*Med. J. Cairo Univ., Vol. 93, No. 3, Accepted 1/6/2025*
*DOI: 10.22608/MJCU. 1431-1447, September 2025*
*www.medicaljournalofcairouniversity.net*

# Antioxidative and Anti-Inflammatory Effects of Matcha Green Tea, the Essential Oils of Thyme and Cinnamon on Chronic Toxoplasmosis: An In Vivo Study

NAGWA I.H. IBRAHIM, M.D.*; DOAA S. FADALY, M.D.**; EMAN A. IBRAHIM, M.D.** and ASMAA M. DARWISH, M.D.*

*The Department of Medical Parasitology* and Pharmacology Department**, Faculty of Medicine, Zagazig University*

## Abstract

*Background:* Toxoplasmosis is a prevalent parasitic infection that leads to significant clinical complications, notably toxoplasmic encephalitis in immuno-compromised individuals. Due to the limitations of current anti-toxoplasmic drugs such as limited effectiveness, resistance development, and potential toxicity, it is essential to identify and investigate new treatment options.

*Aim of Study:* The primary objective of this research was to assess the therapeutic potential of matcha green tea, thyme essential oil, and cinnamon essential oil, both individually and in combination with spiramycin, against chronic toxoplasmosis in mice. The study also aimed to examine their antioxidant and anti-inflammatory properties.

*Material and Methods:* Eighty-one mice infected with the ME49 strain of Toxoplasma gondii were divided into nine groups and treated with various agents for 14 days starting the second week post-infection. Parasitological, biochemical, histopathological, and immunohistochemical analyses were conducted to evaluate the effectiveness of these treatments.

*Results:* The results showed a significant decrease in brain cyst count and viability in all treated groups, with the most substantial reduction observed in the thyme essential oil-treated groups, particularly in combined formulas G5 & G8 (68% & 84% reduction in brain cyst count and 64% & 84% reduction in viability, respectively) compared to the spiramycin-treated group (G3) and the untreated infected group (G2). Additionally, the thyme-treated groups exhibited significant reductions in oxidative (MDA) and inflammatory (IFNγ, iNos, and NSE) parameters, along with increased levels of antioxidants (SOD). Furthermore, these groups' histopathological analysis of the liver and brain showed notable improvement. When combined with spiramycin, matcha green tea and cinnamon essential oil demonstrated synergistic effects.

***Key Words:*** *Toxoplasma gondii – Matcha green tea – Thyme – Cinnamaldehyde – INOS – SOD.*

## Introduction

**TOXOPLASMA** gondii is a prevalent parasite that infects various warm-blooded animals, including humans. Around one-third of the global population is estimated to carry a persistent infection, with symptomatic illness mainly appearing in individuals with weakened immune systems [1]. In individuals with healthy immune systems, the infection is effectively controlled by a strong innate and adaptive immune response, resulting in the elimination of most parasites [2].

The non-lethal infection caused by the ME49 strain is characterized by minor elevations in Th1 cytokines, which aid in managing T. gondii infection while causing minimal harm to the host [3]. Particularly, interferon-gamma (IFN-γ) plays a critical role in preventing toxoplasmic encephalitis (TE) in the later stages of infection in mice by hindering the multiplication of tachyzoites. Nevertheless, the activation of microglia by IFN-γ concurrently may result in tissue damage by producing detrimental substances like nitric oxide (NO) [4].

Multiple studies suggest that decreased levels of the effector molecules IFN-γ and NO are crucial for the persistence of latent T. gondii infection [3].

***Correspondence to:*** Dr. Asmaa M. Darwish,
E-Mail: Asmaadarwish10@gmail.com

Nitric oxide (NO) is recognized as a nitrogenous free radical generated by various mammalian cells, playing essential roles in both helminth and mammalian hosts by enhancing the cytotoxic and microbicidal functions of macrophages. The production of this potent molecule is facilitated by inducible nitric oxide synthase (iNOS) [5]. Noteworthy research reveals that during experimental Toxoplasmic Encephalitis (TE), iNOS is prominently expressed in regions of focal gliosis, as well as in microglia/ macrophages and notably in glial cells linked to the surrounding vasculature [6]. Furthermore, findings indicate that Neuron Specific Enolase (NSE) could potentially serve as a biomarker for assessing neuronal damage post-cerebral ischemia [7].

According to Dincel and Atmaca [8], the expression of NSE plays a crucial role in comprehending TE-associated neuropathology. Oxidative stress (OS) results from an imbalance between pro-oxidant and antioxidant components, primarily instigated by reactive nitrogen species (RNS) and highly reactive oxygen species (ROS) [9]. These species cause harm to neurons by adversely affecting both glial and neuronal cells, with neurons being particularly vulnerable to oxidative damage [8]. It is suggested that oxidative stress induced by Toxoplasma contributes to the mechanisms underlying neuropathology and neurodegeneration. Antioxidant systems play a pivotal role in neutralizing free radicals, supported by enzymes like superoxide dismutase (SOD), glutathione reductase (GR), catalase, and glutathione peroxidase [10].

The current primary therapy for toxoplasmosis faces several obstacles, such as toxicity, the emergence of drug resistance, and the inability to eliminate tissue cysts. This underscores the crucial need to identify novel drug targets for the management or treatment of toxoplasmosis [11]. As a result, natural herbal extracts and medicinal plants are increasingly being employed as alternative remedies for various parasitic illnesses, being considered safer and less harmful than synthetic drugs [12]. Spiramycin, a macrolide antibiotic, is efficacious in treating acute toxoplasmosis, exhibiting lower toxicity levels than other medications and achieving high concentrations in the placenta. It has also demonstrated effectiveness in murine models of Toxoplasma infection [13].

Green tea (Camellia sinensis) is widely consumed, particularly in China and Japan [14]. The polyphenolic catechins found in green tea, such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, are its most abundant biologically active constituents. These compounds are linked to a diverse range of therapeutic benefits, including anti-inflammatory and antioxidant properties. Thyme (Thymus vulgaris) has been acknowledged in traditional medicine for its anti-inflammatory, expectorant, antiseptic, anti-parasitic, antibacterial, and antioxidant characteristics primarily due to thymol and carvacrol [15]. Recent research has also highlighted its antifungal, antiviral, anti-leishmanial, and anticancer effects [16]. In vitro studies have demonstrated the promising efficacy of the essential oils obtained from thyme and carvacrol against C. parvum effects [17]. Cinnamomum zeylanicum, commonly known as cinnamon, is a tropical evergreen tree native to Sri Lanka and southern India. The bark and leaves of C. zeylanicum are extensively used as spices and find multiple applications in the perfumery, food, and pharmaceutical industries, as well as in traditional Ayurvedic medicine for addressing respiratory, digestive, and gynecological disorders [18]. Both laboratory and animal studies have confirmed that the essential oil of C. zeylanicum possesses antioxidant, anticancer, antibacterial, antifungal, and antiparasitic properties [19]. Moreover, Mahmoudvand et al. [20] recently demonstrated the in vitro efficacy of C. zeylanicum essential oil as a natural scolicide.

## Material and Methods

*Mice:*

Eighty-one healthy laboratory bred male Swiss albino mice weighing about 20-25gm each, aged 5 weeks were selected from the Animal House Center of the Faculty of Medicine, Zagazig University was conducted in the study.

*Ethical considerations:*

Mice were reared and sacrificed according to the protocol of The Institutional Animal Care and Use Committee of Zagazig University (ZUIACUC) for Animal Use in Research and Teaching. (Approval No, ZU-IACUC/3/F/112/2024). The study was conducted at pharmacology and parasitology departments, Zagazig University during the period from July 2024 through December 2024.

*Parasites:*

Mice were chronically infected with the non-virulent ME49 strain of T. gondii. This specific strain was housed in the Animal House Center of the Faculty of Medicine at Zagazig University in Egypt after being obtained from the Parasitology Department of the Faculty of Medicine. Following the sacrifice of the infected mice, the brains were obtained and mixed with one milliliter of regular saline. Two drops of each 20µl brain homogenate were placed on slides, and the number of tissue cysts was counted using a light microscope fitted with a 40x magnifying lens. The number of tissue cysts per brain was then calculated by multiplying the count by 20 **[21].**

*Drugs:*

• *Spiramycin (drug control):*

Spiramycin, Medical Union Pharmaceuticals, Cairo, Egypt was used. It was obtained as 200mg film-coated tablets. It was given to mice at a dose of 200mg/kg/day **[13].**

• *Matcha green tea (MGT):*

MGT was purchased in powder form from a local market in Zagazig, Egypt. It was suspended in distilled water and then given to mice at a dose of 3g/kg/day **[22].**

• *Essential oils of thyme and Cinnamon:*

The essential oils of cinnamon and thyme were obtained from the Haraz Company for Medicinal Plants in Cairo, Egypt. The recommended dosage of thyme essential oil was 15µg/kg per day **[23].** A dosage of 100mg/kg/day of cinnamon essential oil was administered **[24].** All drugs were given orally once daily for 14 days starting on the second-week post-infection.

*Experimental design:*

- Group (1): Control non-infected.
- Group (2): Control infected, nontreated
- Group (3): Infected and treated with spiramycin.
- Group (4): Infected and treated with Matcha green tea (MGT)
- Group (5): Infected and treated with Thyme essential oil
- Group (6): Infected and treated with cinnamon essential oil
- Group (7): Infected and treated with both spiramycin and MGT.
- Group (8): Infected and treated with both spiramycin and Thyme essential oil.
- Group (9): Infected and treated with both spiramycin and cinnamon essential oil.

