ABSTRACT

Toxoplasmosis is a cosmopolitan parasitic disease that impacts enormous population sector. In this work we investigated, the antitoxoplasmic therapeutic effect of one synthetic drug; nitazoxanide (NTZ) and another agent of plant origin; *Zingiber officinale* (ginger) as adjuvant to the traditional antitoxoplasmic drugs (pyrimethamine & sulphadiazine) on experimental murine toxoplasmosis (Me 49 avirulent strain). Animals were classified into 7 groups; G I: Non-infected, non-treated (normal control), GII: Infected non-treated mice (infected control), GIII: Infected & treated by a combination of pyrimethamine & sulphadiazine, Group IV: Infected & treated by *Zingiber officinale*, GV: Infected & treated by nitazoxanide (drug control), GVI: Infected & treated by combination of *Zingiber officinale* and pyrimethamine & sulphadiazine, Group VII: Infected & treated by combination of *Zingiber officinale* & nitazoxanide. Brain cyst count and histopathological changes using hematoxylin and eosin (H & E) were used to evaluate the efficacy of tested drugs. In the present study, Group VI achieved the best results among all studied groups as the reduction rate of the mean brain cyst count was 58.8% The corresponding measurements for other groups were lower than group VI as following: 50.9%, 28%, 38% and 40.6% for groups III, IV, V, and VII respectively. Histopathological studies showed obvious correlation with the results of brain cyst counts. This denotes that nitazoxanide could be an acceptable standardized, characterized, already FDA approved and commercially available substitute to traditional antitoxoplasmic synthetic drug. Also *Zingiber officinale* would be a safe and beneficial adjuvant treatment that potentiates the antitoxoplasmic action of the traditional treatment of toxoplasmosis. More studies are needed to address the dose response relationship of both NTZ and *Zingiber officinale* used for treatment for chronic toxoplasmosis.
INTRODUCTION

More than one billion people worldwide are anticipated to harbor Toxoplasma gondii (T.gondii) infection frequently with many lifelong health consequences. Toxoplasmosis is a significant cause of food borne, inflammatory diseases, as well as congenital malformations (Oz, 2014). In Egypt a study by (Kamal et al., 2015) stated that the T. gondii infection seroprevalence among the high risk pregnancy group was 50.8% versus 8.3% among the normal pregnant women. Toxoplasmosis is implicated in post-delivery adverse pregnancy outcomes at a ratio of 80.3% and abortion was the highest detected complication. Ocular toxoplasmosis is a vision-threatening disease and toxoplasmatic retinochoroiditis is the most widespread cause of posterior uveitis in immunocompetent patients (Dukaczewska et al., 2015) Besides, toxoplasmosis is deadly in the immunocompromised individuals such as cancer patients under chemotherapy (Jiang et al., 2015). Presently used therapies are ineffective for persistent chronic disease and congenital toxoplasmosis or have severe side effects which may result in life-threatening complications. There is a pressing need for safe and effective therapies to treat this broad-based contagious and inflammatory disease (Oz, 2014). Spiramycin monotherapy has been used for prophylaxis and treatment of fetomaternal toxoplasmosis. Spiramycin monotherapy is effective in early pregnancy as a preventive measure but not after fetal exposure to the infection. In a prospective cohort trial in Brazil 58% of newborns from spiramycin-treated women, in contrast to over 73% from untreated ones had congenital infection (Avelino et al., 2014). The combination of pyrimethamine and sulphadiazine remains the mainstay for treatment and prophylaxis of the majority of clinical presentations of toxoplasmosis. However, this therapeutic regimen is not always suitable for lengthened treatment because of emergence of undesirable side effects and it may contribute to clinical failure by electing drug-resistant parasite strains. Consequently, new therapies are vitally needed (Reich & Mackensen, 2015). There is a mounting alertness of the therapeutic potential of natural products and medicinal herbs that are habitually believed to be less toxic and seem free from side effects than synthetic drugs, particularly in pregnant women where the administration of empiric antimicrobial therapy is unsafe measurement. (Hökelek and Bronze, 2015). Many herbal plants extracts exhibit anti-Toxoplasma activity including Myrrh (AL-Zanbagi, 2007), Piper nigrum, Capsicum frutescens, Curcuma longa (AL-Zanbagi, 2009), Nigella sativa (Rayan et al., 2011). Ginger (Zingiber officinale) belongs to Zingiberaceae family, it is one of the famous spices globally. It has been used for curing menstruation disorder, cardiac problems, food poisoning, osteoarthritis, cough, nausea, inflammation, motion sickness, epilepsy, cold, menstrual cramps, cancer and many more. Additionally, it also exhibits antimicrobial and antioxidant properties. Medicinal value of ginger and its knowledge supply researchers by a good platform for prospect research studies aiming for protection of human population from several diseases categories (Gupta and Sharma, 2014). Also ginger is an helpful non pharmacological management of nausea and vomiting during early pregnancy.
(Thomson et al., 2014). Ginger is used in parasitological area for treatment of Giardia lamblia (Mahmoud et al., 2014), Anisakis simplex (Lin et al., 2010), Echinococcus granulosus (Baquer et al., 2014), Schistosoma mansoni (Mostafa et al., 2011; Hassan et al., 2016), H. nana (Lin et al., 2014) and blastocystosis (Abdel-Hafeez et al., 2015). Nitazoxanide, 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide is a new nitrothiazolebenzamide compound distinguished for its activity in treating both intestinal helminthic and protozoal infections, besides, it is used as a first line treatment for cryptosporidiosis (Ali et al., 2014). Galván-Ramírez et al. (2013) reported that nitazoxanide reduced T. gondii infection in vitro more than pyrimethamine and found to be not cytotoxic to astrocytes at the administered dose. This in vivo study aimed to explore the possible efficacy of ginger as combined with classical drugs and nitazoxanide treatment against T. gondii infection as a step on the way to a potential synthetic and herbal candidates for effective and safe treatment of toxoplasmosis.

