

Effect of *Punica Granatum* Peels Methanolic Extract on Interactions of *Giardia Intestinalis* with the Intestinal Barrier in Murine Model: Lipid Peroxidation and Oxidative Stress

Hoda A.A. Shaheen¹, Walaa A. Elkholy¹

¹Medical Parasitology department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt

Corresponding author: Hoda Abdel Aziz Shaheen, ORCID number: 0000-0003-3954-8762

E-mail: hudaabdelaziz.medg@azhar.edu.eg, Mobile: 01275160449

ABSTRACT

Background: *Giardia intestinalis* is a flagellate protozoan that infects both humans and animals. Formation of reactive oxygen species (ROS) during several physiological processes in tissues and cells is indicative of the pathogenesis of different parasitic infections involving *Giardia lamblia*.

Objectives: the current study aimed to assess the potential effects of *Punica granatum* on interactions of *G. intestinalis* with the intestinal barrier and its role in ameliorating oxidative stress in a murine model using serum biochemical analysis of both malondialdehyde (MDA) and reduced glutathione (GSH).

Methodology: Five experimental groups were involved: Group I included control healthy, group II included infected untreated, group III included infected-treated with metronidazole, group IV included infected-treated with *P. granatum*, Group V received *P. granatum* for seven days before and also during induction of infection then regular administration of the drug for an additional seven days.

Results: The highest serum levels of MDA (nmol/ml) were expressed in group II 7.03 ± 0.19 followed by group IV that was 5.29 ± 0.62 then group V 4.17 ± 0.28 , while group III had 3.58 ± 0.18 . On the other side, the highest serum levels of GSH (nmol/ml) were expressed in group V 9.93 ± 0.18 , followed by group IV 7.41 ± 0.19 nmol/ml, while in group III was 6.11 ± 0.40 . Group II had 5.00 ± 0.15 nmol/ml. There was significant difference between different groups.

Conclusions: Decreased expression of MDA in prophylactic and treated groups by *P. granatum*, moreover increased expression of GSH in group V prophylactic group explains the protective effect of *P. granatum* against lipid peroxidation and oxidative stress induced by *Giardia* infection.

Keywords: *Giardia intestinalis*; *Punica granatum*; Oxidative stress; Malondialdehyde and Reduced glutathione.

INTRODUCTION

Giardia intestinalis (Syn. *G. lamblia* or *G. duodenalis*), a flagellate protozoan that infects both humans and animals. *G. lamblia* is transmitted through the ingestion of cysts in contaminated water or food or via autoinfection⁽¹⁾. The clinical presentation has a bimodal age distribution, the first peak at ages 0 to 5 years while the second peak occurs at 3rd and 4th decades. *Giardia* has a high occurrence rate in the developing countries⁽²⁾. Reinfections in giardiasis are common this may be attributed to incomplete acquired immunity against *G. lamblia* due to either antigenic variation of the parasite or ineffective immune defenses⁽³⁾.

Regarding the manifestations of giardiasis, may be asymptomatic and vary among patients, varying from acute to chronic manifestations. In case of acute giardiasis patients may complain of foul-smelling explosive watery diarrhea, abdominal pain, steatorrhea, nausea & vomiting. While, in patient with chronic infection the main complain may be in the form of diarrhea, abdominal pain, weight loss, in addition to malabsorption manifestation. Researches on human and animal models have proven that intestinal epithelium not invaded by *Giardia* trophozoites, however the pathophysiology includes partial atrophy of the villi and increased intraepithelial lymphocytes⁽⁴⁾.

Certain factors associated with this immune response may cause the host's body to go into an oxidative

stress state⁽⁵⁾. One term for oxidative stress is an unbalanced state of antioxidants and oxidants. It may be the outcome of multiple processes occurring within the organism's body. Either a reduction in antioxidants or a rise in oxidant production is the cause⁽⁶⁾.

Under physiological conditions, oxidants and antioxidant biomarkers are known to occur. Anyway, even minor changes in the quantities of antioxidants or oxidants may throw them out of balance and cause oxidative damage. When there is insufficient antioxidant system to control oxidative reactions brought on by reactive oxygen species (ROS) and controlled oxidative alterations of proteins, fats, and genomic DNA, the state becomes hazardous. The free radicals are constantly created by regular metabolic activities, however during some parasite illnesses, their production rate rises. The production of ROS in tissues and cells during a variety of regular processes is suggestive of the pathophysiology of certain parasite diseases, such as *Entamoeba histolytica*, *T. gondii*, *G. lamblia*, and *Leishmania sp.* Lipid peroxidation (LPO) is the process that damages cell membranes and releases hepatotoxic marker enzymes as a result, and it has been attributed to the pathophysiology of liver injury caused by derivatives of free radicals. An end product called malonaldehyde (MDA) can often be used as an indicator of lipid peroxidation⁽⁷⁾.

