Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Evaluation of the therapeutic effect of *M. recutita* plant extract against experimental giardiasis

W.A. Mohamed[1], R. El -Sayed[1], H.F. Abdelmaksod[2] and M.A. Farah[1]

[1]Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, [2]Theodor Bilharz Research Institute, Cairo, Egypt

Article information

Article history:
Received 14 April 2025
Accepted 13 August 2025
Published 05 September 2025

Keywords:
Giardia lamblia
M. recutita
Histopathological
Caspase-3

Correspondence:
H.F. Abdelmaksod
pearlhfn@yahoo.com

Abstract

Giardia lamblia (G. lamblia) is a common pathogenic protozoan that frequently causes giardiasis, leading to steatorrhea; management of giardiasis is difficult, and the need to find alternative natural products is needed to avoid the adverse effects of chemotherapeutics. Therefore, the present investigation aimed to assess the impacts of *M. recutita* plant extract in the treatment of experimental giardiasis and evaluate its effect using different parameters of assessment. Mice were classified into: G1 : non-infected, non-managed; G2: infected, non-managed; G3: infected and managed with metronidazole; G4: infected and managed with n-hexane extract of *M. recutita* at 50 % concentration; G5: infected and treated with n-hexane extract of *M. recutita* at 100 % concentration; G6: infected and managed with n-butanol extract of *M. recutita* at 50 % concentration; and G7: infected and managed with n-butanol extract of *M. recutita* at 100 % concentration. The therapeutic efficacy was evaluated by parasitological (cyst and trophozoite count), histopathological, immunohistochemical (Caspase-3), histochemical (PAS staining), and oxidative stress markers (SOD and MDA) parameters. The highest therapeutic outcome was achieved with G3 and G5, showing significant reduction with a percentage of 13.6±3.51 and 13.8±1.92, respectively, and an apparent amendment of pathological insults, immunohistochemical staining, and immunological markers. Results of the present research proved that the *M. recutita* plant extract has a promising therapeutic effect against *Giardia lamblia*. The plant extract from *M. recutita* is readily available and helps prevent the negative side effects of chemotherapy medications.

DOI: 10.33899/ijvs.2025.157756.4147, ©Authors, 2025, College of Veterinary Medicine, University of Mosul.
This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Giardia intestinalis, another name for *Giardia lamblia*, is a parasitic microscopic organism that is a member of the Sarcomastigophora phylum (1), it is a common pathogenic protozoan that frequently is an etiology of giardiasis and infects a variety of vertebrates, including humans (2). It affects nearly 400 million people worldwide diarrhea (steatorrhea), also known as traveler's diarrhea can occur in two forms: trophozoite and cyst (3). With infection rates ranging from 10 to 50%, it is most common in developing nations (4). *G. lamblia* is the dominating intestinal pathogen in Egyptian children suffering from diarrhea (5). Human infection can be contracted by direct fecal-oral contact, ingestion of infected food or water, or even water contamination. Although infections can happen to everyone at any age, children are more likely to have them (6). Weight loss, cramping in the abdomen, watery diarrhea, and malabsorption syndrome are some of the clinical signs of an acute or chronic Giardia infection. The management of giardiasis is difficult. Nowadays medications, including quinacrine, tinidazole, furazolidone, and metronidazole, are

used to treat giardiasis (7). Tetracyclines, such as minocycline, have been shown to have anti-giardiasis properties in addition to their analgesic and anti-inflammatory properties (8). Despite their overall effectiveness, these drugs have side effects that can interfere with the course of treatment, such as nausea, headaches, leukopenia, dizziness, gastrointestinal distress, and foul taste. Additionally, it has been shown that some medications might cause mutagenic, carcinogenic, and toxic effects in lab animals. Consequently, finding a drug with fewer adverse effects is urgently needed. In addition to the benefit of reducing side effects and elevating the safety of their use, some herbs and their derivatives have been reported to have medicinal potential in recent years (9). The demand for economical cultivated crops as well is increasing (10). Several diseases can be managed, prevented, and treated by the natural chemicals found in plants (11). German chamomile, or *Matricaria chamomilla* L. (MC), is a traditional medicinal plant that is a member of the Asteraceae family (12). It is a cold-hardy annual herb that thrives in many types of soil. *M. chamomilla* is indigenous to northern and western Asia in addition to southern and eastern Europe (13). Recently, it has been extensively spread all over the world (14). Traditionally, *M. chamomilla* was utilized in a number of nations to treat a variety of illnesses, such as respiratory, neurological, hepatic, and gastrointestinal issues (15). This plant is also commonly used to treat infections, discomfort, and conditions of the mouth, eyes, and skin. The ability of *M. chamomilla* EOs and extracts to stop the progression of a variety of insects and parasites was the subject of numerous investigations. The leishmanicidal effectiveness of Tunisian *M. chamomilla* essential oils has been assessed in vitro. The findings demonstrated that EOs had a positive impact on Leishmania amazonensis promastigotes, an extracellular and motile form (16). Nepalese *M. chamomilla* essential oils have been tested for their ability to kill glassworms (*Chaoborus plumicornis*), fruit flies (*Drosophila melanogaster*), and nematodes (*Caenorhabditis elegans* and *Artemia salina*) (17). *Acanthamoeba castellanii* was used to test the anti-Acanthamoeba properties of floral removes from Tunisian *M. chamomilla* (18).

In the light of the considerations elevated, this study aimed to evaluate the effects of *M. recutita* in the management of experimental *giardiasis* and evaluation of its effect using different parameters of assessment; this work is the first study to assess the anti-giardial impact of *M. recutita* plant extract.

Materials and methods

Ethical approval

The experimental design was Accepted by the Ethical Committee at the Faculty of Women for Arts, Science, and Education, Ain Shams University, Code: SCI1332405001. All animals in the present investigation have been managed in accordance with internationally recognized principles for experimental animal ethics.

Study design

Mice have been categorized into seven main groups: negative control, positive control and infected treated. Animals' infection has been performed on the 7th day, and have been sacrificed utilizing intraperitoneal anesthesia following 15 days PI. Parasitological, immunological, histopathological and oxidative stress markers parameters have been utilized to achieve the investigation objective.

Plant collection and extractions

M. recutita (chamomile) aerial parts have been obtained from El-Harraz Market, Giza, Egypt. They were kept at the chemistry department of Theodor Bilharz Research Institute (Giza, Egypt) for the removal and fractionation of the plant. The plant-dried powder material (200 g), was extracted using 70% methanol (MeOH), then evaporated under reduced pressure using a rotatory evaporator (BUCHI, Switzerland) under vacuum till dryness to get the crude extract; this was repeated for 5 times. The dried MeOH extract was dissolved in less amount of distilled water, then fractionated using organic solvent including n-butanol and n-hexane (19).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The chemical makeup of *M. recutita* plant extract was examined using a Thermo Scientific Trace Gas Chromatograph Tandem Quadrupole Mass Spectrometer (Austin, TX, USA). For the analysis, a TG-5MS direct capillary column with dimensions of 0.25 millimeters in diameter, 30 meter in length, and 0.25 micrometer in film thickness has been utilized.

The column oven's temperature was raised by 5°C every minute from 50 to 250°C, where it stayed for two minutes. Then, for two minutes, it was raised to 300°C at a pace of 30°C per minute. The mass spectrometry (MS) transfer line and injector were maintained at 260°C and 270°C, respectively. Helium was the experiment's carrier gas, and it flowed at a steady 1 milliliter per minute. An auto-sampler AS1300 connected to a gas chromatograph (GC) in split mode automatically injected 1 µl samples after a 4-minute solvent delay. The temperature of the ion source has been established to 200°C. At 70 electron volts (eV) ionization voltages, electron ionization (EI) mass spectra have been collected in full scan mode, spanning mass-to-charge ratios (m/z) ranging from 50 to 650. By comparing the peak retention duration with standards, the chemical composition of the methanolic extract may be determined. The produced mass spectra were then compared to those found in the WILEY 9 and National Institute of Standards and Technology (NIST) fourteen mass spectral databases (20).

Collection of *Giardia lamblia* cysts

Giardia lamblia cysts have been collected from fresh samples of the stool of heavily infected cases complaining of steatorrhea attending Al-Zahraa University Hospital (El-Abaseya, Egypt). Stool specimens have been promptly processed at the parasitology laboratory. The viability of *Giardia lamblia* cysts has been verified utilizing 0.1 percent eosin vital staining. Cysts have been enumerated in 0.1 milliliters of sediment, and the concentration procedure was repeated with additional samples of stool till the suspension reached approximately ten thousand viable cysts per milliliter of phosphate-protected saline according to (21).

