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THERAPEUTIC EFFICACY OF THYMUS VULGARIS AND *MYRISTICA FRAGRANCE* HOUTT (NUTMEG) ETHANOLIC EXTRACTAGAINST TOXOPLASMOSIS IN MURINE MODEL

By

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Abstract

Toxoplasma gondii is a globally obligate intra-cellular protozoan zoonotic disease. **T**his study evaluated the efficacy of *Thymus vulgaris* and *Myristica fragrance* Houtt (Nutmeg) ethanolic extracts against chronic toxoplasmosis. **A total of** fifty laboratory-bred male Swiss Albino mice were infected with Me-49 *T. gondii* strain and divided into five groups G1:non infected/non-treated control, G2:infected/non-treated, G3: infected/treated with *Thymus vulgaris* extract, G4: infected/treated with nutmeg extract and G5:infected and treated with Spiramycin. Mice were given *Thymus* and *Myristica* ethanolic extract 4 days post infection for 14 days then sacrificed 4 weeks after last dose. Brains and livers were dissected out and processed for histopathological examination. Tissue cyst count evaluated the efficacy of extracts. The results showed that mean number of brain cysts was significantly reduced by 47.5% in mice treated with *Thymus* extract while *Myristica* extract treated group showed a mild reduction (0.8%). *Thymus* effect was near to Spiramycin in cyst reduction (47.5% & 48.89%) respectively. Brain and liver lesions in *Thymus* treated mice showed considerable improvement.

Keywords: Egypt, Toxoplasmosis, *Thymus vulgaris*, *Myristica fragrance*, Ethanolic extracts.

Introduction

Toxoplasma gondii is an obligatory intracellular protozoan parasite affecting about third of human populations (Montoya and Liesenfeld, 2004). Toxoplasmosis is a prevalent zoonotic disease with cats the definitive host (Tenter *et al*, 2000). The standard therapy for acute toxoplasmosis is sulfadiazine and pyrimethamine, but in chronic cases diaminodiphiny-lsulfone, atovaquone, spiramycin, and clindamycin were used (Derouin *et al*, 2002). A substitute consists of a high pyrimethamine dose alone or combined with clindamycin, clarithromycin, azithromycin or atovaquone for patients with sulphonamides hypersensitivity (McLeod *et al*, 2009). Spiramycin is a macrolide antibiotic belongs to the same class of erythromycin, azithromycin and telithromycin but, with poor capacity to cross blood brain barrier as many conventional anti-toxoplasmic drugs (McCarthy *et al*, 2014). *T. gondii* in pregnant women were treated mainly with spiramycin alone or combined with P-S and/or folic acid, and the vertical transmission was 9.9% (95%CI, 5.9%-16.2%) after therapy. Sulfonamides combined with other drugs mostly used in treatment of toxoplasmic encephalitis in AIDS patients; however, the pooled CR was only 49.4% (95%CI, 37.9%-60.9%) in different therapeutic regimes (Wei *et al*, 2015). The pro-longed administration cause bone marrow suppression, folate depletion and both hepatic and renal complications along with emergence of resistant parasite strains which limited their action in chronic toxoplasmosis especially during pregnancy (Gras *et al*, 2005; Cortina-Borja *et al*, 2010). These side effects directed the sight of scientists to safe anti-parasitic agents. Many herbs, plants and natural products have a potential anti-toxoplasmic effect, safe, and inexpensive (Al Nasr *et al*, 2016). WHO (1993) reported that many plant fractions and their dynamic constituents are utilized as traditional medicines by 80% of the world population. Many medicinal plants like *Thy-mus vulgaris*, *Myristica fragrance* showed in-vitro & in-vivo activity against toxoplasmosis (Sharif *et al*, 2016; Hegazy *et al*, 2018).

