

<https://doi.org/10.48047/AFJBS.6.7.2024.4260-4275>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Chemical Characterization and Investigation of the Antioxidant Capacity and the Possible Therapeutic Potential of *Salvia officinalis* Extract against Murine Toxoplasmosis

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Article History

Volume 6, Issue 7, 2024

Received: 05 June 2024

Accepted: 15 July 2024

Published: 20 July 2024

doi: [10.48047/AFJBS.6.7.2024.4260-4275](https://doi.org/10.48047/AFJBS.6.7.2024.4260-4275)

Abstract:

Background and objective: Toxoplasmosis is a parasitic zoonose that is highly prevalent. The development of novel antiparasitic medications is highly challenging since the medications now used to treat toxoplasmosis have many side effects. This study aimed to identify the bioactive components in *Salvia officinalis* extract and their therapeutic efficacy against toxoplasmosis. **Methodology:** A total of 30 albino mice were divided into five groups: GI (non-infected); GII (infected without treatment); GIII, GIV and GV infected and treated groups with spiramycin, *Salvia officinalis* extract and combination of spiramycin and *Salvia officinalis* extract, respectively. Treatment starting from the 6th week post-infection and euthanized on the 8th week. For the study groups, parasitological, histopathological examinations and immunological analysis for IL-10 and TNF-alpha levels was assessed. Moreover, Total phenolics content (TPC) of the extract was evaluated by Folin Ciocalteu assay and the total flavonoids content TFC were estimated using AlCl₃ technique. Determination of the total antioxidant capacity by the phosphomolybdenum method and DPPH assay. Using RP-HPLC, phenolics and flavonoids of the extract were identified. **Results:** The methanol extract show high antioxidant activity IC₅₀ = 6.6µg/ml for DPPH and 400.30 mg AAE/g ext. respectively, this activity could be explained by the extract's high phenolic and flavonoid content. A comparison of the treated groups (GIII, GIV, and GV) with the control positive group (GII) revealed statistically significant variations in the parasite load, along with improvements in the histological changes. Of the test groups, the GV group also demonstrated the best improvement in parasitological and histological indicators. Comparing infected to the uninfected group, there was an increase in IL-10 and decrease in TNF-alpha (P< 0.0001). Significant down regulation of IL-10 and up regulation was seen in all treatment groups (GIII, GIV, and GV). The highest TNF-alpha and lowest IL-10 levels were found in the GV. These results depending on the bioactive compound of *Salvia officinalis*

Conclusion: Spiramycin and *Salvia officinalis* showed a strong synergistic effect in treating toxoplasmosis.

Keywords: *Salvia officinalis*; Toxoplasmosis; Antioxidant; RP-HPLC

1. Introduction

Toxoplasma gondii (*T. gondii*) is an intracellular protozoan parasite that causes the widespread infection known as toxoplasmosis. One-third of people worldwide have anti-Toxoplasma antibodies, making this parasite one of the most prevalent (Mose *et al.*, 2020; de Barros *et al.*, 2022). Oral infection of *T. gondii* cysts occurs when humans consume raw or undercooked meat, produce (fruits and vegetables), or water contaminated with oocysts. Transfusions of blood, organ transplants, and congenital transmission are additional modes of transmission (Almeria and Dubey, 2021). The majority of infections in healthy individuals are asymptomatic. But in immunocompromised individuals, the disease's progression may be fatal (Wesołowski *et al.*, 2023).

The current state of chemotherapeutic options for treating toxoplasmosis is limited, despite a concentrated effort over the past ten years to use high throughput screening techniques to find novel compounds, including natural products, with anti-Toxoplasma activity or to improve or modify already-approved medications. The initial course of treatment for toxoplasmosis is pyrimethamine and sulfadiazine together. Azithromycin or atovaquone, pyrimethamine and clindamycin, or trimethoprim and sulfamethoxazole are combinations of therapies. All treatments, nevertheless, frequently have dangerous adverse effects (Smith *et al.*, 2021; El Sharazly *et al.*, 2023). Thus, there is a pressing need for toxoplasmosis treatment options that are safer, more effective, and more innovative.

Natural medicine has been utilised by people to treat a variety of diseases since ancient times. Because there was not enough knowledge about the causes of diseases, the characteristics of plants, or their therapeutic applications back then, people first used medicinal plants instinctively, taking inspiration from animals (Ranasinghe *et al.*, 2023; Chaachouay *et al.*, 2024). One of the most significant herbal medicines is *Salvia officinalis* L. (family: Lamiaceae). It is commonly known as sage and has been used for centuries to cure a variety of diseases in traditional and folk medicine as well as a dairy condiment in food. Several studies have demonstrated that *S. officinalis* possesses a broad spectrum of useful biological properties. It has been discovered that many chemical components are in responsible for these processes (Ghorbani and Esmaeilzadeh; 2017; Poullos *et al.*, 2020; Amer *et al.*, 2024). The antioxidant activity of *S. officinalis* was attributed to phenolic compounds (Poullos *et al.*, 2020; Rogozinska *et al.*, 2023).

