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EFFICACY OF TOPOISOMERASE INHIBITOR II (LEVOFLOXACIN) COMBINED WITH *ALLIUM SATIVUM* ON EXPERIMENTAL CEREBRAL TOXOPLASMOSIS INFECTED MICE

By

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Abstract

Toxoplasmosis is a worldwide parasitic disease that affects about one third of the population. The infection may range from asymptomatic to severe deadly in immunocompromised patients. Unfortunately, the available drugs are toxic and cannot eradicate bradyzoites in chronic disease. The study evaluated levofloxacin combined with *Allium sativum* compared with trimethoprim + sulphamethoxazole to treat experimental cerebral toxoplasmosis in mice infected with Me49 cystogenic strain. The study included normal control group (GI), infected control group (GII), levofloxacin-treated group (GIII) (90 mg/kg/day starting on the 4th d.p.i. and continued for 7 days), Sutrim-treated group (GIV) (Trimethoprim at a dose 30 mg/kg/day + Sulphamethoxazole at a dose of 150 mg/kg/day starting on 4th d.p.i and continued for 30 days), and the combined LVX+*A. sativum*-treated (GV) (LVX was given as described in GIII and *A. sativum* was given at a dose of 500mg/kg/day started on the 4th d.p.i up to 30 days). The experiment was terminated on the 45th d.p.i. Giemsa-stained impression smears from brain tissues of each mouse were prepared to determine parasitic load. Histopathological and immunohistochemical studies were done. Serum samples were prepared for immunological (IL-10, IL-12, IL-17, IFN-ɤ) and biochemical studies (iNOS, AST, ALT, urea, creatinine). The best results were obtained in GV, with a significant reduction (92.77%) in brain cyst count with improved histopathological findings. There was a significant decrease in IL-10 and significant increases in IL-12, IL-17, IFN-ɤ, iNOS. Liver and renal functions biochemical studies showed safety of this combination.

Keywords: *Toxoplasma gondii*, ME49, Topoisomerase inhibitors, Levofloxacin, *Allium sativum*, autophagy, iNOS

Introduction

Toxoplasma gondii is an obligate intracellular parasite (Abbas *et al*, 2020) that belongs to the phylum Apicomplexa (Kim and Weiss, 2004). This parasite is the causative agent of a worldwide zoonotic disease affecting about one third of the population (Duffy *et al*, 2019).

In Egypt, *T. gondii* infections were highly prevalent in humans and domestic animals, and up to 95% of domestic cats, definitive host, were infected and spread oocysts in the environment (Abbas *et al*, 2020). The risk factors included residency in rural areas, cats' contact, and consumption of undercooked meat and raw or not well washed fruits and vegetables (Taman and Alhusseiny, 2020).

During the acute phase, tachyzoites rapidly invade nucleated cells and begin to replicate. The parasite establishes chronic infection when tachyzoites evade the immune system leading to the formation of tissue cysts containing bradyzoites (Skariah *et al*, 2010) in neurons, microglia, astrocytes and in muscles, where they might persist long-life in the host (Berenreiterová *et al*, 2011). According to the *Toxoplasma* strain and the host immune status, the toxoplasmosis course may range from asymptomatic to severe complications up to fatal (Dupont *et al*, 2012). In the immunocompetent individuals, the disease is usually asymptomatic, but might be fatal in immunocompromised patients such as in the AIDS, cancer, and transplantations (Cong *et al*, 2015).

Despite the great impact of toxoplasmosis, only a few drugs are available to treat the

patients. Treatment choices included pyrimethamine®, sulfadiazine®, atovaquone®, and clindamycin® (Romand *et al,* 1993). But, these drugs were more or less with many side effects and didn't eradicate the bradyzoites (Dittmar *et al*, 2016).

The discovery of apicoplast in apicomplexan parasites had led to the discovery of new targets for therapy against those parasites (Köhler *et al*, 1997). The apicoplast contains many metabolic pathways that are essential for parasite survival (Goodman and McFadden, 2013). Some of these pathways are of prokaryotic origin, which represent interesting targets for the development of specific anti-parasitic compounds with limited toxicity to host cell pathways of eukaryotic origin (Martins-Duarte *et al*, 2015).

Fluoroquinolones are known DNA replication inhibitors that target prokaryotic type II topoisomerases; DNA gyrase & topoisomerase IV (Collin *et al*, 2011). They inhibit subunits A of the apicoplast's DNA gyrase with subsequent inhibition of apicoplast genome replication and parasite viability (Ram *et al*, 2007). The antibiotic ciprofloxacin, a fluoroquinolone inhibitor of type II topoisomerase, was found effective against *T. gondii* tachyzoites *in vitro* (Dubar *et al*, 2011). Besides, the ciprofloxacin derivatives were found to increase the survival of mice infected with the *T. gondii* RH strain as a model for acute toxoplasmosis (Martins-Duarte *et al*, 2015). Ciprofloxacin-loaded with silver nanoparticles proved to be effective against chronic toxoplasmosis (Rashed *et al*, 2022).

