Rev. Biol. Trop., 38(2A): 235-241, 1990

Increase of sulfadiazine effect against *Toxoplasma gondii* by using watermelon or cantaloupe seeds

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(Rec. II-VII-1989. Acep. 24-I-1990)

Abstract: In an experimental model the anti—*Toxoplasma* effect of watermelon and cantaloupe seeds, together with sulfadiazine ($Sd_{7.5}$ and Sd_{15}) given by oral route, were studied. Combination of any of these seeds with the drug was able to increase the survival time and cure some tachyzoite infected mice. The effect was produced either by peeled or complete seeds and more significant results were obtained when $Sd_{7.5}$ and 10^3 tachyzoite inoculum was used in the model. Some Toxoplasma oocyst infected mice were cured with the seeds alone (75% for watermelon, 37,5 or 50% for cantaloupe. Body weight variations are independent of the treatment with Sd alone or in combination with watermelon or cantaloupe seeds.

Key words: Toxoplasma, natural medicines, Cucurbitaceae.

Sulfadiazine (Sd) and pyrimethamine sinergistic effect on experimental toxoplasmosis of mice was demonstrated by Eyles & Coleman (1953, 1955). More recently, Kovacs *et al.* (1987) have found better results with a combination of sulfonamide and trimetrexate. Because the antifolic and antifolinic acid effect of these drugs, it is neccesary to give a parallel treatment with Leucovorin and yeast to avoid abnormal manifestations in the host (Frenkel & Hitchings 1957).

Thinking in all these situations we were looking for any natural compound which could either act directly against *Toxoplasma* or at least increase the Sd antiparasitic effect.

It has been indicated that watermelon seeds have some effect against *Plasmodium* (Nuñez 1978) an Eucoccidiida organism, such as *T. gondii* (Levine 1985). Therefore, using experimental treatment models already described (Frenkel & Hitchings 1957) we tested the action of watermelon and the related cantaloupe seeds together with sulfadiazine against *T. gondii*. This paper gives some promissing information in this interesting field.

MATERIAL AND METHODS

Animals: Wistar mice (20—25 g) were normally caged and used throughout these experiments.

T. gondii strains: The known RH strain and an oocyst producing strain (TCR—1) isolated from an owl *(Glaucidium brasilianum)* were used to infect the animals and test the corresponding treatment. The RH strain is mantained in our laboratory by a twice weekly passage in Wistar mice. TCR—1 strain has been transfered from mouse to mouse, with some cat passages in between, for 5 years.

Sulfadiazine and seeds preparation: Sulfadiazine (ICN Pharmaceuticals Inc.) was diluted to 7.5 ($Sd_{7.5}$) and 15 (Sd_{15}) mg/ liter in destilled water and vital dyes were added to differentiate between the drugs concentrations. Fresh solutions were prepared for each experiment.

Watermelon or cantaloupe seeds were peeled, and pulverized with a morter. The powder was dried at 37^0C and kept in refrigeration for no more than 8 days before use. Amounts of 0.2 or 1 % of seed powder was carefully mixed with grinded food in order to achieve an uniform seed distribution, and animals were feed with these mixtures according to each experiment.

Experimental treatment model: mice were inoculated (subcutaneously) with different tachyzoite concentrations or by the oral route with oocysts. Twenty four hours later, sulfadiazine and seed treatment were started and mantained for 14 days. Daily water consumption, weekly body weight and animal survival time were recorded. After 30

days of infection, survivors were challenged with 10^3 *Toxoplasma* tachyzoites in order to determine chronic infections. Those infected animals that died after challenge due to *T. gondii* infection were considered totally cured. Chronic infected mice were killed and their brains were checked for the presence of *Toxoplasma* cysts. The number of cysts per gram or per brain was determined and blood samples from each animal were studied for antibody production.

RESULTS

Treatment with incomplete watermelon seeds: Survival time percentage was one of the parameters used to determine any concomitant curative effects of watermelon seeds and sulfadiazine. The results obtained in mice infected with three different inocula and treated with several combinations of both substances are shown in figure 1. When mice were infected with 10 tachyzoites and treated with $Sd_{7.5}$, the increase on survival percentage after 30 days of infection was clearly observed if watermelon seeds was added to the food. After 15 days, almost all of the animals treated with Sd_{15} survived, making it impossible to see any effect. In animals infected with 10^3 tachyzoites and then treated, the increased protective effect due to the seeds and $Sd_{7.5}$ treatment was evident 15 days as well as 30 days after infection.

