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RESEARCH ARTICLE

Purslane Extract as a Novel Phytotherapeutic Agent Against Giardiasis in a Rat Model: Mitigating Drug Resistance, Enhancing Intestinal Immunity, And Alleviating Adverse Effects of Conventional Treatments

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ABSTRACT

Giardiasis, caused by the protozoan *Giardia lamblia*, is a prevalent global diarrheal disease associated with significant intestinal pathology, malabsorption, and systemic complications. Current treatments, such as metronidazole (MZ), are limited by side effects and potential resistance, underscoring the need for novel, safe, and effective therapeutic agents. Purslane (*Portulaca oleracea* L.) is a medicinal plant rich in bioactive compounds with documented antioxidant, antimicrobial, and immunomodulatory properties. This study evaluated the therapeutic potential of an aqueous purslane extract (PE), both alone and in combination with MZ, against giardiasis using a comprehensive *in vitro* and *in vivo* approach. Antigiardial efficacy was evaluated *in vitro* (MTT assay on trophozoites/cysts) and *in vivo* using Giardia-infected mice. Albino mice were divided into seven groups: uninfected control, infected control, PE-treated (50, 100, 200mg/kg diet), MZ-treated (200mg/kg), and a PE+MZ combination (200 mg/kg each). LC-MS identified major flavonoids (luteolin-7-O-glucoside, apigenin-7-O-glucoside), phenolic acids, and betalains in PE. PE exhibited substantial DPPH scavenging activity (IC_{50} = 58µg/mL) and notable antimicrobial effects. *In vitro*, PE showed potent antigiardial activity (trophozoite IC_{50} = 38.2µg/mL), surpassing MZ (IC_{50} = 86.5µg/mL), and induced a synergistic effect in combination. PE also triggered a severe oxidative stress response in the parasite, upregulating antioxidant genes more intensely than MZ. *In vivo*, PE treatment caused a dose-dependent reduction in cyst shedding, with the highest dose (200mg/kg) achieving 98.7% clearance. It fully restored growth performance in infected mice (body weight, ADG, FER), normalized serum biochemical and inflammatory markers, and enhanced local immunity. PE significantly downregulated intestinal apoptotic caspases and, in combination with MZ, provided the most complete histopathological recovery of duodenal architecture, reversing villous atrophy, brush border disruption, and mucosal inflammation while supporting immune integrity. Purslane extract demonstrates potent, multi-faceted efficacy against *Giardia lamblia*. Its mechanism involves direct anti-parasitic activity, induction of lethal oxidative stress in the parasite. The synergistic interaction with metronidazole highlights PE's potential as a powerful adjunct therapy, offering a strategy to enhance parasite clearance, accelerate mucosal healing, improve host defense, and optimize clinical outcomes in giardiasis.

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INTRODUCTION

Giardia is a genus of flagellated protozoan parasites, currently comprising six identified species: *G. agilis* in amphibians, *G. ardeae* and *G. psittaci* in birds, *G. muris* and *G. microti* in rodents, and *G. duodenalis* in mammals (Li *et al.,* 2017). Among these, *G. duodenalis* (also referred to as *G. intestinalis* or *G. lamblia*) is the sole species infecting humans, though it has also been detected in various domestic, wild, and farm animals (Ryan and Cacciò, 2013). The present classification of *G. duodenalis* reflects its genetic diversity, encompassing eight assemblages (A–H). Human infections are attributed primarily to assemblages A and B, which are also found in other mammals, highlighting their zoonotic transmission potential (Mircean *et al.*, 2012). Recognized as the etiological agent of human giardiasis, *G. duodenalis* causes gastrointestinal disturbances that can vary from asymptomatic infections to severe watery diarrhea, frequently accompanied by abdominal discomfort, malabsorption, and weight loss.

Giardiasis remains a high-impact, under-recognized parasitic disease with important public health and economic consequences in both humans and animals (Hatam-Nahavandi *et al.*, 2025). It contributes to diarrhoeal illness, child malnutrition and developmental problems, healthcare costs, lost productivity, and livestock losses (Roshidi *et al.*, 2021; Fusaro *et al.*, 2022). These recent studies support framing giardiasis as a poverty-linked, One Health priority, where investments in water, sanitation, hygiene, timely treatment, and animal-health measures can avert substantial, ongoing, and largely preventable costs. Metronidazole remains the primary drug for giardiasis treatment, with other therapeutic alternatives including tinidazole, albendazole, and furazolidone (Hooshyar *et al.*, 2019). Furazolidone and paromomycin are considered effective alternative treatments for giardiasis in certain settings, with paromomycin particularly useful in early pregnancy due to its minimal gastrointestinal absorption (Moron-Soto *et al.*, 2017). Chloroquine has also demonstrated cure rates of approximately 86% in randomized controlled trials, comparable to those of tinidazole. However, furazolidone is associated with risks such as hemolysis, especially in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, as well as other toxicities (Mørch and Hanevik, 2020). Chloroquine can cause a bitter taste, gastrointestinal disturbances, and carries a risk of QT interval prolongation and hemolytic anemia. Consequently, exploring safer and more effective alternatives is essential. Various medicinal plants, such as species from the genera *Artemisia*, *Allium*, *Curcuma*, *Cinnamomum*, *Mentha*, *Origanum*, *Punica*, *Trigonella*, *Satureja*, *Tanacetum*, and *Zingiber*, have been examined as promising natural options (Al-Khfaji, 2017; Al-Megrin, 2017; Dvoodi and Abbasi Maleki, 2017; Najumudin *et al.*, 2018; Scheau *et al.*, 2020). Many of these plants exhibit noteworthy pharmacological properties, including anti-inflammatory, antioxidant, antimicrobial, antiviral, and anticancer activities.

Purslane (*Portulaca oleracea L.*), a globally distributed edible succulent, has garnered significant scientific interest for its dense phytochemical profile and associated therapeutic potentials. Its extract is compositionally rich, containing a diverse array of bioactive compounds confirmed through chromatographic and spectroscopic analyses. These include notable levels of omega-3 fatty acids, flavonoids, polysaccharides, and the unique betalain pigments (betaxanthins and betacyanins), alongside vitamins (A, C, E) and glutathione (Uddin *et al.*, 2014). This composition underpins its potent antioxidant capacity, which has been validated *in vitro* using assays like DPPH, FRAP, and ABTS, showing strong free radical scavenging activity often correlated with its total phenolic and flavonoid content (Uddin et al., 2014). *In vivo* studies in rodent models have demonstrated that purslane extract can mitigate oxidative stress markers (reducing MDA levels and enhancing SOD, CAT, and GSH activity) in tissues affected by hepatotoxicity, neurotoxicity, or metabolic syndrome (Moslemi *et al.*, 2021; Salim and Abdel-Alim, 2025). Regarding its antimicrobial properties, *in vitro* studies report broad-spectrum activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), as well as fungi like *Candida albicans*, with mechanisms attributed to membrane disruption and enzyme inhibition by its flavonoids and alkaloids. Perhaps most promising is its antihelminthic efficacy (Parham *et al.*, 2020; Petropoulos *et al.*, 2021). *In vitro* assays using adult motility and mortality tests against nematodes like *Haemonchus contortus* and cestodes such as *Raillietina echinobothrida* have shown dose-dependent paralysis and death, linked to compounds like noradrenaline and dopamine present in the plant. Crucially, *in vivo* trials in parasitized animals, including poultry and small ruminants, have substantiated these findings, with oral administration of purslane extract significantly reducing fecal egg counts and adult worm burden, suggesting its viability as a complementary anthelmintic agent (Ahmadou *et al.*, 2021; Abubakar *et al.*, 2024). The collective evidence positions purslane not just as a nutritional resource but as a valuable source of multi-functional bioactive agents for potential nutraceutical and pharmaceutical applications.

While purslane's broad-spectrum antimicrobial and anti-inflammatory properties suggest potential, no peer-reviewed study to date has explicitly investigated the *in vivo* anti-*Giardia* activity of standardized purslane extract in a mice model, which is a critical pre-clinical step (Escobedo *et al.*, 2014; Einarsson *et al.*, 2016). This gap encompasses several keys *i.e.,* First, the direct antiprotozoal efficacy remains unquantified; it is unclear if the extract's bioactive compounds (oleraceins, flavonoids) have a cidal or static effect on *Giardia* trophozoites *in vivo*, and what the effective dosage range would be for reducing cyst shedding or trophozoite burden in the intestines of infected mice. Second, the mechanism of action against this protozoan is purely speculative and requires further studies to determine whether it involves disruption of the parasite's membrane,

inhibition of key enzymes, or immunomodulation of the host's response. Third, the impact on pathology and the gut microbiome remains unexplored; research is needed to assess whether treatment ameliorates *Giardia*-induced villous atrophy, inflammation, and microbial dysbiosis in mice (Miao *et al.*, 2019). The bioactivity of purslane compounds must be confirmed in the luminal environment of the small intestine, and studies are needed to determine if encapsulation or other delivery methods are required to protect active constituents from gastric degradation and ensure targeted release. Filling this gap with controlled *in vivo* studies would clarify whether purslane's therapeutic portfolio extends beyond metazoan parasites to include significant protozoan targets, potentially offering a novel, multi-parasitic therapeutic candidate (Alajmi *et al.*, 2019; Saadatmand *et al.*, 2025). Therefore, this study aims to prepare aqueous purslane extract, characterize, and detect the in *vitro* antioxidant, antibacterial, and antigiardia activities. In *vivo* application of purslane extract on Giardia-infected mice compared with metronidazole and the combined effect.