Owing to the drawbacks of the available drugs especially in immunocompromised patients, plant therapy proved effective and helpful in developing immunity (Anand *et al*, 2015). Rivlin (2001) in USA reported that garlic was in use at the beginning of recorded history and was found in Egyptian pyramids and ancient Greek temples, with Biblical references to garlic. He added that ancient medical texts from Egypt, Greece, Rome, China & India each prescribed medical garlic applications. Zugaro *et al.* (2023) in Italy stated that clinical studies of <2000 found that dietary garlic intake has beneficial health effects, such as antioxidant, anti-inflammatory, antitumor, antiobesity, antidiabetic, antiallergic, cardioprotective, antioxidative and hepatoprotective effects, as well as antiparasitic (Aboul-Nour *et al,* 2016).

This study aimed to evaluate Levofloxacin® as one of topoisomerase inhibitors type II combined with *Allium sativum* as an immunomodulator and antiparasitic natural agent compared with trimethoprim® and sulphamethoxazole® combination to treat cerebral toxoplasmosis in male mice experimental infected with Me49 cystogenic strain.

Materials and Methods

Ethical approval: The study was conducted after the International Declaration Guidelines of Helsinki (2008). The study protocol was approved by the Scientific Research Ethical Committee, Faculty of Medicine Menoufia University (IRB: 10/2022PARA4-2).

Experimental animals: Swiss Albino mice aged 6-8 weeks and weighed about 25±0.2g were kept in the experimental room under controlled temperature and humidity conditions (25°C; 70%). They were fed with commercial ration and water *ad libitum* and kept for 15 days adaptation period before being experimented with.

Parasite and infective inoculum: Avirulent *T. gondii* Me49 strain was kindly provided by the National Research Center, Dokki, Giza. Infection was regularly maintained by repeated passage in Swiss albino mice with 0.1ml of brain homogenate of infected mice with about 100 tissue cysts/ml every 8 weeks to establish chronic toxoplasmosis (Djurković-Djaković *et al,* 2002). Each infected mouse received 0.2ml of brain cysts suspension containing 10 cysts.

Tested drugs: 1- Levofloxacin® (LVX), LEVAQUIN® as an oral solution (25 mg/ml, Ortho-McNeil, Titusville, NJ; NDC 0045-1515-01) was used diluted in distilled water to 10.4mg/ml.

2- *Allium sativum* was purchased as 200 mg tablets (Tomex®, Atos Pharma, for prod-

uction of medicinal herbs, Cairo, Egypt) and tablets were dissolved in distilled water.

3- Trimethoprim® (40mg) and Sulphamethoxazole® (200mg) (Sutrim, Memphis for Pharmaceuticals & Chemical Industries, Cairo) oral suspension was diluted in distilled water.

Study design: Clean laboratory breed 45 male mice were divided into five groups: GI: Normal control of five mice served as normal control, each one received 0.2ml of physiological saline orally. GII: Infected control ten infected mice were infected, but not-treated group. GIII**:** Levofloxacin-treated ten infected mice were treated with LVX at a dose of 90 mg/kg every day in 0.2ml given orally started on the 4th days post infection (d.p.i.) and continued for seven successive days (Elliott *et al*, 2015). GIV: Sutrim-treated ten infected mice with Trimethoprim (TMP) at a dose of 30mg/kg/day combined with Sulphamethoxazole (SMX) at a dose of 150mg/kg/day once daily started on the 4th d.p.i and continued for 30 days (Bottari *et al*, 2015). GV: LVX+*A. sativum*-treated ten infected mice with LVX as given in GIII and *A. sativum* at a dose of 500mg/kg/d orally once daily started on the 4th d.p.i and continued for 30 days (Khalil *et al*, 2015).

Sampling: The experiment was terminated on the 45th d.p.i and all mice were anesthetized and sacrificed. For each mouse, blood was collected to separate serum by centrifugation at 3000rpm for 10 minutes and stored at -80°C until required for immunological and biochemical studies.

Histopathological study: Brain from each mouse was fixed in formalin 10%, dehydrated in an ascending series of ethanol, cleared in xylol, for paraffin processing, sectioned (5µm), and stained with hematoxylin & eosin (Drury and Wallington, 1980).