We hypothesized that *S. officinalis* may be a powerful agent to treat *T. gondii* infections based on this understanding as well as the herb's known antioxidant and antiparasitic properties. Taking that in consideration, the objective of this study is to evaluate the therapeutic impact of *S. officinalis* on *T. gondii* (MF49) and identify the bioactive components of *S. officinalis* that provide the antioxidant effect.

2. Material and methods

Plant materials

The *S. officinalis* is native to the Egyptian city Al-Arish in North Sinai was collected during the blossoming stage and identified by Dr. Tearse Labib, El-Orman Botanical Garden, Department of Flora and Taxonomy, Giza, Egypt. A voucher specimen designated (Sa.O March 2021), was placed at the Laboratory of Medicinal Chemistry at the Theodor Bilharz Research Institute in Egypt.

Plant Extraction

The aerial parts of *S. officinalis* were allowed to air dry at room temperature and ground into a powder using a grinder. After that, it was extracted for four hours at 37 °C using 90% methanol. By using a rotary evaporator at 50 °C to remove methanol, the hydro alcoholic extract was produced.

Preliminary phytochemical analysis

To determine the types and distribution of secondary metabolites in the extract, The methanolic extract was submitted to phytochemical investigations through two dimensional paper chromatography (2D-PC) and preliminary phytochemical screening tests (Edeoga *et al.*, 2005).

Estimation of total phenolics content (TPC)

Total phenolics content (TPC) of the extract was evaluated by Folin Ciocalteu assay (Hadjira *et al.*, 2024). A 100 µl extract (100 µg/ml) was combined with 1.5 ml of Na₂CO₃ (20%) and 500 µl of Folin-Ciocalteu reagent, then vortexed for 15 sec and made up to 10 ml using distilled water then allowed to stand for 120 min at 25°C for color development and absorbance was recorded at 765 nm via a spectrophotometer. Total phenolic contents were evaluated in mg of GAE/g extract, milligram Gallic acid equivalent.

Estimation of total flavonoids content (TFC)

Total flavonoids content TFC was estimated using the method designated by Indiarito *et al.*, (2024). Just about 0.5 mg extract in 3 mL methanol was added to, 0.2 mL Potassium acetate solution, 0.2 ml Aluminium chloride solution, and adjusted with distilled water to a 10 ml. It was then incubated for 30 minutes in a dark place. The absorbance was measured at a wavelength of 415 nm using a UV spectrophotometer. TFC was expressed as mg RE / g ext., mg Rutin equivalent.

Estimation of Antioxidant activity

a) Determination of antioxidant activity by DPPH assay

DPPH scavenging potential of the plant extract was assessed according to a method of Manzocco *et al.*, (1998), First, 1 mL of the extract or standard ascorbic acid solutions at varying concentrations (800, 400, 200, 100, 50, 25, 12.5, 6.25 µg/ml) were taken, and then 2 mL of a 0.006% DPPH solution in methanol was added to each test tube to make the final volume 3 ml. The vortex mixer was used to homogenize the tube. After 30 minutes of incubation in a dark room, the sample's absorbance was determined at a wavelength of 517 nm using a UV-Vis spectrophotometer. The results were expressed in IC₅₀ value, the concentration required to inhibit

50% of the DPPH free radicals (Guenane *et al.*, 2024). The absorbance value was used to calculate the DPPH Inhibition percentage according to the equation:

$$\text{DPPH Inhibition percentage} = (1 - \text{Sample absorbance} / \text{Blank absorbance}) \times 100\%$$

b) Determination of the total antioxidant capacity by the phosphomolybdenum method

The evaluation of the total antioxidant capacity (TAC) of *S. officinalis* extract by the phosphomolybdenum assay is a technique based on the reduction of molybdenum (VI) to molybdenum (V) by the constituents contained in the sample. The reaction proceeds in an acidic environment to form greenish-blue colour of reduced phosphomolybdate that are measured at wavelength 695 nm. The measured antioxidants properties can be expressed as equivalents of ascorbic acid. 0.3 ml dilute concentration of *S. officinalis* extract was combined with 3ml of a mixture (28mM Sodium phosphate, 0.6M H₂SO₄ and 4mM Ammonium molybdate) in test tube. Then incubate at 95°C in boiling water bath for ninety minutes. A spectrophotometer was used to test the solution's absorbance at 695 nm after the tubes had been cooled. Ascorbic acid was used as reference standard and in the blank tube; 0.3 ml of methanol was substituted for the extract. The antioxidant capacity of extract was expressed as µg ascorbic acid equivalents (AAE)/mg of extract (Rohan *et al.*, 2014; Ślusarczyk *et al.*, 2021).

