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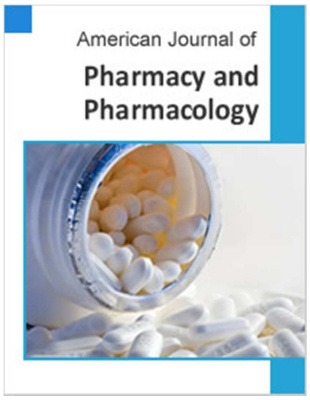

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Chemical Composition and Anti-*Trypanosoma cruzi* Effect of *Thymus vulgaris* L. (Thyme) Essential Oil and Its Main Component, Thymol, in Mice

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Abstract
The present study's aim was to determine the chemical composition of the *Thymus vulgaris* essential oil, and evaluate both activity: the oil and thymol, against *Trypanosoma cruzi* in mice. We used albino mice that were randomly assigned to the following groups (n = 15): infected and untreated (G1); infected and treated with *Thymus vulgaris* essential oil 200 mg/kg (G2); infected and treated with thymol 200 mg/kg (G3); infected and treated with benznidazole 100 mg/kg (G4); untreated and non-infected (G5); and not infected and treated with 200 mg/kg of *Thymus vulgaris* (G6). The treatment was carried out from the 8th post infection day (PID). The parasitaemia was individually verified each 2 days by direct microscopy, counting the parasites in 5 µL of blood. On 14th, 21st and 28th PID, five mice from each group were sacrificed, and hearts were quickly removed and processed for histopathological analysis. *Thymus vulgaris* essential oil and thymol in doses of 200 mg/kg/day by intragastric way produced a significant reduction in parasitaemia in the 22th PID, from 57.60 ± 16.97 to 30.10 ± 15,18 trypomastigotes/mL ($p < 0.05$), and up to 10.90 ± 3.67 trypomastigotes/mL ($p < 0.001$), respectively. The number of amastigotes and inflammatory infiltrates in heart tissue were also reduced at the end of the experiment. These results suggest that the *Thymus vulgaris* essential oil and thymol have an anti-*Trypanosoma cruzi* effect *in vivo* on infected mice.

1. Introduction

Thymus vulgaris L. is a subshrub of 15 to 20 cm of height, member of the Lamiaceae family; it is perennial, aromatic, and is known as Thyme as its vulgar name. Its aroma is due to the essential oil content in which the main component is thymol. Besides, it has been described the presence of several chemotypes according to the geographic area [1]. This plant is widely used in traditional medicine all over the world; in Morocco, it is used for hypertension's treatment [2]; in Iraq, its use is as expectorant, antitussive, anti- bronchiolitis,

antispasmodic, anthelmintic, carminative and diuretic [3]; in Cuba, as stomach stimulant and tonic [4].

The *Thymus vulgaris* essential oil has also shown significant pharmacological properties such as antibacterial activity against *Staphylococcus aureus*, *Enterococcus spp*., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus vulgaris*, *Vibrio spp*,[3, 5]; antifungal effect [6]; besides it has a cytotoxic activity against 3 cell lines of human cancer: human prostate's carcinoma cells (PC-3), human lung carcinoma (A549) and human breast cancer (MCF-7) [5]; and it also has significant antioxidant activity [7].

Thymol had a protective effect with rats who had myocardial infarction induced by isoproterenol, due to its antioxidant properties and lipid antiperoxidation [8]; it showed a significant antiviral activity against herpes simplex type 1 by affecting directly the virion [9]; it demonstrated an anti-inflammatory effect and it improved the wound healing process [10]. Besides, it showed antioxidant, anticlastogenic and radioprotective potential effects in mice, which could be attributed to the normalization of intracellular antioxidant levels and free radical scavenging activities [11]; and it also showed antibacterial and antifungal activity [12, 13].