*Mice inoculation and scarification:*

Mice were orally infected with 10 cysts per mouse using a 19-gauge gavage needle. The mice were sacrificed eight weeks after infection, and the brains were divided into two distinct parts. For histopathology and immunohistochemistry investigations, one brain segment was submerged in 10% formalin, while the other was used to count tissue cysts. Blood samples were collected from the heart and abdominal veins into tubes without anticoagulant: Centrifuged after 1hr and the serum free of hemolysis was separated and used for evaluation of IFNγ levels. One gram of liver tissue was homogenized with 5ml of normal saline and then centrifuged at 4000 r.p.m for 15 minutes. The supernatant fluid was removed and stored at –80 c for quantitative determination of SOD and MDA levels as follows:

Parasitological study:

*Brain cyst and parasite viability quantification:*

Each sacrificed mouse's brain was crushed in a mortar. After that, 5ml of regular saline were added to create brain emulsion homogenates. Two drops of brain homogenate (20µl each) were added to microscopic slides, and the number of cysts in each mouse brain was counted using an X40 objective under light microscopy. The number of tissue cysts in 1ml (1000 microliters) of brain suspension was then calculated by multiplying the count by 25 **[25].** The mean number of cysts in each group was then determined. Using 0.4% trypan blue staining, the average number of living cysts per 100 cysts was determined for the groups under study and compared to the control groups **[26].**

*Histopathology and immunohistochemistry (iNOS and NSE stains):*

To fix the brain and liver tissues from the studied groups, 10% formaldehyde was used. Some sections were produced for immunostaining using Biotin-Streptavidin (BSA) **[27],** while paraffin sections were cut into slices of 5µm thickness and stained with hematoxylin and eosin for histological examination **[28]**. Tissue sections were incubated with an endogenous peroxidase-blocking reagent containing hydrogen peroxide and sodium azide (DAKO peroxidase blocking reagent, Cat. No.S 2001). One to two drops of the supersensitive primary monoclonal antibody [against, Inducible nitric oxide synthase (iNOS, Cat. No. ABN26, Sigma-Aldrich) and Neuron-specific enolase (NSE, Cat. No. AB9698, Sigma-Aldrich) were then applied to the sections. The slides were incubated horizontally in a humid chamber at room temperature for 60 minutes. After blotting off excess buffer, 1-2 drops of the ready-to-use DAKO Envision + system were applied for 20 minutes at room temperature. The sections were then rinsed with PBS as before and blotted. The chromogen used was DAB (diaminobenzidine), 1-2 drops for 10-20 minutes until a desirable brown color was obtained. The slides were then washed in the buffer. Sections were taken to distilled water, and nuclear counterstaining was done using Mayer's hematoxylin (Hx). Immunoreactive intensity was expressed by average grayscale. Values <160 were considered high, 160–170 medium, and 170–180 low [29].

*Evaluation of IFN γ levels:*

Serum samples were tested using the Rat IFN Gamma ELISA Kit PicoKineTM (catalog number: EK0374) sandwich Enzyme-Linked Immunosorbent Assay (ELISA). Following the manufacturer's instructions, the steps were done. IFN-γ levels were calculated using the standard curve. A range of 31.2 to 2000pg/ml was detected.

*Biochemical analysis:*

The levels of malondialdehyde (MDA) and superoxide dismutase (SOD) in liver homogenate were assessed using commercial kits (CAT. No. MD 25 29 and SD 25 21 respectively), following the manufacturer's instructions. Tissue MDA values were represented in nmol/g, whereas SOD activity measurements were expressed in U/g.

*Statistical analysis:*

The data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 25 (IBM, Armonk, NY, USA). Qualitative data is represented as numbers and percentages while quantitative data is represented by mean ± SD. The ANOVA (f) test is a test of significance used for the comparison of three or more groups with quantitative variables. The *p*-value of <0.05 was considered statistically significant & <0.001 for highly considerable results.

## Results

*Parasitological assessment:*

There was a significant decrease in brain cyst count and viability in all treated groups compared with the control infected (G2). The highest reduction was in G8 which received spiramycin + thyme oil (84% and 84%) respectively followed by G7, G5, G9, G3 and G4. The lowest reduction was in G6 received cinnamon oil (45% and 35%) compared with G2 (Table 1).

*Biochemical assessment:*

The highest reduction in levels of IFNγ and MDA among studied groups was observed in animals receiving thyme essential oil either in a combined formula with spiramycin (G8) or as a monotherapy (G5) (222±2.5 & 262±7.6 for IFNY and 22±0.15 & 28±3.2 for MDA) respectively compared with G2 (infected non-treated) followed by G7, G9, G3 and G4 while the lowest reduction was observed in G6 received cinnamon oil (373±7.6 and 42±2.9) compared with G2. Concerning SOD all studied groups showed significant elevation compared with G2 (infected non-treated), the highest elevation was observed in G8 (426±9.4) followed by G7 & G5 while the lowest elevation was detected in G6 (Table 2).

*Immunohistochemical assessment:*

*Morphometric analysis:*

There was a significant decrease in brain iNOS and NSE levels in all treated groups compared to G2 (infected non-treated). The marked decrease was observed in G8 which received Spiramycin+ Thyme oil (2.1±0.044 and 6.2±0.15) which was better than G5 which received Thyme oil (2.7 and 8.9) followed by G7 received Spiramycin + MGT (3.3±0.076 and 11±0.35). G9 Spiramycin + Cinnamon oil (4.2±0.17and 12±0.35), G4 (5.9±0.080 and 14±0.80), G6 (6.6±0.50 and 15±1.0) and G3 (10±0.049 and 37±0.59) revealed decrease in brain iNOS level and brain NSE level compared with G2 (infected non treated) (23±1.0 and 87±1.0) as represented in Table (3).

Table (1): Brain cysts count and viability among studied groups.

| Groups | G1 Control –ve | G2 Control +ve infected | G3 Spiramycin | G4 MGT | G5 Thyme oil | G6 Cinnamon oil | G7 Spiramycin + MGT | G8 Spiramycin + Thyme oil | G9 Spiramycin + Cinnamon oil | F | p |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - Brain cysts count (parasite burden) (x̄± SD) | 0 | 343±40* | 163±15*,# | 173±15*,# | 110±10*,#,a,b | 187±15*,#,c | 90±10*,#,a,b,d | 55±10*,#,a,b,c,d | 117±15 *,#,b,d,f | 89 | <0.0001*** |
| - Reduction percentage R% | % | 0% | 52% | 50% | 68% | 45% | 74% | 84% | 66% | | |
| - Brain cysts viability (number of living cysts/100 cyst) (x̄± SD) | 0 | 96±1.0* | 57±7.6*,# | 52±2.9*,# | 35±5.0*,#,a,b | 62±2.9*,#,c | 30±5.0*,#,a,b,c,d | 15±5.0*,#,a,b,c,d,e | 45±4.6*,#,d,e,f | 120 | <0.0001*** |
| - Reduction percentage | % | 0% | 41% | 46% | 64% | 35% | 69% | 84% | 53% | | |

X : Mean.
SD: Standard deviation.
F : One way ANOVA test.
NS: Non-significant (p>0.05).
***: Highly significant (p<0.001).
Tukey post-hoc test.
*: Significant versus control negative G1.
#: Significant versus control positive G2.
a: Significant versus G3.
b: Significant versus G4.
c: Significant versus G5.
d: Significant versus G6.
e: Significant versus G7.
f: Significant versus G8.

Table (2): Levels of IFNγ, MDA and SOD among studied groups.

| Groups | G1 Control –ve | G2 Control +ve infected | G3 Spiramycin | G4 MGT | G5 Thyme oil | G6 Cinnamon oil | G7 Spiramycin + MGT | G8 Spiramycin + Thyme oil | G9 Spiramycin + Cinnamon oil | F | p |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - IFNγ level (pg/ml) | 211±1 | 494±3.2* | 328±7.6*,# | 350±5*,#,a | 262±7.6*,#,a,b | 373±7.6*,#,a,b,c | 273±7.6*,#,a,b,d | 222±2.5#,a,b,c,d,e | 295±5*,#,a,b,c,d,e,f | 690 | <0.0001*** |
| - MDA level (nmol/g tissue) | 20±0.70 | 62±0.51* | 34±1.1* | 32±2.8*,# | 28±3.2*,# | 43±3.1*,#,a,b,c | 28±2.6*,#,d | 22±0.15#,a,b,d | 42±2.9*,#,a,b,c,e,f | 100 | <0.0001*** |
| - SOD level (U/g tissue) | 348±2.9 | 133±0.46* | 312±13*,# | 313±8.8*,# | 357±7.5*,#,a,b | 286±7.9#,a,b,c | 389±7.6*,#,a,b,c,d | 426±9.4*,#,a,b,c,d,e | 347±7.6#,a,b,d,e,f | 330 | <0.0001*** |

X : Mean.
SD: Standard deviation.
F : One way ANOVA test.
NS: Non-significant (p>0.05).
***: Highly significant (p<0.001).
Tukey post-hoc test.
*: Significant versus control negative G1.
#: Significant versus control positive G2.
a: Significant versus G3.
b: Significant versus G4.
c: Significant versus G5.
d: Significant versus G6.
e: Significant versus G7.
f: Significant versus G8.

Table (3): Levels of iNOS and NSE levels in brain tissues among studied groups.

| Groups | G1 Control –ve | G2 Control +ve infected | G3 Spiramycin | G4 MGT | G5 Thyme oil | G6 Cinnamon oil | G7 Spiramycin + MGT | G8 Spiramycin + Thyme oil | G9 Spiramycin + Cinnamon oil | F | p |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - Brain iNOS level (X ± SD) | 2.5±0.13 | 23±1.0* | 10±0.049*,# | 5.9±0.080*,#,a | 2.7±0.040#,a.b | 6.6±0.50*,#,a,c | 3.3±0.076#,a.b,d | 2.1±0.044#,a.b,d,e | 4.2±0.17*,#,a.b,c,d,f | 910 | <0.0001*** |
| - Brain NSE level (X ± SD) | 4.0±0.025 | 87±1.0* | 37±0.59*,# | 14±0.80*,#,a | 8.9±0.086*,#,a,b | 15±1.0*,#,a,c | 11±0.35*,#,a,b,c,d | 6.2±0.15*,#,a,b,c,d,e | 12±0.35*,#,a,b,c,d,f | 5600 | <0.0001*** |

X : Mean.
SD: Standard deviation.
F : One way ANOVA test.
NS: Non-significant (p>0.05).
***: Highly significant (p<0.001).
Tukey post-hoc test.
*: Significant versus control negative G1.
#: Significant versus control positive G2.
a: Significant versus G3.
b: Significant versus G4.
c: Significant versus G5.
d: Significant versus G6.
e: Significant versus G7.
f: Significant versus G8.