**MATERIALS AND METHODS**

This study was conducted in NRC-zoonotic diseases department. The animal experiment was carried out according to the internationally valid guidelines and the research protocol was approved by Research Ethics Committee, Faculty of Medicine, Benha University, Egypt.

**I. Parasite**

**Me 49 non-virulent strain of T. gondii** (kindly supplied by Parasitology Department, Faculty of Medicine, Alexandria University Egypt) was regularly maintained by repeated inoculation of Swiss albino mouse with 0.1 ml of brain homogenate of previously infected mice containing, approximately 1x10⁷ tissue cysts / ml every 8 weeks to establish chronic toxoplasmosis (Djaković-Djaković et al., 2002).

The mice brains were ground with sterile pestle and mortars and diluted to a concentration of 1x10² cysts / ml obtaining brain cysts suspension.

**II. Experimental animals:**

A total of 65 laboratory-bred male Swiss albino mice, 10 weeks-old, weighing ~ 30-40 g, were selected from the animal house of NRC. They were housed in plastic cages (5 mice/cage) with white wood chips for bedding, fed by commercial complete food mixture and tap water for drinking and maintained under controlled conditions of lighting (12 h light/12 h dark cycle) and temperature (25±2ºC).

**III. Drugs and Plant Materials:**

**Sulfadiazine and pyrimethamine:**

Sulfadiazine: (Dohms Laboratories) and pyrimethamine (Sigma Chemical Co., St.Louis, Mo.), were provided in powder form and prepared daily as liquid suspensions; after brief sonication, the homogenized suspensions were administered orally to mice via tube feeding.

Nitazoxanide:

Nitazoxanide was available as tablets and suspension forms. In this study the suspension form (100mg/5ml) produced by Medizen Pharmaceutical Industries for Utopia Pharmaceuticals was used. Each 5ml suspension contains: Active Ingredients: Nitazoxanide 100mg Inactive Ingredients: Sucrose, pregelati-
nized starch, carboxy methyl cellulose sodium, FD & C red citric acid, acacia gum, microcrystalline cellulose, xanthan gum, sodium benzoate and strawberry flavor.

**Ginger:**

Ginger was available as tablets stored at temperature not exceeding 30°C, each tablet contained 400mg ginger extract (*Zingiber Officinale*). The tablets were crushed and dissolved in distilled water. The form used in this study was a product of Arab Co. for Pharmaceuticals and Medicinal Plants “MEPACOMEDIFOOD”, Egypt.