RP-HPLC- with diode-array detection Conditions

Using RP-HPLC, phenolics and flavonoids of the extract were identified according to Tamer *et al.*, (2022). HPLC analysis was performed using an Agilent 1260 series with Eclipse C18 column (4.6 mm x 250 mm, 5 µm), solvent degasser and quaternary HP pump (series 100) and auto-sampling injector. At 40 °C, the temperature was sustained. The mobile phase was made up of A (water) and B (0.05% CF₃COOH in acetonitrile) with a linear gradient flow rate 0.9 ml/minute. The multi-wavelength detector was setup at 280 nm and the injection volume was 15 µl. The Standards used for the experiment were purchased from Sigma Co. The area under the peak and the retention time were used to identify the compounds and to evaluate the concentration of them by the data of Agilent software.

Animals and experiment design

For this study, Theodor Bilharz Research Institute (TBRI) provided 30 Swiss albino mice aged 6-8 weeks and weighing 20–25 grams. Mice were randomly divided into five groups, each group included 6 mice (Figure 1). Clean wood-chip bedding and well-ventilated plastic cages were provided for the animals, who were kept in temperature-controlled rooms (24±2°C). Fresh bedding was constantly supplied, and in addition to weekly cage cleanings, special water bottles were used to offer water. The experimental animal studies were managed in accordance with TBRI Research Ethics Committee (TBRI-REC) with serial number of the protocol PT (617). TBRI-REC operates in a manner consistent with National Institutes of Health (NIH) guide for care and use of laboratory animals (eighth edition).

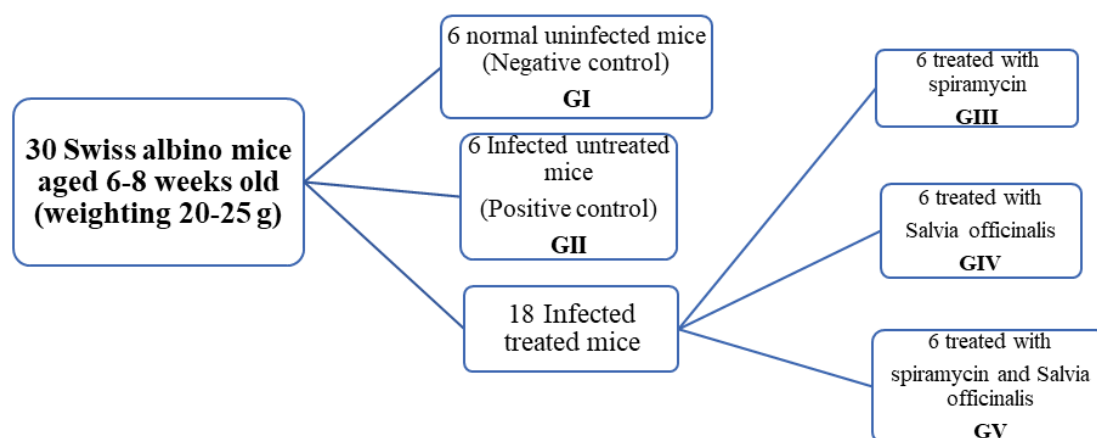


Figure 1: Flow chart of experimental design and animal groups.

Infection

Toxoplasma gondii cystogenic strain ME49 was utilized to infect a male Swiss albino. The organism was provided by the animal house of the Theodor Bilharz Research Institute in Egypt. Every eight weeks, it was maintained by a repeated oral inoculation using a Swiss albino mouse esophageal tube with 100 μ L of brain homogenate from previously infected mice, which contained roughly 1×10^2 tissue cysts/mouse (Djurković-Djaković *et al.*, 2002).

Drugs preparation administration

Spiramycin (3 M.I.U) was manufactured and provided by Pharaonia Pharmaceuticals, Egypt. Mice were administered it orally at a dose of 200 mg/kg/body weight/mouse daily for seven days in a row (Djurković-Djaković *et al.*, 2002). For seven consecutive days, *S. officinalis* alone or in combination with Spiramycin given orally to each mouse in a dose of 150 mg/kg (Kannan *et al.*, 2019).