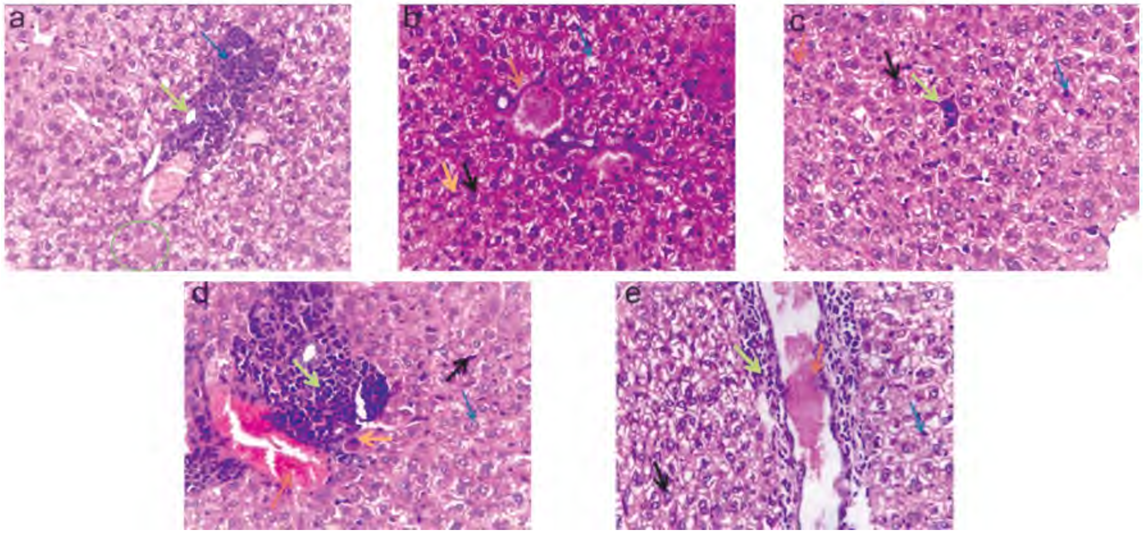


Fig. (1): Histopathological examination of the hepatic sections of studied groups (A): G2 (control positive) showing portal biliary reaction, lymphocytic cholangitis (green arrow), inflammatory infiltrate in the form of lymphocytes and plasma cells (blue arrow), and vague parasitic cysts (green circle) (B): G5 (infected and treated with thyme essential oil) showing normal hepatocytes (black arrow), vascular congestion (red arrow), edema (blue arrow) and Von Kupffer cells were hypertrophied (orange arrow) (C): G7 (infected and treated with combined spiramycin+MGT) showing normal hepatocytes (black arrow), focal mild degenerated hepatocytes with apoptotic nuclei (blue arrow), hypertrophy of von Kupfer cells (red arrow) and reticuloendotheliosis (green arrow). (D): G8 (infected and treated with combined spiramycin+thyme essential oil) showing portal congestion (red arrow), hepato-portal biliary proliferation, and round cell aggregations (green arrow), normal hepatocytes (blue arrow), hypertrophied von Kupfer cells (black arrow) and megakaryocytes (orange arrow) (E): G9 (infected and treated with combined spiramycin + cinnamon essential oil) showing normal hepatocytes (black arrow), mild portal congestion (red arrow) with portal interstitial lymphoplasmacytic aggregations (green arrow), and focal mild degenerated hepatocytes (blue arrow) H&E X 400.

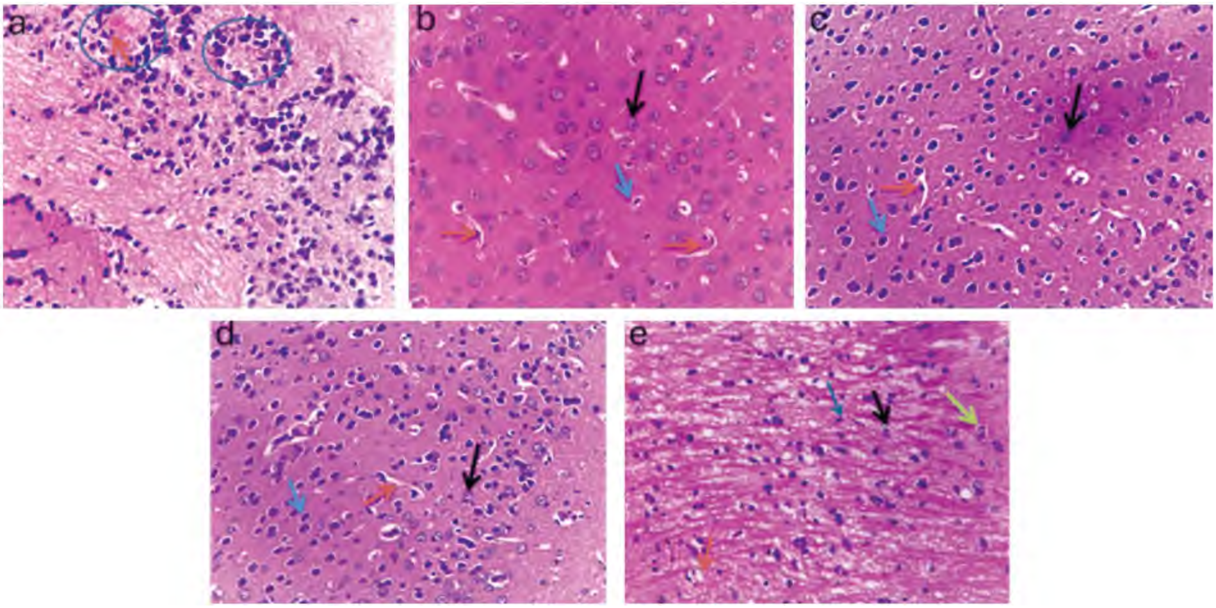


Fig. (2): Histopathological examination of the brain sections of studied groups (A): G2 (control positive) showing encephalitis with varying degrees of severity, the inflammatory cells consisted of lymphocytes and occasional plasma cells, characteristic microglial/lymphocytic nodules (blue circles) with toxoplasmic-like material (maybe recently ruptured cysts) (red arrow). (B): G5 (infected and treated with thyme essential oil) showing normal preserved vascular structures (red arrows), cerebral cortical cells (black arrow), and neuropils and glial cells (blue arrow) (C): G7 (infected and treated with combined spiramycin+MGT) showing normal preserved vascular structures (red arrow), cerebral cortical cells (black arrow), neuropils and glial cells (blue arrow 0) (D): G8 (infected and treated with combined spiramycin + thyme essential oil) showing normal structural configurations including vascular structures (red arrow), cerebral cortical cells (black arrow), neuropils, and glial cells (blue arrow) (E): G9 (infected and treated with combined spiramycin + cinnamon essential oil) showing normal preserved vascular structure (red arrow), cerebral cortical cells (blue arrow), neuropils and glial cells (blue arrow) and few cortical neuronal cells degeneration (green arrow) H&E X 400.

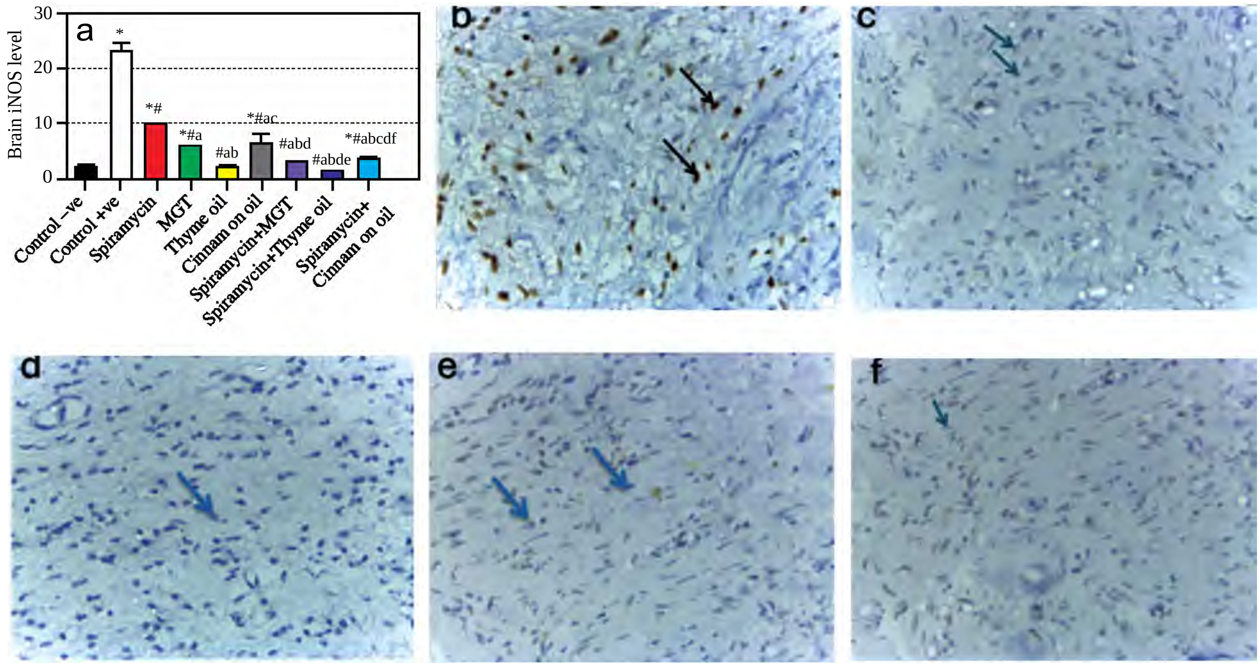


Fig. (3): The iNOS level score (A) of brain sections among the studied groups. Results are representative as means ± SD. Immunohistochemical examination of brain sections (B): G2 (control positive) showing strong positive iNos expression in degenerated neurons (black arrows). (C): G7 (infected and treated with spiramycin + MGT) showing negative expression of iNOS-specific antibodies (blue arrow). (D): G5 (infected and treated with thyme essential oil) showing negative expression of the used markers in different tissue (blue arrow) stained against iNOS-specific antibodies (E): G8 (infected and treated with spiramycin + thyme essential oil) showing negative expression of iNOS in different tissue (blue arrows) (F): G9 (infected and treated with spiramycin + cinnamon essential oil) showing negative expression of iNOS-specific antibodies (blue arrow) X400.