**IV. Experimental infection:**

Mice were inoculated orally with 0.1 ml of the brain cysts suspension (El-Sayed and Aly, 2014).

**V . Experimental Design**

Animals were classified into 7 groups (10 each) except normal control (5 mice); **Group I:** Non-infected, non-treated (normal control) and received 0.1 ml of sterile distilled water. **Group II:** Infected non-treated mice (infected control). **Group III** (drug control): Infected and treated by pyrimethamine (12.5 mg/kg/day) and sulphadiazine at a dose of 200 mg/kg/day. (Romand et al. 1993). **Group IV:** Infected and treated by *Zingiber officinale* 500mg/kg daily (Hassan et al., 2016). **Group V:** Infected and treated by nitazoxanide 200mg/kg (El-Taweel et al., 2016). **Group VI:** Infected and treated by combination of *Zingiber officinale* 150mg/kg (Sanderson et al., 2002) + pyrimethamine and sulphadiazine treatments in the same previous doses. **Group VII:** Infected and treated by combination of *Zingiber officinale* 150mg/kg (Sanderson et al., 2002) and nitazoxanide 100mg/kg (Li et al., 2003). For all treated groups treatments were administered daily at a fixed hour for 15 days starting six weeks post-infection.

**VI. Assessment of anti- Toxoplasma effects of studied drugs:**

At the end of the experiment (8 weeks), all mice were sacrificed and their brains, eyes, liver, spleen & cardiac tissues were obtained. Each brain was divided into 2 halves. One half was used for counting the brain cysts number. Other half of each brain was fixed in 10% formalin for histopathological studies. Counting the brain cysts number was done according to Djaković-Djaković et al. (2002). Then the brain cysts number in each group was calculated according to the following equation: cyst count in 100 μl × 10 × 2. After that, the slides were fixed and stained with trypan blue & examined by light microscope at x100 magnification as seen below.

**Figure (1):** (*) tachyzoites of type II toxoplasma  Arrow: cyst

In histopathological study Haematoxylin and eosin (H & E) staining was performed according to Eraky et al. (2016), then the slides were examined under the light microscope at x10, x40, x100 magnifications for the presence of any pathological changes resulting from T. gondii as necrosis, congestion and hemorrhage.

3. Statistical analysis.

The data were recorded on a report form, tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 20. ANOVA test was used to compare the means of more than 2 groups. Post hoc test (Bonferroni) for pairwise group comparison was used to assess inter-group difference between each 2 groups. The brain cyst reduction rates were assessed using the formula: (Mean value of the infected untreated group - mean value of infected- treated group) X 100 / Mean value of infected - untreated group (Abdel Salam et al., 2008). A P value <0.05 was considered statistically significant (*) while >0.05 statistically insignificant P value <0.01 was considered highly significant (**) in all analyses.

RESULTS

In table 1, a statistically significant difference between the studied groups ($F=37.905$, p value <0.001), which points to the considerable variations between them as regard their activity against chronic Toxoplasma infection. To assess the probable significant difference between groups, Post hoc test (Bonferroni) for pairwise multiple comparison was run between groups and revealed that the difference in the mean brain cyst counts was:

A significant statistical difference when comparing between infected control group and all groups that indicates an observable efficacy of all tested drug regimen.

A significant statistical difference when comparing between drug control group III (treated by combination of pyrimethamine and sulfadiazine) and all other groups except group VI, which empathized the considerable superior performance of pyrimethamine and sulfadiazine combination as antitoxoplasmic drug regimen in the chronic phase and the worthy note excellent antitoxoplasmic effect resulted from just adding ginger to the previous combination in group VI (treated by combination of pyrimethamine and sulfadiazine + ginger) which led to a lower mean brain cyst count than achieved by the drug control group VI by 7.9%.

A significant statistical difference when comparing ginger group (IV) and all other groups its brain cyst count was higher than all groups except infected control one (II).

A significant statistical difference when comparing between group V (Nitazoxanide treated) and that of all other groups except group VII (Nitazoxanide + Ginger). Its mean brain cyst number was higher than all other groups except groups II and IV.

A significant statistical difference when comparing between group VI (Pyrimethamine & sulfadiazine + Ginger) and all other groups. Its mean brain cyst count was the lowest among all studied groups.