Immunohistochemical studies for ATG-5 of brain sections using antibodies related protein-5 (ATG-5) (Anti-APG5L/ATG5 antibody, ab109490, Abcam, USA) as a marker for autophagy. Briefly, paraffin embedded sections were rehydrated and incubated for 20min. in methanol contained H_2O_2 (10%), incubated with the primary antibody, counterstained with Mayer's hematoxylin, dehydrated, cover slipped, and examined by an optical microscope with a photo camera (Leica, Germany).

Measurement of IL-10, IL-12, IL-17 and IFN-γ by ELISA: Serum concentrations of IL-10, IL-12, IL-17 & IFN-γ (Phar Mingen, USA) cytokines were measured by quantitative sandwich ELISAs using specific monoclonal anti-cytokine antibodies kit protocols. The supplied recombinant cytokines were used as standards. The reactions were read using a microplate ELISA reader at 405 nm. The cytokine concentrations were expressed in pg/ml from standard curves.

Biochemical studies: Serum iNOS level was measured by performing the reaction after the standard protocol (Sigma, USA) and the absorbance was read at 540nm. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities were measured in mice sera according to kits protocols (Sigma-Aldrich, USA). The reactions were read at 450nm and the activities were expressed as U/L. Serum urea and creatinine (Abcam, USA) were measured to evaluate renal functions, and the reactions were expressed as mg/dl.

Treatment efficacy: 1- Mortality rate during the study, and 2- Parasite load by examining the brain smears after air-dried, fixed with methanol for 15 min, and stained with 10% of Azur-eosin Giemsa stain (MERCK, Germany) for an hour. Stained slides were washed with water, dried, and examined by a research microscope oil immersion lens. Cysts number was counted as mean of ten fields/mouse, and reductions % were calculated by the following equation: $R\% = \frac{100(C-E)}{C}$

R%= reduction%, C= control group, and E= experimental group (Penido *et al*. 1994)

Statistical analysis: Data were computerized and analyzed by SPSS statistical package version 20. Chi-square (X^2) test was used to assess the relation between two or more qualitative parameters. ANOVA (F-test)

and Kruskal-Wallis test (K-test) were used to assess the significance between quantitative variables, followed by a post Hoc test and $P < 0.05$ was considered significant.

Results

The study was done on 40 infected mice of 4 groups of ten mice each and fifth one of 5 normal control. By the study end, three mice from each of GII & GIII, two from GIV, and one mouse from GV died without significant difference between groups (P=0.54).

Giemsa-stained smears from different all groups showed a significant difference between infected control and all treated groups (P<0.001). LVX+ *A. sativum* gave the highest significant reduction in mean number of cysts count in brain tissues (R= 92.77%) as compared to Sutrim treated group, which showed a reduction of 73.2%, but LVX alone showed least reduction (R= 16.19%).

Examination of normal control brain (GI) showed normal histological architecture. Infected control (GII) showed numerous cysts and mild inflammatory reaction. Also, LVX-treated (GIII) showed brain cysts and mild inflammation. Sutrim-treated (GIV) showed fewer cysts and improvement of brain architecture, while combined LVX+ *A. sativum*-treated (GV) showed an almost normal brain with characteristic glial cells without cysts.

Brain parenchyma by ATG5 of normal control (GI) showed normal architecture. Brain from infected control (GII) showed *T.gondii* cysts. Brain from LVX-treated (GIII) showed a cyst surrounded by strong expression of ATG5 in the adjacent astrocytes. Brain from Sutrim-treated (GIV) showed a devitalized cyst surrounded by strong expression of ATG5 in adjacent astrocytes. Sections from LVX+*A. sativum*-treated (GV) showed neither devitalized cysts nor cysts with normal expression of ATG5.

IL-10, in infected control (GII) showed a highly significant increase (338.3±8.01) as compared to normal control (GI). All treated groups showed significant decrease in IL-10 as compared to infected control (GII), with combined treated (GV) showed lowest value (P< 0.001). IL-12, in infected control (GII) showed a significant increase as compared to normal control (P< 0.001). All treated groups showed elevated levels as compared to GI, with the highest one in combined-treated (GV) and the lowest in the LVX-treated (GIII).

As to IL-17, there were significant increases in infected control (GII) and all treated (GIII, GIV, & GV) as compared to normal control. The highest level was in combined treated (17.13±0.9) with significant difference as compared to all others (P< 0.001).

Regarding IFN-γ, there were significant increases in the infected control group (GII) and all the treated groups when compared to normal control (GI) (P< 0.001). Among treated groups, the lowest (67.51±2.15) was found in LVX-treated (GIII) and the highest level was measured in the combined-treated group (111.2±2.76), but difference was significant as compared to others (P< 0.001).