According to a research by Allain *et al.*⁽⁸⁾ *Giardia* sp parasite produces the enzyme arginine diaminase,

which helps to regulate several variables that lead to oxidative stress in the colon including nitric oxide. As a continuation of our previous work, ⁽⁹⁾ the goal of current study was to assess the potential effects of *Punica granatum* on interactions of *Giardia intestinalis* with the intestinal barrier and its role in ameliorating oxidative stress in a murine model using serum biochemical analysis this was achieved through estimation of both MDA and GSH.

MATERIAL AND METHODS

***Punica granatum* peel extract:** *Punica granatum* was extracted in methanol using the peels. It was purchased at a neighborhood market in Giza, Egypt. It was stored in the Department of Medicinal Chemistry at Theodor Bilharz Research Institute (TBRI). After washing the pomegranate fruit, the skins were carefully removed and cut into small fragments, after drying and then ground turning it into a fine powder. The powdered plant sample was extracted using Soxhlet extraction equipment for 24 hours at 85% methanol. After doing the aforementioned steps three times, the extract was vacuum-concentrated utilizing the rotatory Buchi evaporator and stored in desiccators till needed ⁽¹⁰⁾.

Experimental animals:

The study was conducted from April 2022 to May 2023 at TBRI at the Parasitology Department. The 50 female Swiss albino mice used in this study were purchased and housed in TBRI's animal home. Their ages varied from 8 to 12 weeks and weighted from 20 to 25 g. Stool samples were checked three days before to the start of the experiment to make sure that mice were not harboring intestinal parasites. Upon incorporating D'Antoni's iodine into the smears, they underwent an instant swab ⁽¹¹⁾.

Preparing for *Giardia* infection

The original source of the parasite: Cysts of *Giardia* that prepared to infect mice was collected from patients presenting to Al Zahraa University Hospital's Outpatient Clinic in Cairo, Egypt, with complaints of diarrhea. To extract *Giardia* cysts, samples were washed and sieved several times in normal saline (0.9%).

These cysts were manipulated so that, by oral gavage, each mouse received 1×10^3 cysts in 100 μ of normal saline. After then, fresh pellets were gathered every day to make sure they didn't get contaminated. The bedding was changed and each group's pellets were weighed in order to avoid re-infection.

Design of experiments

Groups of the current study: The animals had been divided up into the following groups. Each group included ten mice: Group I included control healthy,

group II include (Positive control) infected-non treated mice, group III included infected and then treated with Metronidazole (MTZ), group IV that included infected and then treated with *P. granatum* peels extract mice and group V, which included mice that received *P. granatum* for seven days before and also during induction of infection then regular administration of the drug for an additional seven days (prophylactic group).

The administrated treatments:

All studied groups except for negative control group were administered with 1×10^3 cysts of *Giardia*. Group III was treated orally with a suspension of Metronidazole 125 mg/ml (Flagyl, SANOFI Pharmaceutical Industries, Alexandria, Egypt) that was given orally for seven days at a dosage of 0.3 mg/mouse/day ⁽¹²⁾. Groups IV and V were given the plant extract orally. Peel extract was produced and supplied in accordance with **Oshiba et al.** ⁽¹³⁾ with a dose of 3 g/kg body weight.

Collection of samples:

A quick decapitation was used to quickly sacrifice every mouse ten days after the treatment had been completed. Mice in every group had blood samples obtained for hematological testing. The serum sample from each mouse was centrifuged for 10 min at 1000-3000 rpm, then the supernatant was stored at -20 °C to be used for estimation of both lipid peroxidation by measuring liver thiobarbituric acid reactive substances (TBARS) as malondialdehyde (MDA) ⁽¹⁴⁾ and reduced glutathione (GSH) ⁽¹⁵⁾.

Malondialdehyde measurement:

This method depends on colorimetric measurement of reactional products of MDA as an end product of the lipid peroxidation and the Thiobarbituric Acid (TBA). The pink change of MDA-TBA mix was quantified easily by spectrophotometer at OD 532 nm ⁽¹⁶⁾.

Method ⁽¹⁸⁾:

Thiobarbituric Acid (TBA) solution was solubilized in 7.5 ml of glacial acetic acid. Sludge was shifted to another tube after which the final volume was modified to 25 ml with Double distilled water (ddH₂O). All were mixed well to be dissolved and sonicated in a water bath. MDA standard dilution was prepared via dilution of 10 μ l of standard MDA in 490 μ l of ddH₂O. The samples were prepared by slowly mixing 20 μ l of serum with 500 μ l of Sulfuric acid (H₂SO₄) in a micro centrifuge tube, then 125 μ l Phosphotungstic acid solution was added, and all were vortexed, then they were incubated for 5 min. at normal room temperature and centrifuged for 3 min. at 13,000 xg. After gathering, the pellet was reconstituted on ice with 100 μ l ddH₂O. Final volume was adjusted to 200 μ l with ddH₂O. Then 600 μ l of TBA solution were added to 200

μl standard and 200 μl test samples. TBA-standard/TBA-sample mixture was incubated at 95°C for one hour. Cooling to the room temperature in bath of an ice for 10 min. From each 800 μl TBA-standard and TBA-sample reaction mixture, 200 μl were pipetted into a 96 well microplate. Plate was measured promptly at OD 532 nm for colorimetric assay. Finally the concentration of MDA in the samples was estimated as: $MDA \text{ Concentration} = (A/ \text{ml})) \times 4 \times D$.