American Trypanosomiasis, also called Chagas disease, a potentially fatal infection caused by protozoan parasitic *Trypanosoma cruzi*, presents itself in diverse clinical manifestations. In most patients, the early period of *T. cruzi* infection goes virtually unnoticed whereas others develop an acute phase that lasts several weeks and is accompanied by non-specific symptoms such as, for example, fever, tachycardia, weakness, and lymphadenopathy. Localized skin inflammation at the parasite's portal of entry (referred to as a chagoma) may be seen in some patients and some present Romaña's sign. Most chagasic patients survive the acute stage and enter the indeterminate phase, defined by the absence of symptoms which may last years and even decades with cardiomyopathy going undetected in electrocardiograms and chest X-rays. Chronic Chagas disease manifests itself mostly in the form of severe heart pathology leading to arrhythmias, ventricular apical aneurism, and congestive heart failure [14]. It is estimated that there are between 7 and 8 million people infected around the world, the majority of them located in Latin America. Although Chagas disease is mainly find in Latin America, in the last few decades it has been observed more frequently in the United States of America, Canada, many European countries and some of the Western Pacific countries; mostly due to the population's mobility between Latin America and the rest of the world [15].

There is currently no vaccine against the causative agent of Chagas' disease, and the only two medications available in the market for more than 40 years ago are nifurtimox (1972) and benznidazole (1974), both medicines are almost 100% effective in curing the disease if given soon after infection at the onset of the acute phase. However, the efficacy of both diminishes the longer a person has been infected. The potential benefits of medication should be weighed against the long duration of treatment (up to 2 months) and serious adverse effects that often require the patient to leave the treatment [15]. With benznidazole, dermatologic adverse effects occur in approximately 30% of patients and consist of rashes due to photosensitization, rarely progressing to exfoliative dermatitis. The dermatitis is usually mild to moderate; however, the drug should be discontinued immediately in case of severe or exfoliative dermatitis or of dermatitis associated with fever and lymphadenopathy. Approximately 30% of patients experience a dose-dependent peripheral neuropathy. It occurs most commonly late in the treatment course and should trigger cessation of treatment. Bone marrow suppression is rare and should prompt immediate treatment interruption. Additional reported adverse effects include anorexia and weight loss, nausea and/or vomiting, insomnia, and dysgeusia [16]. Benznidazole and nifurtimox should not be taken by pregnant women or by people with kidney or liver failure [15]. It exist protocols for the care of patients with standardized treatments, but they are perceived with low clinical research capacity, therefore, it is also perceived a low therapeutic innovations perspective[17].

Faced to this situation, there is an urgent need of searching for new therapeutic agents for Chagas' disease, and the medicinal plants are a viable alternative; however, the majority of studies of trypanocidal action activity derived from plants, have been made only in vitro. In a study conducted in Brazil by the year 2007, it was demonstrated that the *Thymus vulgaris* essential oil presented in vitro activity against epimastigotes (IC_{50} = 77 ug/mL) and trypomastigotes (IC_{50} = 38 ug/mL) of *Trypanosoma cruzi* [18]. However, at present time there has not been published any study in vivo about this topic in scientific literature; so, motivated by this background, we proposed to assess the activity of *Thymus vulgaris* essential oil and its main component: thymol, against *Trypanosoma cruzi* in a Murine model.

2. Materials and Methods

2.1. Plant Material

The *Thymus vulgaris L*. plant was collected in Lima, Peru. A sample was sent to the Museum of Natural History of the "Universidad Nacional Mayor de San Marcos" for taxonomic identification (voucher N° 078-HSM-USM-2013).

The *Thymus vulgaris* essential oil was obtained by water steam distillation in a Clevenger-type apparatus[19], in which fresh leaves were used. The essential oil was separated and dehydrated with anhydrous Sodium Sulphate (Na_2SO_4), it was filtered and saved in an amber glass bottle under refrigeration at a temperature of 4 °C until its experimental use.

Thymol was acquired from Sigma-Aldrich ®

2.2. Determination of Essential oil's Chemical Composition

We used a Gas Chromatograph Agilent Technologies

7890A with mass detector 5975 C. It was diluted 20 µL of the sample in 1 ml of dichloromethane and it was injected 1 µL. It was used a column DB-5MS, 325 °C; 60 cm x 250 x 0.25 µm; the temperature ramp was 100 °C for 10 min, 1 °C/min to 120 °C, 5 °C/min up to 150 °C, 10 °C/min to 200 °C remaining for 1 minute and finally 10 °C/min to 260 °C. The run time was 48 min, Split 100:1, the carrier gas used was Helium, 20.443 cm/sec. The detector was mass spectrometer.