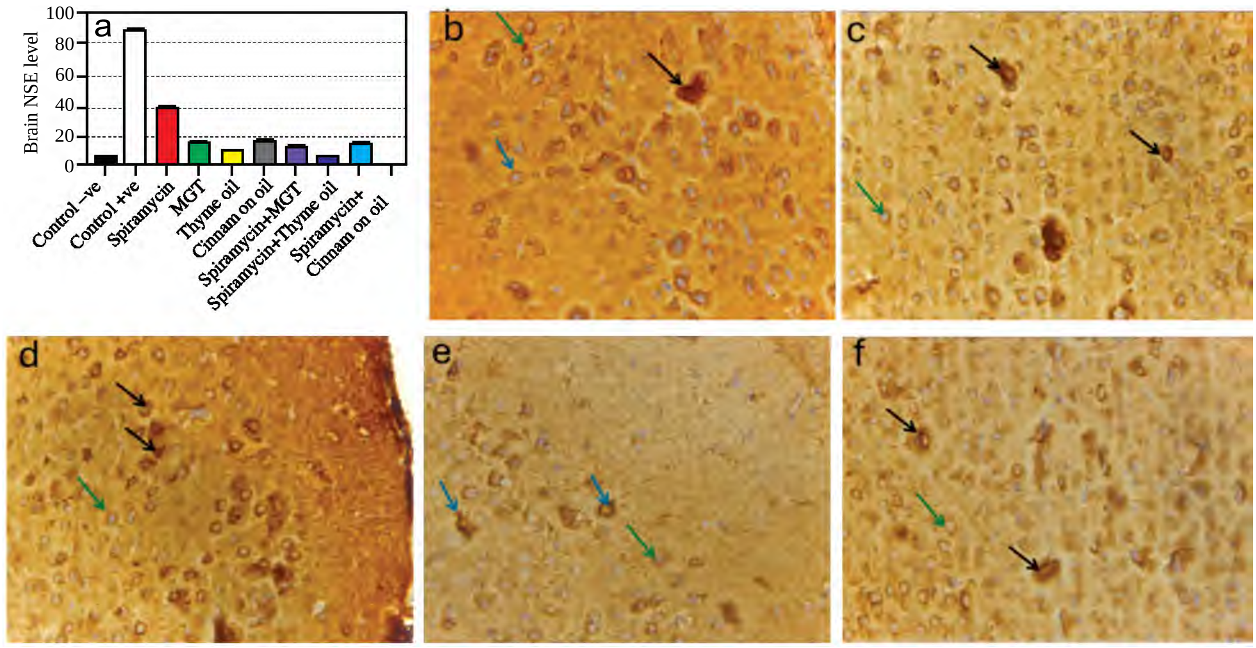


Fig. (4): The NSE level score (A) of brain sections among the studied groups. Results are representative as means ± SD. Immunohistochemical examination of brain sections (B): G2 (control positive) showing a strong positive NSE immunostaining (brownish cytoplasmic stainability) in some degenerated neuronal cells (black arrow) and in reactive glial cells (green arrow), and a weak non-specific reaction was seen in normal neurons (blue arrow) (C): G5 (infected and treated with thyme essential oil) showing a positive reaction to NSE in some of the degenerated cerebral cortical cells (black arrows), other cerebral cells appear unspecifically stained (green arrow) (D): G7 (infected and treated with spiramycin + MGT) showing a positive reaction to NSE in some of the degenerated cerebral cortical cells (black arrows), other cerebral cells were unspecifically stained (green arrow) (E): G8 (infected and treated with spiramycin + thyme essential oil) showing Positive reaction to NSE in some of the degenerated cerebral cortical cells (blue arrows), other cerebral cells were unspecifically stained (green arrow) (F): G9 (infected and treated with spiramycin + cinnamon essential oil) showing Positive reaction to NSE in some degenerated cerebral cortical cells (black arrows), other cerebral cells were unspecifically stained (green arrow) X 400.

## Discussion

Toxoplasma gondii presents a distinctive pathophysiology that poses challenges for pharmacological treatment. The parasite is capable of breaching the blood-brain barrier to establish a persistent infection [30], hindering the achievement of effective drug concentrations and complicating the management of chronic illness [31]. Regrettably, the current medications used for treating and preventing toxoplasmosis, such as spiramycin, pyrimethamine, sulfadiazine, and atovaquone, are associated with significant side effects like increased teratogenicity, bone marrow suppression, and hematologic toxicity [32]. There is a growing interest in examining the efficacy of herbal extracts in treating various parasitic diseases [33]. Natural compounds and traditional herbal remedies are readily accessible and tend to have fewer adverse effects compared to modern anti-toxoplasma therapies [12].

The present research aims to assess the antiparasitic and antioxidant properties of essential oils derived from Thyme (TEO), cinnamon, and matcha green tea in the context of chronic experimental T. gondii infection in mice. The oils were administered both independently and in combination with spiramycin, and the evaluation involved parasitological, biochemical, histopathological, and immunohistochemical analyses.

In terms of parasitological evaluation, our findings indicated a significant reduction in brain cyst burden and viability across all treatment groups when used either as monotherapy or in conjunction with spiramycin. Notably, the groups treated with thyme essential oil (TEO), whether alone or combined with spiramycin (G5 and G8), exhibited the most substantial decrease in brain cyst burden, with reduction percentages of 68% and 84%, respectively, as well as in brain cyst viability, with reduction percentages of 69% and 84%, respectively. The outcomes observed in the TEO-treated groups (G8 and G5) can be attributed to the influence of its active components, such as thymol and carvacrol. While the precise mechanism underlying thyme's antimicrobial actions remains unclear, research suggests that it can enhance immune responses and exhibit potent antioxidant properties [34]. Thyme has been shown to impact cell membrane permeability and modulate membrane organization and surface electrostatics, leading to the release of membrane-associated components and the subsequent elimination of parasites [35]. Essential oils can interfere with membrane-bound enzymes and those involved in energy and protein synthesis, ultimately resulting in cell death [36]. The most recent proposed mechanism of action posits that essential oils and their monoterpenes generate free radicals that may disrupt essential enzymes of the parasite, such as lactate dehydrogenase related to energy metabolism [37].

These findings align with the work of Eraky [38], who demonstrated the prophylactic and therapeutic effects of alcohol extract of thyme against chronic toxoplasmosis by reducing brain cyst numbers compared to the control group in mice infected with Toxoplasma. Moreover, Farag et al. [39] highlighted the therapeutic potential of thyme extract in treating toxoplasmosis, reporting a significant reduction in brain cysts in rats with toxoplasmosis following oral administration for 10 days. Prior studies have documented the anti-parasitic properties of thyme against various parasites. For instance, Santoro et al. [40] illustrated the anti-trypanosomal effects of thyme against T. cruzi, inducing structural abnormalities in the parasite. Additionally, thyme aqueous extract has shown trypanocidal activity against T. evansiby reducing parasite numbers and infectivity [23]. Thyme volatile oil presents promising potential as a natural remedy for bacterial infections and visceral leishmaniasis [41].

Our observations indicated that both MGT and cinnamon essential oil when applied separately demonstrated an anti-parasitic effect like the conventional drug spiramycin. However, using them in conjunction with spiramycin resulted in a synergistic effect. In the groups treated with MGT, there was a reduction in the burden and viability of Toxoplasma brain cysts. This phenomenon is likely due to the antioxidant and anti-inflammatory properties of its biological elements, particularly catechins, which are abundant in epigallocatechin gallate (EGCG) and methylxanthines. The primary impact of green tea catechins on various parasitic infections is the decrease in both parasite growth and population. Additional findings included the fragmentation of the parasite's DNA and diminished synthesis of their fatty acids [42]. EGCG might also inhibit the enzymes necessary for Toxoplasma encystation, namely lactate dehydrogenase and enolases. The work of Fakae et al. [43] demonstrated that the solvent extract of green tea, as well as its chemical compounds, has anti-parasitic properties against Acanthamoeba castellanii, significantly hindering both the replication of trophozoites and encystation by inhibiting the serine proteinases and metalloproteinases involved in the encystation process. Furthermore, Kolodziej et al. [44] observed that EGCG reduced the viability of intracellular Leishmania with minimal induction of nitric oxide synthesis by infected macrophages. Catechins, especially EGCG, display a wide array of antiprotozoal effects by targeting crucial enzymes involved in redox and energy metabolism across parasites like Giardia, Entamoeba, Plasmodium, and Trypanosoma, positioning them as noteworthy candidates for new therapeutic alternatives addressing issues such as side effects and drug resistance against parasitic infections [45]. It was suggested that methanol extracts from green tea have promising prophylactic and therapeutic benefits against C. parvum [46].