Concerning iNOS serum levels, there were significant increases (P< 0.001) in infected and all treated groups as compared to normal control (GI). The highest number was in the combined LVX+ *A. sativum*-treated group (55.37±2.83), but the lowest value was in the LVX-treated (20.56±0.82). The differences between GV and others were statistically significant (P< 0.001).

As to AST &ALT results, infected control showed significant increases (56.63±3.82 & 56.16±3.53, respectively) as compared to normal control (P< 0.001). All treated groups showed a significant increase when compared to normal control and infected control groups. The highest one was in Sutrim-treated group (72.03±2.26 & 77.48±2.61, respectively). Serum urea showed an obvious increase in all treated groups as compared to normal control. Among treated groups, the lowest (42.41±1.33) was in LVX+ *A. sati-v-um*-treated group that was significantly lower than LVX-treated (48.53±2.23) and Sutrim-treated (46.49±2.39) with (P< 0.001), but without significant difference between GV and GI .

There were significant increases in serum creatinine levels in all treated groups as compared to normal control (GI) and infected control (GII) with (P< 0.001), but without significant differences among treated groups (P> 0.05), and within normal range.

Details were given in tables (1, 2, 3 & 4) and figures (1, 2 & 3).

Table 1: Mortality rates among different groups

Variations	GI (n = 5)	GII (n= 10)	GIII (n = 10)	GIV (n = 10)	GV (n = 10)	Total	X^2	*P*- value
Dead mice	0	3	3	2	1	9	3.31	0.54
Percentage %	0	30	30	20	10	20		

Table 2: Brain cyst count and reduction percentages among groups

Variations	GI (n = 5)	GII (n = 7)	GIII (n = 7)	GIV (n = 8)	GV (n = 9)	ANOVA	*P*-value
Cyst count	-	12.17±1.5	10.2±1.47	3.26±0.74 [b c]	0.88±0.18 [b c d]	201.83	<0.001
Reduction%	-	-	16.19%	73.2%	92.77%		

[a] significance when compared to GI, [b] significance when compared to GII, [c] significance when compared to GIII, and [d] significance when compared to GIV.

Table 3: Serum cytokine levels (pg/ml) measured by ELISA

Variations	GI (n = 5)	GII (n = 7)	GIII (n = 7)	GIV (n = 8)	GV (n = 9)	ANOVA	P-value
IL-10	114.7±4.07	338.3±8.01 [a]	161.1±4.67 [a b]	128.4±7.52 [a b c]	125.1±4.43 [a b c]	1606.25	<0.001
IL-12	82.38±8.46	116±3.89 [a]	107.4±7.86 [a b]	112.3±2.94 [a]	127.1±4.27 [a b c]	46.20	<0.001
1L-17	3.32±0.66	8.46±0.54 [a]	8.26±0.59 [a]	13.96±0.72 [a b c]	17.13±0.9 [a b c d]	395.45	<0.001
IFN- ɤ	40.7±6.37	78.91±3.03 [a]	67.51±2.15 [a b]	103.2±6.72 [a b c]	111.2±6.76 [a b c d]	242.95	<0.001

[a] significance when compared to GI, [b] significance when compared to GII, [c] significance when compared to GIII, and [d] significance when compared to GIV.

Table 4: Serum iNOS, AST, ALT, urea, and creatinine

Variations	GI (n = 5)	GII (n = 7)	GIII (n = 7)	GIV (n = 8)	GV (n = 9)	ANOVA	P-value
iNOS (µM)	16.8±1.53	49.97±1.44 [a]	20.56±0.82 [a b]	50.79±1.92 [a c]	55.37±2.83 [a b c d]	717.35	<0.001
AST (U/L)	25.34±1.95	56.63±3.82 [a]	62.33±3.38 [a b]	72.03±2.26 [a b]	62.1±2.62 [a b d]	219.68	<0.001
ALT (U/L)	26.74±2.83	56.16±3.53 [a]	69.91±2.42 [a b]	77.48±2.61 [a b c]	61.31±2.44 [a b c d]	296.47	<0.001
Urea (mg/dl)	40.5±1.62	40.87±1.74	48.53±2.23 [a b]	46.49±2.39 [a b]	42.41±1.33 [c d]	23.79	<0.001
Creatinine (mg/dl)	0.8±0.1	0.84±0.08	1.01±0.11 [a b]	1.09±0.13 [a b]	1.06±0.1 [a b]	10.2	<0.001

[a] significance when compared to GI, [b] significance when compared to GII, [c] significance when compared to GIII, and [d] significance when compared to GIV.