Table 1. *Chemical composition of the Thymus vulgaris L. essential oil.*

Compound (NIST08.L)	Rt (min)	Percentage (relative areas)
α-Thujene	8.56	1.75
α-Pinene	8.89	1.25
Camphene	9.47	1.10
1-Octen-3-ol	9.91	0.33
β-Phellandrene	10.06	0.17
β-Myrcene	10.34	1.91
β-Pinene	10.40	0.30
α-Phellandrene	11.36	0.32
α-Terpinene	11.80	2.84
p-Cymene	12.14	15.8
d-Limonene	12.38	0.60
β-Thujene	12.55	0.19
Eucalyptol	12.64	0.82
γ-Terpinene	13.79	20.27
5-Isopropil-2-metil-biciclo[3.1.0]hexan-2-ol	14.53	0.56
3,7-dimetil-1,3,6-Octatriene	15.98	1.57
Camphor	20.03	0.55
Linderol	21.92	0.38
4-Terpineol	22.53	0.75
m-Thymol	32.20	44.19
p-Thymol	32.85	2.28
Caryophyllene	39.49	0.98
3-Carene	40.76	0.14
Germacrene D	41.34	0.51
γ-Cadinene	42.19	0.17
(-)-β-Cadinene	42.28	0.10
γ-Muurolene	45.05	0.15

2.3. Determination of the Activity on *Trypanosoma cruzi*

2.3.1. Parasites

Blood trypomastigotes were used. They were harvested by cardiac puncture of albino mice infected with *Trypanosoma cruzi* in the parasitemia peak.

2.3.2. Infection in Vivo

Albino male mice Balb/c (25-30 g) were obtained from the National Institute of Health. They were maintained in a light/darkness cycle of 12/12 hours, allowing them a week of acclimatization before the experiment, with food and water ad libitum. The infection was carried out by intraperitoneal injection of 10^4 trypomastigotes from infected blood.

2.3.3. Experimental Groups

The animals were randomly assigned to the following groups (n = 15): infected and untreated (G1), infected and treated with *Thymus vulgaris* essential oil (G2), infected and treated with thymol (G3), infected and treated with benznidazole (G4), uninfected and untreated (G5), and uninfected and treated with the *Thymus vulgaris* essential oil (G6).

2.3.4. Drugs and Treatment Programs

The *Thymus vulgaris* L. essential oil was dissolved in dimethyl sulfoxide (DMSO) to 1 % and it was administrated at a dose of 200 mg/kg/day (G2 and G6); thymol was dissolved in water and DMSO to 1% and it was administrated at a dose of 200 mg/kg/day (G3), the benznidazole was administered at a dose of 100 mg/kg/day (G4), while the control group (G1) received only the vehicle in the same volume. The group G5 did not receive treatment and it served for weight control. The treatment was performed using a gastric tube, from 8^{th} to 28^{th} post infection day (PID).

2.3.5. Parasitemia

The parasitemia was verified individually each 2 days by direct microscopy, counting the parasites in 5 µL of blood collected from the tip of the tail of mice of all experimental groups.

2.3.6. Histopathological Analysis

At 14^{th}, 21^{st} and 28^{th} PID, 5 mice from each group were sacrificed by cervical dislocation and the hearts were quickly removed and processed for histopathological analysis. The hearts were cut longitudinally and they were rinsed in ice-cold phosphate-buffered saline (PBS) and fixed in 10% formaldehyde in PBS. Fixed tissues were dehydrated and embedded in paraffin. Sections (3 µm) were stained with Hematoxylin-Eosin (HE) and analyzed by light microscopy. We determined the number of amastigotes' nests and the number of inflammatory infiltrates (more than 10 mononuclear cells) in 100 fields for each slide. We obtained the average number of amastigotes' nests and inflammatory infiltrates per field, with three sections from each mouse.

2.4. Body Weight

Body weight was measured weekly.

2.5. Statistical Analysis

The data obtained was expressed as mean ± standard deviation and the comparisons between the experimental groups were performed by one way ANOVA followed by a post hoc test of Scheffé. The differences were considered significant with a $p < 0.05$. We used the statistical software GraphPad Prism 6.

2.6. Ethical Aspects

We followed the international guidelines for the management and care of laboratory animals.

3. Results

3.1. Chemical Composition of the Essential Oil

They were identified 27 compounds that included 100 % of the total composition of the *Thymus vulgaris* L. essential oil. The component found in highest amount was thymol, which represented 46.47 % of the total (44.19 % of m-thymol and 2.28 % of p-thymol), followed by γ-terpinene (20.27 %) and *p*-cymene (15.80 %), as shown in Table 1.