The results when Sd_{15} was used were similar, independently of seed treatment.

Infections with 10^5 organisms seemed to be very strong; therefore, treatment with $Sd_{7.5}$ showed a very low survival percentage after 30 days of infection. However, an interesting difference in survival was observed after 30 days of infection in animals treated with Sd_{15} and seeds as compared with those ingesting Sd alone.

Survival time in mice infected with different inocula and treated with Sd and watermelon seeds was recorded. The more convincent results were seen when $Sd_{7.5}$ was used (Fig. 2). Even though differences were observed, in all the inocula the curative effect due to the addition of seeds in the diet was more evident in animals infected with 10^3 *T. gondii* organisms.

More animals treated with Sd and seeds were totally cured as demonstrated by challenge. Thus when mice were treated with Sd_{15} alone, less number of animals were free of *T. gondii* as compared with those with simultaneous treatment with watermelon seeds. The major effect was shown when a 1 % concentration of seeds was used (Fig. 3). None of the animals infected with 10^5 tachyzoites and non-treated were cured. For cantaloupe seeds treatment, a similar protective effect was observed (Fig. 4).

Treatment with complete seeds: To demonstrate if the direct aplication of complete seeds could give better results in *T. gondii* infection treatment, some animals were fed by mouth with complete seeds 1 day after infection with 10^3 organisms. Results are shown in table 1 and underlined data show the most significant differences (t-test were used for all inferential statistics). In fact survival time of mice treated with $Sd_{7.5}$ and 1% seed mixture was longer (27 days) as compared with controls (22.6 days) . Similar results were found in animals treated with Sd_{15} and 0.2 or 1% watermelon seeds: while these animals survived for almost 30 days, mice treated with Sd_{15} alone died after 23 days. Complete cure was observed only in animals treated with Sd_{15} plus complete seeds.

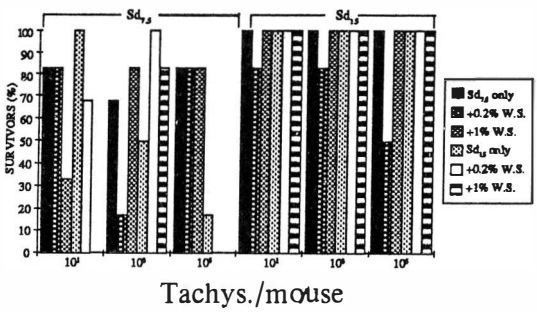

Fig. 1. Percentage of surviving mice infected with T. *gondii* and treated with Sd and watermelon seeds (W.S.) after 15 days or 30 days of infection.

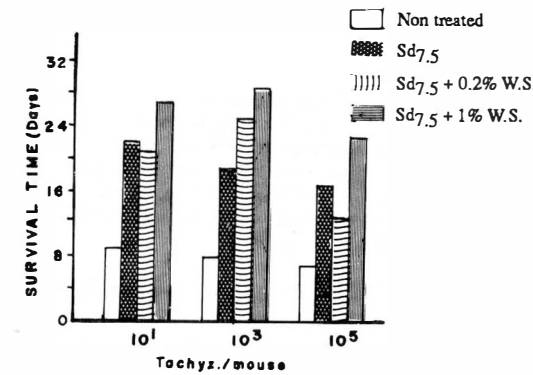

Fig. 2. Survival time of mice infected with *T. gondii* and treated with Sulfadiazine (Sd) and watermelon.

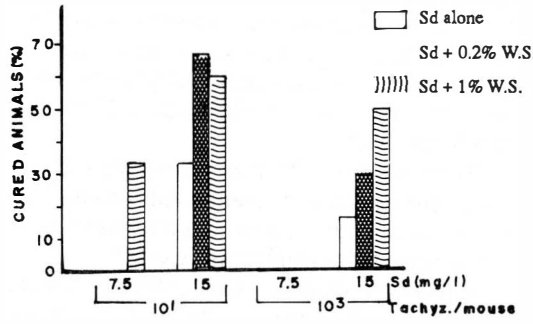

Fig. 3. Percentage of mice infected with *T. gondii* and cured after treatment with Sulfadiazine (Sd) an watermelon seeds.