MATERIALS AND METHODS

Preparation of purslane extract: Fresh aerial parts of purslane (*Portulaca oleracea* L.) were collected, botanically identified, thoroughly washed under running tap water, and rinsed with distilled water to remove debris. The plant material was shade-dried at room temperature and then milled to a fine powder using a laboratory grinder. An aqueous extract was prepared by maceration: briefly, a weighed amount of dried powder (300g) was immersed in 1500mL of distilled water in a sealed glass container and kept at room temperature for 48–72 h with intermittent stirring. The mixture was then filtered through muslin cloth followed by Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 50–55°C to yield a crude aqueous extract, which was subsequently dried in a warm water bath to constant weight and stored at 4°C in airtight containers until use. The yield of the dried extract was 45 g. The extraction method was followed by Tabatabaei *et al*. (2016) with some modifications. For in *vitro* and in *vivo* experiments, the dried extract was reconstituted in distilled water to the working concentrations immediately before use.

LC–MS characterization of purslane extract: The phytochemical profile of the aqueous *P. oleracea* extract was evaluated by liquid chromatography–mass spectrometry (LC–MS). An aliquot of the dried extract was dissolved in methanol–water (80:20, v/v), sonicated for 30 min, and centrifuged at 10,000–10,500 × g for 10 min; the clear supernatant was used for analysis. Chromatographic separation was performed on a UHPLC system equipped with a C18 reversed-phase column (ACQUITY UPLC BEH C18, 2.1 × 100mm, 1.7µm) maintained at 40°C, using a binary gradient of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) at a flow rate of 0.3mL/min. The injection volume was 10µL. Detection was carried out on a high-resolution mass spectrometer (LC–ESI QTOF MS/MS) operated in both positive and negative electrospray ionization modes over an m/z range of 50–1500Da, with data-dependent MS/MS acquisition for structural elucidation. Ion source parameters were set as follows: capillary voltage, 3.0kV (positive) / 2.5kV (negative); cone voltage, 40V; source temperature, 120°C; desolvation temperature, 400°C; desolvation gas flow (N_2), 800L/h; cone gas flow, 50L/h. Mass calibration was performed externally before each batch using a sodium formate solution (0.5mM in 90:10 isopropanol/water) infused at 10µL/min, achieving mass accuracy ≤5 ppm; for real-time mass correction, leucine-enkephalin ($[M+H]^+$ = 556.2771Da) was infused as a lock mass. Data acquisition and processing were performed using MassLynx v4.2 with Compound Discoverer 3.3. Individual compounds were tentatively identified by comparing retention times, accurate masses, fragmentation patterns, and, where available, reference standards with published data for *P. oleracea* phenolics and polar lipids. Compound identification confidence levels were assigned according to metabolomics standards: Level 1 (confirmed) – authentic standards co-analyzed (e.g., chlorogenic acid, quercetin-3-O-glucoside) matching retention time (±0.1 min), accurate mass (±5 ppm), and MS/MS spectra; Level 2 (putative annotation) – accurate mass, isotopic pattern, and MS/MS matched literature or spectral libraries (MassBank, MoNA) with similarity >700; Level 3 (tentative) – exact mass consistent with a known compound class but lacking full confirmation.

Biological activities

DPPH radical scavenging assay: A stock solution of DPPH in methanol (0.1mM) was prepared, protected from light, and diluted to an absorbance of approximately 0.9–1.0 at 517nm. Serial dilutions of the purslane extract were prepared in water to final concentrations of 25, 50, 100, and 200µg/mL, and 1.0mL of each dilution was mixed with 1.0 mL of DPPH solution and incubated in the dark at room temperature for 30 min (Ahmed *et al.*, 2026). The decrease in absorbance was measured at 517 nm using a UV–Vis spectrophotometer against a reagent blank, with ascorbic acid (or Trolox) as a positive control. The percentage of DPPH radical scavenging activity was calculated as follows:

$$\%\,Inhibition = \frac{A0 - A1}{A0}\ x100$$

where $A0$ is the absorbance of the control (DPPH solution without extract), and $A1$ is the absorbance in the presence of the sample. The concentration of extract required to scavenge 50% of DPPH radicals (IC_{50}) was determined from the dose–response curve. The experiment was repeated three times.

Antimicrobial activity: The antimicrobial activity of the aqueous purslane extract was assessed in vitro by the agar well diffusion method against selected bacterial and yeast strains, following standard procedures for plant extracts. Reference strains of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica* serovar Typhi, *Candida albicans*, and *Candida glabrata* were grown in appropriate broth media to mid-

logarithmic phase and adjusted to approximately 10^5–10^6 CFU/mL (0.5 McFarland standard). Mueller–Hinton agar plates (for bacteria) and Sabouraud dextrose agar plates (for yeasts) were surface-inoculated by evenly spreading 0.1 mL of each standardized inoculum. Wells of 6–8 mm diameter were aseptically punched into the agar using a sterile cork borer, and 50–100µL of purslane extract at concentrations of 25, 50, 100, and 200µg/mL were carefully dispensed into each well; solvent alone served as negative control, while standard antibiotics/antifungals (ciprofloxacin for bacteria, and fluconazole for *Candida*) were used as positive controls. Plates were incubated at 35–37°C for 18–24 h for bacteria and 24–48 h for yeasts, after which the diameters of the zones of inhibition (including the well diameter) were measured in millimeters using a digital caliper, and the assay was performed in triplicate. The MIC of purslane extract against pathogens was determined using a microdilution assay (Kowalska-Krochmal and Dudek-Wicher, 2021).

***In vitro* antigiardial activity of PE against trophozoites and cysts:** Trophozoites of *Giardia lamblia* WB strain (ATCC 30957) were routinely maintained through 72-h cultures in TYI-S-33 medium supplemented with 0.5 mg/mL bovine bile and penicillin-streptomycin antibiotics, adjusted to pH 7.1. For encystation studies, cultures were transferred to an encystation medium containing 10mg/mL bovine bile at pH 7.8, with encysting cells harvested at designated intervals by chilling on ice and centrifugation (Kane *et al.*, 1991). Parasite viability was determined via trypan blue exclusion: equal volumes of cell suspension and 0.4% trypan blue (Sigma-Aldrich) were mixed, loaded into a Neubauer haemocytometer, and counted under light microscopy. Viability percentage was calculated as NV/(NV + ND) × 100, where NV represents viable (non-stained) cells and ND represents dead (stained) cells. Total cyst counts were enumerated microscopically in a Neubauer chamber and standardized to an inoculum density of 1×10^6 parasites/mL.

The antigiardial effects of the aqueous extract of purslane (PE) were evaluated against both *G. lamblia* trophozoites and cysts using an MTT-based viability assay. Standardized suspensions (1×10^5 parasites in 0.1 mL) were aliquoted into 96-well microtiter plates. As a negative (vehicle) control, an equal volume (0.1 mL) of distilled water (the same solvent used to reconstitute PE and metronidazole) was added to separate wells, and these untreated control parasites were processed identically to the test groups. Test solutions (0.1 mL) comprising PE (25–200 µg/mL), metronidazole (MZ; 25–200µg/mL), or their combination (MZ at IC_{50} plus PE at IC_{50}) were added, and plates were incubated at 24°C for 24 h (Masoori *et al.*, 2024). Following incubation, 20 µL of MTT (5 mg/mL) was introduced per well, and plates were further incubated for 4 h under identical conditions. Formazan crystals were solubilized with dimethyl sulfoxide (DMSO), and absorbance was measured at 570nm using a microplate reader (STAT-FAX-3200). All assays were conducted in triplicate, and IC_{50} values were derived from dose-response data using Probit analysis.

Evaluation of combination effects: The interaction between purslane aqueous extract (PE) and metronidazole (MZ) was quantified using the fractional inhibitory concentration index (FICI), calculated according to the formula: FICI = [IC_{50} (combination)/IC_{50} (PE alone)] + [IC_{50} (combination)/IC_{50} (MZ alone)] (Odds, 2003). Synergism was defined as FICI ≤ 0.5, partial synergism or additivity as 0.5 < FICI ≤ 1, and antagonism as FICI > 1 (up to 4).