3.2. Effect of the Essential Oil on Parasitemia by *Trypanosoma cruzi*

The treatment from 8th PID with the *Thymus vulgaris* (thyme) essential oil in doses of 200 mg/kg/day by intragastric tube has produced a significant decrease from 57.60 ± 16.97 until 30.10 ± 15.18 trypomastigotes/mL ($p < 0.05$) at the parasitemia peak in the 22nd PID. With thymol in doses of 200 mg/kg/day it was also produced a significant reduction of the parasitemia up to 10.90 ± 3.67 trypomastigotes/mL ($p < 0.001$). Parasitemia was reduced 100% with the treatment with 100 mg/kg/day of benznidazole (Figure 1).

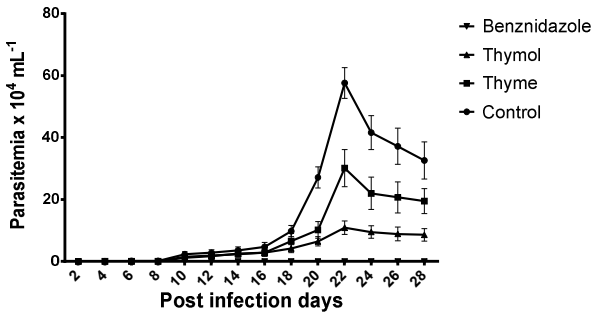

Figure 1. *Effect of oral treatment with Thymus vulgaris (thyme) essential oil and thymol on the parasitemia in mice infected with 10^4 blood trypomastigotes of T. cruzi.*

3.3. Effect of the Essential Oil on Amastigotes of *Trypanosoma cruzi*

The largest number of amastigotes' nests was observed in the 28th PID in the infected group and untreated (21.60 ± 7.54 nests of amastigotes/100 fields), followed by the infected group and treated with 200 mg/kg/day of *Thymus vulgaris* (4.00 ± 2.92 nests of amastigotes/100 fields) producing that treatment a significant reduction ($p < 0.001$) of 81.48%, meanwhile with 200 mg/kg/day of thymol, it was produced a significant decrease up to 2.40 ± 3.29 amastigotes' nests/100 fields ($p < 0.001$), equivalent to 88.89 %. In the group treated with benznidazole 100 mg/kg there was not observed any amastigotes' nests (Figure 2).

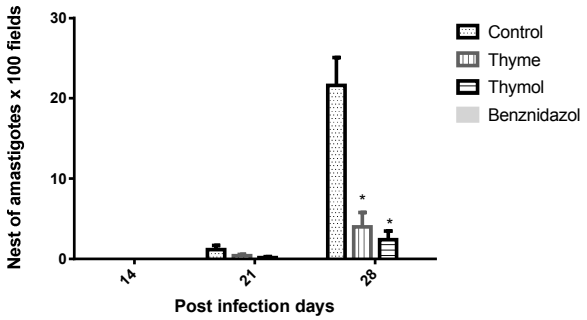

Figure 2. *Amastigotes' nests per 100 fields in mice's hearts infected with 10^4 blood trypomastigotes of T. cruzi and treated with thyme and thymol.*

3.4. Effect of the Essential Oil on Inflammattory Infiltrates

The number of inflammatory infiltrates/100 fields increased progressively in the infected group and no treated, being higher in the 28th PID (36.40 ± 13.45). Treatment with the *Thymus vulgaris* essential oil in doses of 200 mg/kg produced a reduction of inflammatory infiltrates in the heart tissue in the 28th PID up to 28.80 ± 10.64, while they were reduced to 19.20 ± 6.10 with thymol (Figure 3).

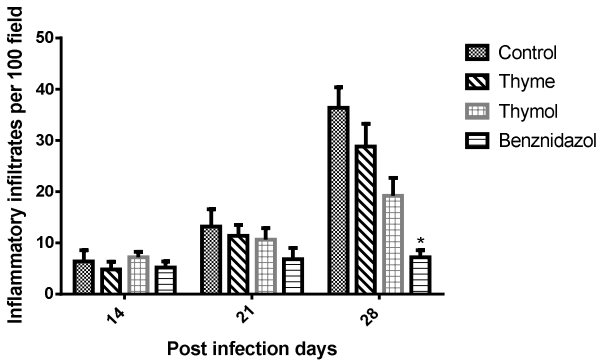

Figure 3. *Inflammatory infiltrates per 100 fields in mice's hearts infected with 10^4 blood trypomastigotes of T. cruzi and treated with thyme and thymol.*

3.5. Effect of the essential oil on Body Weight

Body weight gain of mice in the different experimental groups did not show significant differences when they were compared with the uninfected group and untreated (G5). Table 2.