The biological components of cinnamon oil, especially cinnamaldehyde, the principal active compound in cinnamon known for its diverse medicinal and pharmacological uses including anticancer, cardioprotective, anti-inflammatory, antibacterial, antifungal, and anti-parasitic properties may contribute to the decline in the number and viability of Toxoplasma brain cysts in groups treated with cinnamon oil [47]. Cinnamaldehyde's antimicrobial effectiveness arises from several mechanisms like disrupting the metabolism of vital compounds such as proteins, lipids, and carbohydrates, disturbing the microbial cell membrane, and affecting energy production [48]. Hence, it can be inferred that the compounds found in cinnamon play a significant role in its anti-Toxoplasma effects. This finding aligns withAlanazi and Almohammed [49], who noted that cinnamon exhibits promoting effects on Toxoplasma infection in both in vitro and in vivo studies. In vitro experiments revealed significantly higher mortality rates of tachyzoites at varying concentrations of cinnamon, while in vivo studies indicated a noticeable reduction in the mean number and size of Toxoplasma cysts in infected mice. Similarly, Fabbri et al. [50] found significant impacts of cinnamon essential oil and cinnamaldehyde on Echinococcus granulosus, leading to reduced parasite survival (protoscolicidal effect), with higher amounts of the essential oil causing ultrastructural alterations. Previous studies have examined the anti-parasitic potential of cinnamon, with Salama et al. [51] reporting reduced viability of Trichinella spiralis larvae and adult worms when treated with cinnamon, while Trabelsi et al. [52] explored the anti-parasitic effects of cinnamon extract against Anisakis larvae type 1 in both in vivo and in vitro settings.

Maintaining the equilibrium of oxidation-reduction processes necessitates the controlled generation of reactive oxygen species (ROS) at optimal levels; however, excessive ROS production can lead to deteriorative reactions such as lipid peroxidation, DNA impairment, and enzyme deactivation [53]. ROS plays a pivotal role in regulating immune responses and exerts diverse modulatory effects on inflammation [54]. Essential oils (EOs) and their components can modulate several signaling pathways that are either suppressed or excessively activated during acute or chronic inflammatory responses [55]. The creation of EOs involves various chemical compounds containing conjugated carbon double bonds and hydroxyl groups capable of hydrogen donation, thereby diminishing oxidative stress and thwarting free radicals [56].

Regarding the assessment of oxidant-antioxidant status, all treated mice with infections exhibited a notable rise in superoxide dismutase (SOD) levels and a decrease in malondialdehyde (MDA) levels compared to the infected control group. Notably, a significant enhancement in SOD and MDA levels was observed in groups treated with thyme essential oil, either as a standalone treatment or in combination with spiramycin (G5 and G8). This antioxidant potential of thyme was attributed to its high content of total phenolics and flavonoids like carvacrol and thymol [57]. Studies by Lemos et al. [58] demonstrated the antioxidant activity of thymol and carvacrol through methods such as DPPH, highlighting their strong radical scavenging capacity. Flavonoids exhibit antioxidant properties through various mechanisms that include direct scavenging of ROS, upregulation of antioxidant enzymes, and inhibition of enzymes involved in free radical generation [59]. This finding was supported by Taha et al. [60], who observed a significant increase in SOD levels and a decrease in MDA levels in immunosuppressed mice infected with cryptosporidium and treated with thyme essential oil.

Green tea contains polyphenols (catechins and gallic acid), carotenoids, tocopherols, vitamin C, and essential minerals like Cr, Mn, Se, and Zn. These elements indirectly function as antioxidants by delaying redox-sensitive transcription factors and pro-oxidant enzymes (e.g., lipoxygenases, cyclooxygenases, xanthine oxidase, and nitric oxide synthase) and stimulating antioxidant enzymes such as glutathione transferases (GT) and superoxide dismutases (SOD), which counteract lipid peroxidation, scavenge free radicals, and reduce oxidation by binding to metal ions [61]. Theanine, a key component in matcha, can be converted into glutamine [62]. Glutamate, as per Bornstein et al. [63] can mitigate mitochondrial damage, rejuvenate tricarboxylic acid cycle intermediates, and enhance ATP synthesis through oxidative phosphorylation to mitigate oxidative harm. This finding aligns with Ramez et al. [64] who observed increased levels of antioxidant parameters such as SOD, catalase, total antioxidant capacity, and glutathione peroxidase along with a significant reduction in MDA levels in Schistosoma mansoni-infected mice treated with MGT.

The antioxidant effects of essential oils are likely due to a combination of their constituents, with key compounds playing a significant role in their beneficial biological effects [65]. The primary responsibility for the antioxidant activity of cinnamon essential oil lies with its phenolic and other bioactive compounds, such as (E)-cinnamaldehyde [66], α-pinene [67], eugenol [68], β-caryophyllene [69], and eucalyptol [70]. Ashfaq et al. [71] demonstrated the antioxidant activity of cinnamon essential oil through β-carotene bleaching experiments, showing strong radical scavenging properties and the ability to inhibit lipid oxidation. A study by Sahib [72] revealed that 1000mg of cinnamon over 12 weeks increased serum SOD levels and decreased MDA levels in Type 2 diabetes patients. Hussain et al. [73] found that administering cinnamon at doses of 100 and 200mg/kg for 14 days to acetaminophen-induced rats resulted in reduced MDA levels, increased GSH levels, and enhanced activities of antioxidant enzymes (CAT, SOD, GR, and GPx). The overproduction of chemicals that stimulate reactive oxygen species (ROS) can harm cell structures and disrupt the body's functions in the long term. Inflammatory responses, in addition to their signaling functions that promote inflammation, play a role in various diseases. Anti-inflammatory and antioxidant medications primarily function by scavenging ROS to impede signaling in the inflammatory process [74].

In this investigation, all treated infected mice exhibited a significant decrease in serum IFNγ levels and iNos & NSE (in the brain) in immunohistochemical assessments compared to the infected control group. Notably, a substantial improvement in these levels was observed in groups treated with thyme essential oil either as monotherapy or in combination with spiramycin (G5 and G8). The anti-inflammatory properties of thyme essential oil can be attributed to its active biological constituents, thymol, and carvacrol. Kwon et al. [75] demonstrated that thymol reduced the expression levels of IL-1β, IL-6, TNF-α, IL-8, and MCP-1. Carvacrol exhibited anti-inflammatory actions by suppressing IL-6, IL-8, PGE2, and COX-2 [76].

Gholijani et al. [77] confirmed that both thymol and carvacrol exert their anti-inflammatory effects by reducing IL-1b and TNFα levels at the protein and mRNA levels, with thymol also significantly reducing IL-1b expression. Mahmoodi et al. **[78]** demonstrated that thyme extract at doses of 50-100 mg/kg reduced IFNγ and IL-6 in autoimmune encephalomyelitis. Liang et al. **[79]** showed that thymol inhibited the production of TNF-α and IL-6, and suppressed the expressions of iNos and COX-2 in epithelial cells. Thymol therapy reduced TNF-α, IL-6, IL-17, and IFNγ in rats with rheumatoid arthritis **[80]**.

The physiologically active components of MGT that suppress the gene and protein production of inflammatory cytokines are responsible for its anti-inflammatory properties **[81]**. EGCG, to regulate the inflammatory state, scavenges ROS(82). Matcha, due to higher theanine and rutin levels compared to other green teas, inhibited the growth of liver granulomas by increasing IL10 levels and decreasing TNF-α, IFN-γ, and IL-13 levels **[83]**. Matcha-silver nanoparticles reduced inflammation by enhancing the expression of antioxidant genes (SOD, catalase, GPx) through the reduction of NLRP3 and IL-1β expression and the promotion of SIRT1 levels, which regulate the endogenous antioxidant and anti-inflammatory defense systems **[84]**. Furthermore, SIRT1 inhibits the TLR-4/NF-κB/STAT pathway, reducing the production of inflammatory factors **[85]**.

Cinnamon essential oil's chemical components, particularly cinnamaldehyde and its derivatives contribute to its anti-inflammatory properties by preventing NO production, thereby reducing inflammation. Consequently, cinnamon serves as a novel type of NO inhibitor. Cinnamon hydroalcoholic extract treatment significantly reduced joint swelling and lowered IL-1β and TNF-α levels in rats with CFA-induced arthritis **[86,87]**. Liao et al. **[88]** investigated the anti-inflammatory properties of cinnamon components (cinnamaldehyde, cinnamyl alcohol, cinnamic acid, and coumarin) using mouse macrophage RAW264.7 and a carrageenan-induced mouse paw edema model, revealing that cinnamaldehyde notably inhibited the production of NO, TNF-α, and PGE2 in mouse macrophage RAW264.7.

The study's histopathological findings showed improvements in the liver and brain tissue appearance, manifested by a reduction in inflammatory cells and an enhancement in tissue structure towards normal in various treated groups compared to the severe inflammatory reaction in the untreated infected group (G2). The most significant improvements were observed in the thyme essential oil-treated groups, likely attributed to the antioxidant and anti-inflammatory properties of thymol, carvacrol, and other phenolic and flavonoid components. This is supported byOmidipour et al. **[89]**, who highlighted thyme's ability, due to its high antioxidant content, to improve liver complications and reduce liver destruction. Ahmed et al. **[90]** demonstrated that thyme oil and thymol effectively mitigated experimental hepatotoxicity caused by doxorubicin through their anti-inflammatory, antioxidant, and antiapoptotic characteristics.

In groups treated with matcha, the improvement in the levels of inflammation and the histological appearance of the liver and brain can be attributed to the anti-inflammatory and antioxidant effects. This finding is consistent with the research by Hamed et al. **[91]**, who illustrated the capacity of matcha-silver nanoparticles to repair damage caused by irradiation and restore the cellular structure of the spleen. The presence of potent antioxidant and anti-inflammatory properties in the polyphenolic components of EGCG contributes to reducing cellular damage induced by toxins. Al-Awaida et al. **[92]** observed that mice exposed to smoke and treated with EGCG therapy exhibited preserved tissue morphology.

The amelioration of the histological appearance of the brain and liver in groups treated with cinnamon is due to the antioxidant and anti-inflammatory properties of cinnamon's biologically active components, particularly cinnamaldehyde. This finding aligns with the study by Aioub et al. **[93]**, which demonstrated the therapeutic effect of cinnamon on the adverse histopathological changes in the liver and kidney of rats exposed to acetamiprid, where cinnamon restored the normal histological appearance of these tissues. Similarly, Elkomy et al. **[94]** demonstrated that cinnamon extract improved histological changes in rats administered paracetamol.