Discussion

In the current study, the anti-parasitic efficacy of levofloxacin combined with *A. sativum* in comparison with trimethoprim and sulphamethoxazole (Sutrim) against cerebral toxoplasmosis induced by ME49 avirulent cystogenic strain of *T. gondii* in experimental mice. To study this efficacy at the early stage, treatment was administered orally on the 4th day of infection and continued for 30 days. At this early stage, the drugs suggested to target the newly released bradyzoites, rapid replicating tachyzoite, immature bradyzoites and newly formed brain cysts (Abou-El-Naga and Mogahed, 2021).

In the present study, LVX alone reduced the mean number of *T. gondii* cyst count in mice brain tissues (R%= 16.19), while LVX combined with *A. sativum* resulted in the most significant reduction (R%= 92.77) when compared to Sutrim treatment, which showed a reduction percentage of 73.2%. These results were confirmed by the histopathological findings, which showed corresponding improvements in cyst count and brain inflammation in the treated groups. These histopathological findings in the infected control group were in harmony with other authors (Etewa *et al*, 2018; GabAllah *et al*, 2021; Omar *et al*, 2022). The obtained results in LVX-treated group agreed with Rashed *et al*. (2022). Also, the combined treated group results agreed with utilized medicinal plants against *T. gondii* avirulent Me49 strain, for instance, *Thymus vulgaris* (Eraky *et al*, 2016), curcumin nanoemulsion (Rageh *et al*, 2022), and artemisinin derivatives artemiside and artemisone (Müller *et al*, 2023). Moreover, *Allium sativum* essential oil was effective against *T. gondii* RH strain (Alnomasy, 2021).

The anti-*Toxoplasma* effects of *A. sativum* were related to its organosulfur compounds, which act by disrupting DNA, RNA, and pr-

otein synthesis, and damaging the cell wall and membrane (Bhatwalkar *et al*, 2021). Also, *A. sativum* strengthens the cellular immune response by stimulation of some immune cells, such as lymphocytes, macrophages, and natural killers, as well as modulation of cytokine secretion (Arreola *et al*, 2015).

The present LVX results agreed with several studies which documented the efficacy of fluoroquinolones against apicomplexan parasites. As DNA gyrase inhibitors, fluoroquinolones reduce the religation of cleaved DNA, leading to fragmentation and cell death (Nagano *et al*, 2014). For example, ciprofloxacin resulted in cleavage of apicoplast DNA in *P. falciparum*, without affecting the nuclear DNA (Prusty *et al*, 2010). Also, exposure of *T. gondii* to ciprofloxacin during replication resulted in a decrease in the apicoplast genome copy number (Fichera and Roos, 1997). Moreover, enrofloxacin significantly reduced the parasite load and brain inflammation caused by *T. gondii* (Barbosa *et al*, 2012; Dalhoff, 2015). Furthermore, levofloxacin was found to be effective against lung cancer cells *in vitro* and *in vivo* through inhibition of mitochondrial respiration and ATP production and induction of oxidative damage (Song *et al*, 2016). Additionally, levofloxacin was found to have immunomodulatory actions (Dalhoff and Shalit, 2003).

In the present work, levofloxacin at a dose of 90 mg/kg resulted in a 16.19% reduction in cyst count in treated mice brain. This result was lower than the obtained by Rashed *et al*. (2022) who documented a reduction percentage of 29.5%; however, this could be attributed to using ciprofloxacin at a higher dose in their study (100 mg/kg). The best cyst count reduction (92.77%) was obtained with the combined treatment. This finding may be attributed to a synergistic action between LVX and *A. sativum* which has antiparasitic (Toulah and Al-Rawi, 2007), antioxidative (Banerjee *et al*, 2003), and immunomodulatory (Clement *et al*, 2010) activites. Besides, Sutrim treatment resulted in a 73.2% reduction in cyst count. This agreed with Abou-El-Naga and Mogahed (2021), in their study on experimental cerebral toxoplasmosis. Also, the antiparasitic chemotherapeutic utilized to treat toxoplasmosis only limits the tachyzoites proliferation, but once they convert to bradyzoites, these drugs showed poor effects (Montazeri *et al*, 2017).

Autophagy (meaning exactly self-eating) is an adaptive response controlled by the lysosomal compartment, to provide energy needed for cell homeostasis and repair under stress conditions by degrading long-lived proteins and damaged organelles (Nahdi *et al*, 2010; Besteiro, 2012). Also, it is an important playing defense against microbial pathogens including parasitic protozoa (Gomes and Dikic, 2014). Autophagy allows the delivery of intracellular pathogens to the lysosomes for their degradation in a process called xenophagy. The generated microbial antigens through this process utilized for the activation of innate and adaptive immunity (de Laté *et al*, 2017). However, as *T. gondii* is an intracellular protozoan parasite that replicates inside the parasitophorous vacuole protected from lysosomal fusion, it was able to lead host cell autophagy for its own benefit (Orlofsky, 2009; Lee *et al*, 2013). Autophagy pathway contains several molecules and receptors such as ATG3, ATG5, ATG7, ATG12, & ATG16L1. Moreover, among a series of autophagy proteins, Atg5 was linked to the IFN-γ–mediated anti-*T. gondii* effector mechanisms (Zhao *et al*, 2008).