A significant statistical difference when comparing between group VII...
(NTZ+ Ginger) and all other groups except group V (Nitazoxanide treated). Its mean brain cyst count was higher than group III and group VI only but lower than the rest of studied groups II, IV and V.

Group VI (Pyrimethamine & sulfadiazine + Ginger) achieved the best brain cyst reduction rate among all studied groups (58.8%). The other group brain cyst reduction rates were: 50.9%, 28%, 38% 40.6% for groups III, IV, V and VII (Figure 2) respectively.

Figure (2): The mean brain cyst reduction rate after different treatment regimens of chronic toxoplasmosis
Table (1): Analysis of variance in the mean brain cyst count after different treatment regimens.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Total</th>
<th>Mean no. of brain cysts</th>
<th>Pairwise Group Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Infected control (II) /b</td>
<td>10</td>
<td>602.0</td>
<td>56.73</td>
</tr>
<tr>
<td>Pyrimethamine &amp; sulfadiazine (III) c</td>
<td>10</td>
<td>295.0</td>
<td>40.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ginger (IV) d</td>
<td>10</td>
<td>433.0</td>
<td>41.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitazoxanide (V) e</td>
<td>10</td>
<td>373.0</td>
<td>30.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrimethamine &amp; sulfadiazine + Ginger (VI) f</td>
<td>8</td>
<td>248.0</td>
<td>30.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitazoxanide + Ginger (VII) g</td>
<td>10</td>
<td>357.0</td>
<td>37.13</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

* significant  ** highly significant  
b: significant when compared with infected control group (II)  
c: significant when compared with drug control group (III)  
d: significant when compared with ginger group (IV)  
e: significance when compared with NTZ group (V)  
f: significant when compared with Pyrimethamine & sulfadiazine + Ginger group (VI)  
g: significant when compared with Nitazoxanide + Ginger group (VII)
Figure (3): Histopathology of the brain tissue after chronic infection by *T. gondii*

G I) Section in brain tissue from mice showing no histopathological changes in brain tissue group (H,E x400).

GIIa) Section in brain tissue from mice showing inflammatory cell infiltrate can be seen surrounding necrotic foci (*). Increased number of lymphoid cells can be seen in the meninges (*), suggesting the development of meningitis. The inflammation and parenchymal haemorrhage were the most common lesions found as severe involvement, necrotic foci (black arrow) (H,E x400).

GIIb) Section in brain tissue from mice showing *Toxoplasma gondii* tissue cyst contains bradyzoites, perivascular infiltrate (*) of the inflammatory nature and haemorrhage (yellow arrow), few neurons are shrunken with pyknotic nuclei (red arrow) (H,E x400).

GIII) Section in brain tissue showing no histopathological changes, only very slight Inflammatory cell infiltrate (H,E x400).

GIV) Section in brain tissue from mice showing the meninges are expanded by a cellular infiltrate (*) that occasionally extends into the underlying cerebrum. Increased number of lymphoid cells. The inflammatory cell infiltrate consists of a mixture of lymphocytes & plasma cells seen in the meninges (*), suggesting the development of meningitis & meningeal congestion & haemorrhage (yellow arrow) (H,E x400).

GVa) Section in brain tissue from mice showing mild inflammation (*), slight meningeal hyperemia few neurons are shrunken with pyknotic nuclei (H,E x400).

GVb) Section in brain tissue from mice showing vascular congestion & parenchymal haemorrhage (yellow arrow), very mild inflammation (*) (H,E x400).

GVII) Section in brain tissue from mice showing necrotic foci (black arrow) moderate parenchymal hyperemia (H), (H,E x400).

Figure (4): Histopathological sections in eye retina from mice of different groups.

GI) Preserved cytoarchitecture of retina (no pathological changes) (H, E x 400).

GIIa, b, c) focal retinochoroiditis (arrows), with alterations in the disposition and cytoarchitecture of the layers of the retina; intense inflammatory infiltrate in the outer segment of photoreceptors (FTR), inner plexiform layer (IPL), ganglion cell layer (GCL). Edema (asterisks) were frequently observed (H, E x 400).

GIIId) Regeneration cytoarchitecture of retina (H, E x 400).