Where: A = Amount of MDA in the sample that was determined using the standard curve (nmol) (Figure 1A). ml = The initial plasma volume that was utilized (ml). 4 = correction for using 200 μ of the 800 μl Reaction Mix. D = The factor of sample dilution.

Reduced glutathione (GSH) serum measurement:

The procedure was established on the recycling system of glutathione by dithiobis nitro benzoic acid (DTNB) and glutathione (GSH) reductase. DTNB and glutathione respond to produce 2-nitro-5-thiobenzoic acid that has a yellowish coloration. Consequently, GSH levels can be detected by calculating the amount of absorption at 412 nm⁽¹⁷⁾. For standard preparation, 50, 40, 30, 20, 10, and 0

μl of the 1 $\mu\text{g}/\mu\text{l}$ GSH standard were added into each micro centrifuge tube and 50 μl of 1% Sulfo salicylic acid (SSA) was then added to reach the total volume of 100 $\mu\text{l}/\text{tube}$.

Method:

For plasma sample preparation, the uppermost layer of plasma was moved to a fresh tube to which 50 μl of 5% SSA was added and mixed well then 10 min centrifugation at 8000 x g at 4°C and supernatant was then switched to an entirely new tube. The standard and the test samples were amalgamated in a 96-well plate to make a reaction mix. 160 μl of the reaction mix was added to each well and incubated for 10 min at room temperature to produce Nicotinamide adenine dinucleotide phosphate (NADPH). Then 20 μl of either GSH standard solutions or sample solution was added, and the plate was incubated for 5-10 min at room temperature. 20 μl of the substrate solution were added and let it sit at room temperature for 5 to 10 minutes. With the use of a micro plate reader, the absorbance was determined at 412 nm. The concentration of GSH was determined by using the standard curve of glutathione (figure 1B). All reagent was supplied from (Abcam, cat#118970, China).

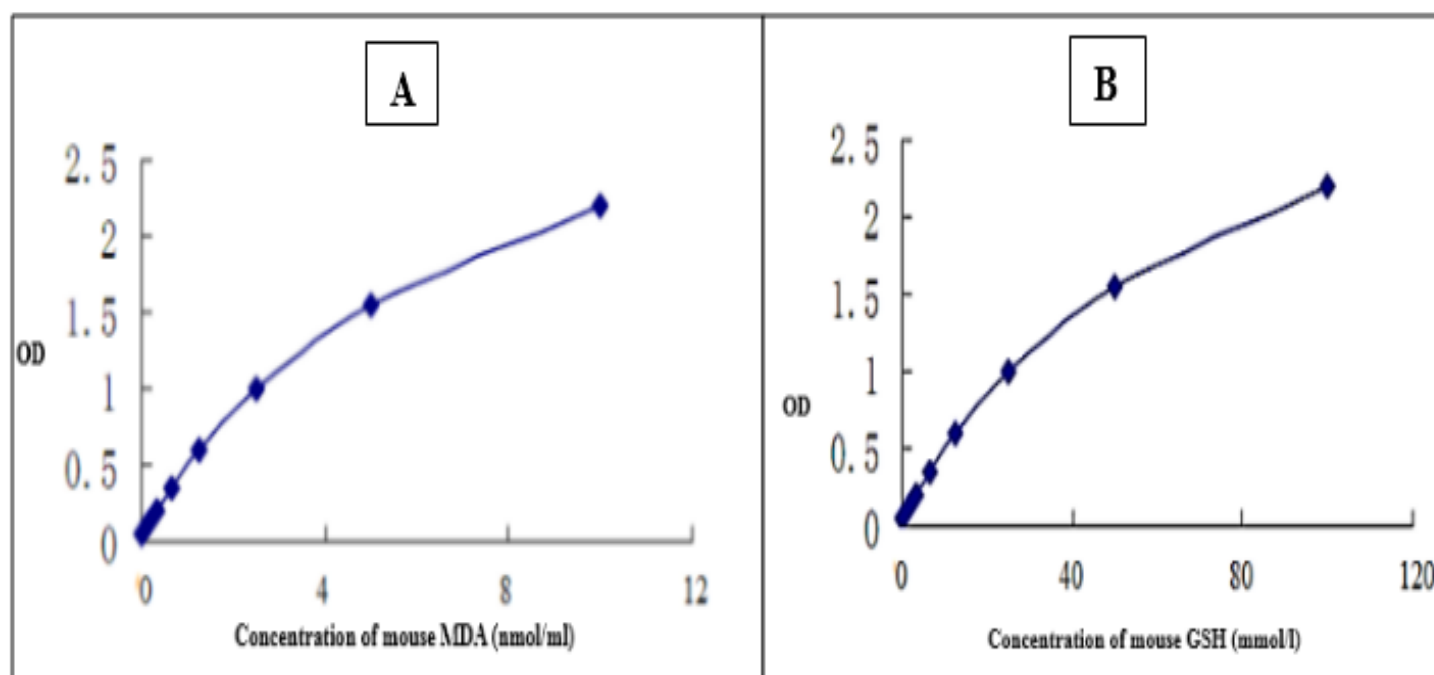


Figure (1): Standard curve of malondialdehyde (plate A) and glutathione (plate B).