In the present study, ATG5 as a marker evaluated autophagy in mice brain tissues by immunohistochemistry. The results showed a strong expression of ATG5 in astrocytes around *T. gondii* cysts in brain tissues from LVX-treated and Sutrim-treated groups. But, the combined-treated group showed normal expression. The infected control group sho wed parasitic cysts with normal ATG5 expression around them. Thus, partial efficacy of LVX alone and Sutrim resulted in an oxidative stress state, which stimulated the autophagy pathway as the host protective mec-

hanism against brain damage. While in the combined group, potentiating antiparasitic and immunomodulatory actions of LVX and *A. sativum* reduced the oxidative stress and inhibited the autophagy process. This agreed with Nahdi *et al*. (2010), who documented cytoprotective effects of crude garlic extract through reducing the iron-induced oxidative stress and autophagy in rats.

Regarding IL-10 and IL-12, the infected control group (GII) showed a significant increase in comparison with the normal control (GI). All treated groups showed elevations of IL-10 when compared to the normal control. Interestingly, the combined-treated group (GV) showed the lowest value among all treated groups. This agreed with Anand *et al*. (2015), who reported high levels of IL-10 in the untreated groups. Also, Dupont *et al*. (2012) reported that the regulatory IL-10 antagonizes the ability of macrophages to kill intracellular parasites, such as *T. gondii*, and IL-10 expression increased with untreated infection suggested that parasite stimula ted IL-10 production evading the immune response.

As regards IL-17, there were significant increases in the infected control (GII) and LVX-treated (GIII) when compared to the normal control (GI). A significant higher increase was found in Sutrim-treated (GIV). The highest level was measured in the combined treated (GV). This agreed with Anand *et al*. (2015), who documented that adaptive cytokines such as IL-2 & 1L-17 were found to be highly expressed in treated groups compared with the untreated control one.

Regarding IFN-γ, there were significant increases in the infected control (GII) and all the treated groups when compared to normal control (GI). Among the treated groups, the lowest was found in LVX-treated (GIII) and the highest level was measured in the combined-treated (GV). Similar results were obtained by Anand *et al*. (2015). The increase in IFN-γ during *T. gondii* infection depended on IL-12 stimulating natural killer (NK) cells and T cells to produce IFN-γ (Gazzinelli *et al*, 1994). Also, increased IFN-γ promoted several intracellular mechanisms inhibiting parasite replication (Dupont *et al*, 2012). Therefore, in the present study, the highest level of this cytokine was measured in the combined treated group that showed the best results as to *T. gondii* cyst count reduction and histopathological improvement. This agreed with Foroutan-Rad *et al*. (2017), who reported that *A. sativum* has immunomodulatory activity by creating a shift in cytokine production pattern from TH2 to TH1 that led to development of a strong cell-mediated immunity and reduced the duration of treatment.

Concerning iNOS serum levels, there were significant increases in the infected and all the treated groups when compared to normal control (GI). The highest was measured in the combined treated group and lowest value among treated ones was in the LVX-treated group. The increased levels of iNOS in the infected and treated groups agreed with Mordue and Sibley (2003), who found that monocytes killed and inhibited the replication of *T. gondii in vitro* by the expression of inducible nitric oxide synthase (iNOS) enzyme stimulated by IFN-γ (Zhao *et al*, 2009). Also, Dincel and Atmaca (2015) rep-orted that nitric oxide triggers the conversion of tachyzoite to bradyzoite with parasiticidal effects on *T. gondii*. In the present work, the highest serum level of iNOS in combined treated group correlated with the significant reduction in cyst count with histopathological improvement in this group.

Regarding AST & ALT liver enzymes levels, the infected control group showed significant increases when compared to normal control group. Moreover, all treated groups showed significant increases when compared to the normal and infected control groups. The highest values were in the Sutrim-treated group. The significant increases in ALT and AST levels in the infected control group agreed with GabAllah *et al*. (2021). The hepatotoxic effect of Sutrim was documented in several studies (Bell *et al*, 2010; Slim *et*

al, 2017; Green *et al*, 2020). Also, The LVX induced hepatotoxicity (Schloss *et al*, 2018). However, lower values were obtained in the combined treated group which could be attributed to *A. sativum* hepatoprotective effect (Chinnala *et al*, 2018; Guan *et al*, 2018).