Infection of animals with *Giardia lamblia* cysts

Animals from various groups have been orally administered two hundred milliliters of phosphate-buffered saline containing ten thousand cysts (22).

Experimental animals

This case-control investigation has been performed on 42 Swiss male albino mice (between three to four weeks old, weighing 20±25 gm). The mice have been collected from Theodor Bilharz Research Institute (Giza, Egypt), and they have been stored in pathogen- free conditions at the animal house of Theodor Bilharz Research Institute (Giza, Egypt). To confirm that mice were free from intestinal parasites, samples of stool have been evaluated for 3 successive days prior to beginning the experiment.

Animal grouping

Animals have been categorized into the following seven groups; each group had 6 mice. G1: non-infected-non managed (Negative control). G2: infected non-managed (Positive control). G3: infected and managed with *Metronidazole* (100 milligrams per kilogram/day for five days). G4: infected and treated with n- hexane extract of *M. recutita* (chamomile) at 50 % concentration (100mg/kg/day for five days). G5: infected and treated with n- hexane extract of *M. recutita* (chamomile) at 100 % concentration (100mg/kg/day for five days). G6: infected and treated with n- butanol extract of *M. recutita* (chamomile) at 50 % concentration (100mg/kg/day for five days). G7: infected and treated with n- butanol extract of *M. recutita* (chamomile) at 100 % concentration (100mg/kg/day for five days). An esophageal tube has been utilized for therapy administration. All animal groups have been then sacrificed two weeks' post-treatment to assess drug efficacy (23).

Parasitological assay

Fecal pellets have been obtained separately from each animal all other day till day 15 post infection. Approximately 0.1 gram of sample of feces was mixed carefully with one milliliter of formol saline, then stained with iodine and cysts were counted utilizing a hemocytometer (24). The rate of animals that developed the infection, the day's number essential to shed the parasite cysts following infection (period of prepatent), and the period for the complete withdrawal of the parasites (patent period) have been documented for every appropriate sample.

Determination of cyst and trophozoites count in different groups of infected mice

Following the sacrifice of mice, the small intestine was extracted, and the duodenal contents underwent parasitological analysis to quantify the *G. lamblia* cysts and trophozoites in five consecutive fields per animal (25).

Histopathological examination

After scarification of mice, the upper six centimeters of the duodenum and proximal jejunum have been promptly excised and tissue samples were fixed in buffered saline formaldehyde (pH 7.2 to 7.4 at 4°C). All fixed specimens underwent dehydration, followed by processing for paraffin wax embedding, and were subsequently sectioned to a thickness of three to five µm utilizing a rotary microtome to identify histopathological alterations. All histological sections were stained with hematoxylin and eosin (H&E) (26).

Immunohistochemistry

Paraffin sections of the small intestine from various groups have been rehydrated and deparaffinized utilizing decreasing levels of ethanol. Sections have been exposed to boiling in citrate buffer (pH 6.0) utilizing a microwave for epitope retrieval. Endogenous peroxidases have been subsequently inhibited using three percent H_2O_2 in ethanol. Sections have been treated with anti-caspase-3 antibodies (Thermo Scientific, Waltham, MA, USA; dilution 1:500). Slices have been washed with TBS supplemented with 0.05 percent Tween-20 (TBS-T. Following washing in TBS-T, color development occurred by incubating segments with 0.05 percent diaminobenzidine (DAB) and 0.01 percent hydrogen peroxide (H2O2) for 3 minutes. Images have been obtained utilizing a Leica light microscope prepared with a digital camera in the Histology and Cell Biology Department at Assiut University, Faculty of Medicine, Assiut, Egypt (27). The cell kinds were identified based on the sizes, shapes of the nuclei, and exterior appearance of the cells during the series of divisions (28).

Histochemistry

Immunocytochemical analysis targeting Giardia trophozoites. Immunocytochemistry has been utilized to identify G. intestinalis trophozoites in 5 µm thick slices. The portions have been rehydrated and deparaffinized. Antigens have been extracted using 0.1 M citrate buffer at pH 6.0, and endogenous peroxidase activity has been suppressed utilizing hydrogen peroxide (1.6 percent in methanol). The sectors have been incubated for two hours in ten

percent normal goat serum (Vector Laboratories, Inc., Orton Southgate, United Kingdom), followed by incubation with a 1ry antibody targeting Giardia trophozoites (1: 1000), established against trophozoites and their excretory products in rats at the Experimental Parasitology laboratory of the National Institute of Pediatrics. Thereafter, the avidin-biotin-peroxidase technique (ABC kit, Vector Laboratories, Inc., Orton Southgate, United Kingdom) has been employed, and the response has been seen using the chromogen 3′ 3′ -diaminobenzidine (Sigma Aldrich, St. Louis, USA). The specimens have been counterstained with hematoxylin (H) and subsequently mounted in resin (Entellan, Merck, Darmstadt, Germany).

Quantification of oxidative stress markers

Oxidative stress markers contain a variety of enzymes that are a part of the antioxidant defence system, which protects against harmful processes caused by oxidative stress and neutralises excess oxygen species (29). Blood samples have been obtained, and sera have been isolated and preserved at −20 ◦C until utilized. Superoxide dismutase (SOD) and malondialdehyde (MDA) levels have been quantified in serum utilizing kits from Biodiagnostic Co., Egypt (30).

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean±standard deviation and ranges when their distribution was parametric (normal) while non-normally distributed variables (non-parametric data) were presented as median with inter-quartile range (IQR). Qualitative variables were presented as number and percentages. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk Test. Independent-samples t-test of significance was used when comparing between two means. One-Way ANOVA test of significance was used when comparing between more than two means. The Comparison between groups with qualitative data was done by using Chi-square test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as Probability (P-value) P value $\leq$0.05 was considered significant, P value $\leq$0.01 was considered as highly significant, P value >0.05 was considered insignificant.

Results

Chemical composition of the essential oil of N-Hexane extracts

GC-MS analysis has been performed for the N-Hexane extract of the chamomile plant. The chromatogram demonstrated the presence of 43 compounds in the N-Hexane extract of chamomile the findings (Table 1). The recognition of the components of each essential oil has been performed by their retention time (RT), molecular formula (MF), molecular weight (MW), and mass peak area%. The major compounds identified in essential oil of N-Hexane extract of the plant were Palmitic Acid, TMS derivative (12.91%),1H-Isoindol-1-one, 2,3-dihydro-4,5-dimethyl-3-(2-methylpropylidene) (10.27%) and D-(-)-Ribofuranose, tetrakis(trimethylsilyl)ether (6.07%). On the contrary, the components which have less concentrations 2-Trimethylsilyloxysebacicacid, bis(trimethylsilyl)- ester (0.12%) and Malic acid, 3TMS derivative (0.31%).

Chemical composition of the essential oil of N-Butanol extracts

GC-MS analysis has been performed for N-Butanol extract of the chamomile plant. The chromatogram revealed the presence of 59 compounds in the N-Butanol of chamomile (Table 2). The recognition of the components of each essential oil was performed by their molecular formula (MF), molecular weight (MW), retention time (RT)and mass peak area%. The major compounds detected in essential oil of N-Hexane extract of the plant were2-(2-Bromo-4-methylphenoxy)-N'-([1-(4-nitrophenyl)-2-pyrrolidinyl]-methylene) acethydrazide (6.88%) and N-Propyl 9,12,15-octadecatrienoate (4.67%). On the contrary, the components that have less concentrations were Uridine, 3TMS derivative (0.12%) and 1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene (2.97%). Trimethylsilyl derivatives (TMS) resulting from the synthesis process are listed as natural compounds as they are derived from the chemical derivatization process necessary for the analysis of non-volatile or low-volatile compounds, facilitating spectroscopic analysis by increasing their volatility.

Determination of cyst count in distinct groups of infected mice

The number of *Giardia* cysts and the percentage of decrease in the stool of infected mice, two weeks following the administration of drugs were counted. G3 (group Infected and treated with metronidazole) showed the highest percentage of reduction 5.4+2.07 (Figure 1).

Determination of trophozoites count in distinct groups of infected

Comparison between the studied groups regarding trophozoite level, the *Giardia* number of trophozoites and the percentage of decrease in the stool of infected mice, 2 weeks following administration of drugs. Group 3 showed significant reduction with a percentage of 13.6±3.51 (Figure 1).