Analysis of antioxidant enzyme gene expression: The impact of PE (at sublethal concentrations of 1/3 IC_{50}, 1/2 IC_{50}, and IC_{50}), MZ (at equivalent sublethal doses), and their combination (MZ at IC_{50} plus PE at IC_{50}) on the expression of key antioxidant genes, NADH oxidase (NADH), peroxiredoxin 1a (PXR1a), and superoxide reductase (SOR), was examined in *G. lamblia* trophozoites (Argüello-García *et al.*, 2015). Total RNA was isolated from treated samples using the RNX-plus kit (Cinagene, Iran). Complementary DNA (cDNA) was synthesized from purified RNA using the cDNA synthesis kit (Parstous Biotechnology, Iran) following the manufacturer's instructions. Primer sequences used for target genes are listed in Table 1. Primer validation was performed prior to quantitative real-time PCR. Standard curves were generated using serial dilutions of pooled cDNA for each target gene and the housekeeping gene beta-actin. Amplification efficiencies (E) were calculated from the slope of the standard curve (E = 10^(−1/slope) − 1) and ranged from 91% to 104% for all primers, with correlation coefficients (R^2) > 0.99, confirming that efficiencies were within the acceptable range for the 2^(−ΔΔCt) method. Quantitative real-time PCR was performed on the StepOne™ Real-Time PCR System (Applied Biosystems, USA) with the following thermal cycling conditions: initial denaturation at 95°C for 6 min, followed by 30 cycles of 95°C for 30 s and 62°C for 60 s, and a final extension at 73°C for 7 min. Relative mRNA expression levels were determined using the 2^(-ΔΔCt) method, normalized to the housekeeping gene *β-actin*.

Table 1: Quantified major bioactive compounds in aqueous purslane extract (LC-MS)

Compound class	Specific compound	Concentration (mg/g DW)
Flavonoids	Luteolin-7-O-glucoside	12.45±1.32
	Apigenin-7-O-glucoside	8.21±0.87
	Kaempferol-3-O-rutinoside	5.67±0.64
	Myricetin	3.12±0.41
	Quercetin	0.96±0.12
Phenolic Acids	Caffeic Acid	4.33±0.55
	Ferulic Acid	2.89±0.38
	Gallic Acid	1.24±0.18
Betalains	Portulacaxanthin II (Betaxanthin)	1.58±0.22
	Betanin (Betacyanin)	0.87±0.15
Alkaloids	Oleracein E	2.05±0.31

Data are presented as mean±SD (*n*=3). Statistical significance (P<0.05) was determined by the appropriate test as detailed in the methods.

In *vivo* efficacy of purslane extract (PE) against *G. lamblia* infection in mice: A total of 210 Albino mice (6–8 weeks old; 22–26g body weight) were housed under controlled conditions (24±1°C, 12:12h light/dark cycle, 40–70% humidity) with free access to standard

rodent feed and water. Infected mice were randomly divided into seven groups (n = 10 per group with 7 replicate), receiving daily oral treatments for 7 consecutive days as follows: Group 1 (Negative control receive control diet), Group 2 (infected control, vehicle only); Group 3, 4, and 5 (PE alone at determined 50, 100, and 200 mg/kg diet); Group 6 (MZ/metronidazole at 200 mg/kg); and Group 7 (PE + MZ combination at 200 mg/kg) (Fig. 1).

***Giardia lamblia* cyst preparation, oral inoculation, and infection confirmation**: *Giardia lamblia* (syn. *Giardia duodenalis*) was used for all infection experiments. Trophozoites were axenically cultured in modified TYI-S-33 medium supplemented with 10% adult bovine serum and 0.1% bovine bile (pH 7.1) at 37°C. To obtain infective cysts, confluent trophozoites were induced to encyst *in vitro* by transferring to encystation medium (TYI-S-33 adjusted to pH 7.8 with excess bile and iron) for 72 hours. Cysts were harvested, purified by Percoll density gradient centrifugation (specific gravity 1.09–1.12), washed three times in sterile phosphate-buffered saline (PBS), and counted using a hemocytometer. For short-term preservation, purified cysts were suspended in PBS with antibiotics (100 U/mL penicillin, 100µg/mL streptomycin) and stored at 4°C for up to 4 weeks. For long-term cryopreservation, cysts were resuspended in cold medium containing 10% dimethyl sulfoxide (DMSO), cooled at −1°C/min, and stored in liquid nitrogen (−196°C). Prior to use, preserved cysts were thawed rapidly at 37°C, diluted into pre-warmed PBS, and viability (>70%) was confirmed by excystation assay (HCl-trypsin treatment, pH 2.0, followed by neutralization to pH 7.4 at 37°C).

To promote *G. lamblia* colonization, 210 female mice received an antibiotic preconditioning regimen (0.5 mg/mL vancomycin, 1mg/mL neomycin, and 1 mg/mL ampicillin in drinking water *ad libitum*) for 4 days prior to inoculation. Experimental giardiasis was then induced by oral gavage of 20,000 viable cysts per mouse (Fig. 2). Successful infection was confirmed daily by microscopic stool examination using the formalin- ether sedimentation method, with cyst shedding typically evident 3–5 days post-inoculation. For collection of *Giardia* cysts from infected animals, fecal pellets were homogenized in distilled water, sieved, and purified by sucrose flotation (specific gravity 1.13) followed by washing in sterile PBS. Cysts obtained from feces were preserved using the same cryopreservation protocol described for *in vitro*-derived cysts. All animal procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. The experimental protocol received approval from the Institutional Animal Care and Use Committee (IACUC), ensuring compliance with standard ethical oversight for animal research.

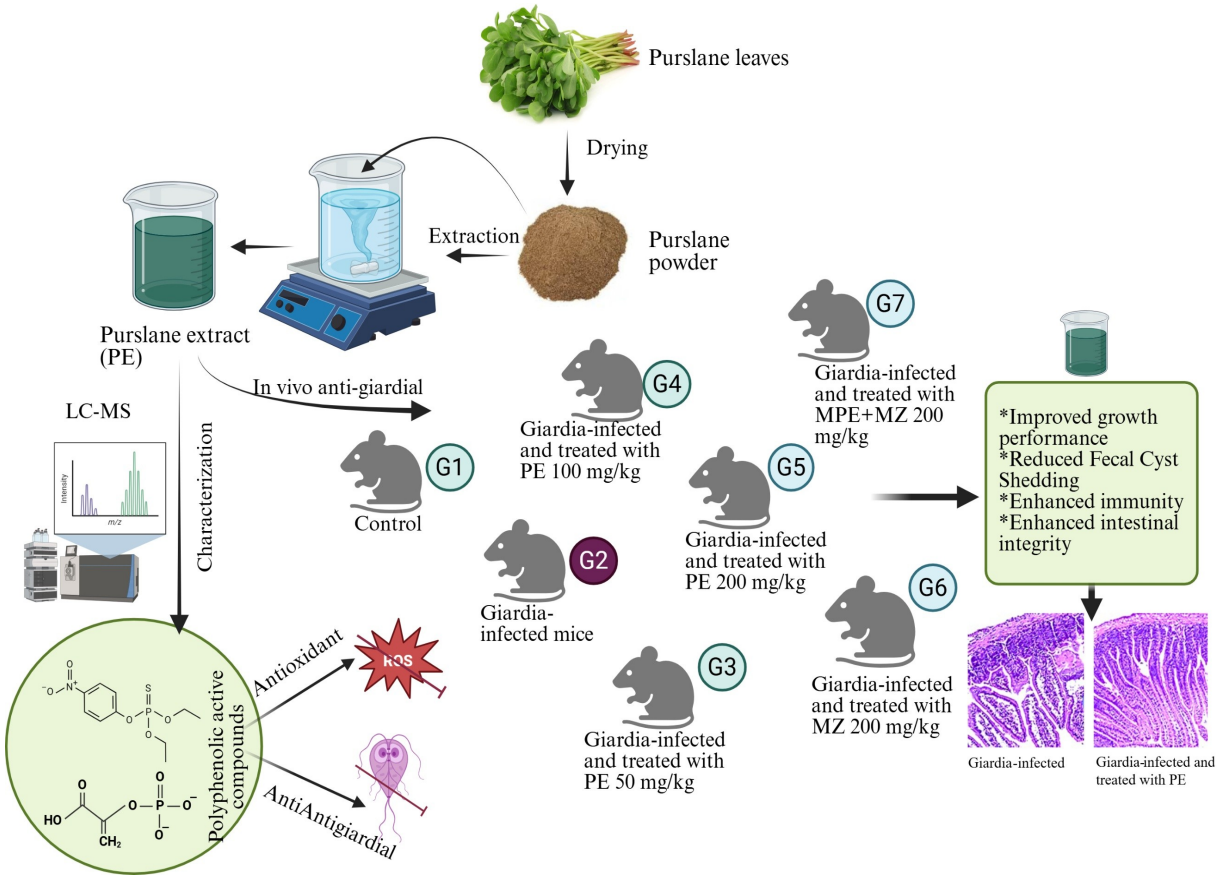

Fig. 1: Experimental design of the in vivo application of purslane extract (PE) against *Giardia* infection in mice.