As regards to serum urea results, there were obvious increases in all treated groups when compared to normal control group. Among the treated groups, the lowest was recorded in the combined-treated group. There were significant increases in serum creatinine values in all treated groups when compared to normal and infected control ones. However, these increases, the values were in the normal range indicating the safety of all drugs on kidney function tests. Also, lower values were obtained in combined treated group, which could be attributed to the renal prot-ective properties of *A. sativum* (Shang *et al*, 2019; Dorrigiv *et al*, 2020).

Conclusion

The study showed that levofloxacin® as a replication inhibitor acts synergistically with *A. sativum* cytoprotective effect on experimental Me49 strain cerebral toxoplasmosis during early stage. The combination was better than the conventional drug Sutrim (trimethoprim and sulphamethoxazole) regarding all assessed parameters.

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Authors's contribution: The authors equally contributed in the theoretical and practical study.

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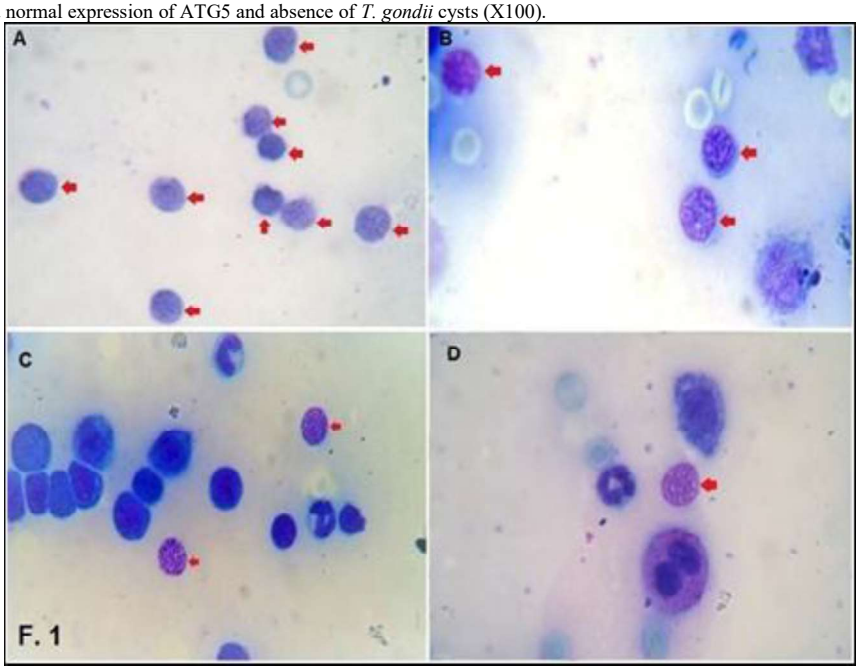
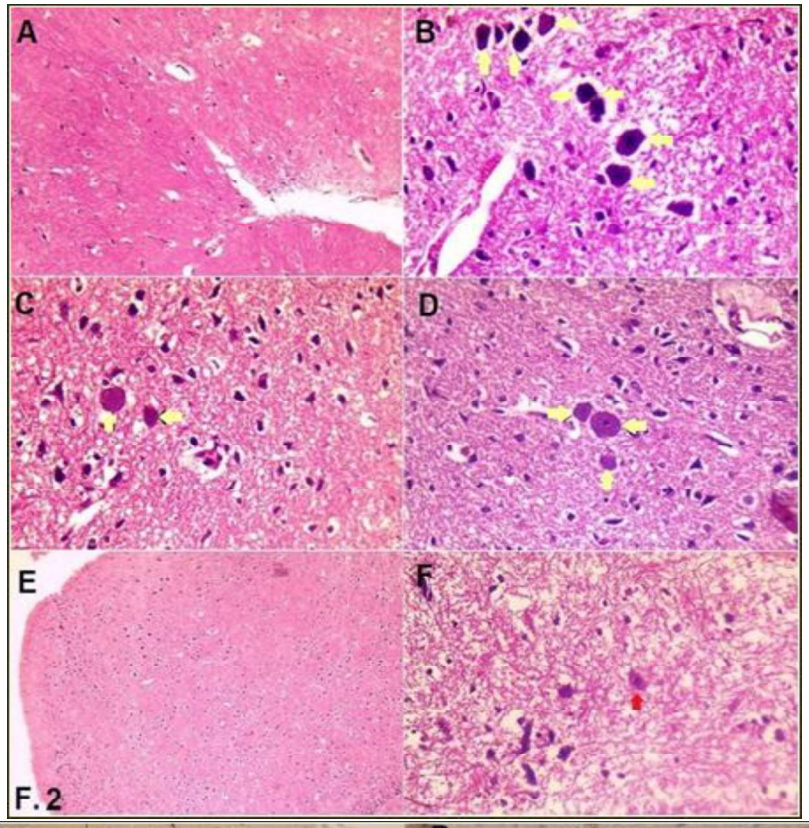
Explanation of figures

Fig.1**:** Brain impression smears showing *T.gondii* tissue cysts (red arrows) containing bradyzoites. A) from infected control (GII), B) from LVX-treated group (GIII), C) from sutrim-treated group (GIV), D) from combined LVX and *A. sativum*- treated group (GV) (Giemsa stain, X100).