Thymus vulgaris (garden thyme) is a well-known medicinal plant with anti-inflamma-

tory and antioxidant properties. Its ethanolic extract has a hepato-protective activity against *T. gondii* by improving pathological lesions and liver functions reducing the genotoxic damage (El-Sayed and Ramadan, 2017). Its essential oils especially thymol and carvacol are responsible for its medicinal properties, showing very strong antibacterial, antifungal, and antioxidant activities (Fachini-Queiroz*et al*, 2012). Also, *Myristica fragrance* Houtt (= nutmeg) is a tropical evergreen tree, the seed kernel of inside its fruits which contain many monoterpenes and phenyl propane derivatives (Matan and Nisoa, 2014). It has anti-inflammatory, analgesic, antibacterial, antifungal activities, anticonvulsant and anti-diabetic properties (Cao *et al*, 2015). As anti-parasites, this plant exhibited not only a strong anti-*Toxoplasma gondii* activity (Pillai *et al*, 2012) but also, showed lethal effect on *Anisakis simplex* (López *et al*, 2015).

The present study aimed to investigate the potential anti-*Toxoplasma gondii* activity of alcoholic extracts of both *T. vulgaris* and *M. fragrance* in comparison with spiramycin.

Materials and Methods

Animal ethics protocol: All mice were reared and sacrificed according to the protocol of The Institutional Animal Care and Use Committee according to Zagazig University (ZU-IACUC) for animal Use in Research and Teaching.

Plant material and extraction: *Thymus vulgaris* aerial parts were collected from experimental farm of Faculty of Agriculture, Zagazig University in April 2018. The Nutmeg fruits, *Myristica fragrance*, were purchased from the local Egyptian market. Voucher specimens were ensured to be not exposed to insecticides. Species was kindly confirmed by Ass. Prof. Dr. Mohamed Ahmed Abdulkader, Faculty of Agriculture, Zagazig University.

Alcoholic extracts of the fresh aerial parts of *T. vulgaris* and the dried fruits of *M. fragrance* were obtained by using ethanol (Attia *et al*, 2015).

Parasite: Me 49 *T. gondii* strain was obtained from the National Research Centre, Giza, and maintained in Medical Parasitology Department, Zagazig Faculty of Medicine by successive passage in Swiss Albino mice using 0.1ml homogenate of brain at weekly interval (Djurković-Djaković *et al*, 2002).

Animal infection and treatment: A total of 50 laboratory-bred Swiss Albino male mice (6-8 weeks, ~25 g) were housed in a suitable environment in the Scientific and Medical Research Centre, Zagazig University (ZSMRC). Mice were divided to 5 groups: (10 mice/g), G1: non-infected, non-treated (control -ve). G2: infected, not treated with any remedies or drugs (control +ve), G3: infected and treated with thymus extract (400mg/kg/day), G4: Infected and treated with nutmeg extract (500 mg/kg/ day), G5: infected and treated by spiramycin (200mg /kg /day) for 3 weeks (Grujic *et al*, 2005).

Mice were infected by gavage (100cysts/ mouse), treatment with remedies started 4 days post infection for 14 days. Mice were sacrificed 4 weeks after last dose of therapy, brain and liver were removed and divided into two parts, one for cysts counting and the second was preserved in formalin 10% and processed (Abdel Wahab *et al*, 1989) for histopathological examination.

Parasitological study: Brain cysts were microscopically counted, mean number of cysts/mouse and mean number of cysts/ groups were calculated (Djurkovic-Djakovic and Milenkovic (2001).

Statistical analysis: Data were analysed by (SPSS) program version 11.0 for Windows. All values are expressed as the mean ± S.D. and analysed using F-test (ANOVA) to compare the studied groups. Significance was assumed as $p < 0.05$.

Results

In infected untreated group, mean number of cysts was 637.7±10. *Thymus* extract reduced cysts to 334.6±4.57 (47.5%), Spiramycin reduced to 325.9±5.4 (48.89%), without complete cysts eradication. *Nutmeg* extract reduced cysts count to 632.5±8.4 (0.8%).