*Conclusion:*

This research study evaluates the anti-toxoplasmic efficacy of MGT and essential oils of thyme and cinnamon against chronic toxoplasmosis. Our results highlight the potential therapeutic potential of these natural compounds, especially thyme essential oil, as adjunctive therapies for chronic toxoplasmosis. The observed synergistic interactions with spiramycin alongside the antioxidant and anti-inflammatory properties indicate that these compounds may provide a novel and more efficacious strategy for disease management. Our future studies will be directed to test the active ingredients of these compounds and load them on different forms of nanoparticles.

*Acknowledgments:* We are grateful to the Parasitology Department at the Faculty of Medicine, Zagazig University, Egypt for support in providing us with the non-virulent ME49 strain of T. gondii that induced chronic infection in mice and to the Animal House Center of the Faculty of Medicine at Zagazig University, Egypt to sustain the cycle.

*Author contribution:* NIHI, DSF, EAIMA, and AMD contributed to the study design and the work plan; NIHI, DSF, EAIMA, and AMD prepared the materials, collected the data, and conducted the analysis; AMD wrote the first draft of the manuscript, all named authors read and approved the final manuscript; and all authors approved the order of authors listed.

*Financial support:* No financial support was obtained.

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

## References

1- ALMERIA S., MURATA F.H., CERQUEIRA-CÉZAR C.K., KWOK O.C., SHIPLEY A. and DUBEY J.P.: Epidemiological and public health significance of Toxoplasma gondii infection in wild rabbits and hares: 2010–2020. Microorganisms, 9(3):59, 2021.

2- SASAI M., PRADIPTA A. and YAMAMOTO M.: Host immune responses to Toxoplasma gondii. International Immunology, 30 (3): 113-9, 2018.

3- MORDUE D.G., MONROY F., LA REGINA M., DINARELLO C.A. and SIBLEY L.D.: Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. The Journal of Immunology, 167 (8): 4574-84, 2001.

4- ROZENFELD C., MARTINEZ R., SEABRA S., SANT'ANNA C., GONÇALVES J.G.R., BOZZA M., et al.: Toxoplasma gondii prevents neuron degeneration by interferon-γ-activated microglia in a mechanism involving inhibition of inducible nitric oxide synthase and transforming growth factor-β1 production by infected microglia. The American journal of pathology, 167 (4): 1021-31, 2005.

5- DA SILVA A.S., MUNHOZ T.D., FARIA J.L., VARGAS-HÉRNANDEZ G., MACHADO R.Z., ALMEIDA T.C., et al.: Increase nitric oxide and oxidative stress in dogs experimentally infected by Ehrlichia canis: Effect on the pathogenesis of the disease. Veterinary microbiology, 164 (3-4): 366-9, 2013.

6- KARAMAN U., CELIK T., KIRAN T.R., COLAK C. and DALDAL N.U.: Malondialdehyde, glutathione, and nitric oxide levels in Toxoplasma gondii seropositive patients. The Korean journal of parasitology, 46 (4): 293, 2008.

7- HATFIELD R.H. and MCKERNAN R.M.: CSF neuron-specific enolase as a quantitative marker of neuronal damage in a rat stroke model. Brain Research, 577 (2): 249-52, 1992.

8- DINCEL G.C. and ATMACA H.T.: Role of oxidative stress in the pathophysiology of Toxoplasma gondii infection. International journal of immunopathology and pharmacology, 29 (2): 226-40, 2016.

9- MARIANI E., POLIDORI M., CHERUBINI A. and MECOCCI P.: Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview. Journal of Chromatography B, 827 (1): 65-75, 2005.

10- FANG Y-Z., YANG S. and WU G.: Free radicals, antioxidants, and nutrition. Nutrition, 18 (10): 872-9, 2002.

11- CHENG L., TIAN Y., WANG Y., WANG T., YAO Y., YU H., et al.: Toxoplasma TgAtg8-TgAtg3 interaction primarily contributes to apicoplast inheritance and parasite growth in tachyzoite. Microbiology Spectrum, 10 (1): e01495-21, 2022.

12- EBRAHIMZADEH M.A., TAHERI M.M., AHMADPOUR E., MONTAZERI M., SARVI S., AKBARI M., et al.: Anti-Toxoplasma effects of methanol extracts of Feijoa sellowiana, quercus castaneifolia, and Allium paradoxum. Journal of Pharmacopuncture, 20 (3): 220, 2017.

13- GRUJIĆ J., DJURKOVIĆ-DJAKOVIĆ O., NIKOLIĆ A., KLUN I. and BOBIĆ B.: Effectiveness of spiramycin in murine models of acute and chronic toxoplasmosis. International journal of antimicrobial agents, 25 (3): 226-30, 2005.

14- EMOTO Y., YOSHIZAWA K., KINOSHITA Y., YUKI M., YURI T., YOSHIKAWA Y., et al.: Green tea extract-induced acute hepatotoxicity in rats. Journal of toxicologic pathology, 27 (3+ 4): 163-74, 2014.

15- BURT S.: Essential oils: Their antibacterial properties and potential applications in foods a review. International journal of food microbiology, 94 (3): 223-53, 2004.

16- KOWALCZYK A., PRZYCHODNA M., SOPATA S., BODALSKA A. and FECKA I.: Thymol and thyme essential oil new insights into selected therapeutic applications. Molecules, 25 (18): 4125, 2020.

17- GAUR S., KUHLENSCHMIDT T.B., KUHLENSCHMIDT M.S. and ANDRADE J.E.: Effect of oregano essential oil and carvacrol on Cryptosporidium parvum infectivity in HCT-8 cells. Parasitology international, 67 (2): 170-5, 2018.

18- RANASINGHE P., JAYAWARDENA R., PIGERA S., WATHURAPATHA W.S., WEERATUNGA H.D., PREMAKUMARA G.S., et al.: Evaluation of pharmacodynamic properties and safety of Cinnamomum zeylanicum (Ceylon cinnamon) in healthy adults: A phase I clinical trial. BMC complementary and alternative medicine, 17: 1-9, 2017.

19- DORRI M., HASHEMITABAR S. and HOSSEINZADEH H.: Cinnamon (Cinnamomum zeylanicum) as an antidote or a protective agent against natural or chemical toxicities: A review. Drug and chemical toxicology, 41 (3): 338-51, 2018.

20- MAHMOUDVAND H., MAHMOUDVAND H., OLIAEE R.T., KARESHK A.T., MIRBADIE S.R. and AFLATOONIAN M.R.: In vitro protoscolicidal effects of Cinnamomum zeylanicum essential oil and its toxicity in mice. Pharmacognosy magazine, 13 (Suppl 3): S652, 2017.

21- JOHNSON L.L., BERGGREN K.N., SZABA F.M., CHEN W. and SMILEY S.T.: Fibrin-mediated protection against infection-stimulated immunopathology. The Journal of experimental medicine, 197 (6): 801-6, 2003.

22- HAMDEN K., CARREA U.S., AYADI MARKI F., MASMOUDI H. and EL FEKI A.: Positive effects of green tea on hepatic dysfunction, lipid peroxidation and antioxidant defence depletion induced by cadmium. Biological Research, 41 (3): 331-9, 2008.

23- FARRAG H.M.M., YONES D.A., HASSANIN E.S.A., IBRAHEIM Z.Z. and HUSSEIN E.E.H.: Thymus vulgaris, Mentha piperita and Elettaria cardamomum against Trypanosoma evansi in vitro and in an animal model with new insights for the treatment of trypanosomosis. Annals of Parasitology, 67 (1), 2021.

24- SOROUR S.S., ABOU ASA S., ELHAWARY N.M., GHAZY E.W., ABD EL LATIF A., EL-ABASY M.A., et al.: Anticoccidial and hepatoprotective effects of artemisinin liquid extract, cinnamon essential oil and clove essential oil against Eimeria stiedae infection in rabbits. Tropical biomedicine, 35 (4): 926-43, 2018.

25- DJURKOVIĆ-DJAKOVIĆ O., MILENKOVIĆ V., NIKOLIĆ A., BOBIĆ B. and GRUJIĆ J.: Efficacy of atovaquone combined with clindamycin against murine infection with a cystogenic (Me49) strain of Toxoplasma gondii. Journal of Antimicrobial Chemotherapy, 50 (6): 981-7, 2002.

26- CROWLEY L.C., MARFELL B.J., CHRISTENSEN M.E. and WATERHOUSE N.J.: Measuring cell death by trypan blue uptake and light microscopy. Cold Spring Harbor Protocols, 2016 (7): pdb. prot087155, 2016.

27- HSU S-M., RAINE L. and FANGER H.: A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. American journal of clinical pathology, 75 (5): 734-8, 1981.

28- SUVARNA K.S., LAYTON C. and BANCROFT J.D.: Bancroft's theory and practice of histological techniques: Elsevier health sciences, 2018.

29- HASHISH H.A.: Protective Role of Ginger against Metformin Induced Alteration in Bcl2 Expression in the Spleen of Normoglycemic Albino Rat. Journal of Histoloy and Histopathology, 6 (4): 1-5, 2019.

30- HAMED E.F.A., MOSTAFA N.E., FAWZY E.M., IBRAHIM M.N., ATTIA R. and SALAMA M.A.: The delayed death-causing nature of Rosmarinus officinalis leaf extracts and their mixture within experimental chronic toxoplasmosis: Therapeutic and prophylactic implications. Acta Tropica, 221: 105992, 2021.

31- FAUCHER B., MOREAU J., ZAEGEL O., FRANCK J. and PIARROUX R.: Failure of conventional treatment with pyrimethamine and sulfadiazine for secondary prophylaxis of cerebral toxoplasmosis in a patient with AIDS. Journal of antimicrobial chemotherapy, 66 (7): 1654-6, 2011.

32- LAPINSKAS P.J. and BEN-HARARI R.R.: Perspective on current and emerging drugs in the treatment of acute and chronic toxoplasmosis. Postgraduate Medicine, 131 (8): 589-96, 2019.

33- THILLAIVANAN S. and SAMRAJ K.: Challenges, constraints and opportunities in herbal medicines-a review, 2014.

34- ROTA M.C., HERRERA A., MARTÍNEZ R.M., SOTOMAYOR J.A. and JORDÁN M.J.: Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. Food control, 19 (7): 681-7, 2008.