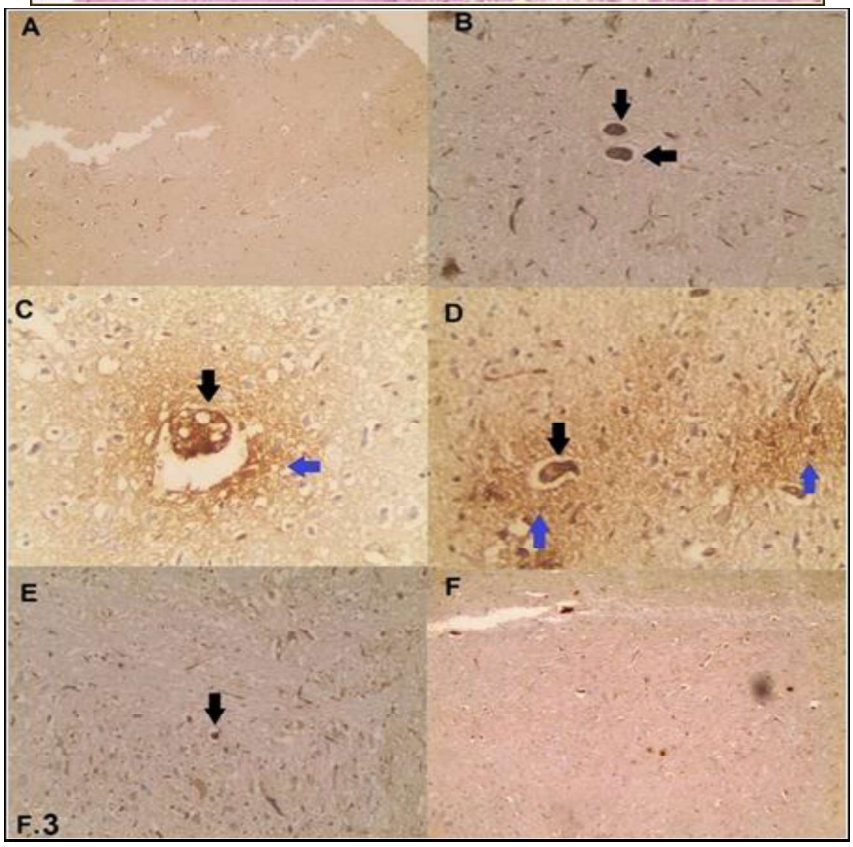
Fig. 2: A) Mouse brain of mice from normal control (GI) showed normal histological structure (H & E, X100). B) Mouse brain from infected control (GII) infected showed numerous *T. gondii* cysts (yellow arrows) (H & E, X400). C) Mouse brain treated with LVX (GIII) showed few *T. gondii* cysts (yellow arrows) (H & E, X400). D) Mouse brain treated with Sutrim (GIV) showed few *T. gondii* cysts (yellow arrows) (H & E, X400). E) Mouse brain treated with combined LVX and *A. sativum* (GV) showed almost normal brain tissue free of cysts (H & E, X100). F) Higher power view of section E showing normal brain parenchyma with characteristic glial cells (red arrow) (H & E, X400).

Fig. 3: Sections of brain parenchyma immunostained by ATG5. A) Mouse brain from normal control (GI) showed normal architecture (X100). B) mouse brain from infected control (GII) showed *T.gondii* cysts (arrows) (X200). C) mouse brain from LVX-treated (GIII) showed *T. gondii* cyst (black arrow) surrounded by strong expression of ATG5 in adjacent astrocytes (blue arrow) (X400). D) mouse brain from Sutrim-treated (GIV) showed devitalized cyst (black arrow) surrounded by strong expression of ATG5 in adjacent astrocytes (blue arrow) (X400). E) mouse brain from LVX and *A. sativum*-treated (GV) showed devitalized small cyst (black arrow) (X100). F) mouse brain from GV showed normal expression of ATG5 and absence of *T. gondii* cysts (X100).





F. 2



F.3