GIV) focal retinochoroiditis with moderate alterations in the disposition and cytoarchitecture of the layers of the retina (red line); moderate inflammatory infiltrate (arrow). Edema (asterisks) were moderately observed in the inner INL and outer ONL. and inflammatory infiltrate in the IPL, GCL and vitreous and edema in ONL and INL (asterisks) (H, E x 400).

GV) Very mild alterations in the disposition and cytoarchitecture of the layers of the retina; mild inflammatory infiltrate (arrows). Edema (asterisks) were less frequently observed (H, E x 400).

GVId) Regeneration of cytoarchitecture of the layers of the retina. Edema (asterisks) were less frequently observed (H, E x 400).

GVII) Very mild alterations in the disposition and cytoarchitecture of the layers of the retina; mild inflammatory infiltrate (arrows). Edema (asterisks) were less frequently observed (H, E x 400).
Figure (5): Histopathological sections in liver tissue from mice of different groups.

GI) Liver of mice in group I showing no pathological changes (H, E x400)
GII) Liver of mice in group II showing a) *Toxoplasma gondii* tissue cyst contains bradyzoites, b) severe inflammatory cellular infiltration and haemorrhage (head arrow) in the portal area and degeneration in the hepatocytes (arrow), dilatation of the central vein (CV) with focal inflammatory cells' aggregation forming granuloma (*) especially in GIIc and diffuse infiltration in the hepatic parenchyma (*) (H, E x400).
GIII) Liver of mice in group III showing very mild inflammatory cellular infiltration in the portal area and complete regeneration in the hepatocytes, mild diffuse infiltration in the hepatic parenchyma (*) (H, E x400).
GIV) Liver of mice in group IV showing inflammatory cellular infiltration in the portal area and minimal regeneration but still some fatty necrosis in some cells (arrow) with focal inflammatory cells' and moderate diffuse infiltration in the hepatic parenchyma (*) (H, E x400).
GV) Liver of mice in group V showing mild inflammatory cellular infiltration in the portal area (*) and moderate regeneration in the hepatocytes, mild to moderate diffuse infiltration in the hepatic parenchyma (*) (H, E x400).
GVI) Liver of mice in group VI showing very mild inflammatory cellular infiltration in the portal area and complete regeneration in the hepatocytes, mild diffuse infiltration in the hepatic parenchyma (H, E x400).
GVII) Liver of mice in group VII showing mild inflammatory cellular infiltration in the portal area and some regeneration in the hepatocytes (arrow), moderate diffuse infiltration in the hepatic parenchyma (*) (H, E x400).
Figure (6): Histopathological Sections in spleen tissue from mice of different groups.

GI: Spleen of mice showing no pathological changes. (H, E x400).
GII(a,b,c): Spleen of mice showing disorganised white and red bulb (R), haemorrhage, congestion (green arrow) & multiple number of megakaryoblasts multinuclear giant cell (Astrexis) infarction (yellow arrow) perivascular congestion (head arrow). (H, E x400).
GIII: Spleen of mice showing no or minimal pathological changes. (H, E x400).
GIV: Spleen of mice showing disorganised white and red bulb, some megakaryoblasts multinuclear giant cell (*). (H, E x400).
GV: Spleen of mice showing less disorganised white and red bulb and less number of megakaryoblasts multinuclear giant cell (*). (H, E x400).
GVI: Spleen of mice showing no or minimal pathological changes. (H, E x400).
GVII: Spleen of mice showing few number of megakaryoblasts, multinuclear giant cell and other inflammatory cells (*). (H, E x400).
G I Section in heart tissue from mice normal control show no pathological changes (H&E x400).

G II Section in heart tissue from mice showing moderate to severe congested B.V (Arrow head) together with parasites perivascular lymphohistiocytic infiltrates dense granulomas can be observed in some areas (astrexis) some degeneration of architecture (arrow) (H, E x 400).

G III Section in heart tissue from mice showing regeneration of architecture (H, E x400).

G IV Section in heart tissue from mice showing mild mononuclear cellular infiltrate (astrexis) and severe injury in cardiac tissue (arrow). (H, E x400)

GV Section in heart tissue from mice showing mild mononuclear cellular infiltrate in cardiac tissue. (H, E x400).

G VI Section in heart tissue from mice showing some regeneration of architecture and myocytes (H, E x400).