Treatment with Sd and incomplete cantaloupe seed. More animals survived when they were treated with Sd_{15} plus cantaloupe seeds (Fig. 4). This effect was seen for low (10 tachyzoites) as well as for high (10^3 tachyzoites) *Toxoplasma* infection.

TABLE 1

*Effect of Sd and watermelon seeds treatment on Toxoplasma infected mice**

Treatment		% of survivors after		Survival time	% of cured mice
Sd (mg/% body wieght)	Watermelon seeds (g/%)	15 d	30 d	day	
0	0	100	100	30	0
0	0	0	0	9.6	0
7.5	0	62.5	62.5	22.6	0
15.0	0	75	25	23.0	12.5
0	0.2	0	0	8.9	0
0	1	0	0	8.9	0
7.5	0.2	25	50	23.3	0
7.5	1	75	75	27.0	0
15	0.2	100	100	29.8	37.5
15	1	100	100	29.8	25

* 10^3 tachyzoites infection

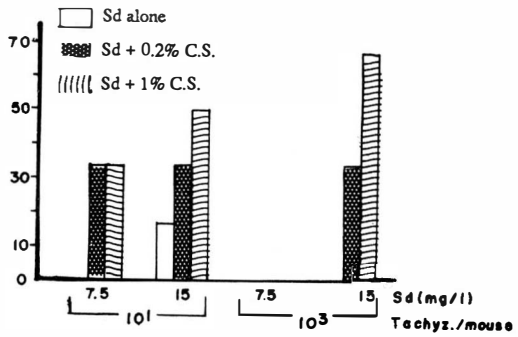

Fig. 4. Percentage of mice infected with *T. gondii* and cured after treatment with Sulfadiazine (Sd) and cantaloupe seeds (C.S.)

Even when the results were not very convincent, a major number of mice survived from *T. gondii* infection when they were treated with Sd^{15} plus 0.2% (87.5%) or plus 1% cantaloupe seeds (100%) as compared with those treated with Sd^{15} alone in which 75% of mice were cured (Table 2).

Treatment of oocyst infected animals (Table 3): Most of the animals inoculated with 10^3 *T. gondii* oocysts survived when they were treated with Sd alone or with cantaloupe or watermelon seeds without Sd. However it is interesting to observe that treatment with seeds without Sd, produced a cure percentage: 75% for watermelon and 37.5% or 50% for cantaloupe besides the combined effect with Sd that produces a 100% cure.

Body weight variation (Table 4) Animals treated with Sd_{15} showed a higher body weight increase after 2 weeks of infection as compared with those non treated or treated with $Sd_{7.5}$. Four weeks later, the increase in their body weight was lower but no significant differences were observed between animals treated with seeds and those treated with Sd alone.

Animals treated with Sd—watermelon seeds combination showed a higher decrease of body weight after 30 days (Fig. 5) as compared with mice treated with the Sd—cantaloupe seeds combination.

DISCUSSION

Due to the toxic effect of drugs used in malaria and toxoplasmosis treatment, researchers are returning to the natural products (Peters *et al.* 1986). Artemisin and related sesquiterpenes as ginghaosu and others are some of the materials on study (Klayman 1985, Chang & Pechre 1988). Cucurbitacea fruits seeds have been considered also to have some curative effect in malaria (Nuñez 1978). Since *T. gondii* is an Eucoccidiida such as *Plasmodium* (Levine 1985) we expected that watermelon and cantaloupe seeds could be useful in toxoplasmosis treatment. These seeds increased the Sd effect since survival time was higher in animals treated with both products (Figs. 1-2). This effect was more evident where $Sd_{7.5}$ was used in animals infected with 10^3 tachyzoites, which was the most adequate inoculum to demonstrate the effect of the treatment. This can be explained because this low Sd concentration is not enough to inhibit the parasite multiplication as Sd_{15} does. However, addition of the seeds apparently produces a higher anti-Toxoplasma effect as demonstrated not only by mice survival time but by the cure observed in