Drugs Assessment

Parasitological examination

The brains of every mouse that was sacrificed were homogenised in one millilitre of saline and sieved. Using an x40 objective and 10 μ L of brain homogenate on microscopic slides, the number of tissue cysts per mouse brain was counted under light microscopy. The number of tissue cysts per millilitre (1000 μ L) of brain suspension was determined by taking the average of three counts and multiplying it by 100 (Djurkovic-Djakovic *et al.* 2002). Each group's mean number of cysts per brain was calculated.

Histopathological examination

After being preserved in 10% (v/v) buffered formalin, specimens of brain tissue from various groups were prepared for paraffin block embedding. Slices of tissue measuring roughly 5 μ m in thickness were cut out, stained with Haematoxylin and Eosin (H&E) stains, and examined under a light microscope (Fischer *et al.*, 2008; Drury & Wallington 1980).

Immunological analysis Interleukin 10 and TNF Alpha

Blood samples were collected in plain tubes during scarification, after centrifuging blood samples for five minutes at 4500 rpm, serum was kept at -20 °C until analysis. In accordance with the manufacturer's instructions for the ELISA assays, which were provided by (INNOVA BIOTECH Co., Ltd., Cat. No. In-Mo 1242) and (INNOVA BIOTECH Co., Ltd., Cat. No. In-Mo 1920), respectively, the levels of IL10 and TNF alpha were measured.

Statistical analysis

IBM Corp., Armonk, NY, USA, SPSS Statistics version 26 was used for the data analysis. The data was shown as mean $\pm$ SD with a 95% confidence interval. ANOVA testing was used to compare normally distributed variables between groups, and Dunnett T3 was used as a post-hoc test. $P > 0.05$ indicates no significance, $P < 0.05$ indicates significance, while $P < 0.001$ indicates highly significance

Results

Total phenolics content (TPC) and total flavonoids content (TFC) of *S. officinalis* extract

The Preliminary phytochemical tests showed that the methanolic extract of *S. officinalis* has a great number of bioactive phytoconstituents as phenolics, flavonoids, carbohydrate, tannins, sterols and saponins. The TPC of *S. officinalis* extract was TPC= 521 ± 0.98 mg GAE / g ext. Sage extract also contain flavonoids which evaluated as TFC = 119.7 ± 1.1 mg RE / g ext., (Table 1).

Antioxidant activity of *S. officinalis* extract

S. officinalis extract, was screened for antioxidant activities by DPPH radical scavenging and TAC methods, showing promising results of antioxidant activity. *S. officinalis* extract has some degree of electron donation capacity, the total antioxidant capacity is 400.30 mg AAE/g extract while DPPH IC_{50} = 9.6 μ g/ml compared with ascorbic acid as a positive control with IC_{50} value of 3.21 μ g/ml (Table 1).

Table 1: Total antioxidant capacity, DPPH free radical antioxidant activity, total phenolic content and total flavonoids content of *S. officinalis* methanol extract.

Sample	TAC	DPPH	TPC	TFC
	mg AAE/g ext.	(IC_{50} μ g/ml)	(mg GAE/g ext.)	(mg RE / g ext.)
Methanol extract	400.30 ± 1.08	9.6 ± 0.71	521 ± 0.98	119.7 ± 1.1
Ascorbic acid	-	3.21 ± 0.55	-	-

RP-HPLC finger print with diode-array detection of *S. officinalis* extract

S. officinalis methanol extract was subjected to further analysis via RP- HPLC with diode-array detection at 280 nm to identify their chemical constituents at different retention times (Table 2, Figure. 2). Rosmarinic acid (at 11.11 min) and Carnosic acid (at 10.9 min) are the predominant constituents in the extract.

Table 2: The constituents of *S. officinalis* methanol extract by RP-HPLC finger print with diode-array detection.

Name of the compound	Retention time (min)	Area%
Nubenoic acid	2.541	6.962
Carnosol	2.797	5.696
Quinic acid	3.215	0.438
Gallic acid	3.438	2.238
Salvianolic acid	3.591	2.046
β -Sitosterol	3.797	0.995
Chlorogenic acid	4.287	2.550
Catechin	4.765	0.046
Caffeic acid	6.252	3.188
Syringic acid	6.591	0.866
Rosmanol	8.233	0.471
Ellagic acid	8.826	0.202
Coumaric acid	9.393	11.542
Ferulic acid	10.065	6.823
Naringenin	10.471	1.816
Carnosic acid	10.918	21.264
Rosmarinic acid	11.117	23.074
Daidzein	12.288	1.619
Luteolin	12.643	0.253
Querectin	12.888	0.640
Diosmetin	13.380	2.349
Cinnamic acid	14.090	3.136
Apigenin	14.540	0.092
Kaempferol	14.993	0.989
Hesperetin	15.527	0.122