G VII Section in heart tissue from mice showing very mild mononuclear cellular infiltrate and hyperemia in cardiac tissue. (H, E x400)
DISCUSSION

The treatment of *T. gondii* infection emphasizes the problematic issue of the inadequate effectiveness of the existing antiparasitic agents and their side effects and also, the possible appearance of resistant *Toxoplasma* strains (EL-Sayed and Safar, 2014). Discovery of low toxicity compounds and competent to prevent and treat *T. gondii* would be extremely beneficial for treatment of infections in immunocompromised patients (Gomes et al., 2012). The medicinal herbs value as sources of natural product bioactive molecules to medicine is ascribed to not only in their pharmacological or chemotherapeutic outcome, but also in their role as parent molecules for the manufacture of new drug substances (EL-Sayed and Safar, 2014). The rhizomes of the ginger (*Zingiber officinale*) are regularly used as a flavor or food supplement. There are some antioxidants and anti-inflammatory constituents in ginger rhizomes (Ahui et al., 2008 & Haniadka et al., 2013). In the parasitological field, previous studies have confirmed that ginger and its constituents have significant nematocidal, cestocidal and anti-protozoal activities *in vitro* and *in vivo* (El-Sayed and El-Saka,2015). Nitazoxanide is a broad-spectrum anti-parasitic and broad-spectrum antiviral drug that is indicated in medicine for the management of diverse helminthic, protozoal and viral infections (Di Santo and Ehrisman, 2013 and Rossignol et al., 2014). In this study we assessed the therapeutic antitoxoplasmic effect of both ginger which was mentioned before to have antitoxoplasmic activity in some handful studies (Choi et al., 2008 and Choi et al., 2013) and nitazoxanide which was reported before to have an invivo antitoxoplasmic activity (Galván-Ramírez et al., 2013), but up to our knowledge, no *in vivo* studies addressed its antitoxoplasmic yet. Including traditional mainstay antitoxoplasmic treatment (pyrimethamine and sulpha diazine), we designed versatile drug regimens to explore the synergistic and/or antagonistic activities of newly studied drugs as mentioned in material and methods section. In the present work, group VI (treated by combination of *Zingiber officinale*, pyrimethamine and sulphadiazine) accomplished the best therapeutic efficacy in treating chronic toxoplasmosis infections among studied groups as with this treatment regimen the brain cyst reduction rate was 58.8%. Inversely was the *Zingiber officinale* monotherapy treated group (group IV) which showed the lowest rate for the mean brain cyst mean reduction among all groups (28%). In-between, other groups lied in the grey area with the best performance within those medium acting drug regimens was for group III, treated by pyrimethamine and sulphadiazine (drug control group) as it achieved 50.9% brain cyst reduction rate. In nitazoxanide treated mice the corresponding rate was 38%. This rate was slightly increased upon adding *Zingiber officinale* as adjuvant to nitazoxanide treatment to reach 40.6%. (Tables 1 & Figures 1-7). In this study, ginger exhibited some activity against *T. gondii* in experimentally infected mice when used alone in 500mg/kg per mice as compared by infected control group, this agrees to Choi et al. (2013) who evaluated the anti-parasitic effect of GEF1 (fraction 1 obtained from ginger extract) against *T. gondii in vitro* and *in vivo*. They demonstrated that GE-
F1 not only triggers anti-"T. gondii" effects leading to the inactivation of apoptotic proteins in infected host cells by direct inhibition of "T. gondii" but also possesses anti-parasitic properties which hinder inflammatory cytokine secretion in vivo so as to the parasite activity was strongly affected after GEF1 treatment, this anti-inflammatory properties of ginger which were also mentioned before by Kuo et al. (2011), Wang et al. (2011), Ha et al. (2012) and Ajayi et al. (2015) may explain the performance superiority that tinged the results of group VI which was treated by combination of "Zingiber officinale" and pyrimethamine & sulfadiazine, as we can hypothesize that the potentiating antitoxoplasmic action exerted by ginger to pyrimethamine & sulfadiazine may be due to those anti-inflammatory properties. Also, the direct antiparasitic inhibitory action mentioned before by Choi et al. (2013) could be the leading cause of the observed decrease in brain cysts number like what happens with the classical anti-Toxoplasma drugs which when started promptly after infection caused rapid resolution of retinocchoroiditis lesions prevented wide spread tissue destruction decreased the chances of the parasite disseminatio and reduced lesion size and vitreal inflammation as well as improved visual acuity. (Soheilian et al., 2011). However, in this study, using ginger alone even in its high dose didn’t give a satisfactory effect on the parasite yield so as to its mean brain cyst count was significantly higher than all other groups except infected control group, a performance ranked it as the closest one to the untreated group yield (Table 1), the difference between our results of ginger treated group from that reported by Choi et al. (2013) could be understood in view of knowing that their results about ginger antitoxoplasmic activity were verified by using GEF1 (fraction 1 obtained from ginger