35- SÁNCHEZ M.E., DEL V TURINA A., GARCIA D.A., NOLAN M.V. and PERILLO M.A.: Surface activity of thymol: implications for an eventual pharmacological activity. Colloids and Surfaces B: Biointerfaces, 34 (2): 77-86, 2004.

36- ELSHAFIE H.S., ARMENTANO M.F., CARMOSINO M., BUFO S.A., DE FEO V. and CAMELE I.: Cytotoxic activity of Origanum vulgare L. on hepatocellular carcinoma cell line HepG2 and evaluation of its biological activity. Molecules, 22 (9): 1435, 2017.

37- WITOLA W.H., ZHANG X. and KIM C.Y.: Targeted gene knockdown validates the essential role of lactate dehydrogenase in Cryptosporidium parvum. International journal for parasitology, 47 (13): 867-74, 2017.

38- ERAKY M.A., EL-FAKAHANY A.F., EL-SAYED N.M., ABOU-OUF EA-R. and YASEEN D.I.: Effects of Thymus vulgaris ethanolic extract on chronic toxoplasmosis in a mouse model. Parasitology research, 115: 2863-71, 2016.

39- FARAG T.I., SALAMA M.A., YAHIA S.H. and ELFEQY R.A.: Therapeutic efficacy of thymus vulgaris and myristica fragrance houtt (nutmeg) ethanolic extractagainst toxoplasmosis in murine model. Journal of the Egyptian Society of Parasitology, 49 (1): 73-9, 2019.

40- SANTORO G.F., DAS GRAÇAS CARDOSO M., GUIMARÃES L.G.L., SALGADO A.P.S., MENNA-BARRETO R.F. and SOARES M.J.: Effect of oregano (Origanum vulgare L.) and thyme (Thymus vulgaris L.) essential oils on Trypanosoma cruzi (Protozoa: Kinetoplastida) growth and ultrastructure. Parasitology research, 100: 783-90, 2007.

41- AGHA M. and BAAJ RJJoEOR.: Contribution study to the chemical composition of the volatile oil in the leaves of Thymus vulgaris L. and determination of the anti-bacterial activity and anti-leishmanial vitality. J. Journal of Essential Oil Research, 30 (1): 10-6, 2018.

42- REYGAERT W.C.: Green tea catechins: Their use in treating and preventing infectious diseases. BioMed research international, 2018 (1): 9105261, 2018.

43- FAKAE L.B., HARUN MS., TING D.S.J., DUA H.S., CAVE G.W., ZHU X-Q., et al.: Camellia sinensis solvent extract, epigallocatechin gallate and caffeine confer trophocidal and cysticidal effects against Acanthamoeba castellanii, 237: 106729, 2023.

44- KOLODZIEJ H., RADTKE O.A. and KIDERLEN A.F.: Stimulus (polyphenol, IFN-γ, LPS)-dependent nitric oxide production and antileishmanial effects in RAW 264.7 macrophages. Phytochemistry, 69 (18): 3103-10, 2008.

45- ARGÜELLO-GARCÍA R. and QUIÑONEZ-BASTIDAS G.N.: Catechins as emerging and promising antiparasitic agents. Biomed J. Sci. Technol. Res., 30: 23065-71, 2020.

46- EL-WAKIL E.S., ALI MOHAMED E., EL-WAKIL E.A., ABOUSHOUSHA T.S. and MOUSA AMER N.: Anti-cryptosporidial activity of Camellia sinensis (green tea extract) in experimentally infected immunocompromised mice. Acta Protozoologica, 61: 23-34, 2022.

47- DOYLE A.A. and STEPHENS J.C.: A review of cinnamaldehyde and its derivatives as antibacterial agents. Fitoterapia, 139: 104405, 2019.

48- NOWOTARSKA S.W., NOWOTARSKI K., GRANT I.R., ELLIOTT C.T., FRIEDMAN M. and SITU C.: Mechanisms of antimicrobial action of cinnamon and oregano oils, cinnamaldehyde, carvacrol, 2, 5-dihydroxybenzaldehyde, and 2-hydroxy-5-methoxybenzaldehyde against Mycobacterium avium subsp. paratuberculosis (Map). Foods, 6 (9): 72, 2017.

49- ALANAZI A.D. and ALMOHAMMED H.I.: Therapeutic potential and safety of the Cinnamomum zeylanicum methanolic extract against chronic Toxoplasma gondii infection in mice. Frontiers in Cellular and Infection Microbiology, 12: 900046, 2022.

50- FABBRI J., MAGGIORE M.A., PENSEL P.E., DENEGRI G.M. and ELISSONDO M.C.: In vitro efficacy study of Cinnamomum zeylanicum essential oil and cinnamaldehyde against the larval stage of Echinococcus granulosus. Experimental parasitology, 214: 107904, 2020.

51- SALAMA M.A.M., MOSTAFA N.E., ABD EL-AAL N.F., MOAWAD H.S.F., HAMMAD S.K., ADEL R., et al.: Efficacy of Zingiber officinale and Cinnamomum zeylanicum extracts against experimental Trichinella spiralis infection. Journal of Parasitic Diseases, 46 (1): 24-36, 2022.

52- TRABELSI N., NALBONE L., MAROTTA S.M., TAAMALI A, ABAZA L. and GIARRATANA F.: Effectiveness of five flavored Tunisian olive oils on Anisakis larvae type 1: Application of cinnamon and rosemary oil in industrial anchovy marinating process. Journal of the Science of Food and Agriculture, 99 (10): 4808-15, 2019.

53- TONI M., MASSIMINO M.L., DE MARIO A., ANGIULLI E. and SPISNI E.: Metal dyshomeostasis and their pathological role in prion and prion-like diseases: The basis for a nutritional approach. Frontiers in Neuroscience, 11: 3, 2017.

54- GUZIK T., KORBUT R. and ADAMEK-GUZIK T.: Nitric oxide and superoxide in inflammation. J. Physiol Pharmacol., 54 (4): 469-87, 2003.

55- DE FAZIO L., SPISNI E., CAVAZZA E., STRILLACCI A., CANDELA M., CENTANNI M., et al.: Dietary geraniol by oral or enema administration strongly reduces dysbiosis and systemic inflammation in dextran sulfate sodium-treated mice. Frontiers in Pharmacology, 7: 38, 2016.

56- RAFIE H., SOHEILA H. and GRANT E.: Rosmarinus officinalis (rosemary): A novel therapeutic agent for antioxidant, antimicrobial, anticancer, antidiabetic, antidepressant, neuroprotective, anti-inflammatory and anti-obesity treatment. J. Herbal Med., 3 (2): 8, 2017.

57- ALDOSARY S., EL-RAHMAN S., AL-JAMEEL S. and ALROMIHI N.: Antioxidant and antimicrobial activities of Thymus vulgaris essential oil contained and synthesis thymus (Vulgaris) silver nanoparticles. Brazilian Journal of Biology, 83: e244675, 2021.

58- LEMOS M.F., LEMOS M.F., PACHECO H.P., GUIMARÃES A.C., FRONZA M., ENDRINGER D.C., et al.: Seasonal variation affects the composition and antibacterial and antioxidant activities of Thymus vulgaris. Industrial Crops and Products, 95: 543-8, 2017.

59- DIAS M.C., PINTO D.C. and SILVA A.M.: Plant flavonoids: Chemical characteristics and biological activity. Molecules, 26 (17): 5377, 2021.

60- TAHA N.M., ZALAT R.S., KHALED E. and ELMANSORY B.M.: Evaluation of the therapeutic efficacy of some essential oils in experimentally immunosuppressed mice infected with Cryptosporidium parvum. Journal of Parasitic Diseases, 47 (4): 733-43, 2023.

61- NAKAGAWA T. and YOKOZAWA T.: Direct scavenging of nitric oxide and superoxide by green tea. Food and chemical Toxicology, 40 (12): 1745-50, 2002.

62- UNNO K., FURUSHIMA D., HAMAMOTO S., IGUCHI K., YAMADA H., MORITA A., et al.: Stress-reducing function of matcha green tea in animal experiments and clinical trials. Nutrients, 10 (10): 1468, 2018.

63- BORNSTEIN R., MULHOLLAND M.T., SEDENSKY M., MORGAN P. and JOHNSON S.C.: Glutamine metabolism in diseases associated with mitochondrial dysfunction. Molecular and Cellular Neuroscience, 103887, 2023.

64- RAMEZ A.M., ELMAHALLAWY E.K., ELSHOPAKEY GE., SALEH A.A., MOUSTAFA S.M., AL-BRAKATI A., et al.: Hepatosplenic protective actions of Spirulina platensis and matcha green tea against Schistosoma mansoni infection in mice via antioxidative and anti-inflammatory mechanisms. Frontiers in Veterinary Science, 8: 650531, 2021.

65- SINGH G., KAPOOR I., SINGH P., DE HELUANI C.S., DE LAMPASONA M.P. and CATALAN C.A.: Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (Curcuma longa Linn.). Food and chemical toxicology, 48 (4): 1026-31, 2010.

66- TEPE A.S. and OZASLAN M.: Anti-Alzheimer, anti-diabetic, skin-whitening, and antioxidant activities of the essential oil of Cinnamomum zeylanicum. Industrial Crops and Products, 145: 112069, 2020.

67- PAGONI A., MARINELLI L., DI STEFANO A., CIULLA M., TURKEZ H., MARDINOGLU A., et al.: Novel anti-Alzheimer phenol-lipoyl hybrids: Synthesis, physico-chemical characterization, and biological evaluation. European Journal of Medicinal Chemistry, 186: 111880, 2020.

68- GÜLÇIN İ.: Antioxidant activity of eugenol: A structure–activity relationship study. Journal of medicinal food, 14 (9): 975-85, 2011.

69- DAHHAM S.S., TABANA Y.M., IQBAL M.A., AHAMED M.B., EZZAT M.O., MAJID A.S., et al.: The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. Molecules, 20 (7): 11808-29, 2015.