Table1: Mean numbers of *Toxoplasma* brain cysts in groups

Experimental groups	Mean cyst count	Percentage
Infected not treated (G2)	637.7±10	-
Infected & treated by *Thymus* (G3)	334.6±4.57	47.5%
Infected & treated by *Myristica* (G4)	632.5±8.4	0.8%
Infected and treated by spiramycin (G5)	325.9±5.4	48.89%

Table2: *T.gondii* brain cyst count and the probability values in groups

Groups	G2	G3	G4	G5
G5	.000	.013	.000	
G4	.127	.000		
G3	.000			
G2				

*Significant at 0.05 level.

Histopathological changes in liver and brain of infected mice were evident as compared to uninfected control (Fig.1a, b; Fig.2 a, b). Mice treated with thymus extract showed normal neuronal cells with scanty perivascular aggregates of inflammatory cells (Fig.2c) with few *Toxoplasma* cysts (Fig.3c).Brain of mice treated with nutmeg extract showed minimal improvement with dilated congested vascular spaces surrounded by heavy aggregates of inflammatory cells (Fig.2d). Liver of infected mice treated with thymus extract showed return of liver tissue to normal state and absence of inflammatory cell (Fig. 1c) with few toxoplasma cyst (Fig.3 a). on the other hand, liver of mice infected and treated with nutmeg extract showed minimal effect with dilated congested central vein surrounded by aggregation of inflammatory cell (Fig.1d) with many *Toxoplasma* cysts (Fig.3 b).

Discussion

Toxoplasmosis has serious impacts on both animals and human health. Several field studies have been conducted on *Toxoplasma gondii* parasite using different herbs with many types of extracts such as *Artemisia annua* (De Oliveira *et al*, 2009), *Eurycomalongifolia* (Kavitha *et al*, 2012), *Ginkgo biloba* (Chen *et al*, 2008), *Zingiber officinale* (Choi *et al*, 2013), *Jatrophacurcas* seed cake (Soares *et al*, 2015) and *Vanilla* extract (Oliveira *et al*, 2014). Although ignoring their chemical composition, *T. vulgaris* and *M. fragrance* have been valued as a natural home remedies for many diseases and succeeded to gain the trust of public as a good spices because of their unique aroma, availability in the market and their cheap prices. Here in our study we tried to assess the effect of both *T.vulgaris* and *M.Fragrance* as potential anti-Toxoplasma agents vs. spiramycin on chronic toxoplasmosis.

The ME49 strain of *Toxoplasma* used in our work was selected because it is less virulent, this gives us more time to evaluate different treatment regimens (Oliveira *et al*, 2014).

Thymus extract was evaluated in this study for its anti-toxoplasma activity, compared with spiramycin. It recorded significant results with a percentage of 47.5% & 48.89% respectively on the cysts in brain of mice. This is not the first time that Thymus was reported to exert anti-toxoplasmic activity. Erakey *et al*. (2016) found a potent prophylactic effect of ethanolic thymus against establishment of chronic toxoplasmosis when administered 5 days before infection, they added that the extract revealed a remarkable therapeutic effect when administered orally 6 weeks post infection.

In the present study, a plenty of inflammatory cells are invading tissues with congestion of minute blood vessels. Aided by inflammation, the parasite causes death of infected neurons via the production of nitric oxide and other toxic oxygen products. This agreed with Carruthers and Suzuki (2007).

Based on the fact that the effect of a drug is evaluated by its ability to permit the survival of infected treated animals after a given time

(Chang and Pechère, 1988), using thymus (400mg/kg/day) and spiramycin (200mg/ kg/ day) helped the survival of mice. The exact mechanism of action of spiramycin on toxoplasmosis is not well clarified (McCarthy *et al*, 2014), however its action on *T. gondii* can be explained on bacteriological basis, it is believed to act as an inhibitor of protein synthesis by binding to the 50S subunit of bacterial ribosomes inducing rapid breakdown of polyribosomes (McCarthy *et al*, 2014; Pestka, 1977; Tenson *et al*, 2003). Although the significant results obtained in our work reflects the effective action of spiramycin as anti-toxoplasmic drug, it was not surprising and comes in accordance with other researches that document the effective action of spiramycin against toxoplasma especially in pregnancy (Grujić *et al*, 2005; Dunay *et al*, 2018).

