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Full Length Research Paper

Effect of Combined Albendazole and Sutrim in Experimental Toxoplasmosis

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This work is a trial to elucidate the prophylactic effect of a combination of a broad spectrum antihelmintic: Albendazole, and sutrim (Sulphame- thoxazole +Trimethoprin) on experimental *Toxoplasma gondŭ* infection. A group of 20 female albino rats were infected orally with 50 toxoplasma trophozoites per rat. This group was further subdivided into four subgroups: subgroup I constituted infected control rats given 50 Toxoplasma trophozoites / rat orally, then sacrificed two months later. Subgroup II: animals infected with 50 Toxoplasma trophozoites/rat 24 hours after being treated with one third the dose of albendazole orally/rat (0.4 mgm). Sacrifice was done two months later. Subgroup III: Infected rats given the same infective dose 24 hours after being treated with combined Trimethoprim and Sulphamethoxazole 1.2mgm orally/rat. Sacrifice was done two months later. Subgroup IV: Infected rats with 50 trophozoites per rat, after being given half the doses of both drugs orally: 0.2 mgm Albendazole + 0.6 mgm sutrim (Sulphamethoxazole+ Trimethoprim). Again, animals were sacrificed two months post infection. In all animal treated groups, treatment was initiated 24 hours prior to infection. Parasitological count of the Toxoplasma organisms was done in Hx & Eosin and Giemsa stained brain and liver tissues. It was found that animals given the combination regimen revealed less parasite count in the liver and brain tissue (7.14+3.94) and (14.17+4.83) respectively, when compared to the infected untreated control group. Pathological staining of the liver and brain of these rats revealed diminution of the parasitic count in respect to the control infected untreated group (26.79+7.02) and (72.61+7.69) respectively .

This study may be in value in endemic areas where drug resistance to the usual anti-Toxoplasma compounds may be commonly encountered.

Keywords: Albendzole, Trimethoprim, Sulphamethoxze, Experimental toxoplasmosis, parasitological examination, Histopathological examination, Sutrim (Spiramycin).

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by infection with *Toxoplasma gondii*. The later is a coccidian intracellular protozoon belonging to genus Isospora and is prevalent in temperate climates, it is worldwide in distribution (Johns and Munday, 2007).Infection of humans occurs through three principal routes: a newly infected pregnant woman passing infection to her fetus, consumption of undercooked infected meat, and ingestion of *T. gondii* oocysts in food, through accidental contamination from cat litter (Mc Leod, 2008).An infected rat can excrete up to 20 million oocysts over two weeks, even a single oocyst is infectious and they can remain infectious in water for up to 6 months and in warm moist soil for up to a year (Mui, 2008). According to Mui (2008) congenital toxoplasmosis (1/50,000 births a year in the

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United States) can cause severe vision loss, brain damage and even death. Mc leod, (2008), stated that people at increased risk are immunecompromised patients, such as those with cancer, auto-immune disease, AIDS or transplant recipients. People with normal immune system can suffer major organ damage from chronic infections, eye disease that may lead to loss of sight (Mc Leod, 2008). Again, Mui (2008) and Mc Leod (2008) reported that there's an association between chronic brain infection with Toxoplasma, and diseases such as schizophrenia and epilepsy.

It is known that, the usual drug used to treat toxoplasmosis is Sutrim (Trimethoprim + Sulphamethoxazde). Albendazole is a commonly used broad spectrum anthelmentic with a potent antigiardial effect. Being also a protozoon, Toxoplasma was tested wether it responds to the deleterious antiprotozoal effect of Albendazole or not. The aim of this study is to elucidate the prophylactic effect of Albendazole anthelmentic, alone and/or in combination with the usual antitoxoplasma drug (Trimethoprim+ Sulphamethoxazde) in experimental toxoplasmosis. The parameters of assessment were both the parasitological and histopathological ones using liver and brain tissues of the animals.

MATERIAL AND METHODS

A group of 20 female albino rats (three months old, 60 gm/rat) were bred on a standard diet and water in Theodor Bilharz Research Institute. Animals were divided into four groups:

Group I: Constituted infected control rats given 50 Toxoplasma trophozites / rat orally / then sacrificed two months later.

Group II: animals infected with 50 trophozotes orally/ rat 24 hours after being treated with one third dose of Albendozole orally/ rat (0.4 mgm) sacrifice was done two months later.

Group III: infected rats were given Sutrim orally (Trimethoprim + sulphamethoxazde) 1.2 mgm orally/ rat 24 hours prior to infection, then sacrificed 2 months later.

Group VI: infected rats with 50 trophozoites / rats orally given half the doses of both drugs orally (0.2 mgm Albendazole orally / rat + 0.06 mgm Trimethoprim + sulphamethoxazole orally). Treatment was initialed 24 hours prior to infection. Again, animals were sacrificed 2 months past infection.