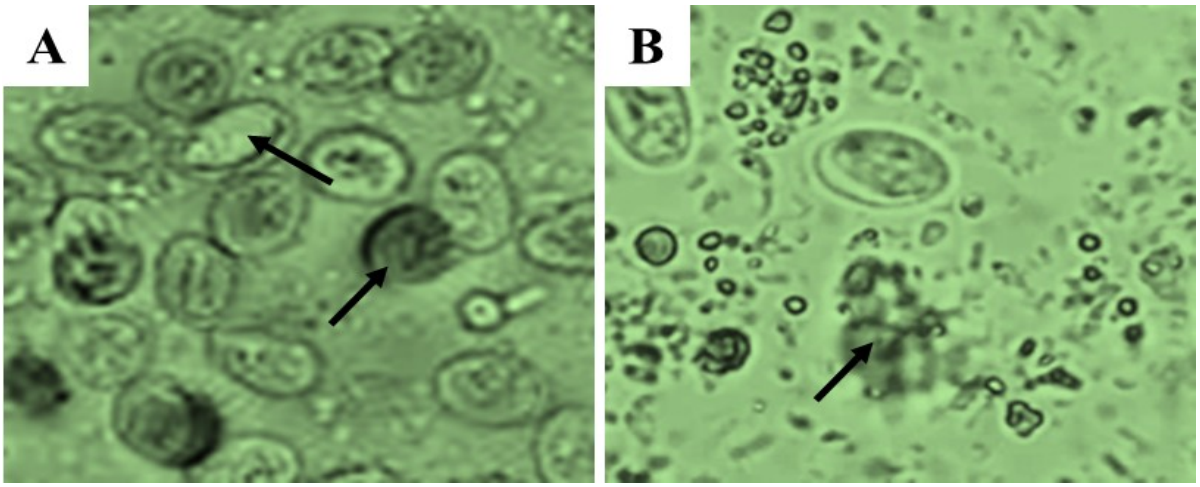

Fig. 2: (A) Fecal smear stained with Trypan blue showing a viable *G. lamblia* cyst. (B) A Trypan blue–stained fecal smear showing a dead *G. lamblia* cyst.

Growth performance: Body weight was recorded individually for all mice at baseline (pre-infection) and daily thereafter, using a digital precision balance (accuracy±0.01 g), throughout the treatment phase: daily weighing for 7 days. Post-infection follow-up phase: weighing at 3-day intervals from day 8 onward, ending between day 14 and day 30. Growth performance metrics were calculated as follows: percent body weight change = [(final weight - initial weight) / initial weight] × 100; average daily gain (ADG) = (final weight - initial weight) / number of days; and feed intake = total feed consumed per group divided by number of mice and days. Feed efficiency ratio (FER) was determined as ADG / average daily feed intake (Iñigo-Figueroa *et al.*, 2013).

Concomitantly, fecal cyst shedding was quantified daily from individual fresh stool samples (approximately 0.1–0.2g) collected post-treatment initiation. Cysts were enumerated microscopically using the formalin-ether concentration technique or iodine-stained haemocytometer direct count after flotation/ sedimentation, with results expressed as cysts per gram of feces (cysts/g). Infection clearance was defined as three consecutive negative cyst counts.

Serum biochemical analysis in *Giardia*-infected mice: At the endpoint of the experiment (day 7 or 14 post-treatment), mice were humanely euthanized under anesthesia, and blood was collected via cardiac puncture or retro-orbital bleeding into serum separator tubes. Samples were allowed to clot at room temperature for 30 min, centrifuged at 3000 × g for 10 min at 4°C, and the resulting serum was aliquoted and stored at −80 °C until analysis following the method of Alsharif *et al.* (2023).

Key biochemical parameters were quantified using commercial diagnostic kits on a semi-automated clinical chemistry analyzer (5010 V5+ photometer). Liver function markers included alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by kinetic UV-IFCC methods; kidney function was assessed via blood urea nitrogen (BUN) and creatinine (kinetic Jaffé method); protein status encompassed total protein (Biuret colorimetric), albumin (bromocresol green), and globulin (calculated as total protein minus albumin); glucose was measured enzymatically by glucose oxidase; and lipid profile included total cholesterol and triglycerides (CHOD-PAP and GPO-PAP methods, respectively). Inflammatory markers, pro-inflammatory cytokines (TNF-α, IL-6 via ELISA), were assayed.

Effects of purslane extract (PE) and metronidazole (MZ) on apoptosis-related gene expression: To evaluate how PE alone or combined with MZ influences the expression of apoptosis-associated genes (*caspase-3*, *-8*, and *-9*) in the intestinal tissue of mice infected with *G. lamblia*, total RNA was isolated from treated samples using the RNX-plus extraction kit (Sigma, USA) (Liu *et al.*, 2020). Complementary DNA (cDNA) was then synthesized from the purified RNA using the cDNA synthesis kit (Sigma, USA) according to the manufacturer's protocol. Target-specific primer sequences are provided in Table 1. Quantitative real-time PCR amplification was conducted on the StepOne™ Real-Time PCR System (Applied Biosystems, USA) using these thermal cycling parameters: initial denaturation at 95°C for 6 min, followed by 30 cycles of 95°C for 30 s and 62°C for 60 s, with a final extension at 73°C for 7 min. Relative quantification of mRNA levels was performed via the 2^(-ΔΔCt) method, using *beta-actin* as the reference housekeeping gene for normalization.

Histopathological study: To prepare tissue for histopathological analysis, segments of duodenum and proximal jejunum (measuring 1–2 cm) were collected from each rat. The samples were fixed in 10% formalin and embedded in paraffin for sectioning. Following the protocol described by Ammar *et al.* (2014), tissue sections were stained with hematoxylin and eosin (H&E). In a blinded evaluation, histological alterations were assessed using a semiquantitative grading scale adapted from Dann *et al.* (2018). Parameters scored included: inflammatory cell infiltration within the villous core (graded 0–3, from normal to severe), epithelial hyperplasia (graded 0–3, from normal to extensive), the presence or absence of epithelial erosions/ulcerations (scored 0 or 1), goblet cell hyperplasia (scored 0 or 1), and muscle layer thickening (graded 0–3, from normal to severe) (Hossain *et al.*, 2024).

Statistical analysis: For statistical analysis of the histopathological scores, data are presented as mean±standard deviation (SD), as appropriate. Normality was assessed using the Shapiro-Wilk test. For comparison between two groups, an independent-samples t-test was used for normally distributed data, while the Mann-Whitney U test was used for non-normally distributed data. For comparisons among experimental groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (or your specific version, e.g., 28.0).

RESULTS

Profile of bioactive compounds in aqueous purslane extract by LC-MS analysis: Characterization of the aqueous purslane (*Portulaca oleracea* L.) extract via Liquid Chromatography-Mass Spectrometry (LC-MS) revealed a quantifiable profile of its principal bioactive compounds implicated in its therapeutic effects. The major flavonoids were quantified as follows: luteolin-7-O-glucoside at 12.45±1.32mg/g of dry extract, apigenin-7-O-glucoside at 8.21±0.87mg/g, kaempferol-3-O-rutinoside at 5.67±0.64mg/g, and myricetin at 3.12±0.41mg/g. Notable phenolic acids included caffeic acid (4.33±0.55mg/g) and ferulic acid (2.89±0.38mg/g). The distinctive betalain pigments, portulacaxanthin II (a betaxanthin) and betanin (a betacyanin), were present at 1.58±0.22mg/g and 0.87±0.15mg/g, respectively. Furthermore, the alkaloid oleracein E was detected at a concentration of 2.05±0.31mg/g. Minor constituents such as gallic acid (1.24±0.18mg/g) and quercetin (0.96±0.12mg/g) were also identified (Table 1).

Biological activity

Antioxidant: The dose-dependent DPPH radical scavenging activity of purslane extract (PE) and the standard antioxidant ascorbic acid (AsA) is presented in Fig. 3. While both substances demonstrated increasing antioxidant capacity with higher concentrations, AsA exhibited greater potency across all tested doses. This difference in efficacy is quantified by the half-maximal inhibitory concentration (IC_{50}), which was 58 µg/mL for PE and 32µg/mL for AsA ($P>0.05$). Nevertheless, the substantial activity of the crude purslane extract (IC_{50} of 58 µg/mL) validates its role as a rich source of antioxidant phytochemicals.

Antimicrobial: The antimicrobial profile of purslane extract (PE), as presented in Table 2, reveals activity that is remarkably competitive with conventional antibiotics at higher test concentrations. At 100µg/mL, PE produced inhibition zones against key pathogens that approached or, in the case of *S. aureus*, nearly matched those of the pure antibiotic standards. Specifically, the zones for PE against *S. aureus* (26.3mm) and *B. subtilis* (24.7mm) were comparable to those of ciprofloxacin (28.1 mm and 26.9 mm, respectively). Furthermore, the clear dose-dependent response with zones at 100mg/mL significantly larger than those at 50µg/mL. While the Minimum Inhibitory Concentration (MIC) values for PE remain in the mg/mL range, indicating a lower intrinsic potency than µg/mL-level antibiotics, the comparable zone data is significant.

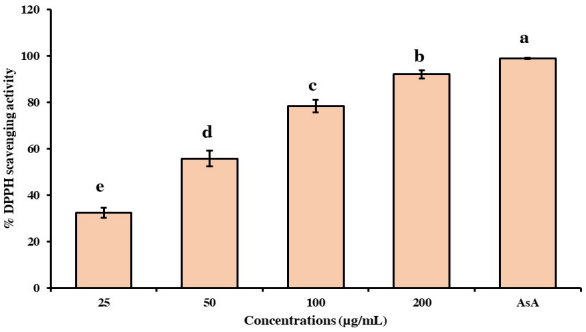

Fig. 3: DPPH scavenging activity percentage of aqueous purslane extract (PE). Lowercase letters above columns indicate significant differences at $P<0.05$.