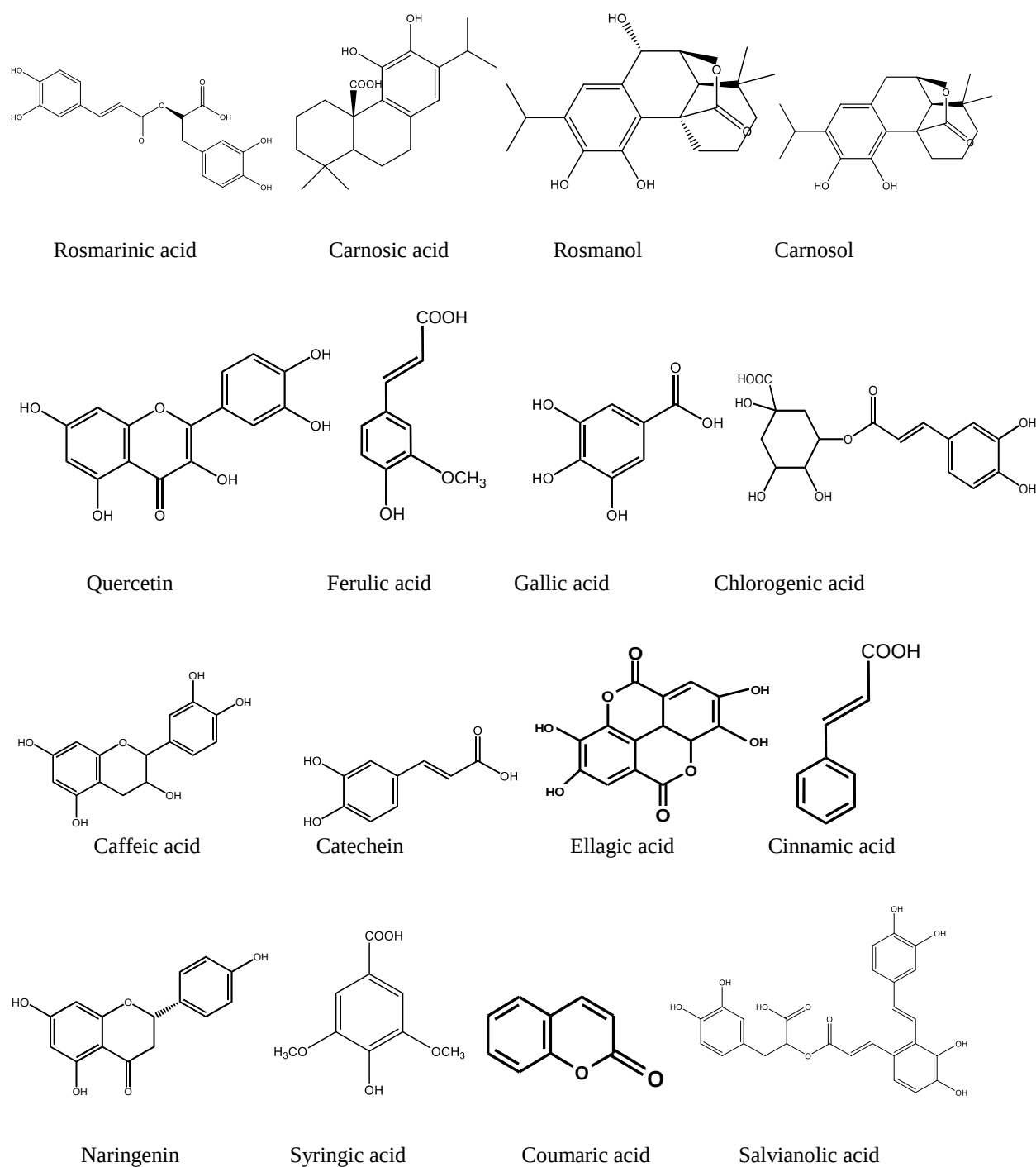


Figure 2: Two-dimensional structures of the identified compounds of *S. officinalis* methanol extract by RP-HPLC finger print with diode-array detection.

Parasitological Results

In brain mice weighing 0.01 g, the number of *Toxoplasma* cysts was significantly reduced in treated groups GIII, GIV and GV than in GII, with drug efficacy percentages of 61%, 71% and 80%, respectively. The treated group GV (received combined treatment with spiramycin and *S. officinalis*) showed the best results 80%. **Table 3** displays the multiple comparisons (Dunnett T3) for the tissue cyst count mean values across the various study groups.

Table 3: *Toxoplasma* cyst mean in the brains of mice from all experimental groups by post hoc test multiple comparisons (Dunnett T3).