Histopathological and immunohistochemical examination

Histopathological results of the intestine (Figure 2). Immunohistochemical marker caspase 3 was used, sections

of the intestinal tissue were examined: The results of Caspase-3 staining showed that the G1 control group showed faint positivity for Caspase 3 (+), G2 showed high expression of Caspase 3 and G3& G4 showed lower expression of Caspase 3 (Figures 3-9).

Table 1: Chemical composition of the essential oil of N-Hexane extracts

Peak no	RT	Compound name	M.F	M.Wt	Peak area
1	9.26	Glycerol, 3TMS derivative	C12H32O3Si3	308	0.55
2	9.36	Silanol, trimethyl-, phosphate (3:1)	C9H27O4PSi3	314	0.15
3	10.87	Decanoic acid, TMS derivative	C13H28O2Si	244	0.06
4	11.2	Malic acid, 3TMS derivative	C13H30O5Si	350	0.31
5	11.61	L-5-Oxoproline, 2TMS derivative	C11H23NO3Si2	273	0.07
6	13.34	two, three-bis[(trimethylsilyl)oxy]propyl ester,bis(trimethylsilyl), Phosphoric a`		460	0.06
7	13.39	D-(-)-Ribofuranose,tetrakis(trimethylsilyl)ether (isomer two)	C17H42O5Si4	438	0.34
8	13.59	D-(-)-Ribofuranose,tetrakis(trimethylsilyl)ether (isomer two)	C17H42O5Si4	438	6.07
9	13.63	Tetrachloro-o-benzoquinone	C6Cl4O2	245	1.81
10	14.16	3-pentulosonic a`, trimethylsilyl ester, 2,4,5-tris-O-(trimethylsilyl)-	C17H42O5Si4	438	0.47
11	14.21	Altronic acid, Gamma.-lactone, 4TMS derivative	C18H42O6Si	466	0.05
12	14.34	Benzoic a, 2,3-dicyano-4-(pentyloxy)phenyl ester`, four-(four-ethylcyclohexyl)-	C29H34N2O3	194	1.856
13	14.43	Hexadecenoic acid, methyl ester	C17H34O2	270	1.62
14	14.62	2,3-dihydro-four,five-dimethyl-3-(2-methylpropylidene)- 1H-Isoindol-1-one	C10H9NO	161.	10.27
15	14.76	D-Glucopyranose, 5TMS derivative	C21H52O6Si	541	1.43
16	14.90	D-Gluconic acid, 6TMS derivative	C24H60O7Si	629	1.78
17	15.18	Palmitic Acid, TMS derivative	C19H40O2Si	328	12.91
18	15.34	Pyridine, 4-[3',4'-dimethoxyphenyl]-	C13H13NO2	215	0.98
19	15.37	D-Arabinopyranose, 4TMS derivative (isomer 2)	C17H42O5Si	438	0.77
20	15.44	Myo-Inositol, 6TMS derivative	C24H60O6Si	613	2.41
21	16.17	Oleic Acid, (Z)-, TMS derivative	C21H42O2Si	354	5.64
22	16.21	alpha.-Linolenic acid, TMS derivative	C21H38O2Si	350	1.08
23	16.26	Stearic acid, TMS derivative	C21H44O2Si	356	1.08
24	16.54	Methyl 2-hydroxytetradecanoate, TMS derivative	C18H38O3Si	330	0.29
25	16.67	Palmitic Acid, TMS derivative	C19H40O2Si	328	0.22
26	16.69	Trimethylsilyl(3Z)-seven-oxo-three-{two-[(trimethylsilyl)oxy]ethylidene}-four-oxa-1-azabicyclo[3.2.0]heptane-two-carboxylate	C8H9NO5	199	0.16
27	17.09	octakis(trimethylsilyl) ether,Beta. -Gentiobiose,, methyloxime (isomer two)	C37 H89NO11Si 8	948	5.31
28	17.22	1-Phenyl-3-[bis(methoxycarbonyl)methyl]hex-1-en-4-yne	C7H10	94.1	2.27
29	17.72	Morphine, 2TMS derivative	C23H35NO3Si	429	0.70
30	17.78	d-Glucose, six-O-[6-deoxy-2,3,4-tris-O-(trimethylsilyl)-alpha-l-mannopyranosyl]-2,3,4,5-tetrakis-O-(trimethylsilyl)-	C22H55NO6Si	570	0.54
31	17.84	2-Trimethylsilyloxysebacicacid, bis(trimethylsilyl)- ester	C19H42O5Si	434	0.12
32	17.90	Pregnane-3,20-dione, sevnteen-[(trimethylsilyl)oxy]-, bis(O-methyloxime), (five.alpha.)-	C21H32O2	316	0.44
33	18.06	2-Bromosebacic acid, 2TMS derivative	C16H34O4Si	346	0.45
34	18.24	1-Monopalmitin, 2TMS derivative	C9H20O4Si2	248	2.70
35	18.30	2,4-Quinolinedicarboxylic acid	C32H20N2O4	496	1.05
36	18.39	(3-Amino- [1,2,4] triazol-1-yl)-(2,2-dichloro-1-methyl-cyclopropyl)-methanone	C2H4N4O	102	7.07
37	18.48	Sucrose, 8TMS derivative	C36H86O11Si8	919	0.53
38	18.60	3-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer two)	C36H86O11Si8	919	0.12
39	18.63	L-(+)-Threose, tris(trimethylsilyl) ether, trimethylsilyloxime (isomer one)	C16H41NO4Si4	423	0.09
40	19.68	octakis(trimethylsilyl) ether,D-(+)-Cellobiose,, methyloxime (isomer two)	C36H86O11Si8	948.	4.42
41	19.72	octakis(trimethylsilyl) ether, Maltose, methyloxime (isomer two)	C37H89NO11Si8	948	5.11
42	20.24	3-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer two)	C36H86O11Si8	919.	6.79
43	20.82	octakis(trimethylsilyl) ether (isomer one), 2-.alpha.-Mannobiose	C36H86O11Si8	919	1.06