extract) which may have different pharmacological properties than the whole herb extract used in this experiment. Concerning nitazoxanide results, they were in contrast with those of Galvan-Ramirez et al. (2013) who said that nitazoxanide decreased "T. gondii" infection in vitro more than pyrimethamine and was not cytotoxic to astrocytes at the administered dose. This controversy could be explained by that they evaluated the nitazoxinide efficacy in an in vitro experiment while ours was in vivo one, where several host metabolic factors may impact the drug yield, additionally the drug's own pharmacokinetics; absorption, degradation to active metabolites with different activity, excretion and bioavailability surely influences the therapeutic action which is not the case in in vitro studies in which only the parent drug is tested with elimination of the possible effect of all other in vivo host mentioned factors. However, the overall efficacy rates of NTZ as antitoxoplasmic agent was so close to what has been reported in other in vivo studies against Cryptosporidia parasites in which treatment with NTZ as a powder or as an injectable formulation administered orally yielded only moderate efficacy; 42 or 26% of the oocyst output in controls, respectively, (Blagburn et al., 1998). The NTZ possible effective mechanism of action might be attributed to being a noncompetitive inhibitor of the pyruvate: ferredoxin/flavodoxin oxidoreductases (PFORs) which catalyze the oxidative decarboxylation of pyruvate to acetyl...
coenzyme A (acetyl-CoA) and CO\textsubscript{2}, with reducing equivalents transferred to either ferredoxin or flavodoxin. These enzymes are present in the amitochondriate eukaryotic human protozoa; \textit{Trichomonas vaginalis}, \textit{Entamoeba histolytica}, and \textit{Giardia intestinalis}, \textit{Cryptosporidium parvum}, (Hoffman et al., 2007) and \textit{Toxoplasma} apicoplast, where it operates as a general electron switch at the bifurcation step of many different electron transfer pathways (Singh and Bhakuni, 2008). Consequently NTZ using this mechanism, might hinder the crucial parasitic respiratory processes leading to their death and diminishing invasion of new host cells thus alleviating the parasitic yield for the sake of affected host tissues. It is worthy to mention that no mice died after administration of ginger and nitazoxanide throughout the chronic toxoplasmosis experiment, which at least proves the safety of these tested drugs at the given doses. Another valuable fact that favors using NTZ as antitoxoplasmic agent is being better as regard safe use during pregnancy as it is classified by FDA as B category which means that animal studies show no risks, but there are no controlled studies on pregnant women are available, so it is allowed after the 1\textsuperscript{st} trimester of pregnancy Product Information. Alinia (nitazoxanide), (2005), while the traditional anti \textit{Toxoplasma} treatment (pyrimethamine & sulphanadiazine) are classified by FDA as C category, which means that animal studies have shown risk to the fetus and there are no controlled studies in women (CDC report, 1997). Since the main toxoplasmonic health hazards are happening due to fetomaternal transmission, so availability of new drug with better safety during pregnancy and having comparable therapeutic antitoxoplasmic effect is believed to be considerably valuable for prevention or reducing the risk of \textit{Toxoplasma} fetomaternal transmission. In the same line, histopathological examination of brain, eye, liver, spleen and cardiac tissue sections revealed more or less correlated results to the parasitological work as group VI always showed least degree of histopathological alterations. Brain sections of mice of infected group showed meningeal inflammatory reaction mainly by mononuclear cells, congestion of meninges and necrosis of neurons. \textit{Toxoplasma} cysts were found in meninges and implanted in the brain tissue. These observations were in agreement with Warce, (2008) Also, retinal degeneration and necrosis in the eyes tissue with appearance of \textit{Toxoplasma} cysts were detected as what was stated by Fangli et al., (2005). Theoretically, these pathological changes might be attributed to intracellular propagation of the parasite, leading to host cell disruption due to liberated parasites that invade and destroy adjacent cells, forming increasingly larger focal lesions. So, consequently if a therapeutic agent has a direct antiparasitic effect as mentioned before, we can easily understand the differences in the histopathologies amplitude found among the treated groups as compared with the infected group one. The antioxidant effect of ginger reported previously by Verma and Asnani. (2007), Sakr, (2007) and Mostafa et al. (2011) might be a potential cause for its antitoxoplasmic effect in this study. In conclusion, this study demonstrated, the efficacy of both nitazoxanide monotherapy and \textit{Zingiber officinale}+ pyrimethamine and sulfaedazine triple therapy in the treatment of...
chronic toxoplasmosis. Further studies are recommended in order to investigate the effectiveness of this NTZ regimen against *T. gondii* on a wider scale before approval for treatment of human infection. Also, introduction of ginger as a routine food supplement for offering safe prophylaxis against *T. gondii* infection should be considered.