Drug Administration	Infected Model	Infected Treated Groups			P-value
		Spiramycin	<i>Salvia officinalis</i>	Combined Spiramycin + <i>Salvia officinalis</i>	
Mean ± SE	945 ± 33.7	365.8 ±18	277.8 ±12.9	185 ± 4.6	0.000**
P- value		0.000**	0.000**	0.000**	
Tukey HSD @ P. value	Infected untreated	0.000**	0.000**	0.000**	
	Spiramycin	-	0.029*	0.000**	
	<i>Salvia officinalis</i>	-	-	0.020*	
Reduction percentage		61%	71%	80%	

Brain cyst count is represented as Mean ± SE, *P*<0.05 * significant difference; *P*<0.001** highly significant difference.

Histopathological results

Histopathological examination of brain sections of GI (Normal uninfected) showing almost normal neuronal pattern with tangles, normal vasculature and pericellular and interstitial oedema. The sections of GII (infected untreated group) revealed profound histopathological alterations in cerebral cortex as a result of infection with *T. gondii* exhibited by deformed neurons with deeply stained nuclei. Multiple *T. gondii* cysts embedded in the tissue and massive deformed neurons surrounded by haloes were also detected in GII. The sections from groups treated with spiramycin showing moderate neuronal degeneration with many scattered lymphocytes and focal pericellular oedema. While the sections from treated with *S. officinalis* showing mild neuronal degeneration with a few scattered lymphocytes normal vascular pattern and interstitial oedema. The sections from group treated with combined Spiramycin and *S. officinalis* showing almost intact neurons preserved capillary pattern and focal pericellular oedema after drug administration (Figure 3A-3E).