Table 2: Chemical composition of the essential oil of N-Butanol extracts

Peak no	RT	Compound name	M.F	M.wt	Peak area
1	9.36	1-(3-Methylbutyl)-2,3,5,6-tetramethylbenzene	C15H24	162	3.87
2	9.51	Silanol, trimethyl-, phosphate (3:1)	C9H27O4PSi3	314	0.12
3	11.60	D-Arabinose, tetrakis(trimethylsilyl)ether, pentafluorobenzyloxime (isomer 1)	C20H51NO5Si5	526	0.09
4	11.75	Xylopyranose,3-O-methyl-1,2,4-tris-O-(trimethylsilyl)-	C15H36O5Si3	380	0.42
5	12.45	D-Arabino-Hexonic a`, 3-deoxy-2,5,6-tris-O-(trimethylsilyl)-, γ-lactone	C15H34O5Si3	378	0.03
6	12.97	L-(-)-Arabitol,5TMS derivative	C20H52O5Si5	513	0.11
7	13.59	5-(1-Naphthyl)tricycle[4.1.0.0(2,7)]hept-3-ene	C17H14	218	5.53
8	13.80	1,5-Anhydrohexitol, 4TMS derivative	C18H44O5Si4	452	0.50
9	13.88	Methyl galactoside (1S,2R,3S,4R,5R)-, 4TMS derivative	C19H46O6Si4	482	3.07
10	14.04	Molybdenum, tricarbonyl[(1,2,3,4,5,6-.eta.)-1,4-dimethylbenzene]-	C10H8MoO3	272	1.01
11	14.30	3-pentulosonic a`, 2,4,5-tris-O-(trimethylsilyl)-, trimethylsilyl ester	C17H40O6Si4	452	3.53
12	14.39	1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene	C15H24	204	2.97
13	14.55	Pentadecanoic a`, 14-methyl-, methyl ester	C17H34O2	270	2.88
14	14.64	D-Chiro-Inositol, 6TMS derivative	C24H60O6Si6	613	1.11
15	14.67	Scyllo-Inositol, 6TMS derivative	C24H60O6Si6	613	1.28
16	14.70	1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)-	C13H12O2	200	1.53
17	14.82	Beta.-D-Glucopyranose, 5TMS derivative	C21H52O6Si5	541	1.48
18	14.91	(3,5-Dimethoxynaphthalen-2-yl)(7-methyl-3,4-dihydro-2H-quinolin-1-yl)methanone	C23H23NO3	361	0.22
19	14.98	D-(+)-Cellobiose, octakis(trimethylsilyl) ether, methyloxime (isomer 2)	C37H89NO11Si8	948	0.17
20	15.08	1,2,3,4,6,7,8,8a-Octahydronaphthalene-6,7-diol, 5,8a-dimethyl-3-isopropenyl-, cyclic carbonate, trans-	C16H22O3	262	0.03
21	15.22	Palmitic a`, TMS derivative	C19H40O2Si	328	3.87
22	15.28	Benzenepropanoic a`, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	C19H36O5Si3	428	0.12
23	15.66	Aciphyllene	C15H24	204	0.09
24	15.81	9(1H)-Phenanthrone, 2,3,4,4a,4b,5,6,7,8,8a-decahydro-	C14H20O	204	0.42
25	15.90	Pentenoic a`, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	C11H24O3Si2	260	0.03
26	16.10	Melibiose, octakis(trimethylsilyl)-	C36H86O11Si8	919	2.53
27	16.32	9-Octadecenoic acid, (E)-, TMS derivative	C21H42O2Si	354	0.02
28	16.60	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer two)	C21H52O6Si5	541	4.26
29	16.76	Glyceryl-glycoside TMS ether	C27H66O8Si6	687	0.03
30	17.13	1,3,12-Nonadecatriene	C19H34	412	0.09
31	17.19	N-Propyl 9,12,15-octadecatrienoate	C20H34O2	306	4.67
32	17.28	2-(2-Bromo-4-methylphenoxy)-N'-([1-(4-nitrophenyl)-2-pyrrolidinyl]methylene)acethydrazide	C8H6BrNO	242	6.88
33	17.33	D-(+)-Xylose, tetrakis(trimethylsilyl) ether, trimethylsilyloxime (isomer one)	C20H51NO5Si5	526	1.74
34	17.37	Levoglucosan, 3TMS derivative	C15H34O5Si3	378	0.03
35	17.44	Uridine, 3TMS derivative	C18H36N2O6Si3	460	0.12
36	17.51	D-(+)-Xylose, 4TMS derivative	C20H51NO5Si5	526	0.41
37	17.58	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	C13H16N2O2S	264	6.08
38	17.64	D-(+)-Cellobiose, octakis(trimethylsilyl) ether, methyloxime (isomer two)	C37H89NO11Si8	948	1.50
39	17.83	Maltose, 8TMS derivative, isomer 1	C36H86O11Si8	919	2.54
40	18.14	1-Monopalmitin, 2TMS derivative	C25H54O4Si2	474	0.52
41	18.20	D-Lactose, octakis(trimethylsilyl) ether, methyloxime (isomer 2)	C37H89NO11Si8	948	0.15
42	18.30	Bicyclo[2.2.1]heptane-2-acetamide, N-(2,3-dimethyl-6-quinoxalinyl)-	C9H12	136	1.21
43	18.48	DL-Arabinose, tetrakis(trimethylsilyl) ether, benzyloxime (isomer 1)	C24H49NO5Si4	543	1.46
44	18.55	2-(2-Bromo-4-methylphenoxy)-N'-([1-(4-nitrophenyl)-2-pyrrolidinyl]methylene)acethydrazide	C8H6BrNO	242	0.60
45	18.71	two-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer two)	C36H86O11Si8	919	1.80
46	18.81	D-(+)-Turanose, octakis(trimethylsilyl) ether, methyloxime (isomer one)	C37H89NO11Si8	948	0.04
47	18.89	D-Fructose,5TMS derivative	C21H52O6Si5	541	0.38
48	19.07	3-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer 2)	C36H86O11Si8	919	0.95
49	19.17	Palatinose,-heptakis(trimethylsilyl)-ether, methyl	C22H55NOSi5	570	0.58
50	19.26	9(1H)-Phenanthrone, 2,3,4,4a,4b,5,6,7,8,8a-decahydro-	C14H20O	204	0.07
51	19.63	2-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer 2)	C36H86O11Si8	919	0.25
52	19.73	Maltose, 8TMS derivative	C36H86O11Si8	919	2.82
53	19.82	Maltose, octakis(trimethylsilyl) ether, methyloxime (isomer 1)	C37H89NO11Si8	948	1.73
54	20.12	,octakis(trimethylsilyl) ether 3-.alpha.-Mannobiose (isomer 2)	C36H86O11Si8	919	0.03
55	20.17	3-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer 2)	C36H86O11Si8	919	0.01
56	20.32	3-.alpha.-Mannobiose, octakis(trimethylsilyl) ether (isomer 1)	C36H86O11Si8	919	5.27
57	20.53	octakis(trimethylsilyl) ether, (isomer 2) 3-.alpha.-Mannobiose	C36H86O11Si8	919	0.51
58	20.89	Sucrose, 8TMS derivative	C36H86O11Si8	919	0.72
59	20.89	Pyridine-2,5-dicarboxylic acid bis-cyclopropylamide	C13H15N3O2	245	1.06

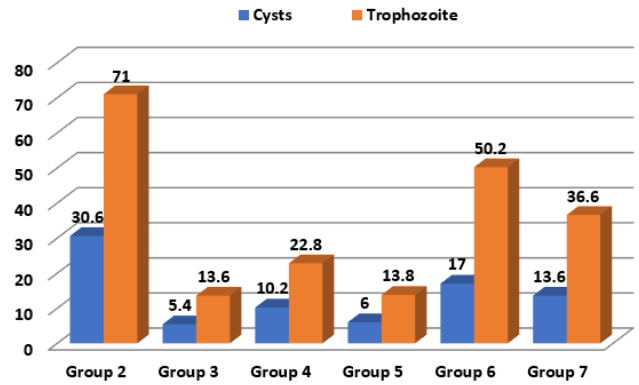

Figure 1: Showing the mean count of *giardia* trophozoites and cysts in comparison between each group.

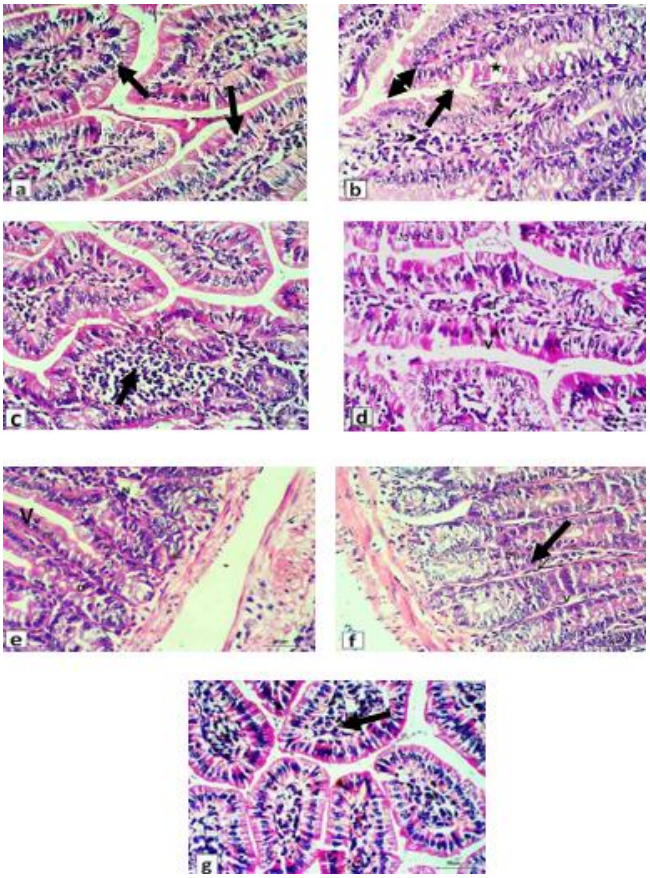

Figure 2: Photomicrograph of section in the duodenum of the small intestine stained with H&E (x400) from: (a) negative control mice showing normal villi lined with simple ciliated columnar epithelial.(arrow). (b) positive control showing *Giardia lambelia* trophozoite in the lumen of the duodenum (arrow), blunted and shortening of villi (double head arrow), pyknotic nuclei in the enterocyte (head of arrow), atrophy and vacuolar degeneration erosion in some villi (star). c)Infected and treated with metronidazole showing nearly normal structure with a mild increase of lymphocyte infiltration (arrow). d) Infected and treated with 50% hexane showing long villi with normal microvilli of the epithelial cell (v). (e) Infected and treated with 100% hexane showing normal villi with microvilli (V) and mild degeneration and necrotic in villi (D,N). f) Infected and treated with 50% N-butanol showing long villi and loss of it's brush border (v) and hemorrhage and infiltration in the lumen (arrow). (g) Infected and treated with 100% N-butanol showing mild lymphocyte (arrow) and few necrotic cell depress in the lumen (N).