Ethical considerations:

This study was approved according to TBRI Research Ethics Committee (TBRI-REC) international valid guidelines and approved by the Ethics Board of Al-Azhar University. Animals were maintained under suitable conditions at animal house in TBRI.

Statistical analysis

The statistical package of social science (SPSS) program for Windows (version 22) was used to code, process, and analyze the data. Based on the independent t-test, the p-value indicates a significant difference when comparing each treated group with the control group.

P-value varies significantly between groups based on the results of the One-way ANOVA test. When comparing groups using a post hoc test (Tukey HSD), P-values ≤ 0.05 were considered significant, while those less than 0.01 were regarded as highly significant.

RESULTS

Biochemical analysis:

In the current study, the highest serum levels of MDA were expressed in group II (Infected-non-treated), 7.03 ± 0.19 nmol/ml followed by group IV (infected and treated with *P. granatum*) was 5.29 ± 0.62 nmol/ml, and then group V (prophylactic group) 4.17 ± 0.28 nmol/ml. While the serum levels of MDA: In group III (Infected and treated with MTZ) was 3.58 ± 0.18 nmol/ml. On the other side, the highest serum level of GSH was expressed in group V (prophylactic group, 9.93 ± 0.18 nmol/ml) followed by group IV (Infected and treated with *P. granatum*) that was 7.41 ± 0.19 nmol/ml. While, in group III infected and treated with MTZ, it was 6.11 ± 0.40 nmol/ml and in group II (Infected-non-treated), GSH was 5.00 ± 0.15 nmol/ml. There was significant differences existed between the various groups (Chart 1 and table 1). Using Independent t test showed a high level of significance between MDA and GSH in each group separately (Chart 2 and table 2).