4. Discussion

The main component of the *Thymus vulgaris* essential oil, identified in our study, was thymol (46.47 %), followed by γ-terpinene (20.27 %) (Table 1). In different parts of the world it has been found differences in the chemical composition; in Poland, thymol (38.1 %), carvacrol (2.3 %) and p-cymene (29.1 %) [9]; in Brazil, thymol (46.6 %) and *p*-cymene (38.9 %) [12]; in Serbia, thymol 49.1 % and *p*-cymene (20 %) [20]; in Spain, linalool (44 %) and terpineol-4 (11.88 %) [21]; in Iran, carvacrol (46.62 %) and γ-terpinene (9.70 %) [22].

Table 2. *Body weight change (g) of mice treated with thyme and thymol.*

GROUP	DAY				
	0	**7**	**14**	**21**	**28**
Infected + untreated	23.13 ± 1.46	25.00 ± 1.69	26.07 ± 2.43	27.70 ± 3.02	27.60 ± 3.36
Infected + thyme	24.13 ± 1.55	25.33 ± 1.95	26.07 ± 2.12	26.90 ± 2.96	27.00 ± 3.00
Infected + thymol	24.87 ± 1.77	24.20 ± 2.46	26.27 ± 2.46	27.10 ± 1.79	25.20 ± 2.68
Infected+ Benznidazole	23.33 ± 1.40	24.40 ± 1.68	26.07 ± 2.31	26.90 ± 2.23	28.00 ± 1.22
Uninfected + untreated	23.67 ± 1.84	23.73 ± 2.52	25.80 ± 2.78	26.30 ± 2.79	28.20 ± 1.48
Uninfected + thyme	24.30 ± 2.00	25.80 ± 1.48	27.10 ± 2.28	26.70 ± 2.41	26.60 ± 2.17

Values expressed as mean ± SD. No significant difference in the groups vs uninfected group and untreated. Data processed by one-way ANOVA and Scheffe post hoc test.

In a study conducted by Thompson et al., 2003, at 15 sites in different areas of the south of France, it was found a chemical polymorphism of *Thymus vulgaris* with six different chemotypes, distinguished on the basis of the dominant monoterpene produced in the glandular trichomes on the leaves' surface. These chemotypes were: geraniol chemotype (49.9-73.1% of geraniol, 4.2-18.7% of linalool, 2.8-3.9% of β-caryophyllene); α-terpineol chemotype (58.1-74.2% of α-terpineol, 2.7-10.4% of thymol, 1.6-6.2% of linalool); thujanol-4 chemotype (19.0-28.1% of thujanol-4, 14.7-15.1% of ternipen-4-ol, 8.8-14.0% and 3.7-13.1% of mircenol-8); linalool chemotype (63.4-81.8% of linalool and 1.9-2.4% of thymol); carvacrol chemotype (30.9-57.2% of carvacrol, 5.0-8.7% of γ-terpinene and 3.2-14.1 % of thymol); and, thymol chemotype (40.1-52.5% of thymol, 9.7-9.9% of p-cymene and 7.2-14.1 % of γ-terpinene). This chemical polymorphism was associated with genetic and ecological differences in the species between sites like altitude, temperature, soil type, and moisture content [(1)]. According to this characterization, the essential oil of our sample from Lima-Peru belongs to the thymol chemotype.

In the search for new alternative drugs for the treatment of Chagas' disease, the essential oils of some plant species showed *in vitro* activity against epimastigotes and amastigotes of *Trypanosoma cruzi* [18, 23], and in vivo activity in mice that were experimentally infected with this parasite [24, 25].