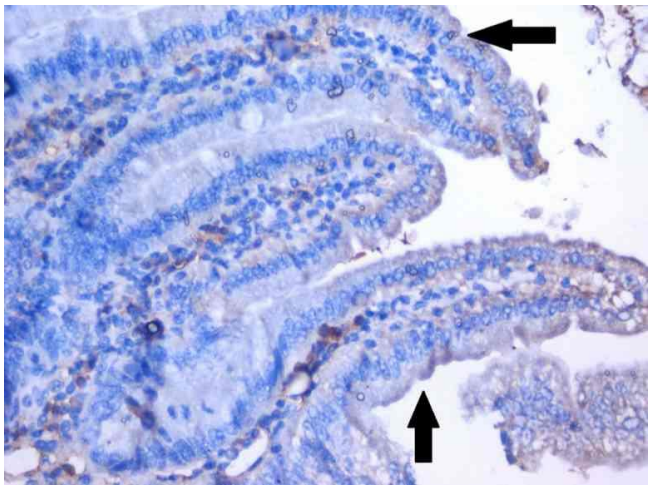

Figure 3: Showing Section in the intestine of the Control non-infected group (G1) showing negative expression of Caspase-3 in villous epithelial cells (arrows) (IHC, Caspase-3, DAB, X200).

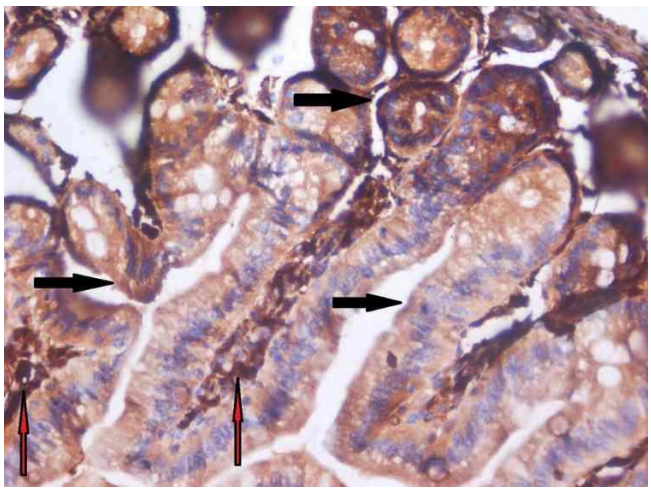

Figure 4: Showing Section in the intestine of the group (G2) showing strong diffuse positive expression of Caspase-3 in mucosal epithelial (black arrows) and inflammatory cells (red arrow) (IHC, Caspase-3, DAB, X200).

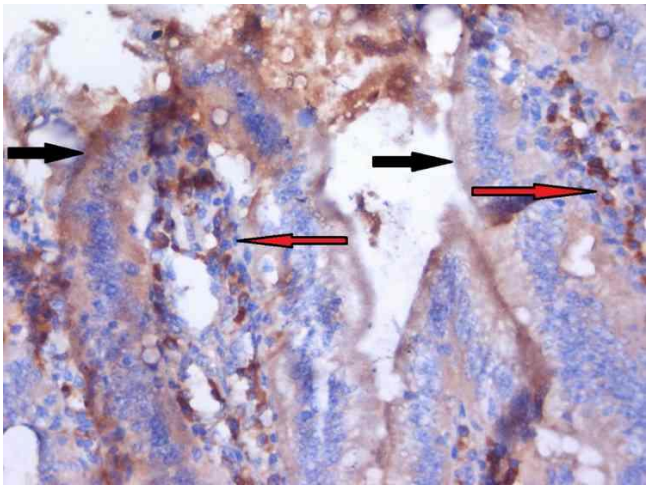

Figure 5: Showing Section in the intestine of the group (G3) showing mild positive expression of Caspase-3 in mucosal epithelial (black arrows) and in scattered inflammatory cells (red arrows) (IHC, Caspase-3, DAB, X200).

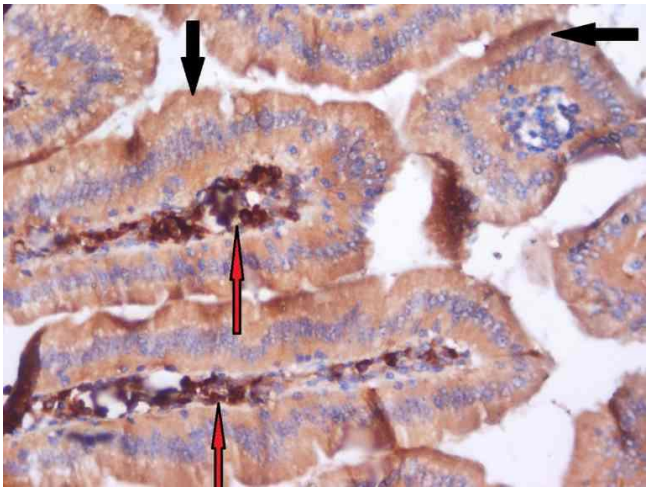

Figure 6: Showing Section in the intestine of the group (G4) showing moderate diffuse positive expression of Caspase-3 in mucosal epithelial (black arrows) and moderate number of inflammatory cells (red arrow) (IHC, Caspase-3, DAB, X200.

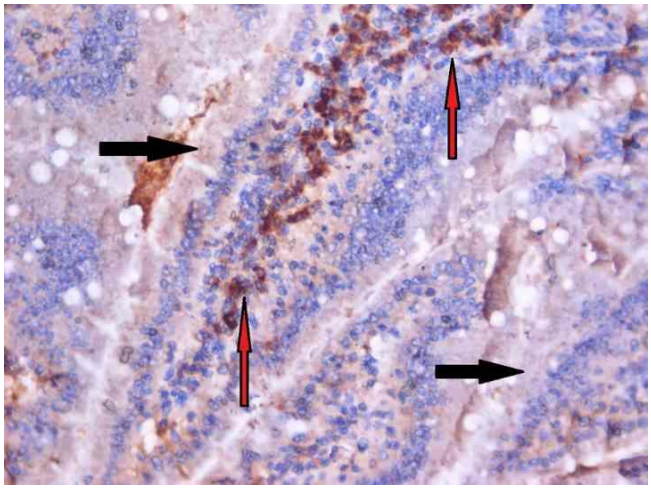

Figure 7: Showing Section in the intestine of the group (G5) showing faint positive expression of Caspase-3 in mucosal epithelial (black arrows) andpositive expression in some scattered inflammatory cells (red arrows) (IHC, Caspase-3, DAB, X200).

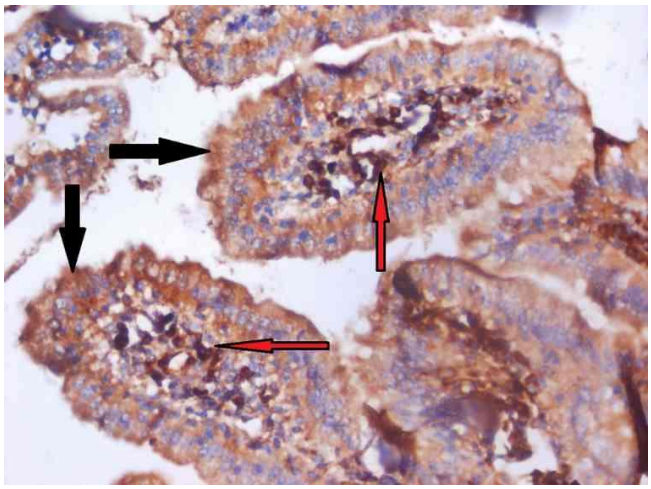

Figure 8: Showing Section in the intestine of the group (G6) showing moderate diffuse positive expression of Caspase-3 in mucosal epithelial (black arrows) and moderate number of inflammatory cells (red arrow) (IHC, Caspase-3, DAB, X200).

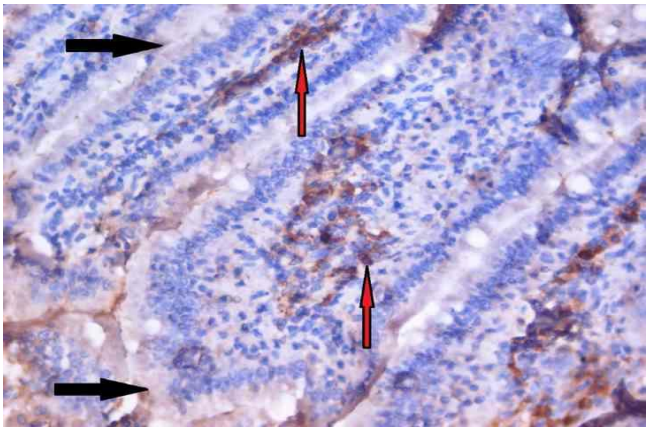

Figure 9: Showing section in the intestine of the group (G7) showing faint positive expression of Caspase-3 in mucosal epithelial (black arrows) and positive expression in some scattered inflammatory cells (red arrows) (IHC, Caspase-3, DAB, X200).