Animals were kept on a standard diet under 24C° temperature for three weeks in the biological unit of TBRI. The experiment was carried under the internationally valid guidelines in the Theodor Bilharz Research institute.

All animals were sacrificed 8 weeks post infection (Mahmoud et al., 2006).

Parasitological studies

Rats were weighed and then scarified. The brain was extracted and weighed, then ground in phosphate buffer saline (preservative). Two CMs samples were taken with a 20µ pipette, then counted on graduated slide this procedure is repeated for both control untreated and treated animals (Mahmoud et al., 2006).

Histopathological studies of liver and brain tissues

After sacrifice of the animals, five sections (5 microns in thickness each) were taken from the liver and brain. Each section is at a distance of at least 50mm from the preceding one .Sections were stained with Hx & Eosin (Von Lichtenberg, 1962) and Giemsa stain (E Gupta, et al.; 2009). Microscopic examination for the *Toxoplasma gondii* tachyzoites was carried out using low and high power (X200 and X400).

Statistics

Comparison was done between each treated group and its respective untreated control. The percentage change between each two groups to be compared was assessed using this formula

$$\frac{\text{Mean value of the first group - mean value of the second group}}{\text{Mean value of the first group}} \times 100$$

Differences between the mean scores of any of the two groups to be compared were tested for significance using an unpaired two tailed student's t-test. The data were considered significant if P values were less than < 0.05.

RESULTS

Parasitological parameter (Brain and liver smear examination)

The mean parasitic count by field microscopy in the control and treated groups I, II and III as shown in table (1) was (26.79 ± 7.02), (12.95 ± 5.62), (6.6+2.06) and (7.14 ± 3.94) respectively for the liver samples. Again this parasitic count reached (72.61 ± 7.69), (24.42 ± 4.74) ,(11.21+3.29) and (14.17 ±4.83) respectively for the brain samples.

Pathological parameter

Histopathological examination of liver and brain sections with toxoplasmosis revealed granulomas , inflammation , necrosis & calcifications (Cosme Alvarado-Esquivel**,** et

Table 1 Prophylactic effect of treatment with Sutrim and/or Albendozole on the number of tachyzoites 24 hours prior to infection.

Animal group	**No. of toxoplasma in contents of 10 successive power fields (40x10) mean ± SE**	
liver	**brain**	
Group I - control	26.79+7.02	72.61+7.69
Group II - Albendozole	12.95+ 5.62***	24.42 + 4.74***
Group III - Sutrim	6.6+2.06***	11.21+3.29***
Group IV - Albendozole + sutrim	7.14 +3.94***	14.17+ 4.83***

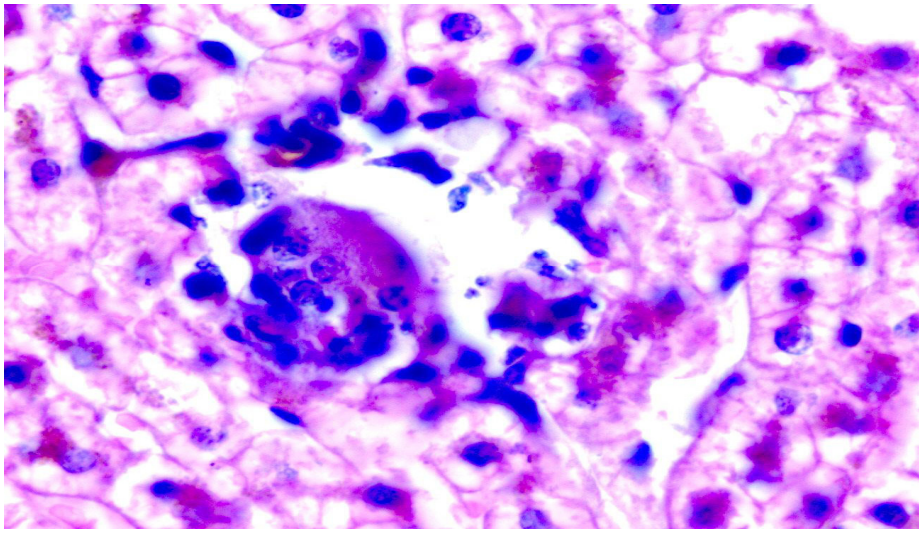

Figure 1 Liver shows toxoplama cyst containing multiple basophilic dot-like bradyzoites (arrow) . H&E x1000.