Anti-parasitic activity: The results presented in Table 3 provide a quantitative assessment of the direct anti-parasitic activity of purslane extract (PE) against *Giardia lamblia*. The data reveal a clear and concentration-dependent reduction in both trophozoite and cyst viability for both PE and the standard drug metronidazole (MZ). As expected, PE demonstrated greater potency, with an IC_{50} of 38.2µg/mL against trophozoites, compared to 86.5 µg/mL for MZ, showing the superiority of PE. However, the significant finding is the substantial anti-giardial activity of the crude plant extract, which achieved over 91.5% inhibition of trophozoites at 200µg/mL. Notably, PE also exhibited considerable activity against the more resistant cyst form, reducing its viability to 28.4% at the highest concentration. The most promising result is the synergistic effect observed in the combination therapy. When PE was combined with MZ, each at its individual IC_{50} concentrations, the resulting trophozoite viability (10.2%) was lower than that achieved by either agent alone at those same concentrations, or even by MZ at double its IC_{50}.

Table 2: Enhanced *In vitro* antimicrobial activity of purslane extract (PE) compared to standard antibiotics

Test Microorganism	Zone of Inhibition of purslane extract (PE)/ concentrations µg/mL				Ciprofloxacin	Fluconazole	PE MIC (µg/mL)
	25	50	100	200			
			Gram-negative Bacteria				
Escherichia coli	8.5±1.0	12.2±0.9	15.5±1.1	20.8±1.3	24.8±0.7	–	12.5
Pseudomonas aeruginosa	7.2±1.1	10.5±1.2	13.8±1.3	18.2±1.4	22.3±0.8	–	25.0
			Gram-positive Bacteria				
Staphylococcus aureus	11.3±0.8	15.8±0.7	20.3±0.9	25.1±1.0	28.1±0.5	–	6.25
Bacillus subtilis	10.1±0.9	14.4±0.8	18.7±1.0	23.5±1.1	26.9±0.6	–	12.5
			Fungi				
Candida albicans	6.8±1.0	9.3±1.0	12.2±1.2	16.5±1.3	–	22.5±0.9	25.0
Aspergillus niger	5.5±1.2	8.1±1.2	10.5±1.4	14.2±1.5	–	20.8±1.1	>25.0

Purslane extract (PE) was tested at four concentrations (25, 50, 100, and 200 mg/mL; 100 µL/well). Ciprofloxacin (5 µg/disc) was the standard antibacterial agent, and fluconazole (25 µg/disc). Concentration (MIC) was determined via broth microdilution for PE. Zone of Inhibition values are mean±standard deviation (SD) of triplicate experiments (n=3).

Table 3: *In vitro* antigiardial activity of purslane aqueous extract (PE), metronidazole (MZ), and their combination against *Giardia lamblia*

Treatment	Concentration (µg/mL)	Trophozoite Viability (%)	Cyst Viability (%)	Calculated IC_{50} (µg/mL)
Purslane Extract (PE)	25	72.1±2.9	88.5±3.5	
	50	48.6±3.4	70.2±4.2	
	100	22.8±2.1	45.3±3.9	
	200	8.5±1.7	28.4±4.5	38.2 (Trophozoites)
Metronidazole (MZ) - Standard Drug	25	85.4±3.2	92.1±4.1	
	50	67.8±4.5	80.3±3.8	
	100	42.5±3.9	65.2±4.7	
	200	21.3±2.8	48.6±5.0	86.5 (Trophozoites)
Combination (MZ + PE)	PE (38.2) + MZ (86.5)	10.2±1.5	35.1±3.8	–

Viability was assessed via MTT assay after 24 h of incubation. Values are presented as mean percentage of the untreated control±standard deviation (SD) (n=3). The IC_{50} (half-maximal inhibitory concentration) for trophozoites was determined by Probit analysis. The combination treatment used each agent at its individual IC_{50} concentration.

Gene expression of antioxidant genes induced by PE on *G. lamblia* trophozoites: The data in Fig. 4A-C reveal a striking mechanistic profile where purslane extract (PE) demonstrates a more potent disruptive effect on the oxidative stress defense of *Giardia lamblia* than the standard drug metronidazole (MZ). Both treatments induced a dose-dependent upregulation of the key antioxidant genes *NADH oxidase (nox)*, *peroxiredoxin 1a (prx1a)*, and *superoxide reductase (sor)*, indicating a compensatory response to pro-oxidant insult. Critically, at equivalent sublethal doses, PE triggered a significantly more intense transcriptional response than MZ. At their respective IC_{50} concentrations, PE (38.2 µg/mL) induced a 6.85 to 8.30-fold increase in gene expression, whereas MZ (86.5 µg/mL) induced only a 3.68 to 4.92-fold increase. This indicates that PE provokes a more severe oxidative challenge within the parasite per unit of concentration, aligning with its lower, more potent IC_{50} value from viability assays. The combination therapy yielded the most profound result. Administering MZ and PE together at their individual IC_{50} concentrations resulted in a dramatic, super-additive upregulation, with *prx1a* expression exceeding 11-fold. This synergistic overstimulation suggests that the two agents work through complementary mechanisms to overwhelm the parasite's antioxidant defenses.

Growth performance: Table 5 demonstrates the significant restorative effect of purslane extract (PE) on the growth performance of *Giardia*-infected mice, which was compromised by the infection. As expected, the infected control group (G2) exhibited severe stunting, with significantly lower final body weight, body weight change (4.5%), average daily gain (ADG), and feed efficiency ratio (FER) compared to the uninfected control (G1). This reflects the malabsorptive and catabolic state induced by giardiasis. Treatment with PE alone (G3-G5) elicited a clear, dose-dependent recovery in all parameters. At the highest dose of 200 mg/kg (G5), PE effectively normalized final body weight, ADG, and FER to levels statistically comparable to the uninfected control, demonstrating its ability to fully counteract the infection's growth-suppressing effects. The standard drug metronidazole (MZ, G6) also restored growth parameters effectively.

The most notable finding was the performance of the combination therapy (G7: PE + MZ). This group not only matched but slightly exceeded the uninfected control in final weight, percent weight change, ADG, and FER, achieving the highest numerical values across all therapeutic groups.

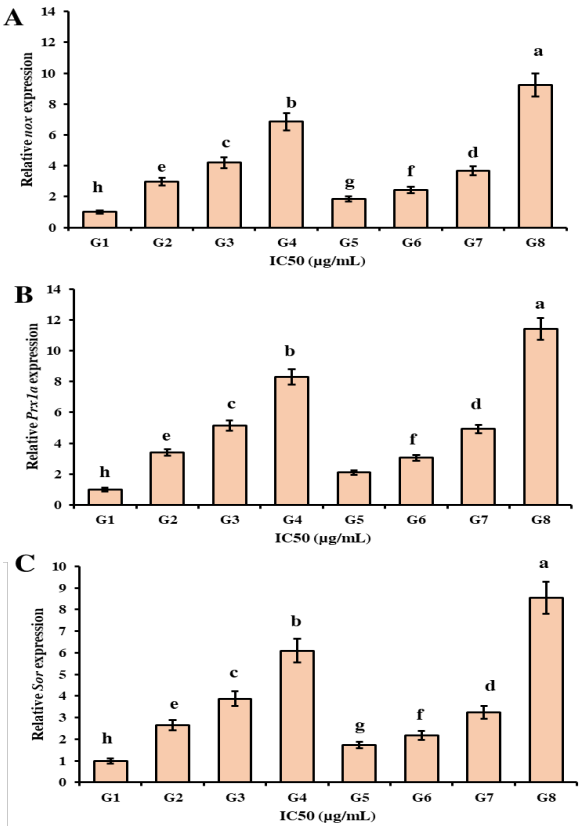

Fig. 4: Gene expression of antioxidant genes (A) Nox, (B) Prx1a, and (C) Sor induced by purslane extract (PE) against *Giardia* cysts normalized to β-actin. Data are presented as mean fold-change relative to the untreated control±standard deviation (SD) from three independent biological replicates (n=3). IC_{50} values used for PE and MZ are based on trophozoite viability, Group 1 (Negative control receive control diet), Group 2 (infected control, vehicle only); Group 3, 4, and 5 (PE alone at determined 50, 100, and 200 mg/kg diet); Group 6 (MZ/metronidazole at 200 mg/kg); and Group 7 (PE + MZ combination at 200 mg/kg).

Fecal cyst shedding count: The fecal cyst shedding data presented in Table 5 provide direct evidence of the potent *in vivo* anti-giardial efficacy of purslane extract (PE). A clear dose-dependent therapeutic effect was observed in PE-treated groups (G3-G5), with the highest dose (200 mg/kg, G5) achieving a 98.7% reduction in cyst output by Day 7 and leading to infection clearance in an average of 6.5 days. This demonstrates that PE alone can effectively suppress parasite reproduction and transmission in the host. Notably, while the standard drug metronidazole (MZ, G6) acted more rapidly, clearing infections by Day 5 on average, the combination therapy (G7) yielded the most striking outcome. The PE+MZ

group exhibited the fastest reduction in cyst counts from the first day of treatment, achieved 100% cyst elimination by Day 7, and cleared infections in a mean of only 4.2 days. This accelerated clearance, significantly faster than either MZ or high-dose PE alone, strongly indicates a synergistic chemotherapeutic effect. The combination likely disrupts the parasite's viability through complementary mechanisms, MZ causing direct nitro-oxidative damage and PE's phytochemicals potentially impairing redox defense and membrane integrity, resulting in superior parasite elimination and a dramatic reduction in environmental contamination risk.