Histochemistry results

PAS Ab staining was used; sections of the intestinal tissue were examined PAS stain showed goblet cell depletion in the infected untreated group (G1) compared to the normal pattern found in the control non-infected group. There was restoration of Goblet cells in all other groups with variable degrees (Figures 10-16).

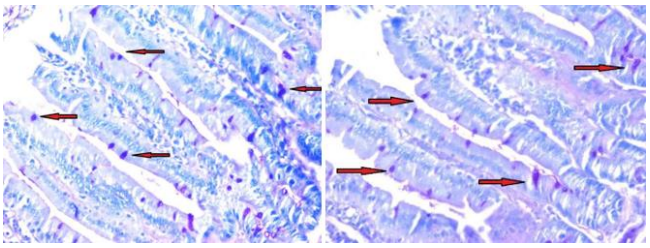

Figure 10: Sections in the intestine of G1 control group showing the average number of goblet cells (red arrows) within the villous epithelial cells (PAS stain, X200).

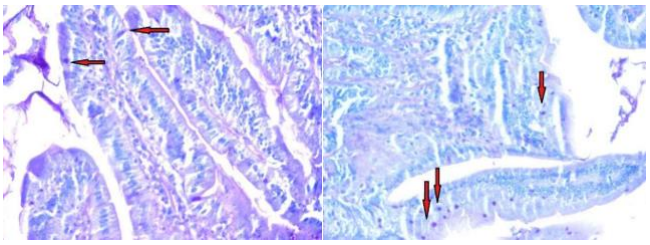

Figure 11: Sections in the intestine of group (G2) showing patchy decrease in number of goblet cells (red arrows) within the villous epithelial cells PAS compared to the normal pattern found in the control non-infected group (PAS stain, X200).

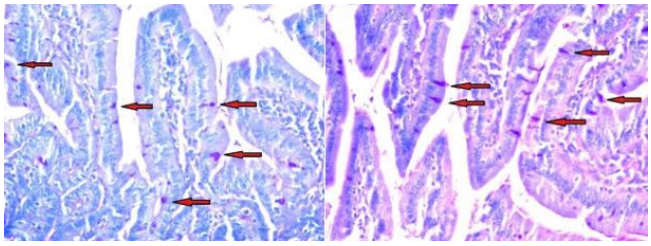

Figure 12: Sections in the intestine of the group (G3) showing nearly restoration of the number of goblet cells (red arrows) within the villous epithelial cells like the control non-infected group (PAS stain, X200).

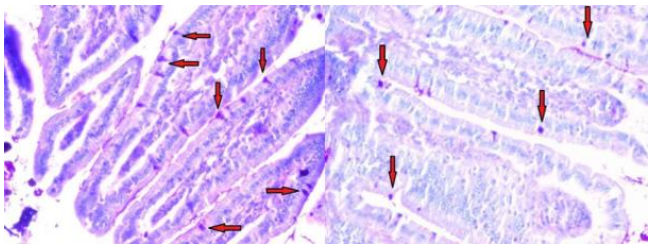

Figure 13: Sections in the intestine of group (G4) showing focal restoration of the number of goblet cells (red arrows) within the villous epithelial cells like the control non-infected group (PAS stain, X200).

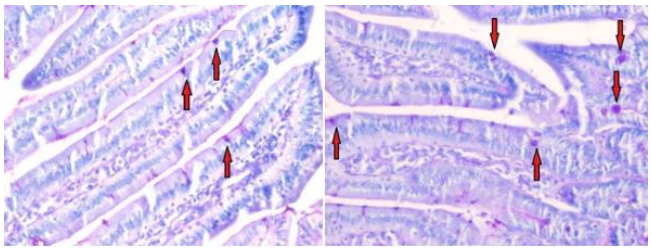

Figure 14: Sections in the intestine of group (G5) showing focal restoration of the number of goblet cells (red arrows) within the villous epithelial cells like the control non-infected group (PAS stain, X200).

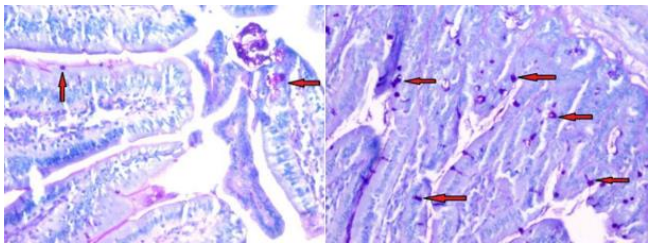

Figure 15: Sections in the intestine of group (G6) showing restoration of the average number of goblet cells (red arrows) within the villous epithelial cells like the control non-infected group (PAS stain, X200).

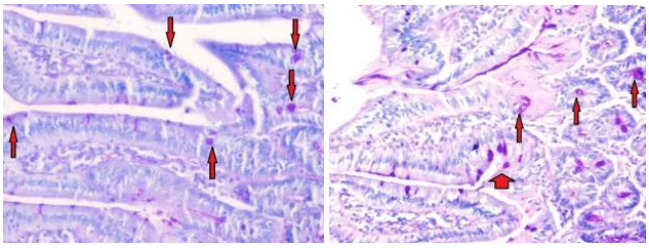

Figure 16: Sections in the intestine of group (G7) showing restoration of the average number of goblet cells (red arrows) within the villous epithelial cells like the control non-infected group (PAS stain, X200).

Oxidative stress markers results (Quantification of SOD and MDA)

MDA comparison between the studied groups regarding MDA (nmol/mg/protein), where G7 showed the lowest expression (Figure 17). SOD comparative analysis among the examined groups regarding SOD (u/mg/protein), where G1 showed the lowest expression levels (Figure 18).

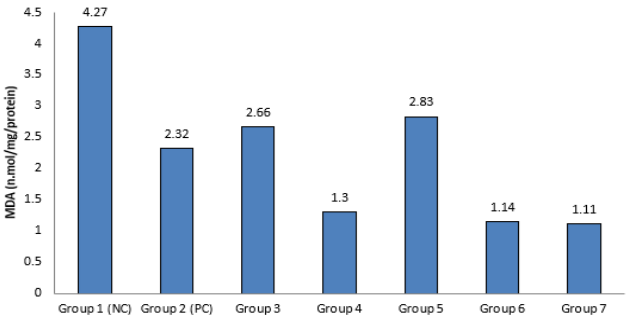

Figure 17: Comparison between the studied groups regarding MDA (nmol/mg/protein).

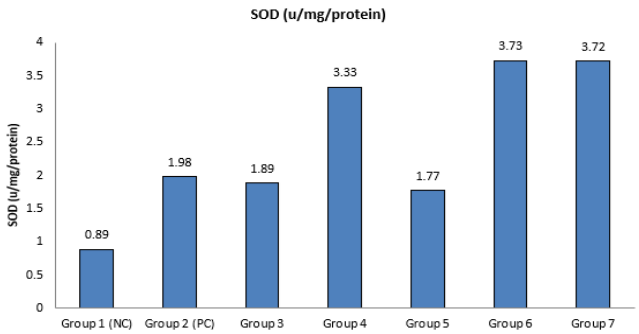

Figure 18: Comparison between the studied groups regarding SOD (u/mg/protein).