70- HORVATHOVA E., NAVAROVA J., GALOVA E., SEVCOVICOVA A., CHODAKOVA L., SNAHNICANOVA Z., et al.: Assessment of antioxidative, chelating, and DNA-protective effects of selected essential oil components (eugenol, carvacrol, thymol, borneol, eucalyptol) of plants and intact Rosmarinus officinalis oil. Journal of agricultural and food chemistry, 62 (28): 6632-9, 2014.

71- ASHFAQ M.H., SIDDIQUE A. and SHAHID S.: Antioxidant activity of Cinnamon zeylanicum:(A review). Asian Journal of Pharmaceutical Research, 11 (2): 106-16, 2021.

72- SAHIB A.S.: Anti-diabetic and antioxidant effect of cinnamon in poorly controlled type-2 diabetic Iraqi patients: A randomized, placebo-controlled clinical trial. Journal of intercultural ethnopharmacology, 5 (2): 108, 2016.

73- HUSSAIN S., ASHAFAQ M., ALSHAHRANI S., SIDDIQUI R., AHMED R.A., KHUWAJA G., et al.: Cinnamon oil against acetaminophen-induced acute liver toxicity by attenuating inflammation, oxidative stress and apoptosis. Toxicology Reports, 7: 1296-304, 2020.

74- CHU C., DENG J., MAN Y. and QU Y.: Green tea extracts epigallocatechin-3-gallate for different treatments. BioMed research international, 2017 (1): 5615647, 2017.

75- KWON H.I., JEONG N.H., KIM S.Y., KIM M.H., SON J.H., JUN S.H., et al.: Inhibitory effects of thymol on the cytotoxicity and inflammatory responses induced by Staphylococcus aureus extracellular vesicles in cultured keratinocytes. Microbial pathogenesis, 134: 103603, 2019.

76- WIJESUNDARA N.M., LEE S.F., DAVIDSON R., CHENG Z. and RUPASINGHE H.V.: Carvacrol suppresses inflammatory biomarkers production by lipoteichoic acid-and peptidoglycan-stimulated human tonsil epithelial cells. Nutrients, 14 (3): 503, 2022.

77- GHOLIJANI N., GHARAGOZLOO M., FARJADIAN S. and AMIRGHOFRAN Z.: Modulatory effects of thymol and carvacrol on inflammatory transcription factors in lipopolysaccharide-treated macrophages. Journal of immunotoxicology, 13 (2): 157-64, 2016.

78- MAHMOODI M., AYOOBI F., AGHAEI A., RAHMANI M., TAGHIPOUR Z., HOSSEINI A., et al.: Beneficial effects of Thymus vulgaris extract in experimental autoimmune encephalomyelitis: Clinical, histological and cytokine alterations. Biomedicine & Pharmacotherapy, 109: 2100-8, 2019.

79- LIANG D., LI F., FU Y., CAO Y., SONG X., WANG T., et al.: Thymol inhibits LPS-stimulated inflammatory response via down-regulation of NF-κB and MAPK signaling pathways in mouse mammary epithelial cells. Inflammation, 37: 214-22, 2014.

80- GOLBAHARI S. and FROUSHANI S.M.A.: Synergistic benefits of nicotine and thymol in alleviating experimental rheumatoid arthritis. Life sciences, 239: 117037, 2019.

81- OHISHI T., GOTO S., MONIRA P., ISEMURA M. and NAKAMURA Y.: Anti-inflammatory action of green tea. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents), 15 (2): 74-90, 2016.

82- SHAN D., FANG Y., YE Y. and LIU J.: EGCG reducing the susceptibility to cholesterol gallstone formation through the regulation of inflammation. Biomedicine & pharmacotherapy, 62 (10): 677-83, 2008.

83- KOCHMAN J., JAKUBCZYK K., ANTONIEWICZ J., MRUK H. and JANDA K.: Health benefits and chemical composition of matcha green tea: A review. Molecules, 26 (1): 85, 2020.

84- YANG Y., LIU Y., WANG Y., CHAO Y., ZHANG J., JIA Y., et al.: Regulation of SIRT1 and its roles in inflammation. Frontiers in Immunology, 13: 831168, 2022.

85- WICIŃSKI M., SOCHA M., WALCZAK M., WÓDKIEWICZ E., MALINOWSKI B., REWERSKI S., et al.: Beneficial effects of resveratrol administration Focus on potential biochemical mechanisms in cardiovascular conditions. Nutrients, 10 (11): 1813, 2018.

86- SHARMA H., CHAUHAN P. and SINGH S.: Evaluation of the anti-arthritic activity of Cinnamomum cassia bark extract in experimental models. Integrative medicine research, 7 (4): 366-73, 2018.

87- LEE S.H., LEE S.Y., SON D.J., LEE H., YOO H.S., SONG S., et al.: Inhibitory effect of 2-hydroxycinnamaldehyde on nitric oxide production through inhibition of NF-κB activation in RAW 264.7 cells. Biochemical pharmacology, 69 (5): 791-9, 2005.

88- LIAO J-C., DENG J-S., CHIU C-S., HOU W-C., HUANG S-S., SHIE P-H., et al.: Anti-inflammatory activities of Cinnamomum cassia constituents in vitro and in vivo. Evidence-Based Complementary and Alternative Medicine, 2012 (1): 429320, 2012.

89- OMIDIPOUR R., ZAREI L., BOROUJENI M.B. and RAJABZADEH A.: Protective effect of thyme honey against valproic acid hepatotoxicity in Wistar rats. BioMed Research International, 2021 (1): 8839898, 2021.

90- AHMED O.M., GALALY S.R., MOSTAFA M-AM, EED E.M., ALI T.M., FAHMY A.M., et al.: [Retracted] Thyme Oil and Thymol Counter Doxorubicin-Induced Hepatotoxicity via Modulation of Inflammation, Apoptosis, and Oxidative Stress. Oxidative Medicine and Cellular Longevity, 2022 (1): 6702773, 2022.

91- HAMED N.S., TAHA E.F. and KHATEEB S.: Matcha-silver nanoparticles reduce gamma radiation-induced oxidative and inflammatory responses by activating SIRT1 and NLRP-3 signaling pathways in the Wistar rat spleen. Cell Biochemistry and Function, 41 (8): 1115-32, 2023.

92- AL-AWAIDA W., GOH K.W., AL-AMEER H.J., GUSHCHINA Y.S., TORSHIN V.I., SEVERIN A.E., et al.: Assessing the protective role of epigallocatechin gallate (EGCG) against water-pipe smoke-induced toxicity: A comparative study on gene expression and histopathology. Molecules, 28 (22): 7502, 2023.

93- AIOUB A.A., ABDELNOUR S.A., HASHEM A.S., MAHER M., ABDEL-WAHAB S.I., ALKERIDIS L.A., et al.: Cinnamon nanoemulsion mitigates acetamiprid-induced hepatic and renal toxicity in rats: Biochemical, histopathological, immunohistochemical, and molecular docking analysis. BMC Veterinary Research, 20 (1): 256, 2024.

94- ELKOMY A., ABOUBAKR M., SOLIMAN A., ABDEEN A., ABDELKADER A. and HEKAL H.: Paracetamol induced hepatic toxicity and amelioration by cinnamon in rats. Int. J. Pharmacol. Toxicol., 4: 187, 2016.

# التأثيرات المضادة للأكسدة والالتهابات لشاى الماتشا الأخضر والزيوت العطرية للزعتر والقرفة على داء المقوسات المزمن: دراسة حية

داء المقوسات (Toxoplasmosis) هو عدوى طفيلية منتشرة تؤدى إلى مضاعفات سريرية كبيرة، لاسيما التهاب الدماغ المقوسة فى الأفراد الذين يعانون من ضعف المناعة. وبسبب محدودية الأدوية المضادة للتوكسوبلازما الحالية مثلا لفعالية المحدودة، وتطورالمقاومة، والسمية المحتملة، فمنا لضرورى تحديد ودراسة خيارات علاج جديدة. كان الهدف الرئيسى من هذا البحث هو تقييم الإمكانات العلاجية لشاى الماتشا الأخضر، زيت الزعترالعطرى، وزيت القرفة العطرى، سواء بشكل فردى أو بالاشتراك مع السبراميسين، ضد داء المقوسات المزمن فيا لفئران. كما تهدف الدراسة إلى فحص خصائصها المضادة للأكسدة والالتهابات. تم تقسيم ٨١ فأرًا مصابًا بسلالة ME ٤٩ من المقوسة الغوندية إلى تسع مجموعات تمت معالجتها بعوامل مختلفة لمدة ١٤ يومًا ابتداءً من الأسبوع الثانى بعدالعدوى. تم إجراء تحليلات الطفيليات، الكيمياء الحيوية، الهيستوباثولوجية، والمناعية النسيجية الكيميائية لتقييم فعالية هذه العلاجات. وأظهرت النتائج انخفاضا كبيراً فى عدد أكياس الدماغ وقابليتها للحياة فى جميع المجموعات المعالجة، مع حدوث انخفاض كبير فى المجموعات المعالجة بزيت الزعتر الأساسى، وخاصة فى المجموعتين الخامسه والثامنة (٦٨ ٪ ٨٤ ٪ انخفاض فى عدد أكياس الدماغ و٦٤ ٪ و ٨٤ ٪ انخفاض فى القابلية للحياة، على التوالى) مقارنة بالمجموعة الثالثة المعالجة بسبيراميسين فقط والمجموعة الثانية المصابة غيرالمعالجة. بالإضافة إلى ذلك، أظهرت المجموعات المعالجة بالزعتر انخفاضاً كبيراً فى المعاملات التأكسدية (MDA) والالتهابية (IFNγ, iNos and NSE)، إلى جانب زيادة مستويات مضادات الأكسدة (SOD). وعلاوة على ذلك، أظهرتحليل هذه المجموعات للأنسجة المرضية للكبد والدماغ تحسناً ملحوظاً. عندما تم الجمع بين الشاى الأخضر والقرفة والزيت العطرى مع سبيراميسين، أظهرت آثار تآزرية.