Blood biochemistry: The biochemical and inflammatory profile presented in Table 6 reveals the systemic restorative effects of purslane extract (PE) in *Giardia*-infected mice. Infection (G2) induced a state of metabolic dysfunction characterized by elevated liver enzymes (ALT, AST), increased uremic markers (BUN, Creatinine), hypoalbuminemia, reduced total protein, and dysregulated glucose and lipid metabolism, alongside a marked systemic inflammatory response (elevated TNF-α, IL-6). Treatment with PE, particularly at 200 mg/kg (G5), effectively normalized these perturbations in a dose-dependent manner. Notably, PE treatment restored liver and kidney function markers to levels statistically equivalent to the uninfected control (G1), corrected protein and metabolic profiles, and significantly attenuated pro-inflammatory cytokine levels. The effect of high-dose PE on reducing TNF-α and IL-6 was comparable to that of the standard drug metronidazole (MZ, G6), highlighting PE's potent anti-inflammatory properties. The combination therapy (G7: PE+MZ) consistently yielded the most favorable biochemical outcomes, with all parameters reflecting a complete return to homeostatic balance. This group exhibited the lowest inflammatory cytokine levels, underscoring a superior anti-inflammatory synergy.

Table 4: Impact of purslane extract (PE) and metronidazole (MZ) on growth performance in *Giardia*-infected mice

Treatment group	Dose (mg/kg diet)	Final body weight (g)	Body weight change (%)	Average daily gain (ADG, g/day)	Average daily feed intake (ADFI, g/day)	Feed efficiency ratio (FER)
G1	–	32.5±1.8 a	30.2±2.1 a	0.32±0.02 a	5.1±0.3 a	0.063±0.004 a
G2	–	26.1±2.1 b	4.5±1.8 b	0.05±0.02 b	4.8±0.4 ab	0.010±0.003 b
G3	50	28.4±1.9 c	13.8±2.0 c	0.14±0.02 c	4.9±0.3 a	0.029±0.003 c
G4	100	29.8±1.7 ac	19.2±1.9 d	0.20±0.02 d	5.0±0.3 a	0.040±0.004 d
G5	200	30.9±1.6 a	23.8±2.2 e	0.25±0.02 e	5.0±0.2 a	0.050±0.003 e
G6	200	31.5±1.5 a	27.1±1.8 af	0.28±0.02 af	5.2±0.3 a	0.054±0.004 af
G7	200 (each)	32.8±1.4 a	31.5±1.7 a	0.33±0.02 a	5.2±0.3 a	0.064±0.005 a

Values are presented as mean±standard deviation (SD) (n=8 mice per group). Different superscript letters (a, b, c, d, e, f) within a column indicate statistically significant differences (P<0.05) as determined by one-way ANOVA followed by Tukey's post hoc test. Body Weight Change (%) = [(Final Weight – Initial Weight) / Initial Weight] × 100. Average Daily Gain (ADG) = (Final Weight – Initial Weight) / experimental days. Feed Efficiency Ratio (FER) = ADG / Average Daily (Rahman *et al*., 2025). Group 1 (Negative control receive control diet), Group 2 (infected control, vehicle only); Group 3, 4, and 5 (PE alone at determined 50, 100, and 200 mg/kg diet); Group 6 (MZ/metronidazole at 200 mg/kg); and Group 7 (PE + MZ combination at 200 mg/kg).

Table 5: Daily fecal cyst shedding (cysts/g) in *Giardia*-infected mice following treatment

Treatment Group	Dose (mg/kg diet)	Day 1 Post-Treatment (cysts/g)	Day 3 Post-Treatment (cysts/g)	Day 5 Post-Treatment (cysts/g)	Day 7 Post-Treatment (cysts/g)	Mean Clearance Day
G2	–	18,500±2,340 a	20,100±2,890 a	22,500±3,150 a	19,800±2,760 a	(No clearance)
G3	50	16,200±2,110 a	12,500±1,980 b	9,300±1,450 b	6,800±1,220 b	>7.1
G4	100	15,800±1,950 a	9,800±1,540 c	5,200±980 c	2,100±650 c	8.2±1.1
G5	200	14,500±1,870 a	6,300±1,120d	1,800±520 d	250±120 d	6.5±0.8
G6	200	12,100±1,650 b	3,200±850 e	450±150 e	50±40 ^e	5.0±0.5
G7	200 (each)	9,800±1,420 c	1,500±480 f	120±60 f	0±0 f	4.2±0.4

Cyst counts are presented as mean cysts per gram of feces±standard deviation (SD) (n=8). Different superscript letters (a, b, c, d, e, f) within a column indicate statistically significant differences (P<0.05) by one-way ANOVA and Tukey's post hoc test. Clearance was defined as three consecutive negative cyst counts. Group 2 (infected control, vehicle only); Group 3, 4, and 5 (PE alone at determined 50, 100, and 200 mg/kg diet); Group 6 (MZ/metronidazole at 200 mg/kg); and Group 7 (PE + MZ combination at 200 mg/kg).

Table 6: Effect of purslane extract (PE) and metronidazole (MZ) on serum biochemical and inflammatory parameters in *Giardia*-infected mice

Parameter (Unit)	G1	G2	G3	G4	G5	G6	Reference
Liver Function							
ALT (U/L)	32.5±3.2 a	58.7±6.1 b	45.2±4.8 c	38.1±4.0 ac	35.8±3.7 a	33.1±3.5 a	30-65
AST (U/L)	85.4±8.5 a	142.3±14.2 b	112.6±11.0 c	98.5±9.8 ac	91.2±9.0 a	87.8±8.6 a	70-150
Kidney Function							
BUN (mg/dL)	18.2±1.8 a	28.9±2.9 b	23.5±2.3 c	20.1±2.0 ac	19.5±1.9 a	18.8±1.8 a	15-30
Creatinine (mg/dL)	0.42±0.04 a	0.68±0.07 b	0.55±0.05 c	0.48±0.05 ac	0.45±0.04 a	0.43±0.04 a	0.2-0.8
Protein Status							
Total Protein (g/dL)	6.5±0.6 a	5.1±0.5 b	5.6±0.5 c	6.0±0.6 ac	6.3±0.6 a	6.4±0.6 a	5.0-7.0
Albumin (g/dL)	3.8±0.4 a	2.9±0.3 b	3.2±0.3 c	3.5±0.3 ac	3.7±0.4 a	3.7±0.4 a	3.0-4.5
Globulin (g/dL)	2.7±0.3	2.2±0.2	2.4±0.2	2.5±0.2	2.6±0.3	2.7±0.3	1.5-3.5
A/G Ratio	1.41±0.14 a	1.32±0.13 ab	1.33±0.13 ab	1.40±0.14 a	1.42±0.14 a	1.37±0.14 ab	1.0-2.0
Metabolic Profile							
Glucose (mg/dL)	125±12 a	98±10 b	110±11 ac	118±12 ac	122±12 a	124±12 a	90-140
Total Cholesterol (mg/dL)	102±10 a	78±8 b	88±9 c	95±10 ac	99±10 a	101±10 a	70-120
Triglycerides (mg/dL)	85±9 a	62±7 b	72±7 c	79±8 ac	82±8 a	84±8 a	50-100
Inflammatory Cytokines							
TNF-α (pg/mL)	15.2±1.5 a	45.6±4.6 b	32.8±3.3 c	22.1±2.2 d	20.5±2.1 d	17.8±1.8 ad	–
IL-6 (pg/mL)	10.5±1.1 a	38.2±3.8 b	25.4±2.5 c	16.3±1.6 d	15.1±1.5 d	12.9±1.3 ad	–

Values are expressed as mean±standard deviation (SD) (n=8 mice per group). Different superscript letters (a, b, c, d) within a row indicate statistically significant differences (P<0.05) as determined by one-way ANOVA followed by Tukey's post hoc test. A/G Ratio = Albumin/Globulin Ratio. ELISA: Enzyme-Linked Immunosorbent Assay. Group 1 (Negative control receive control diet), Group 2 (infected control, vehicle only); Group 3, 4, and 5 (PE alone at determined 50, 100, and 200 mg/kg diet); Group 6 (MZ/metronidazole at 200 mg/kg); and Group 7 (PE + MZ combination at 200 mg/kg).