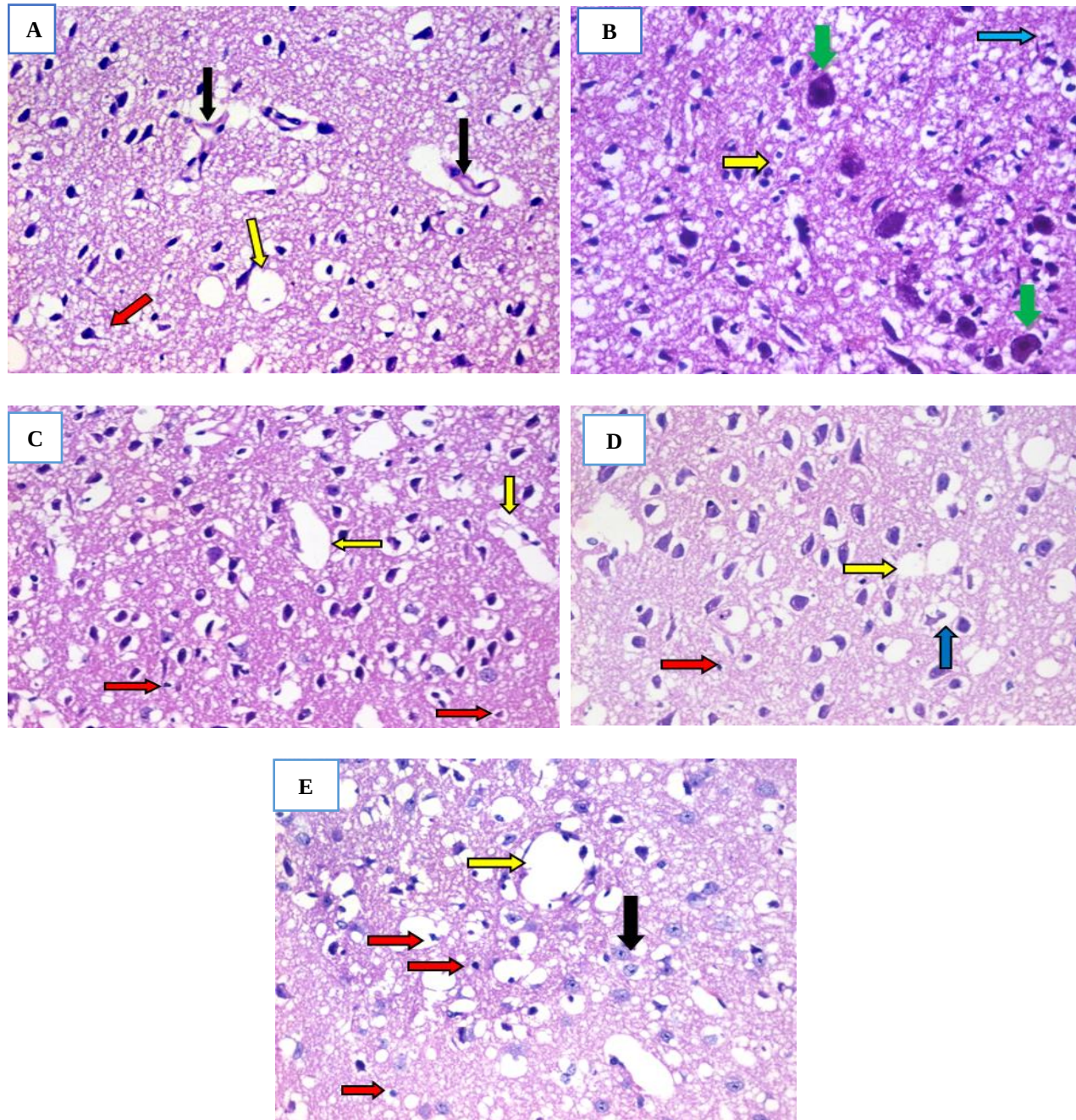


Figure 3: **A:** A section photomicrograph of brain tissue from **GI** with almost normal neuronal pattern with tangles (red arrow), normal vasculature (black arrows) and pericellular and interstitial oedema (yellow arrows); **B:** sections from **GII** showing some *Toxo* cysts (green arrows), marked neuronal degeneration, perivascular cuffing by mononuclear inflammatory cells (yellow arrows), and gliosis (blue arrow) as well as obvious pericellular and interstitial oedema; **C:** Sections from **GIII** showing moderate neuronal degeneration (neurophagia)(black arrows) few scattered lymphocytes (red arrows)and obvious patchy pericellular and interstitial oedema (yellow arrows); **D:** Sections from **GIV** showing mild neuronal degeneration (blue arrows), few lymphocytes (red arrows) and moderate interstitial oedema (yellow arrows); **E:** Sections from **GV** showing almost intact neurons (black arrows), scattered lymphocytes (red arrows) and focal interstitial oedema (yellow arrows).

Immunological results

TNF Alpha

TNF Alpha levels were highly significantly decreased in GII infected untreated positive control compared to GI, the non-infected negative control (P< 0.0001). An induction of TNF Alpha levels was detected in all treated groups GIII, GIV and GV compared to infect untreated GII (P< 0.0001). The highest TNF Alpha levels were detected in GV, who received combined treatment with combined Spiramycin and *S. officinalis* (Table 4).

Interleukin 10

IL-10 level was highly significantly increased in Group II infected untreated positive control compared to GI, the non-infected negative control (P< 0.0001). A reduction of IL-10 levels was detected in all treated groups GIII, GIV, GV compared to infect untreated GII (P< 0.0001). The lowest IL-10 level was detected in GV, received combined treatment with combined Spiramycin and *S. officinalis* (Table 5).

Table 4: Serum level of TNF-α in different study groups

Groups	TNF-α	
	Mean ± SE	ANOVA P. value
Normal (Negative control)	451.6 ± 3.9	0.001**
Infected untreated (Positive control)	209.1 ± 2.6	
Spiramycin	282.3 ± 3	
<i>Salvia officinalis</i>	351.9 ± 2	
Combined Spiramycin + <i>S. officinalis</i>	395.8 ± 2.9	

Table 5: Serum level of IL-10 in different study groups

Groups	IL-10	
	Mean ± SE	ANOVA P. value
Normal (Negative control)	48 ± 2	0.001**
Infected untreated (Positive control)	238.9 ± 2.8	
Spiramycin	131.2 ± 2.2	
<i>Salvia officinalis</i>	92.1 ± 2.8	
Combined Spiramycin + <i>Salvia officinalis</i>	73.3 ± 3.2	

Discussion

Despite this, *Toxoplasma gondii* infections are still very common in humans and can cause fatal disease in certain cases. The restricted range of treatment choices available for people with toxoplasmosis highlights the pressing need to find new, more potent treatments for both the latent and acute forms of the infection. It has previously been documented how pro- and antioxidant mechanisms contribute to toxoplasmosis treatment (Bosch *et al.*, 2015; Dincel and

Atmaca, 2016; Szewczyk-Golec *et al.*, 2021). Chemicals that alter redox state have the potential to be anti-Toxoplasma medications since they can decrease the vitality of the parasite. Therefore, this study aimed to evaluate the antioxidant effect of *S. officinalis* on *T. gondii* activity. Moreover, determine the chemical compounds in the *S. officinalis* methanol extract that responsible for the antioxidant effect.