**Acknowledgments** The authors are grateful to NRC zoonotic diseases staff members. Our utmost gratitude to Prof. Dr. Ashraf Barakat for compliance with ethical standards.

**Conflict of interest** The authors declare that they have no conflict of interest. Human and animal rights informed consent. This study was approved by the Scientific Research Ethical Committee, Faculty of Medicine, Benha University. The experimental animal studies were conducted in accordance with international guidelines for animal care. All experimental mice were housed under standard laboratory conditions with an average temperature of 20–25 °C and were given drinking water and regular mouse diet.

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دراسة التأثير العلاجي لعشب الزنجبيل وعقار النيتازوكسانيد على الفئران المصابة بداء المقوسات (التوكسو بلازما) المزمنة

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داء المقوسات هو مرض طفيلي عالمي يؤثر على قطاع هائل من السكان. في هذا العمل، نقوم بتقييم التأثير العلاجي لدواء النيتازوكسانيد وآخر من أصل نباتي (الزنجبيل) على الفئران المصابة بداء المقوسات التجريبية (السلالة الغير فتاكة) مقابلة بالأدوية التقليدية المستخدمة حالياً في علاج داء المقوسات للإنسان (بيريميثامين والسلفاديازين). تم تصنيف الحيوانات إلى 7 مجموعات، المجموعة الأولى: الفئران المصابة الفارغة المعالجة المجموعة الثانية: الفئران المصابة الفارغة المعالجة، المجموعة الثالثة: المصابات وعلاجها من خلال مزيج من بيريميثامين والسلفاديازين، المجموعة الرابعة: المصابات وعلاجها بالزنجبيل، المجموعة الخامسة: المصابات وعلاجها بدواء النيتازوكسانيد، المجموعة السادسة: المصابات وعلاجها بمزيج من الزنجبيل وبيريثامين سلفاديازين، المجموعة السابعة: المصابات وعلاجها بمزيج من الزنجبيل ودواء النيتازوكسانيد. لتقسيم فعالية الأدوية التي تم اختبارها، تم حساب عدد الأكياس في المخ والتغيرات التشريحية المرضية باستخدام صبغة هيماتوكسلين ويوسين. في هذه الدراسة، حققت المجموعة السادسة أفضل النتائج من بين مجموعات الدراسة وذلك لأن بها انخفض معدل عدد الأكياس إلى 58.8% بالمقارنة مع الفئران المصابة غير المعالجة وكانت قياسات المجموعات الأخرى جميعها تقل عن المجموعة السادسة كالتالي: 70.9%، 52.8%، 38.2%، وأخيراً 4.6% للمجموعات.

Chronic Toxoplasmosis

The third, fourth, and fifth study showed a clear correlation between the results of the tissue tests for chronic toxoplasmosis and the data obtained from the fluid samples of the brain. This indicates that the use of nitenoxan also as a replacement drug for chronic toxoplasmosis is acceptable. In addition, ginger can be an auxiliary treatment and safe against chronic toxoplasmosis if added to the traditional medicines used in treating chronic toxoplasmosis. There is still the need for more studies to determine the relationship between the different doses of ginger and the response of patients with chronic toxoplasmosis to treatment with it.