Discussion

Giardia lamblia is a flagellated protozoan parasite that infects people causing giardiasis and gastrointestinal illness (31). It leads to worldwide waterborne epidemics of both acute and chronic fatty diarrhea across all demographics. Furthermore, it can be exacerbated by the malabsorption of fats and carbohydrates, chloride hypersecretion, and expedited intestinal transit. Chemotherapeutics include MTZ, nitroimidazoles, benzimidazoles were utilized as primary therapies for giardiasis (32). Although their effectiveness, recurrences and established carcinogenicity in investigational animals continue to hinder their application. Adverse side effects, including leukopenia and neurotoxic manifestations such as ataxia, convulsions, and vertigo, may causes the discontinuation of chemotherapy (33). Management failures can similarly occur. Thus, extended treatment protocols, elevated dosages, or the modification of pharmacological classes can be advantageous in decreasing the risk of cross-resistance. In this context, mixture treatment (CT) is emerging as a promising alternative for refractory giardiasis; however, elevated parasite resistance to these therapies underscores the urgent need for the discovery of novel, effective, non-chemical, and safe medicines for giardiasis management (34). This investigation aims to assess the effects of *M. recutita* in the management of experimental giardiasis and evaluate its effect using different parameters of assessment. This was the first trial to assess the role of *M. recutita* in management of giardiasis. We compared our results with other studies that used natural products. Our parasitological results showed that the group treated with flagyl gave the best results, followed by the group infected and treated with n- hexane extract of *M. recutita* (chamomile) at 100 % concentration. This goes in agreement with (35) who found that the natural product Olibanum of ten, fifteen, and twenty milligrams per kilogram per day ibited *G. lamblia* multiplication in vivo in a dose-dependent method. Our outcomes didn't go in agreement with Al-Ghandour who proved that the combination of the natural product of propolis and olibanum produce better findings compared to utilizing them separately and near-by outcomes to MTZ (36). Our histopathological results showed improvement of the intestinal wall when treated with *M. recutita*. This agreed with (37) who proved that Olibanum and propolis medicinal extracts improved the histological alterations produced by infection in the duodenum and jejunum. These findings stated with those of Thabet and Abdel-Fattah, who investigated the inhibitory impact of propolis medicinal extracts on *G. lamblia* trophozoites in vitro, revealing that a concentration of 250 µg/ml resulted in growth reduction following seventy-two hours (38). This also agreed with Abd-Elhamid who found that managing infected animals with A. annua extract significantly reduced desquamated enterocytes, resulting in approximately normal villi coating (39). In our study, the immunohistochemical marker, caspase 3, sections of the intestinal tissue and the liver tissue were examined. Our results were in accordance with Abd-Elhamid who proved that *A. muricatta* showed lower expression of Caspase-3 (39). This also went in agreement with (40) whose

study showed no apoptotic expression of caspase-three has been detected in the group managed with mixed prebiotic and probiotic supplements of probiotic in addition to nitazoxanide. PAS stain of the intestine showed best restoration of Goblet cells in G7. PAS Ab stain of the liver showed mild patchy decrease in magenta color within the hepatocytes representing decrease in their collagen content in G2. Our parameters included quantification of SOD and MDA. Our results showed that group 7 had the lowest MDA and the highest SOD. This was the first study to use MDA and SOD as oxidative stress markers.

Conclusion

Results of the current investigation proved that the *M. recutita plant extract* has a promising therapeutic effect against *Giardia lamblia*. The plant extract from *M. recutita* is readily available and helps prevent the negative side effects of chemotherapy medications.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Sarah KN. Giardiasis: A review study in Iraq. Al-Ameed J Med Res Hlth Sci. 2024;2(1):1-4. DOI: 10.61631/3005-3188.1010
2. Zoghroban HS, Ibrahim FM, Nasef NA, Saad AE. The impact of L-citrulline on murine intestinal cell integrity, immune response, and arginine metabolism in the face of *Giardia lamblia* infection. Acta Trop. 2023;237(1):106748. DOI: 10.1016/j.actatropica.2022.106748
3. Vicente B, Freitas AD, Freitas M, Midlej V. Diagnostic approaches for human giardiasis: unveiling optimal strategies. Diagnostics. 2024;14(4):364. DOI: 10.3390/diagnostics14040364
4. Tembo SJ, Mutengo MM, Sitali L, Changula K, Takada A, Mweene AS, Simulundu E, Chitanga S. Prevalence and genotypic characterization of *Giardia duodenalis* isolates from asymptomatic school-going children in Lusaka, Zambia. Food Waterborne Parasitol. 2020;19:e00072. DOI: 10.1016/j.fawpar.2020.e00072
5. El-Badry A, Mohammed F, Abdul Gawad E. Predominance of *Giardia intestinalis* assemblage B in diarrhoeic children in Sharkia, Egypt. PUJ. 2017;10(1-2):39-43. DOI: 10.21608/PUJ.2017.4735
6. Lagunas-Rangel FA. *Giardia* telomeres and telomerase. J Parasitol Res. 2024;123(4):179. DOI: 10.1007/s00436-024-08200-6
7. Escobedo AA, Hanevik K, Almirall P, Cimerman S, Alfonso M. Management of chronic *Giardia* infection. Expert Rev Anti Infect Ther. 2014;12(9):1143-57. DOI: 10.1586/14787210.2014.942283
8. Edlind TD. Tetracyclines as antiparasitic agents: lipophilic derivatives are highly active against Giardia lamblia in vitro. Antimicrob Agents Chemother. 1989;33(12):2144-2145. DOI: 10.1128/AAC.33.12.2144
9. Alnomasy SF, Alotaibi BS, Mujamammi AH, Hassan EA, Ali ME. Microbial aspects and potential markers for differentiation between bacterial and viral meningitis among adult patients. PLoS One. 2021;16(6):e0251518. DOI: 10.1371/journal.pone.0251518
10. Salih M, Kka N. Evaluation of the efficiency of onion (*Allium cepa* L.) sets treated with ascorbic acid and salicylic acid in stimulating growth and seed production. Mesop J Agric. 2022;50(1):52-64. DOI: 10.33899/magrj.2022.132858.1163
11. Neiva A, Vanessa R, Maria N, Flavia C, Maria C, Denise A Plant species used in giardiasis treatment: Ethnopharmacology and in vitroevaluation of anti-Giardia activity. Rev Brasileira de Farmacognosia. 2014;24:215-224. DOI: 10.1016/j.bjp.2014.04.004
12. Alnomasy S, Al-Awsi GRL, Raziani Y, et al. Systematic review on medicinal plants used for the treatment of Giardia infection. Saudi J Biol Sci. 2021;28(9):5391-5402. DOI: 10.1016/j.sjbs.2021.05.069
13. Lim TK. Edible medicinal and non-medicinal plants. Netherlands: Springer; 2012. DOI: 10.1007/978-94-007-4053-2_45
14. Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (*Matricaria chamomilla* L.): an overview. Phcog Rev. 2011;5(9):82. DOI: 10.4103/0973-7847.79103
15. Neves JM, Matos C, Moutinho C, Queiroz G, Gomes LR. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern Portugal). J Ethnopharmacol. 2009;124(2):270-283. DOI: 10.1016/j.jep.2009.04.041
16. Hajaji S, Sifaoui I, López-Arencibia A, Reyes-Batlle M, Jiménez IA, Bazzocchi IL, Valladares B, Akkari H, Lorenzo-Morales J, Piñero JE. Leishmanicidal activity of α-bisabolol from Tunisian chamomile essential oil. J Parasitol Res. 2018;117:2855-2867. DOI: 10.1007/s00436-018-5975-7
17. Satyal P, Shrestha S, Setzer WN. Composition and bioactivities of an (E)-β-farnesene chemotype of chamomile (*Matricaria chamomilla*) essential oil from Nepal. Nat Prod Commun. 2015;10(8):1934578X1501000835. DOI: 10.1177/1934578X1501000835
18. Hajaji S, Sifaoui I, López-Arencibia A, Reyes-Batlle M, Jiménez IA, Bazzocchi IL, Valladares B, Pinero JE, Lorenzo-Morales J, Akkari H. Correlation of radical-scavenging capacity and amoebicidal activity of *M. recutita* L. (Asteraceae). Exp Parasitol. 2017;183:212-217. DOI: 10.1016/j.exppara.2017.09.011
19. Selim Y, Helmy H, Hamed MA, Abdel-Latif N. Chemical and biological studies of thyme based on different solvents extraction. Egypt J Chem. 2023;66(6):247-253. DOI: 10.21608/ejchem.2022.155704.6721
20. Mohamed EA, Alrasheid AA, Elshazly MA, Elwakil ES, Abdel-Hameed ES, Aly IR, Ayoub SM, Osman EE, Shemis MA, Mohamed SS. Phytochemical analysis and anti-cryptosporidial effects of *Zingiber officinale* extracts: in-vivo and in-silico studies. Egypt J Med Microbiol. 2025;34(1):183-194. DOI: 10.21608/ejmm.2024.332077.1366
21. Dyab AK, Yones DA, Ibraheim ZZ, Hassan TM. Anti-giardial therapeutic potential of dichloromethane extracts of *Zingiber officinale* and *Curcuma longa* in vitro and in vivo. J Parasitol Res. 2016;115:2637-2645. DOI: 10.1007/s00436-016-5010-9
22. Aly E, Sabry H, Fahmy Z, Zalat R. Efficacy of combination therapy (metronidazole and/or artemether) in experimental giardiasis and its impact on nonenzymatic oxidative stress biomarkers. PUJ. 2014;7(1):68. [available at]
23. Shukla G, Bhatia R, Sharma A. Prebiotic inulin supplementation modulates the immune response and restores gut morphology in *Giardia duodenalis*-infected malnourished mice. J Parasitol Res. 2016;115:4189-4198. DOI: 10.1007/s00436-016-5196-x
24. Benamrouz S, Guyot K, Gazzola S, Mouray A, Chassat T, Delaire B, Chabé M, Gosset P, Viscogliosi E, Dei-Cas E, Creusy C. *Cryptosporidium parvum* infection in SCID mice infected with only one oocyst: qPCR assessment of parasite replication in tissues and development of digestive cancer. PLoS One. 2012;7(12):e51232. DOI: 10.1371/journal.pone.0051232
25. Montironi ID, Campra NA, Arsaute S. *Minthostachys verticillata* Griseb (Epling.) (Lamiaceae) essential oil orally administered modulates gastrointestinal immunological and oxidative parameters in mice. J Ethnopharmacol. 2022;290:115078. DOI: 10.1016/j.jep.2022.115078
26. Azadbakht M, Chabra A, Saeedi A, Motazedian MH, Monadi T, Akbari F. Anti-parasitic activity of some medicinal plants essential oils on giardia lamblia and *Entamoeba histolytica*, In Vitro. Res J Pharmacol. 2020;7(1):41-47. DOI: 10.22127/rjp.2019.168142.1462
27. Ross MH, Pawlina W. Histology: A text and atlas with correlated cell and molecular biology. UK: Wolters Kluwer; 2016. DOI: 10.26641/1997-9665.2019.4.76-89