The *Thymus vulgaris* L. hydroalcoholic extract and the essential oil, have shown a potential antiprotozoal activity, characterizing an effectiveness against trophozoites of *Entamoeba histolytica* [26], as well as being effective in cutaneous leishmaniasis in mice with better activity than glucantime [27]. In the present investigation, the *Thymus vulgaris* L. essential oil and its main component, thymol, produced a significant reduction of the parasitemia peak in infected mice with *Trypanosoma cruzi* (Figure 1).

In fungi and bacteria, the *Thymus vulgaris* essential oil produced an extensive lesion and permeabilization of the plasma membrane [6, 28]; while in vitro studies with epimastigotes and trypomastigotes of *Trypanosoma cruzi*, it has been shown by transmission electron microscopy that the *Thymus vulgaris* essential oil and thymol produced cytoplasmatic swelling with occasional morphological alterations in plasma and flagellar membrane [18], which could indicate a direct effect on the parasite.

In mammalian hosts, *T. cruzi* (amastigote form) replication occurs in the cytoplasm of different types of cells, including macrophages, fibroblasts, skeletal and heart muscle cells, and endothelial cells, but heart, esophagus, or intestinal pathologies are the most common and severe forms of chronic chagasic disease, often with fatal consequences. Invasion and destruction of host cells represents the earliest documented manifestation of tissue damage associated with *T. cruzi* infection [14]. The treatment of the infected mice with *T. cruzi*, with the *Thymus vulgaris* essential oil and thymol, significantly decreased the number of amastigotes' nests in the mice's cardiac tissue (Figure 2). This is related to the decrease of the parasitemia by the trypanocidal action, but it could also be related to an immunomodulator effect, since it has been demonstrated a potent role of thymol in modulation of macrophage activity with a significant increase in the proliferation of splenocytes, the uptake capacity of macrophages was enhanced due to increased membrane fluidity and it also increases lysosomal activity of macrophages [29].

Inflammation is indeed a key characteristic of the pathology of Chagas disease. There is ample evidence that the most severe forms of the disease are defined by damage to vital tissues generally framed by inflammation, strongly suggesting that *T. cruzi* and damaged tissue can constitute suitable stimuli for the recruitment of inflammatory cells. Activated neutrophils and eosinophils are rather aggressive inflammatory-type cells known to destroy *T. cruzi* and mammalian cells [14]. The decrease in inflammatory infiltrates in the mice's hearts (Figure 3), might be related to the presence of a smaller number of parasites in this tissue, the trypanocidal action, as well as the anti-inflammatory effect both of the *Thymus vulgaris* essential oil as its main ingredient, thymol. In this regard, it has been shown that the *Thymus vulgaris* essential oil significantly decreased the secretion of pro-inflammatory cytokines TNFα, IL-1β and IL-8 [30]; also, it significantly reduced production and gene expression of the proinflammatory mediators TNFα, IL-1β, and IL-6 and highly increased these parameters on the anti-inflammatory IL-10 cytokine [31]. On the other hand, the thymol showed anti-inflammatory activity by inhibiting the

release of human neutrophil elastase [32], decreasing the influx of leukocytes to the injured area [10], and by decreasing the secretion of proinflammatory cytokines TNFα, IL-1β and PGE2 [29]. It has recently been reported that the inflammation and mortality during the acute infection by *T. cruzi* are reduced by regulatory mechanism that involves the IL-17RA-mediated recruitment of suppressive IL-10-producing neutrophils [33]. Any extrapolation of these findings to human Chagas disease would be premature and unwarranted, given that the transition from acute to chronic Chagas disease in most susceptible mouse strains occurs in a matter of few weeks, as opposed to the many years that it takes human patients to come out of the indeterminate phase and present overt cardiomyopathy. Furthermore, there are notable differences in both the susceptibility, pathology, and rates of progression of Chagas disease in human and murine hosts [14].

The corporal weight is an important toxicity indicator since it is involved in a series of organic changes, when a significant change of its value suggests some adverse effect of drugs or chemicals. In our experiment, the mice's gain of corporal weight during the treatment period with the *Thymus vulgaris* essential oil and thymol, did not change significantly (Table 2), which would indicate they lack of toxicity. This observation keeps relation with other investigations where thymol did not show any cytotoxic effect in normal human peripheral blood mononuclear cells [34], no cytotoxic effects were recorded for thymol at any concentration and time of exposure on the intestinal cells line Caco-2 after 24 and 48 h of exposure [35], thyme essential oil had no detectable effects on embryo development in mice [36]. Although, in another study it has been informed that thymol induced both the structural chromosome aberration and frequency of micronucleus at all concentrations in human peripheral lymphocytes [37].It is recommended in vivo tests of acute and sub chronic toxicity to establish the safety of the *Thymus vulgaris* essential oil and of thymol.