In the present study, the effect of nutmeg extract against *T. gondii* was low when compared with other works. The nutmeg alcoholic extract possessed a weak action and showed only slight effects on *Toxoplasma* brain cyst when compared with spiramycin (632.5±8.4). This effect was marked in different tissue specimens especially brain, it showed little improvement but with obviously dilated congested vascular spaces surrounded by heavy aggregates of inflammatory cells. This effect comes in contrary to our hypothesis which supposes the efficacy of nutmeg as anti-toxoplasmic agent, because of the variable amounts of the active components that exerts antimicrobial activity in different ways. Pillai *et al*, (2012) investigated anti-toxopla-smic effect of nutmeg essential oil found an effective action against *Toxoplasma* with very low cytotoxic activity. Whereas, ethanolic extracts of *M. fragrance* showed potent anti-malarial activity against *P. falciparum* (Thiengsusuk *et al*, 2013) and its crude suspension prevented diarrhea (Grover *et al*, 2002). It was concluded that the thymus extract effectively reduced the number of tissue cysts.

Conclusion

The outcome results showed that *Thymus vulgaris* extract was near in effect when compared to spiramycin® in reducing the number of tissue cysts, on the other hand nutmeg extract showed minimal effectiveness.

Recommendation

Further studies are still needed to establish the optimum formula and dose of *Thymus vulgaris* extract that could be safely used as a natural substitute in treatment of human toxoplasmosis.

Conflict of interest: The authors declared that they have no conflict of interest

