensity of lesions. The results shown by the GGEE and found more effective in comparison to GGPE.

The present study confirms that treatment with GGPE and GGEE reduces indomethacin-induced enterocolitis, as evidenced by macroscopic, histological, and biochemical data. Furthermore, GGEE exhibits more pronounced protective effects against indomethacin-induced enterocolitis compared to GGPE. The roots of Glycyrrhiza glabra contain numerous polyphenolic components, acting as potent antioxidants [38]. Particularly, Licochalcones B and D exhibit significant activity by inhibiting microsomal lipid peroxidation. Retrochalcones were found to be effective against mitochondrial lipid peroxidation, preventing oxidative hemolysis of red blood corpuscles. Isoflavones like glabridin, hispaglabridin A, and 3′hydroxy-4-O-methylglabridin found in *G. glabra* demonstrate substantial antioxidant activity [39]. Additionally, glycyrrhizin in *G. glabra* roots has proven anti-inflammatory and anti-ulcer activity [40]. Phytochemical analysis indicates the presence of polyphenolic compounds in GGEE. Therefore, the strong protective effects observed against indomethacin-induced enterocolitis by GGEE are likely attributable to these phytoconstituents.

CONCLUSION

In conclusion, this study affirms the safety of a single oral dose of 2000 mg/kg for both GGPE and GGEE. Significantly, GGEE demonstrated a more robust and dose-dependent efficacy in mitigating various parameters associated with indomethacin-induced enterocolitis when compared to GGPE. This heightened effectiveness is likely attributed to the presence of potent pharmacologically active compounds, specifically phenolic compounds, and flavonoids, in GGEE. These compounds, commonly found in various fruits, vegetables, and medicinal plants, are renowned for their diverse biological activities, encompassing antioxidant and anti-inflammatory properties. The present findings indicate that GGEE exerts a substantial antioxidant effect, evidenced by its ability to restore altered levels of SOD, catalase, reduced glutathione, and lipid peroxidation. Additionally, its anti-inflammatory activity, demonstrated by MPO inhibition, a marker of inflammation and oxidative stress, further underscores its potential therapeutic benefits. The interconnected nature of antioxidant and anti-inflammatory processes suggests a holistic mechanism of action for GGEE. While these results are promising, further investigations are imperative to unravel the precise and comprehensive mechanisms underlying the observed therapeutic effects. This study lays a foundation for future research aimed at unlocking the full potential of GGEE as a viable candidate for addressing inflammatory and oxidative stress-related conditions.

CONFLICT OF INTEREST

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

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