Our findings suggest that the *Thymus vulgaris* essential oil and thymol have an anti-*Trypanosoma cruzi* effect in vivo in infected mice.

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References

[1] Thompson J, Chalchat JC, Michet A, Linhart Y, Ehlers B. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. J Chem Ecol. 2003;29(4):859-880.

[2] Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). J Ethnopharmacol. 2007;110:105-117.

[3] Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J Ethnopharmacol. 2008;116(3):403-406.

[4] Roig y Mesa J. Plantas medicinales, aromáticas o venenosas de Cuba. 2ª. ed. Tomo II. La Habana: Editorial Científico-Técnica; 2012. Spanish

[5] Zu Y, Yu H, Liang L, Fu Y, Efferth T, Liu X, Wu N. Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules. 2010; 15(5):3200-3210.

[6] Pina-Vaz C, Gonçalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, et al. Antifungal activity of *Thymus* oils and their major compounds. J Eur Acad Dermatol Venereol. 2004; 18:73-78.

[7] Chizzola R, Michitsch H, Franz C. Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes. J Agric Food Chem. 2008; 56(16):6897-904.

[8] Meeran MF, Prince PS. Protective effects of thymol on altered plasma lipid peroxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. J Biochem Mol Toxicol. 2012; 26(9):368-73.

[9] Lai WL, Chuang HS, Lee MH, Wei CL, Lin CF, Tsai YC. Inhibition of herpes simplex virus type 1 by thymol-related monoterpenoids. Planta Med. 2012; 78(15):1636-8.

[10] Riella KR, Marinho RR, Santos JS, Pereira-Filho RN, Cardoso JC, Albuquerque-Junior RL, et al. Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from *Lippia gracilis*, in rodents. J Ethnopharmacol. 2012; 143(2):656-63.

[11] Archana PR, Nageshwar Rao B, Satish Rao BS. In vivo radioprotective potential of thymol, a monoterpene phenol derivative of cymene. Mutat Res. 2011; 726(2):136-45.

[12] Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against Escherichia coli. Lett Appl Microbiol. 2008;47(3):174-9.

[13] Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur J Clin Microbiol Infect Dis. 2011; 30(1):41-50.

[14] Kierszenbaum F. Mechanisms of pathogenesis in Chagas disease. Acta Parasitologica. 2007;52(1):1-12.

[15] World Health Organization. Chagas disease (American Trypanosomiasis). Fact sheet N° 340. Accessed 5 december 2014. available: http://www.who.int/mediacentre/factsheets/fs340/es/

[16] Bern C, Montgomery SP, Herwaldt BL, Rassi A, Marin J, Dantas RO, et al. Evaluation and Treatment of Chagas Disease in the United States: A Systematic Review. JAMA. 2007; 298(18):2171-2181.

[17] WHO/ TDR. Report on Chagas disease, 2007. Accessed 25 november 2014. available: http://whqlibdoc.who.int/hq/2007/TDR_SWG_09_spa.pdf.

[18] Santoro G, Das Graças M, Guimaraes L, Salgado A, Mena R, Soares M. Effect of orégano (Origanum vulgare L.) and Thyme (*Thymus vulgaris* L.) essential oils on *Trypanosoma cruzi* (Protozoa: Kinetoplastida) growth and ultraestructure. Parasitol Res. 2007; 100(4):783-790.

[19] Lahlou M. Methods to study the phytochemistry and bioactivity of essential oils. J Phytother Res 2004; 18:435-448.

[20] Nikolic M, Glamoclija J, Ferreira I, Calhelha R, Fernandes A, Markovi´c T, et al. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. Industrial Crops and Products. 2014; 52: 183-190.

[21] Ballester-Costa C, Sendra E, Fernández-López J, Pérez-Álvarez J, Viuda-Martos M. Chemical composition and in vitro antibacterial properties of essential oils of four Thymus species from organic growth. Industrial Crops and Products. 2013; 50: 304-311.