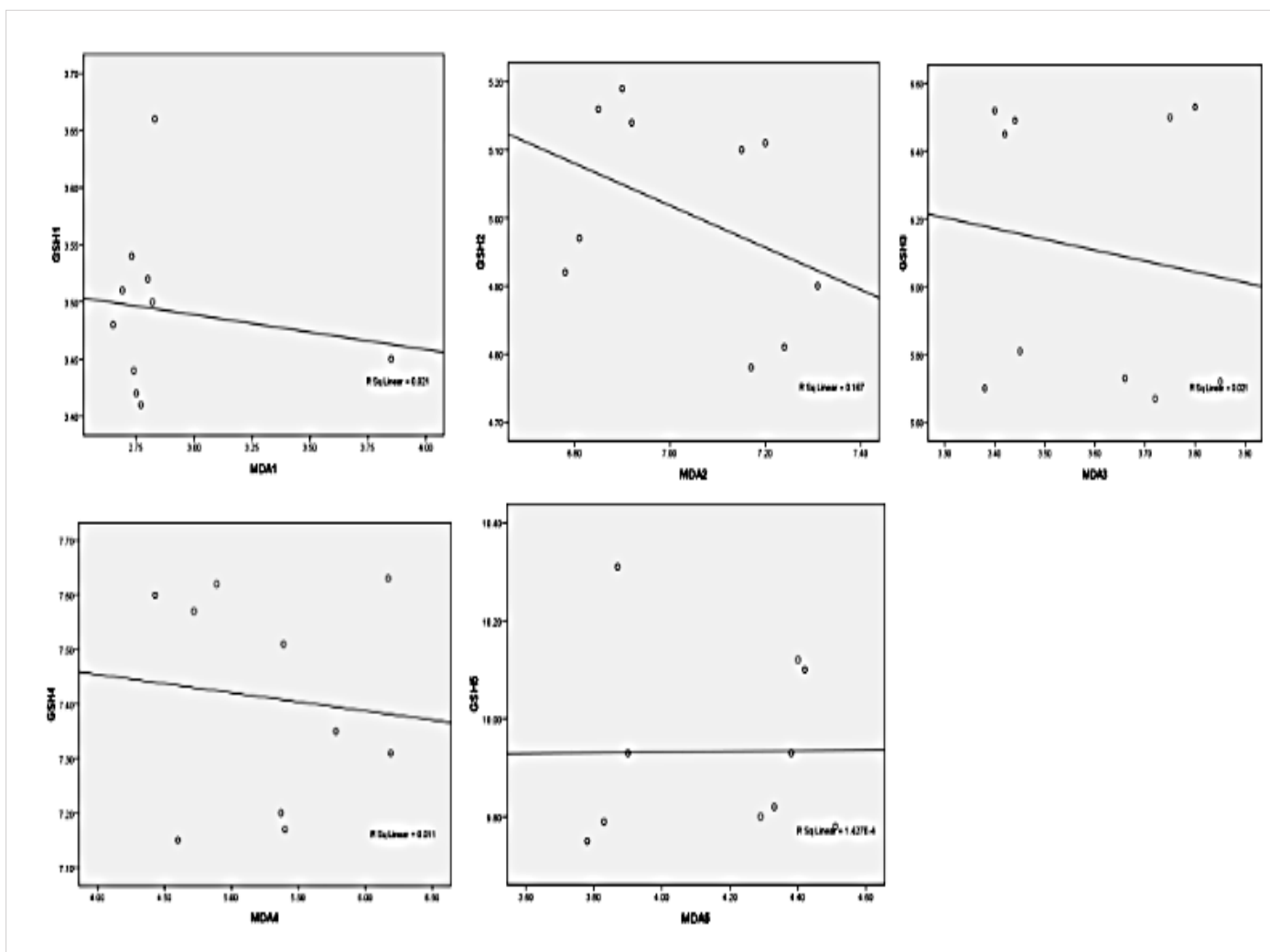


Chart (1): The expression of both MDA and GSH in different studied groups

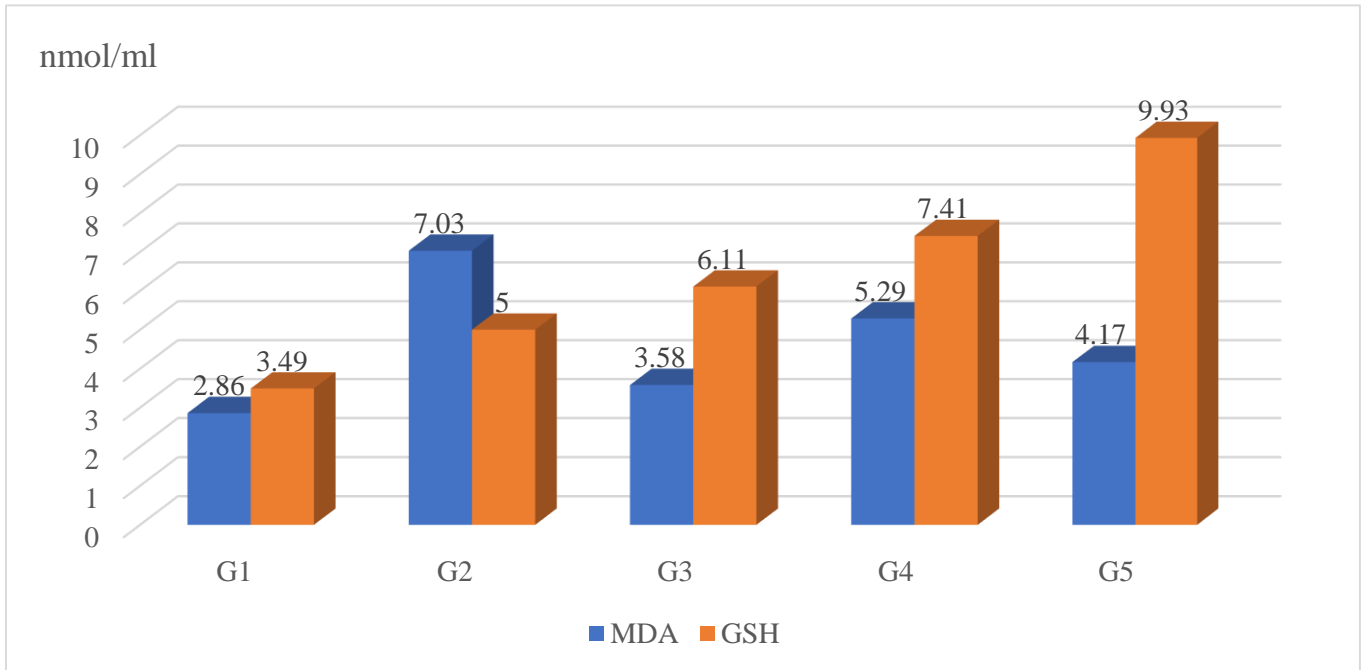


Chart (2): Correlation of MDA with GSH in various study groups.

Table 1: Serum expression of both MDA and GSH in various study groups

	Group I	Group II	Group III	Group IV	Group V
MDA (nmol/ml), Mean± SD	2.86± 0.35 ^a	7.03± 0.19 ^b	3.58± 0.18 ^c	5.29±0.62 ^d	4.17±0.28 ^e
ANOVA test	F= 196.7				
P value	0.000*				
GSH (nmol/ml), Mean± SD	3.49±0.07 ^a	5.00±0.15 ^b	6.11±0.40 ^c	7.41±0.19 ^d	9.93±0.18 ^e
ANOVA test	F=111.6				
P value	0.001*				

*Significant difference (p value < 0.05). NB. a, b, c, d different superscript letter indicates significant difference between different groups.