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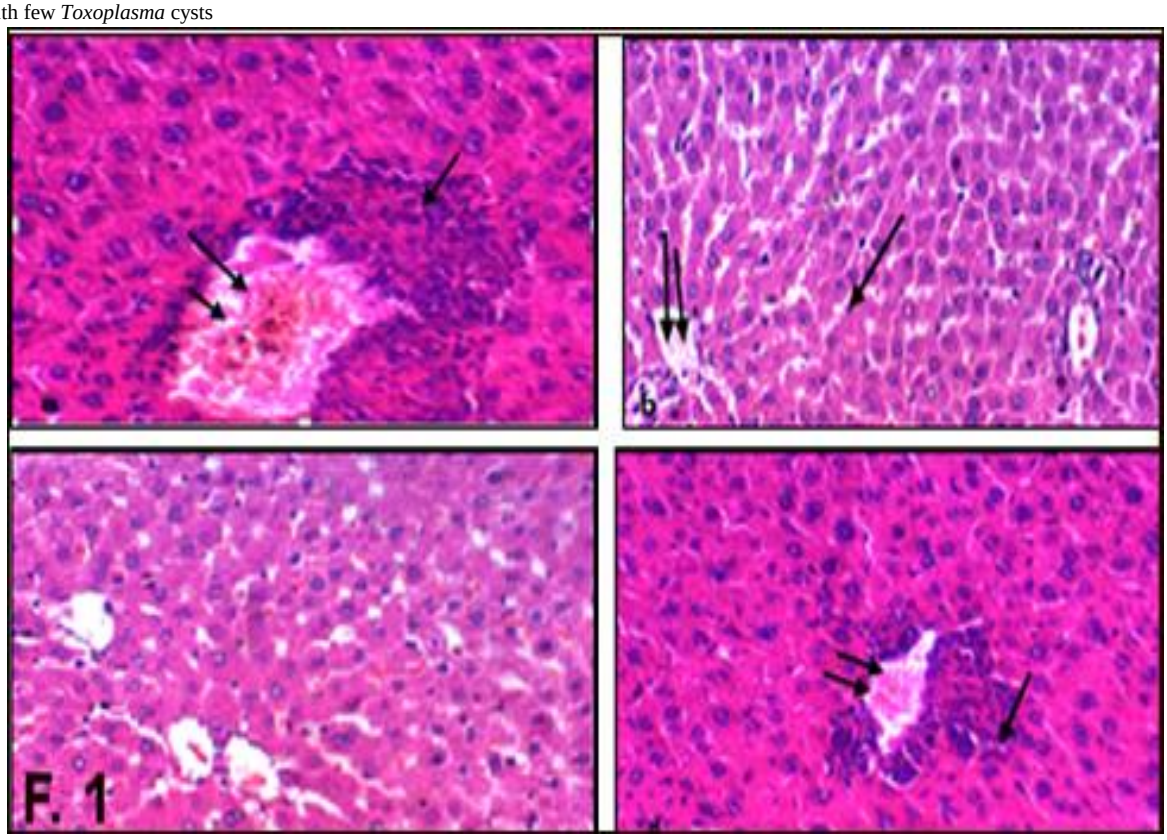
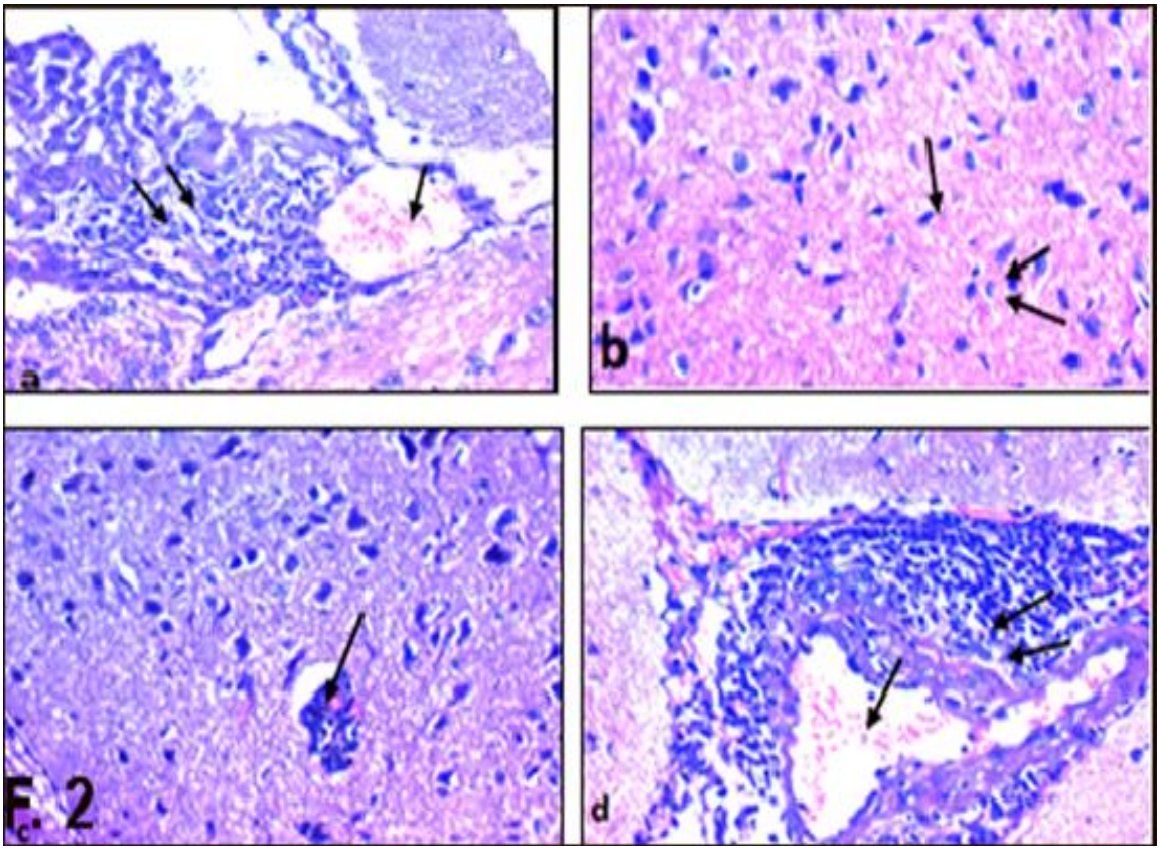
Explanation of figures