[22] Hosseini M, Ghasemi Y, Javad M, Faridi P, Moradli G, Montazeri N. Volatile oil composition and antimicrobial activity of two Thymus species. Pharmacognosy Journal. 2013;5:77-79.

[23] Rojas J, Solís H, Palacios O. Actividad anti-Trypanosoma cruzi in vitro de aceites esenciales de diez plantas medicinales. An Fac Med. 2010;71(3):161-165. Spanish

[24] Rojas J, Palacios O, Ronceros S. Efecto del Aceite Esencial de *Aloysia triphylla* Britton (cedrón) sobre el *Trypanosoma cruzi* en ratones. Rev Peru Med Exp Salud Publica. 2012;29(1):61-68. Spanish

[25] Rojas J, Ronceros G, Palacios O, Sevilla C. Efecto anti-*Trypanosoma cruzi* del aceite esencial de *Cymbopogon citratus* (DC) Stapf (hierba luisa) en ratones Balb/c. An Fac Med. 2012; 73(1):7-12. Spanish

[26] Behnia M, Haghighi A, Komeylizadeh H, Javad S, Abadi A. Inhibitory Effects of Iranian *Thymus vulgaris* Extracts on in Vitro Growth of *Entamoeba histolytica*. Korean J Parasitol. 2008; 46(3):153-156.

[27] Nilforoushzadeha M, Shirani-Bidabadia L, Zolfaghari-Baghbaderania A, Saberia S, Siadata A, Mahmoudib M. Comparison of *Thymus vulgaris* (Thyme), *Achillea millefolium* (Yarrow) and propolis hydroalcoholic extracts versus systemic glucantime in the treatment of cutaneous leishmaniasis in balb/c mice. J Vector Borne Dis. 2008; 45:301-306.

[28] Nguefack J, Budde B, Jakobsen M. Five essential oils from aromatic plants of Cameroon: their antibacterial activity and ability to permeabilize the cytoplasmic membrane of *Listeria innocua* examined by flow cytometry. Letters in Applied Microbiology 2004; 39: 395-400.

[29] Chauhan AK, Jakhar R, Paul S, Kang SC. Potentiation of macrophage activity by thymol through augmenting phagocytosis. International Immunopharmacology. 2014; 18(2):340-346.

[30] Tsai ML, Lin Ch, Lin W, Yang Ch. antimicrobial, antioxidant, and anti-inflammatory activities of essential oils from five selected herbs. Biosci. Biotechnol. Biochem. 2011;75(10):1977-1983.

[31] Ocaña A, Reglero G. Effects of Thyme Extract Oils (from*Thymus vulgaris, Thymus zygis*, and *Thymus hyemalis*) on Cytokine Production and Gene Expression of oxLDL-Stimulated THP-1-Macrophages. Journal of Obesity. 2012, doi:10.1155/2012/104706.

[32] Braga PC, Dal Sasso M, Culici M, Bianchi T, Bordoni L, Marabini L. Anti-Inflammatory Activity of Thymol: Inhibitory Effect on the Release of Human Neutrophil Elastase. Pharmacology 2006; 77: 130-136.

[33] Tosello Boari J, Amezcua Vesely MC, Bermejo DA, Ramello MC, Montes CL, et al. IL-17RA Signaling Reduces Inflammation and Mortality during Trypanosoma cruzi Infection by Recruiting Suppressive IL-10-Producing Neutrophils. PLoS Pathog 2012;8(4):e1002658. doi:10.1371/journal.ppat.1002658.

[34] Deb DD, Parimala G, Saravana Devi S, Chakraborty T. Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. Chem Biol Interact. 2011; 193(1):97-106.

[35] Ruiz-Cabello M, Gutiérrez-Praena D, Pichardo S, Moreno FJ, Bermúdez JM, Aucejo S, et al. Cytotoxicity and morphological effects induced by carvacrol and thymol on the human cell line Caco-2. Food and Chemical Toxicology. 2014; 64: 281-290.

[36] Domaracky M, Rehák P, Juhás Š, Koppel J. Effects of Selected Plant Essential Oils on the Growth and Development of Mouse Preimplantation Embryos *In Vivo*. Physiol. Res. 2007;56:97-104.

[37] Buyukleyla M, Rencuzogullari E. The effects of thymol on sister chromatid exchange, chromosome aberration and micronucleus in human lymphocytes. Ecotoxicol Environ Saf. 2009; 72(3):943-7.