Table (2): Correlation between MDA and GSH in different studied groups using independent t test

	Group I	Group II	Group III	Group IV	Group V
MDA/nmol/ml: Mean± SD	2.86± 0.35	7.03± 0.19	3.58± 0.18	5.29±0.62	4.17±0.28
GSH/nmol/ml: Mean± SD	3.49±0.07	5.00±0.15	6.11±0.40	7.41±0.19	9.93±0.18
Independent t test	t=5.5	t=25.56	t=-17.8	t=-10.2	t=-53.1
P value	0.001*	0.001*	0.001*	0.001*	0.001*

There was significant difference between both markers in different studied groups using independent t test. *Significant difference (p value < 0.05).

Using correlation coefficient, there was no correlation between MDA and GSH among all treated groups ($p > 0.05$) (Table 3).

Table 3: Correlation of MDA with GSH in different groups using Correlation coefficient.

Correlation of MDA with GSH in:	Correlation coefficient (r)	P value
Group I	-0.146	0.686
Group II	-0.409	0.241
Group III	-0.145	0.689
Group IV	-0.106	0.770
Group V	0.012	0.974

DISCUSSION

G. lamblia, an intestinal protozoan, causes diarrhoea in people and animals worldwide. It adheres to mucosa and, its appearance is subtle^(2,3). Due to its mutagenic and carcinogenic properties of MTZ and nitroimidazole, albendazole and nitazoxanide are alternative treatments for giardiasis⁽¹⁸⁾. Unfortunately, most utilized drugs are contraindicated as they have significant adverse reactions⁽¹⁹⁾. Also, *Giardia* may acquire drug resistance to these drugs⁽²⁰⁾. Consequently, this research was directed to find the effect of alternative herbal regimens against giardiasis. In murine giardiasis, glutathione and malondialdehyde were used as an indicator of the oxidative stress prevalence.

In the present study, the highest serum levels of MDA were expressed in group II (Infected-non-treated, 7.03 ± 0.19 nmol/ml), reflecting the role of *Giardia* infection in lipid peroxidation and oxidative stress, while in group III it was 3.58 ± 0.18 nmol/ml and in group IV, it was 5.29 ± 0.62 nmol/ml. Group V (4.17 ± 0.28 nmol/ml) was the nearest result to the group infected and treated with MTZ. On the other side, the highest serum levels of GSH were expressed in group V (9.93 ± 0.18 nmol/ml). While, in group III it was 6.11 ± 0.40 nmol/ml and in group IV it was 7.41 ± 0.19 nmol/ml. Consequently, this finding supports the antioxidant and protective effect of *Punica granatum*. Supporting our study, serum MDA was substantially greater in *Giardia*-positive individuals than in controls, but serum total antioxidant capacity was significantly lower⁽²¹⁾.

People with giardia had higher MDA and lower glutathione levels in their serum than in healthy. In accordance with our results on natural compound like *Punica granatum*, **El-Derbawy et al.**⁽²²⁾ reported that chitosan nanoparticles loaded with aqueous ginger extract may reduce liver peroxidation and boost liver function in *S. mansoni*-infected mice, reflecting GSH's antioxidant defense mechanism. This agrees with **Ahmed et al.**⁽²³⁾ who reported that in rats with experimentally-induced oxidative stress, feeding of diet with ginger (1% w/w) showed preserving antioxidant enzymes; catalase,

superoxide dismutase, and glutathione peroxidase that reduced lipid peroxidation. Also, **Ahmed et al.**⁽²⁴⁾ reported that ginger-fed rats reduced lindane-induced lipid peroxidation via modulating oxygen free radical scavenging enzymes; glutathione (GSH) and its dependent enzymes, glutathione reductase, peroxidase, and S-transferase.

Other research has connected oxidative stress to parasite species, supporting the present finding as a study by **Abd Al-Wahab et al.**⁽²⁵⁾ who found that control group had considerably lower levels of serum MDA than in parasitic-infected patients, including giardiasis. Also, the study of **Demirci et al.**⁽²⁶⁾ reported that children with chronic giardiasis had lower serum zinc and iron levels, higher MDA levels, and lower superoxide dismutase enzyme effect on red blood cell walls compared to uninfected children. Chronic giardiasis in children promotes protein-energy malnutrition, vitamin A deficiency, iron deficiency anaemia, and poor academic and cognitive performance according to other study⁽²⁷⁾.