Fig. 1:*a*-liver of infected mice showing dilated congested central vein surrounded by heavy aggregates of chronic inflammatory cells**, b**-normal liver tissue formed of normal sized central veins surrounded by rows and cords of normal hepatocytes separated by blood sinusoids, **c-** liver of infected mice and treated with thymus extract showing return of liver tissue to its normal state and absence of inflammatory cells,d-liver of mice infected and treated with nutmeg extract,minimal effect with dilated congested central veinsurrounded by aggregation of inflammatory cell**.**

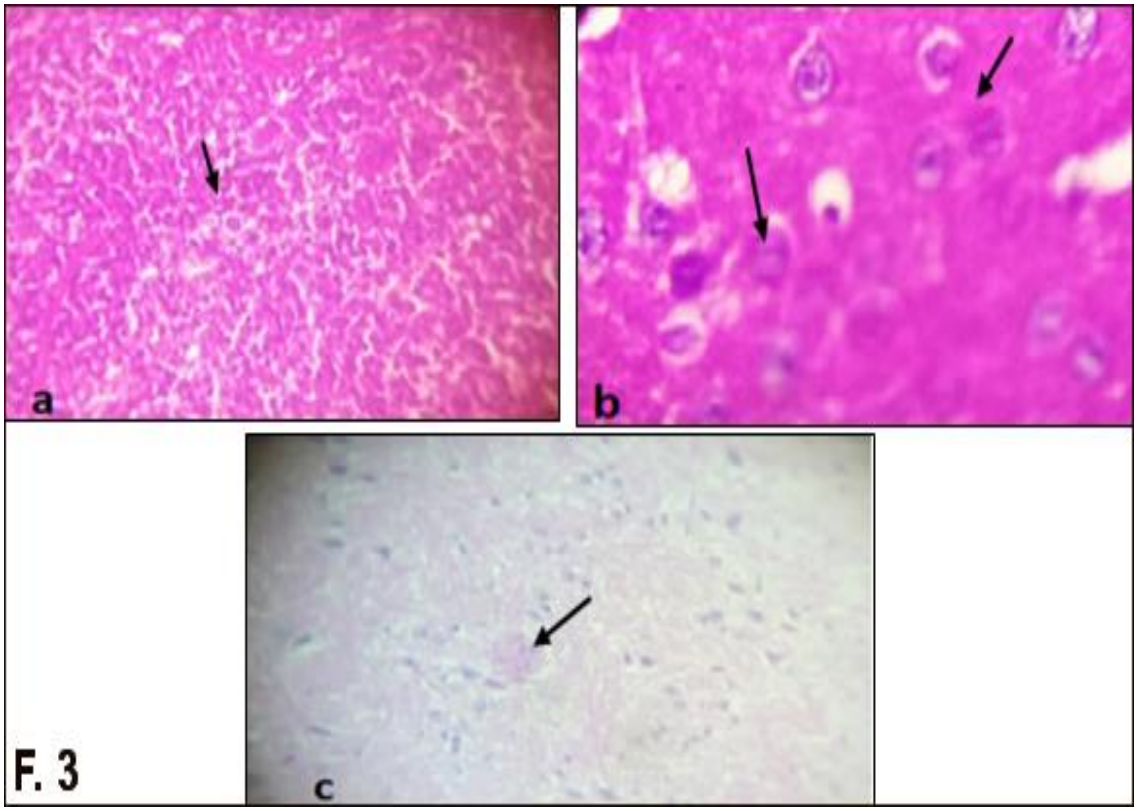
Fig. 2: a- brain of infected mice showing brain tissue separated by dilated congested vascular spaces and heavy aggregates of inflammatory cells,b- normal brain tissue formed of numerous variable sized neuronal cells intercommunicated by thin eosinophilicfibrillary background,c-mice treated with thymus extract showing normal neuronal cells and fibrillary background with scanty perivascular aggregates of inflammatory cells, d- mice treated with nutmeg extract showing minimal improvement with dilated congested vascular spaces surrounded by heavy aggregates of inflammatory cells .

Fig. 3: a: liver of thymus group with few toxoplasma cyst, b: liver of nutmeg group with many *Toxoplasma* cysts, c: brain of thymus group with few *Toxoplasma* cysts





F. 2



F. 3