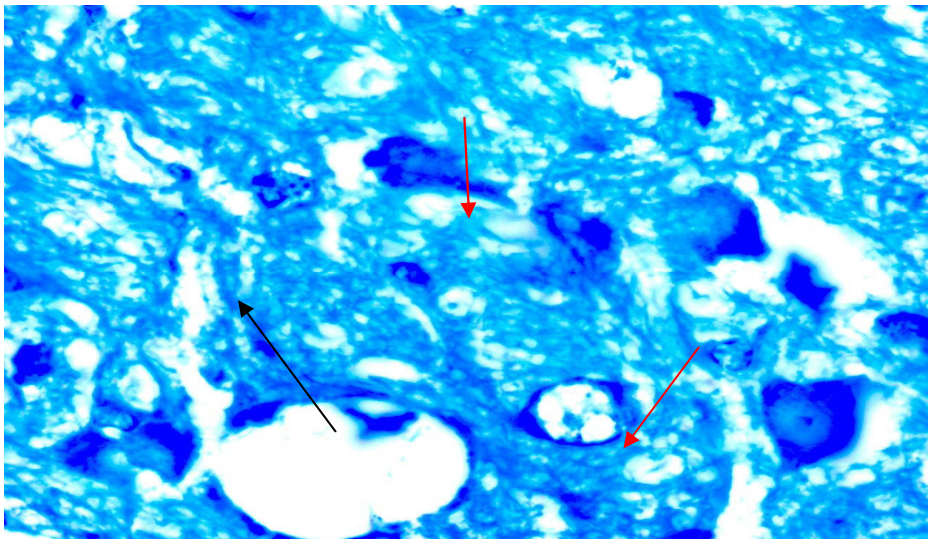

Figure 2 Brain shows a bradyzoite cyst (arrow) and a vague granuloma with multinucleated giant cells (red arrows). Giemsa x1000

al.; 2011) as well as the toxoplasma protozoa form featuring cysts containing multiple basophilic dot-like parasites called (bradyzoites) (Yezid Gutierrez, 2000) .

DISCUSSION

Chemotherapy of *toxoplasmosis* has been hampered by the advent of drug resistance to the commonly used anti-*Toxoplasma* drugs. From this study, it could be concluded that, the combination between Sutrim (Trimethoprim + Sulphamethoxazde) and Albendazole (broad spectrum anthelmentic), exerts a (potent) prophylactic effect when given to experimental animals 24 hour prior to infection. This is evidenced by the lower level of parasite count recovered in the treated groups in

comparison with the control group.

Previously, combs and Muller (2002) stated that coccida provide a rich hunting ground for drug designers, as there are significant biochemical differences between the parasites and their hosts. Recent years, have brought the discovery of the plastid and its possible metabolic machinery, characterization of acido-calcisomes, reports on the apparent absence from some coccida of a typical mitochondrion, and the discovery of the mannitol cycle and shikimate pathway in the parasites (Coombs and Mu ller, 2002). Moreover, modern technologies such as genomics and proteomics are bringing new insights into the biochemistry of coccidia and highlighting possible drug targets in abundance. A major issue for drug discoverers is to decide upon the targets to prioritise (Coombs and mu ller, 2002).

Mc Leod (2008) reported that a newly developped anti-malarial medicine treats *toxoplasmosis*. This drug is known as JPC. It shows ten times efficacy than the current gold- standard treatment for toxoplasmosis, without toxicity JPC_2056 is taken orally, easily absorbed and bioavailable. In tissue cultures, and in mice, the drug was rapidly effective, markedly reducing parasite numbers within just a few days after the injection (Mc Leod, 2008).This was evident by their ruffled fur and hunched shoulders. While treated mice appeared well (Mc Leod, 2008).

Mui (2008) added that studies in tissue culture found no evidence of parasite plaques produced 52 days after treatment. Therefore, the authors concluded that absence of growth indicates that this compound is cidal and not merely “static” for the active form of *T. gondii*. The drug inhibits the action of an enzyme dilhydrofolate reductase (DHFR) produced by the family of the parasites that includes those that cause *Toxoplasmosis* and malaria.

Last, but not the least, the authors stated that the new drug was effective against all malaria parasites, even those with multiple mutations that make them resistant to other anti-folate medicines (Mc Leod, 2008).

CONCLUSION

The aim of this study was to testify the prophylactic effect of the combination between the broad spectrums anthelmintic: Albendazole and Sutrim (Trimethoprim + sulphamethoxazde) which is the usual anti-*Toxoplasma* drug, in experimental *Toxoplasma Gondii* infection. The low dose combination gathers or acts as a double weapon including lower cost expenditure and minimal side-effects of the drugs.

This study could be of help in endemic areas like Egypt, where multiple parasitic infections could occur concurrently with *Toxoplasmosis*.

Further trials are being recommended to discover new potent anti-*Toxoplasma* compounds with minimal side-effects and easy availability.

This experiment complies with the current laws of the country in which they were performed which is Egypt.

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