Reduction in manganese and zinc in the studies mentioned previously due to *G. lamblia* infection may cause free radicals increase, which induce MDA compound formation and deplete or reduce GSH as these minerals act as cofactors for some antioxidant enzymes⁽²⁸⁾. The results of **Kadhim and Naemy**⁽²¹⁾ indicated that levels of MDA increase with age. This is supporting the theory of the correlation between oxidative stress and aging⁽²⁹⁾. **Dkhil**⁽³⁰⁾ also found a significant antioxidant status increase and host tissue protection against pomegranate extract-induced damage. In an Iraqi investigation by **Jasim and Al-Azzaui**⁽³¹⁾ on Kala-azar patients, malondialdehyde levels in erythrocytes were higher than in healthy controls. **Kadhim et al.**⁽³²⁾, also reported low GSH and high MDA serum level in amoebic dysentery cases versus the control group.

Immune suppression may cause oxidative stress and lipoprotein composition alterations in the host response to giardiasis, which may disrupt metabolic processes and decrease nutritional status⁽³³⁾. Thus, the invasion of parasites stimulates immune cells to produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) to defend against the parasite, but excessive production of these free radicals instead of killing the parasite causes more damage to the body by oxidizing many compounds⁽³⁴⁾.

CONCLUSION

G. lamblia infection, showed considerably higher serum MDA that explains the relationship between *G. lamblia* infection and oxidative stress. While decreased expression of MDA in prophylactic and treated groups by *P. granatum*, and increased expression of GSH in prophylactic group explains the protective effect of *P.*

granatum against lipid peroxidation and oxidative stress induced by *Giardia* infection.

Funding source: No funding

Conflicts of interest: No conflicts of interest.