The DPPH test is a common, affordable, rapid and easy approach for the evaluation of antioxidant capacities that use the free radicals for assessing the potential of substances to serve as free-radical scavengers or hydrogen donor (Baliyan *et al.*, 2022). The high phenolic content in *S. officinalis* extract (TPC= 521 mg GAE / g ext and TFC = 119.7 mg RE / g ext) contributes to its strong antioxidant activity. An optimized reversed phase high-performance liquid chromatography with diode array detection (RP-HPLC-DAD) approach was used to identify twenty five compounds, including phenolic acids and flavonoids. These results were consistent with previous studies (Gligor *et al.*, 2019; Boufadi *et al.*, 2021).

Phenolic compounds of *S. officinalis* extract play a key role in its antioxidant, antimicrobial, anti-parasitic and anti-inflammatory effects due to the existence of the conjugated unsaturation and phenolic hydroxyl groups that enables the delocalization of the free radicals, reducing oxidative stress thereby protecting cells from oxidative damage. The phenolic acid's anti-inflammatory effects are thought to arise from its ability to inhibit pro-inflammatory enzymes and cytokines (Direito *et al.*, 2021). Additionally, phenolic acids have been shown to possess anti-parasitic activity, making it a potential candidate for the development of novel therapeutic agents. It has been reported that several herbal extract such as *Raphanus sativus*, *Ceratonia siliqua*, and *Cuminum cyminum* methanol extracts could be promising sources of new medicament for toxoplasmosis (Elazab and Arafa, 2022). The effect of *S. officinalis* against *T. gondii* is attributed to its bioactive compounds, primarily rosmarinic acid and caffeic acid, which exhibit anti-parasitic properties. These polyphenolic compounds disrupt the parasite's membrane structure and function, leading to the inhibition of *T. gondii*'s growth and replication. Specifically, bioactive interfere with the parasite's mitochondrial function, causing a depletion of ATP and ultimately leading to apoptosis. Additionally, *S. officinalis* compounds have been shown to modulate the host's immune response, enhancing the production of pro-inflammatory cytokines and activating immune cells, such as macrophages and neutrophils, to combat *T.gondii* infection. In the present study, the efficacy of the combined use of spiramycin and *S. officinalis* could be roughly estimated through assessing the parasite load parasitologically and histopathologically. According to our findings, *S. officinalis* separately and in combined form can significantly reduce the parasite load in the examined treated group (GIV and GV), revealing that the animals treated with *S. officinalis* exhibited a significant reduction in the parasite load compared to the positive control group with a drug efficiency (71%). While, the animals treated with combined spiramycin and *S. officinalis* exhibited a significant reduction in the parasite load compared to the positive control group with drug efficiency (80%). In respect to the histopathological examination, the stained brain tissue smears obtained from groups treated with *S. officinalis* alone showed mild neuronal degeneration with a few scattered lymphocytes normal vascular

pattern and interstitial oedema, while the group treated with combined Spiramycin and *S. officinalis* showed almost intact neurons preserved capillary pattern and focal pericellular oedema.

Overproduction of pro-inflammatory cytokines causes tissue injury, which triggers the release of anti-inflammatory cytokines, which promotes *Toxoplasma* growth (Sana *et al.*, 2022). One important cytokine that helps hepatocytes manufacture acute phase proteins is tumour necrosis factor alpha (TNF alpha) (Atmaca and Atmaca, 2022). In this study, TNF Alpha levels were significantly decreased during infection ($P < 0.0001$). All treated groups GIII, GIV and GV showed a significant increase in TNF Alpha levels compared to infected untreated group. On the other hand, the cytokine (IL-10) serum level was significantly elevated during infection and a reduction of IL-10 levels was detected in all treated groups GIII, GIV, GV. This result was in the line with Denis *et al.*, (2022) who studied different 11 cytokines and reported an elevation of all cytokine levels with acute infection.

The herb's anti-inflammatory properties can help regulate the immune system, potentially improving the body's ability to fight off the infection. Additionally, the current study indicates that the extract from sage may have a direct parasitological effect, meaning they can kill the parasite directly. This is likely due to the combined action of various bioactive compounds within the plant. The highest TNF Alpha levels were detected in GV, who received combined treatment with combined Spiramycin and *S. officinalis*. The lowest IL-10 level was detected in GV, received combined treatment with combined Spiramycin and *S. officinalis*.

Conclusion

In this respect, the potential use of *S. officinalis* as natural antioxidants is worth considering as possible novel anti-*Toxoplasma*. Furthermore, *S. officinalis* anti-parasitic properties may be exploited in the development of novel therapeutic strategies targeting *T. gondii*. Additionally, the use of *S. officinalis* in animal feed or as a natural pesticide could help reduce the transmission of *T. gondii* in livestock and the environment, ultimately minimizing the risk of human infection.

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