تقييم التأثير العلاجي لمستخلص نبات البابونج الألماني ضد الجيارديا التجريبية

ولاء أحمد محمد[1]، رضوى احمد السيد[1]، هاجر فتحي عبد المقصود[2] و منى أحمد فرح[1]

[1]قسم علم الحيوان، كلية البنات للآداب والعلوم والتربية، جامعة عين شمس، [2]معهد تيودور بلهارس للأبحاث، القاهرة، مصر

الخلاصة

جيارديا لامبليا هي كائن حي أولي ممرض شائع يسبب داء الجيارديا بشكل متكرر، مما يؤدي إلى الإسهال الدهني؛ إن إدارة داء الجيارديا صعبة، وهناك حاجة إلى إيجاد منتجات طبيعية بديلة لتجنب الآثار الضارة للعلاج الكيميائي. لذلك، يهدف البحث الحالي إلى تقييم آثار مستخلص نبات ماتريكاريا ريكوتيتا في علاج داء الجيارديا التجريبي وتقييم تأثيره باستخدام معايير تقييم مختلفة. تم تصنيف الفئران إلى المجموعة الأولى غير مصابة، غير معالجة، المجموعة الثانية مصابة، غير معالجة؛ المجموعة الثالثة مصابة ومعالجة بالميترونيدازول؛ المجموعة الرابعة مصابة ومعالجة بمستخلص ن-هكسان من ماتريكاريا ريكوتيتا بتركيز ٥٠٪؛ المجموعة الخامسة مصابة ومعالجة بمستخلص ن-هكسان من م. ريكوتيتا بتركيز ١٠٠٪؛ المجموعة السادسة مصابة ومعالجة بمستخلص ن-بيوتانول من م. ريكوتيتا بتركيز ٥٠٪؛ والمجموعة السابعة تم علاجها باستخدام مستخلص ن-بيوتانول من طفيلية ماتريكاريا ريكوتيتا بتركيز ١٠٠٪. قيمت الفعالية العلاجية من خلال معايير الطفيليات (عدد الأكياس والأغذية)، والنسيجية المرضية، والكيميائية المناعية (كاسباس-٣)، والكيميائية النسيجية (صبغة حامض البريوديك وكاشف شيف)، ومؤشرات إجهاد تأكسدي المالوندالديهايد والسوبر أوكسيد ديسميوتيز. وتحققت أعلى نتيجة علاجية مع المجموعة الثالثة والخامسة، حيث أظهرتا انخفاضًا ملحوظا بنسبة ١٣,٦±٣,٥١ و١٣,٨±١,٩٢ على التوالي، وتعديلًا واضحًا في الإصابات المرضية، والصبغة الكيميائية المناعية، والعلامات المناعية. أثبتت نتائج هذا البحث أن مستخلص نبات ماتريكاريا ريكوتيتا له تأثير علاجي واعد ضد طفيلية الجيارديا لامباليا. يتوفر مستخلص نبات ماتريكاريا ريكوتيتا بسهولة، ويساعد في الوقاية من الآثار الجانبية السلبية لأدوية العلاج الكيميائي.

28. Al-Mahmood SS, Khalil KW, Edreesi AR. Histopathology and Immunohistochemistry of tumors in animals attending veterinary teaching hospital. Iraqi J Vet Sci. 2022;36(2):309-314. DOI: 10.33899/ijvs.2021.130114.1733
29. Al-Ajeli RR, Al-Qadhi AS, Al-Mahmood SS, Alkattan LM. Pathological study of neoplasms surgically excised from animals attended the veterinary teaching hospital. Iraqi J Vet Sci. 2021;35(1):9-14. DOI: 10.33899/ijvs.2019.126188.1260
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358. DOI: 10.1016/0003-2697(79)90738-3
31. Groudan K, Gupta K, Chalhoub J, Singhania R. *Giardia lamblia* diagnosed incidentally by duodenal biopsy. J Investig Med High Impact Case Rep. 2021;9:23247096211001649. DOI: 10.1177/23247096211001649
32. Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, Kasuga F, Rokni MB, Zhou XN, Fèvre EM, Sripa B, Gargouri N. World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. PLoS Med. 2015;12(12):e1001920. DOI: 10.1371/journal.pmed.1001920
33. Campanati L, Monteiro-Leal L. The effects of the antiprotozoal drugs metronidazole and furazolidone on trophozoites of *Giardia lamblia* (P1 strain). Parasitol Res. 2002;88:80-85. DOI: 10.1007/s004360100502
34. De Andrade EC, Leite IC, Rodrigues VO, Cesca MG. Parasitoses intestinais: uma revisão sobre seus aspectos sociais, epidemiológicos, clínicos e terapêuticos. Rev APS. 2010;13(2):231-240. DOI: 10.25110/arqsaude.v27i5.2023-082
35. Escobedo AA, Lalle M, Hrastnik NI, Rodríguez-Morales AJ, Castro-Sánchez E, Cimerman S, Almirall P, Jones J. Combination therapy in the management of giardiasis: what laboratory and clinical studies tell us, so far. Acta Trop. 2016;162:196-205. DOI: 10.1016/j.actatropica.2016.06.026
36. Sanad M, Abo El Maaty D, Abd El-Aal N, Al-Ghandour A, Farag Y, Farag T. Therapeutic efficacy of probiotics against experimental giardiasis: histopathological, histochemical and ultrastructural changes. J Egypt Soc Parasitol. 2020;50(1):21-32. DOI: 10.21608/jesp.2020.88747
37. Shahinaz FA, Nashwa IR, Anhar AM, Hala KED, Faisal MAK, Abeer FB. A study on the effect of *Myrtus communis* and olibanum on *Giardia lamblia* infection in Egypt. Microbes Infect Dis. 2020;1(3):209-220. DOI: 10.21608/mid.2020.47108.1075
38. Thabet H, Abdel Fattah N. In vitro effect of propolis versus wheat germ agglutinin on growth and adherence of an Egyptian *Giardia lamblia* trophozoite strain. New Egypt J Med. 2006;35(1):30-37. DOI: 10.1016/j.phymed.2004.07.008
39. Abd-Elhamid TH, Abdel-Rahman IA, Mahmoud AR, Allemailem KS, Almatroudi A, Fouad SS, Abdella OH, Elshabrawy HA, El-Kady AM. A complementary herbal product for controlling giardiasis. Antibiotics. 2021;10(5):477. DOI: 10.3390/antibiotics10050477
40. Shaaban Y, Hassan Z, Hussein R, Hassan A, Salama D. Evaluation of the role of combined prebiotic and probiotic supplements as prophylactic and therapeutic agents against experimental giardiasis. PUJ. 2021;14(2):193-203. DOI: 10.21608/puj.2021.83828.1124