REFERENCES

1. **Dixon B (2021):** *Giardia duodenalis* in humans and animals - Transmission and disease. Res. Vet. Sci., 135: 283-289. doi: 10.1016/j.rvsc.2020.09.034.
2. **Tuba K, Ebru S, Rabia B et al. (2019):** C-kit Expression and potential role of iNOS, COX-2, and ICAM-3 in Giardiasis. Ann. Gastroenterol Dig. Syst., 2 (1): 1011. ISSN: 2637-4501.
3. **Müller N, Von Allmen N (2005):** Recent insights into the mucosal reactions associated with *Giardia lamblia* infections. Int. J. Parasitol., 35 (13): 1339 - 1347. doi: 10.1016/j.ijpara.2005.07.008.
4. **Cotton J, Beatty J, Buret A (2011):** Host-parasite interactions, and pathophysiology in *Giardia* infections. Int. J. Parasitol., 41: 925-933. DOI: 10.1016/j.ijpara.2011.05.002
5. **Chen L, Deng H, Cui H et al. (2017):** Inflammatory responses and inflammation-associated diseases in organs. Oncotarget., 9 (6): 7204-7218. doi: 10.18632/oncotarget.23208.
6. **Pizzino G, Irrera N, Cucinotta M et al. (2017):** Oxidative Stress: Harms and Benefits for Human Health. <https://doi.org/10.1155/2017/8416763>
7. **Alajmi R, AL-Megrin W, Metwally D et al. (2019):** Anti-*Toxoplasma* activity of silver nanoparticles green synthesized with Phoenix dactylifera and Ziziphus spina-christi extracts which inhibits inflammation through liver regulation of cytokines in Balb/c mice Bioscience Reports, 39: 1-16. doi: 10.1042/BSR20190379.
8. **Allain T, Amat C, Motta J et al. (2017):** Interactions of *Giardia* sp. with the intestinal barrier: Epithelium mucus and microbiota, 5: 1. e1274354, DOI: 10.1080/21688370.2016.1274354.
9. **Elkholy W, Elkholy M, Elsokary A et al. (2022):** Therapeutic and prophylactic effects of Punica granatum peel extract versus metronidazole in murine Giardiasis intestinalis. Journal of King Saud University Science, 34(8):102321 <https://doi.org/10.1016/j.jksus.2022.102321>
10. **Rowayshed G, Salama A, Abul-Fadl M et al. (2013):** Nutritional and Chemical Evaluation for Pomegranate (*Punica granatum* L.) Fruit Peel and Seeds Powders by Products. Middle East. J. Appl. Sci., 3 (4): 169-179.
11. **Subbannayya K, Babu MH, Kumar A et al. (1989):** *Entamoeba histolytica* and other parasitic infections in south Kanara district Karnataka. J. Commun. Dis., 21 (3): 207-213.
12. **Brandelli C, Giordani R, De Carli G et al. (2009):** Indigenous traditional medicine: in vitro anti-giardial activity of plants used in the treatment of diarrhea. Parasitol. Res., 104 (6): 1345-1349. DOI:10.1007/s00436-009-1330-3
13. **Oshiba S, Yaseein R, El-Shennawy A et al. (2018):** *In vivo* effect of pomegranate (*Punica granatum*) extracts versus Nitazoxanide drug on the ileum of experimentally infected mice with *Cryptosporidium parvum* oocysts. J. Am. Sci., 14 (2): 27-39. doi:10.7537/marsjas140218.04.
14. **Ohkawa H, Ohishi N, Yagi K (1979):** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-8. doi: 10.1016/0003-2697(79) 90738-3.
15. **Schmidit E, Schmidit F (1993):** Kline Enzyme Fiebel Schriftenreihe. Diagnostic Boehringer Mannheim. [Enzyme diagnosis of liver disease - ScienceDirect](https://doi.org/10.1016/j.jksus.2022.102321)
16. **Bahrami S, Shahriari A, Tavalla M et al. (2016):** Blood levels of oxidant/antioxidant parameters in rats infected with *Toxoplasma gondii*. <http://dx.doi.org/10.1155/2016/8045969>.
17. **Guilford T (2012):** What Every Doctor Should Know About Glutathione. H.P.C., 17: 13.
18. **El-Gendy A, Hamed M, Ghallab M et al. (2021):** Therapeutic Effect of Chitosan Nanoparticles and Metronidazole in Treatment of Experimentally Giardiasis Infected Hamsters. Iran. J. Parasitol., 16 (1): 32-42. DOI: 10.18502/ijpa.v16i1.5509
19. **Long K, Rosado J, Montoya Y et al. (2007):** Effect of vitamin A and zinc supplementation on gastrointestinal parasitic infections among Mexican children. Pediatrics, 120 (4): e846-e855. doi: 10.1542/peds.2006-2187
20. **Ismail A, Abdel-Magied A, Elhenawy A et al. (2022):** Association Between *Giardia* Genotype and Oxidative Stress Biomarkers Among *Giardia*-Infected Children: A Case-Control Study. Acta Parasitol., 67 (3): 1145-1151. doi: 10.1007/s11686-022-00548-y..
21. **Kadhim R, Al -Naemy N (2020):** Giardiasis and oxidative stress: a case-control study. Eurasia J. Biosci., 14: 5679-5683
22. **El-Derbawy M, El Kholy W, Baiuomy I et al. (2019):** A study of the potential therapeutic effect of ginger (*Zingiber officinale*) loaded nanoparticles on murine schistosomiasis mansoni. Journal of the Egyptian Society of Parasitology, 49 (1): 123-128. 10.21608/JESP.2019.68294
23. **Ahmed R, Seth V, Pasha S et al. (2000):** Influence of dietary ginger (*Zingiber officinale* Rosc) on oxidative stress induced by malathion in rats. Food Chem. Toxicol., 38 (5): 443-50. doi: 10.1016/S0278-6915(00)00019-3.
24. **Ahmed R, Suke S, Seth V et al. (2008):** Protective effects of dietary ginger (*Zingiber officinale* Rosc.) on lindane-induced oxidative stress in rats. Phytotherapy research, 22 (7): 902-906. <https://doi.org/10.1002/ptr.2412>
25. **Abd Al-Wahab S, Mahdi J et al. (2009):** Oxidative stress among patients with some different parasitic infections. Med. J. Basrah. Univer., 27: 66-70. <https://doi.org/10.33762/mjbu.2009.49171>
26. **Demirci M, Delibas N, Altuntas I et al. (2003):** Serum iron, Zinc and Copper levels and lipid peroxidation in children with chronic Giardiasis. J Health popul Nutr., 21 (1): 72-75.
27. **Al-Mekhlafi H, Surin J, Sallam A et al. (2010):** Giardiasis and poor vitamin A status among aboriginal school children in rural Malaysia. Am. J. Trop. Med. Hyg., 83: 523-527. doi: 10.4269/ajtmh.2010.09-0371
28. **Olechnowicz J, Tinkov A, Skalny A et al. (2018):** Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J. Physiol. Sci., 68: 19-31. doi: 10.1007/s12576-017-0571-7.
29. **Schöttker B, Brenner H, Jansen E et al. (2015):** Evidence for the free radical/oxidative stress theory of ageing from the chances consortium: a meta-analysis of individual participant data. BMC Medicine, 13: 300. doi: 10.1186/s12916-015-0537-7.
30. **Dkhil M (2013):** Anti-coccidial, anthelmintic and antioxidant activities of pomegranate (*Punica granatum*) peel extract. Parasitol. Res., 112: 2639-46. doi: 10.1007/s00436-013-3430-3.
31. **Jasim B, Al-Azzaay A (2009):** Oxidative Stress and Antioxidant Status in Human Infected with Kala-azar. AJPS., 6 (1): 116-122. DOI: <https://doi.org/10.32947/ajps.v6i1.365>
32. **Kadhim R, Jawad R, Shaalan N (2019):** Amoebic dysentery patients and two important signs (Malondialdehyde and Glutathione) of oxidative stress. Annals of Tropical Medicine and Public Health, 22 (6):72-78.
33. **Ventura L, Caliari M, Santos J et al. (2014):** Changes in oxidative stress and lipoprotein in malnourished gerbils infected with *Giardia lamblia*. Free Radic. Antioxid., 4: 62-66. DOI: 10.5530/fra.2014.1.10
34. **Agarwal A, Said T (2005):** Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. B.J.U. International, 95: 502-507. doi: 10.1111/j.1464-410X.2005.05328.x.