Apoptosis gene expression: Fig.s 5A-C illustrate the dose-dependent protective effect of purslane extract (PE) against *Giardia*-induced apoptosis in the intestinal epithelium. Infection alone (G2) triggered a significant pro-apoptotic environment, upregulating caspase-3, caspase-8, and caspase-9 by approximately 3-fold. This molecular response correlates with the histopathological damage observed in the intestine. Treatment with PE demonstrated a clear and graded therapeutic response. The lowest dose of PE (50mg/kg, G3) provided a modest reduction in caspase expression, while intermediate (100 mg/kg, G4) and high (200mg/kg, G5) doses produced progressively stronger suppression of all apoptotic markers. At 200mg/kg, PE reduced caspase expression to levels near those achieved by the standard drug metronidazole (MZ, G6), indicating that a sufficiently concentrated PE regimen can effectively protect intestinal cells from infection-driven programmed cell death. This effect is likely mediated by the extract's antioxidant and anti-inflammatory phytochemicals, which mitigate the cellular oxidative stress and inflammatory signaling that trigger apoptosis. Notably, the combination therapy (G7: PE + MZ) produced the most protective outcome, returning all caspase expression levels closest to the uninfected baseline. This synergistic effect suggests that while MZ eliminates the parasitic source of injury, PE simultaneously fortifies the host tissue against the downstream apoptotic cascade, leading to optimal mucosal preservation. This mechanistic insight supports the superior clinical and histological recovery seen in the combination group and underscores the value of PE as a mucosal-protective adjunct in the treatment of giardiasis.

Histopathology: Fig. 6A (G1: Uninfected Control) represents the normal histological architecture of the duodenum. The intestinal villi (V) are tall, slender, and well-organized with intact epithelial lining. The brush border (BC) is clearly defined, and the mucosal layer (ML) shows a normal thickness with no evidence of inflammatory cell infiltration. This serves as the baseline for healthy tissue morphology. Fig. 6B (G2: Infected Control) demonstrates severe pathological alterations characteristic of untreated giardiasis. Marked villous atrophy (V) is evident, with shortened, blunted, and fused villi. The brush border (BC) appears disrupted and irregular. The mucosal layer (ML) is notably thickened and edematous, with a prominent infiltration of inflammatory cells (predominantly lymphocytes and plasma cells) into the lamina propria and submucosa. These changes correlate with the malabsorptive state observed in growth parameters.

Fig. 6C (G3: PE, 50 mg/kg) shows initial signs of mucosal recovery. Villous height (V) remains reduced but exhibits less blunting compared to G2. The brush border (BC) continuity shows partial improvement, though irregularities persist. Inflammatory infiltration in the mucosal layer (ML) is moderately reduced, indicating the beginning of an anti-inflammatory effect at this low dose, consistent with the modest improvement in clinical and molecular parameters.

Fig. 6D (G4: PE, 100 mg/kg) displays significant histological amelioration. Villi (V) are notably taller and more organized, approaching normal morphology. The brush border (BC) is more uniform and continuous. The mucosal layer (ML) thickness is decreased, and inflammatory cell infiltration is substantially reduced, reflecting the dose-dependent efficacy of PE in mitigating infection-induced damage. Fig. 6E (G5: PE, 200 mg/kg) illustrates near-complete restoration of mucosal integrity. Villous architecture (V) is well-preserved and comparable to the uninfected control. The brush border (BC) is distinct and intact. The mucosal layer (ML) appears normal in thickness with minimal residual inflammation, demonstrating that high-dose PE alone can effectively reverse the histopathological consequences of giardiasis.

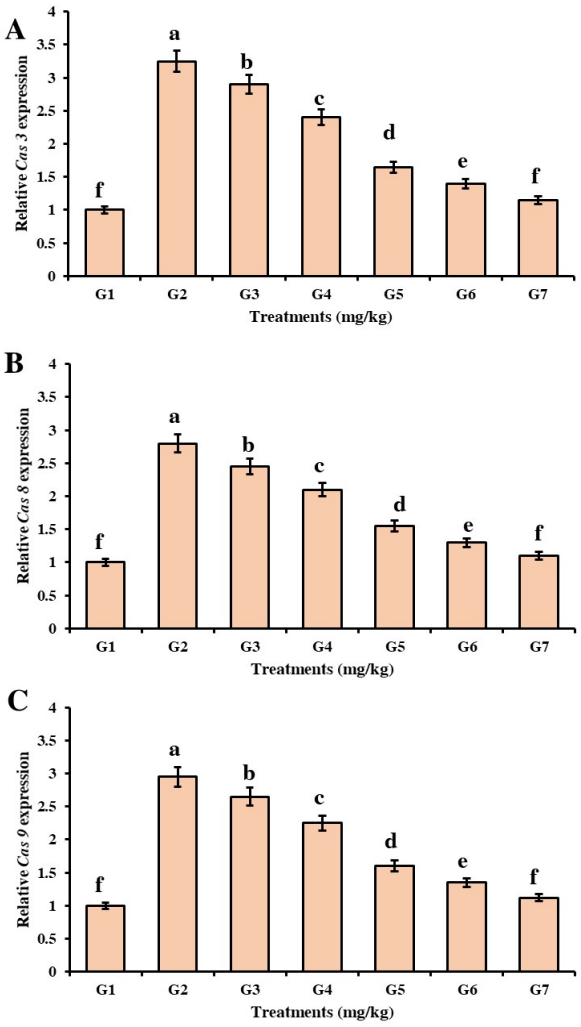

Fig. 5: Effect of Purslane Extract (PE) and Metronidazole (MZ) on the Relative Expression of Apoptosis-Associated Genes in the Intestinal Tissue of Giardia-Infected Mice. Lowercase letters above columns indicate significant differences at P<0.05. Group 1 (Negative control receive control diet), Group 2 (infected control, vehicle only); Group 3, 4, and 5 (PE alone at determined 50, 100, and 200 mg/kg diet); Group 6 (MZ/metronidazole at 200mg/kg); and Group 7 (PE + MZ combination at 200mg/kg).

Fig. 6F (G6: MZ, 200 mg/kg) shows effective tissue recovery, similar to G5. Villi (V) are well-formed, the brush border (BC) is restored, and the mucosal layer (ML) exhibits only mild, focal inflammation. This confirms the therapeutic efficacy of the standard drug in resolving structural damage post-parasite clearance.

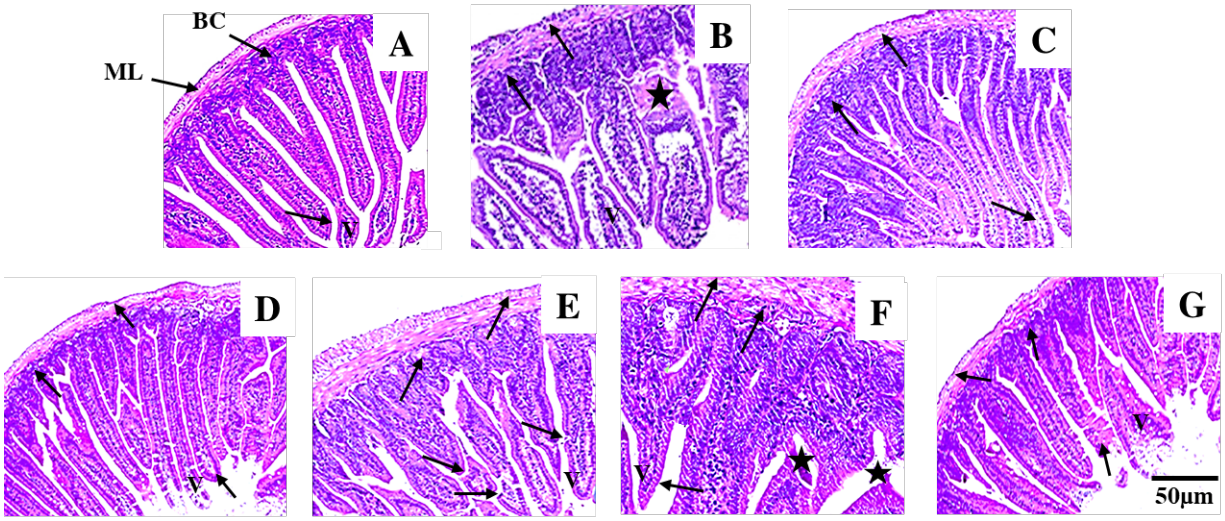

Fig. 6: Representative photomicrographs of duodenal histoarchitecture showing the effects of purslane extract (PE) and Metronidazole (MZ) treatment on *Giardia lamblia*-induced mucosal damage in mice. (H&E staining, 200x magnification). A: Uninfected control (G1) showing intact, tall villi (V), a distinct brush border (BC), and normal mucosal layer (ML). B: Infected control (G2) exhibiting severe villous atrophy (*), disrupted BC, and marked inflammatory thickening of the ML. C–E: Mice treated with PE at 50, 100, and 200 mg/kg diet (G3–G5), demonstrating dose-dependent restoration of villous height, BC integrity, and reduced inflammation. F: Standard MZ treatment (G6, 200 mg/kg) showing effective mucosal recovery but exhibiting mild villous atrophy (*). G: Combination therapy (PE+MZ, G7) revealing optimal architectural restoration, with villous morphology and ML comparable to the healthy control. Scale bar: 50 µm.

Parameters scored included: inflammatory cell infiltration within the villous core (graded 0–3, from normal to severe), epithelial hyperplasia (graded 0–3, from normal to extensive), the presence or absence of epithelial erosions/ulcerations (scored 0 or 1), goblet cell hyperplasia (scored 0 or 1), and muscle layer thickening (graded 0–3, from normal to severe). Fig. 6G (G7: PE + MZ Combination, 200 mg/kg each) represents the optimal histological outcome, achieving normal (grade 0) scores across all parameters: no inflammatory cell infiltration, no epithelial hyperplasia, absence of erosions/ulcerations (score 0), no goblet cell hyperplasia (score 0), and no muscle layer thickening (grade 0). The villous architecture (V) is indistinguishable from that of the healthy control, with tall, slender villi. The brush border (BC) is exceptionally well-defined and continuous. The mucosal layer (ML) is normal in thickness and completely devoid of pathological inflammatory infiltration. Thus, the combination treatment not only eliminated the parasite but also fully reversed each of the five scored histopathological alterations, corresponding to the lowest possible severity grades. This visual evidence corroborates the synergistic effect observed in all other assays, showcasing superior and accelerated tissue healing when antioxidant/anti-inflammatory plant therapy is combined with direct antiparasitic chemotherapy.

DISCUSSION

At present, plant-derived bioactive compounds are increasingly recognized for their therapeutic promise as natural antiparasitic agents. Although synthetic antigiardial drugs remain effective, their administration is frequently accompanied by undesirable side effects, potential mutagenic risks, and the growing problem of drug resistance (Kim *et al.*, 2016; Vivancos *et al.*, 2018). Thus, efforts have shifted toward identifying plant-based alternatives with improved safety profiles and fewer adverse reactions. In recent years, a research trend has emerged focusing on synergistic combinations of phytochemicals and conventional drugs, which can enhance antiparasitic efficacy while reducing toxicity (Malongane *et al.*, 2017). In this context, we evaluated the antigiardial potential of purslane extract (PE), both alone and in combination with metronidazole (MZ), against *Giardia lamblia* infection. The findings revealed that PE, either individually or co-administered with MZ, significantly suppressed the viability of *G. lamblia* trophozoites and cysts in *vitro* compared with untreated controls. The fractional inhibitory concentration index (FICI) values below 0.5 indicated a strong synergistic interaction between PE and MZ. In infected mice, the oral administration of PE at 100 and 200 mg/kg markedly reduced cyst counts and fecal excretion rates after seven days of treatment. Notably, the combined PE + MZ therapy completely eliminated cysts, demonstrating full recovery from infection.

Portulaca oleracea (purslane) is rich in flavonoids, alkaloids, omega-3 fatty acids, and phenolic acids, bioactive compounds known for their antimicrobial, anti-inflammatory, and antiparasitic actions. Several investigations have confirmed that purslane exhibits broad-spectrum antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* (Fouda *et al.*, 2022; Abdel-Rahman *et al.*, 2024). Its antiparasitic properties have also been documented against *Plasmodium berghei*, *Leishmania major*, and *Toxoplasma gondii*, where aqueous and ethanolic extracts of purslane significantly inhibited parasite multiplication and induced morphological alterations, without causing notable host toxicity (Eskandari *et al.*, 2016; Liyanage, 2022). These findings support purslane's capacity as a promising natural antiparasitic candidate. A previous study found

that aqueous *Portulaca oleracea* extract was effective against *Giardia lamblia* trophozoites from sheep. A 15 mg/mL stock was diluted to 1.5–7.5mg/mL and incubated for 48 h. The extract showed concentration-dependent inhibition, with 7.5 mg/mL significantly reducing parasite viability, though complete eradication was not achieved and no cytotoxicity testing was done (Suad *et al.*, 2025). The same study notes that previous *in vitro* work had already shown strong anti-Giardia activity of aqueous and alcoholic purslane extracts against protozoa including *G. lamblia* (Suad *et al*., 2025).

The antigiardial mechanism of PE appears linked to its ability to induce oxidative stress in parasites while augmenting host antioxidant defenses (Mostafa *et al.*, 2024). Polyphenolic constituents in purslane, such as quercetin, caffeic acid, and kaempferol, are known to promote regulated production of reactive oxygen species (ROS), leading to alterations in the parasite's redox homeostasis and apoptosis signaling (Ghorani *et al.*, 2023). The findings demonstrated that PE treatment dose-dependently elevated intracellular ROS production in *G. lamblia* trophozoites, accompanied by upregulating of redox-regulating genes, *NADHox*, *PXR1a*, and *SOR*. Similar findings were reported in *Leishmania* species, where oxidative stress induced by purslane extract disrupted mitochondrial function, triggering apoptosis-like death of parasites (Shaha, 2025). Thus, ROS-mediated oxidative imbalance may be a critical antiparasitic mechanism through which PE operates. Giardiasis commonly disrupts ion transport and reduces the absorptive surface area of intestinal villi, resulting in diarrhea and electrolyte imbalance (Khlaf, 2022; Ma'ayeh and Svärd, 2024). In the present study, PE, especially when co-administered with MZ restored serum sodium and potassium levels in infected mice, suggesting improved intestinal function and epithelial repair. This effect may be attributed to the high content of omega-3 fatty acids and flavonoids in purslane, which can enhance membrane fluidity and stabilize intestinal epithelial transport systems (Kumar *et al.*, 2022; Kureshi *et al.*, 2025).

Previous studies have also reported that purslane extracts modulate cytokine responses by stimulating macrophage activity, increasing immunoglobulin A (IgA) antibody levels, and enhancing the production of antimicrobial peptides in mucosal tissues (Lin *et al.*, 2021; Lun *et al.*, 2025). These findings align with our observations, suggesting that the immune-modulating properties of PE play a key role in augmenting antigiardial immunity.

Giardia infection can induce apoptosis of intestinal epithelial cells through caspase pathway activation and BAX-associated mitochondrial signaling (Roxström-Lindquist *et al.*, 2005; Girola *et al.*, 2015). Consistent with this mechanism, the present work found that PE significantly reduced the expression of caspase-3, −8, and −9, indicating that it protects intestinal epithelial integrity by preventing apoptosis (Miao *et al.*, 2025). These anti-apoptotic properties agree with previous research demonstrating that purslane's bioactive compounds, particularly polysaccharides and betacyanins can downregulate apoptotic genes and upregulate anti-apoptotic factors in intestinal and hepatic tissues (Li *et al.*, 2022).

Long-term administration of PE at therapeutic doses did not significantly alter hepatic or renal biochemical markers, confirming its safety profile. These findings concur with prior toxicological studies indicating that purslane extracts have a high safety margin and do not produce chronic toxicity in rodent models, even at elevated doses (Aboulthana *et al.*, 2022). In randomized double-blind trials in NAFLD patients, 300–700 mg/day purslane extract for 8–12 weeks improved or did not worsen liver function tests, with significant reductions in ALT, AST, GGT and no safety signal on liver enzymes compared with placebo (Darvish Damavandi *et al.*, 2021; Milkarizi *et al.*, 2024). In another 12-week trial, purslane as adjuvant therapy was considered safe, with no indication of hepatic toxicity in reported outcomes (Damavandi *et al.*, 2023). The observed synergistic effect between PE and MZ highlights the potential role of PE as a natural adjunctive therapy in Giardiasis management. To validate these findings, further research should explore bioavailability, pharmacodynamic interactions, and metabolic fate in *vivo*, alongside well-designed clinical trials evaluating its efficacy and safety in human populations. Also, complete in vitro profiling with cytotoxicity and mechanism studies; move into standardized animal giardiasis models with detailed parasitological and histological endpoints; and rigorously evaluate safety, dosing, and possible synergy with metronidazole, using frameworks already applied to other antigiardial plant extracts.

Conclusions: Based on the collective *in vivo* evidence, purslane extract demonstrates significant potential as a therapeutic agent against giardiasis in mice models. The anti-giardial efficacy, evidenced by a substantial reduction in fecal cyst output and duodenal trophozoite burden, is likely mediated by a synergistic combination of its bioactive compounds. These compounds are proposed to exert direct antimicrobial effects by disrupting the protozoan's membrane integrity and vital enzymatic pathways. Concurrently, the extract's potent antioxidant constituents, including omega-3 fatty acids, vitamins, and betalains, mitigate the infection-induced oxidative stress in the intestinal mucosa, which is crucial for resolving the pathological sequelae. This dual action is reflected in improved growth parameters, such as the restoration of body weight gain and feed efficiency in treated mice, indicating a reversal of the malabsorptive and catabolic state caused by the parasite. Crucially, the therapeutic outcome is histologically corroborated by a marked improvement in duodenal histoarchitecture. Treatment with purslane extract leads to a significant amelioration of villous atrophy, reduction in inflammatory cell infiltration, normalization of epithelial hyperplasia, and restoration of goblet cell populations. Purslane extract operates through a multifaceted mechanism, combining direct antiprotozoal activity, antioxidant protection, and anti-inflammatory support to effectively control *Giardia* infection, alleviate its clinical and metabolic consequences, and promote the structural healing of the infected intestine in mice. These findings position purslane as a promising source of novel compounds for the development of complementary anti-giardial therapies.

Conflict of interest: The authors declare that there is no conflict of interest.

Authors contribution: Conceptualization, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT, formal analysis, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT, investigation, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT, data curation, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT, writing original draft preparation, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT, writing final manuscript and editing, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT, visualization and methodology, AMA, RSA, LAA, HHA, SAK, MAA, FSA, HSA, MAA, MNA, and RT. All authors have read and agreed to the published version of the manuscript.

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