TABLE 2

Effect of Sd and cantaloupe seeds treatment on Toxoplasma *infected mice*

Treatment		% of survivors after		Survival time	% of cured
Sd (mg/% body wieght)	Cantaloupe seeds (g/%)	15 d	30 d	day	mice
0	0	100	100	30	0
0	0	0	0	6.9	0
7.5	0	100	87.5	**29.9**	**25**
15.0	0	100	87.5	29.6	75
0	0,2	0	0	7.1	0
0	1	0	0	6.8	0
7.5	0,2	100	87.5	29.9	0
7.5	1	100	71.4	26.7	**25**
15	0,2	100	100	30	**87.5**
15	1	100	100	30	**100**

* 10^3 tachyzoites infection

TABLE 3

Effect of Sd and two Cucurbitacea seeds on mice infected with 103 Toxoplasma *oocysts*

Treatment		% of survivors		% cured
Sd (mg/% body weight)	Watermelon g %	15 d	30 d	animals
0	0	87.5	87.5	0
7.5	0	100	100	100
15	0	100	100	100
0	0,2	100	100	**75**
0	1	100	100	**75**
7.5	0,2	100	100	100
7.5	1	100	100	100
15	0,2	100	100	100
15	1	100	100	100
cantaloupe				
0	0,2	100	100	**50**
0	1	100	87.5	**37.5**
7.5	0,2	100	100	100
7.5	1	100	100	**87.5**
15	0,2	100	100	100
15	1	100	100	100

frequently acquired by oocyst ingestion (Frenkel & Ruiz 1980).

Body weight decrease is usually observed in *Toxoplasma* infected mice, but we could not find any differences between Sd plus seed treated mice and those treated with Sd alone (Table 4). It means that there are many others factors, difficult to control, that play important roles in animal nutrition. An apparent higher increase in body weight was found for Sd—watermelon seeds treated animals as compared with those in which the combination Sd—cantaloupe seeds was used (Fig. 5), but the results present a random distribution. This suggests that food ingestion is the same, independently of the type of seeds added to it. Therefore the curative effect is probably similar for both Cucurbitacea fruits.

TABLE 4

Variation of body weight in mice

Inoculum (Tachyz/ Mouse)	Treatment Sd	Seed (%)	Increase or decrease (%) watermelon 2*	watermelon 4	cantaloupe 2	cantaloupe 4
10^1	-	-	25	25	-0.5	12
			18	-	2	10
	7.5	-	30	14	5	2
	7.5	0.2	33	-	14	6
	7.5	1	28	1	15.5	-3.5
	15	-	48	-3	4.5	4
	15	0.2	34	25	16	4
	15	1	42	4	9	11
10^3	-	-	-	-	-	-
	7.5	-	20	21	2.5	-3
	7.5	0.2	19	41	14.5	7
	7.5	1	38	-20	6	-3
	15	-	43	2	1	-12
	15	0.2	19	-25	14	3
	15	1	43	-5	3.5	15
10^5	-	-	-	-	-	-
	7.5	-	-	-	-	-
	7.5	0.2	10	-	0.4	11
	7.5	1	38	12	10	-14
	15	-	49	5	3.5	1
	15	0.2	44	-25	12.5	10
	15	1	39	-7	4	3

* Two or 4 weeks after infection

some of the animals treated with Sd and seeds (Fig. 3 y 4). At least in these experiments no significant differences were observed for watermelon and cantaloupe treatment; this could indicate that the effective factor is similar, probably because of their taxonomic relation.

Treatment with complete seeds (Table 1 and 2) gave some controversial results, probably because of difficult distribution of seed material. However the presence of a helper effect of these natural products for Sd treatment was evident, since more animals were free of *Toxoplasma* infection after treatment with Sd_{15} plus any of both kind of seeds (see underlined data) than after treatment with Sd alone. Comparing these results with those using peeled seeds, we did not find any important differences in the curative effect.

Oocysts are very important in human infection in Costa Rica because they are very resistant to environmental conditions (Frenkel *et al.* 1975) and there are many infected cats that shed large amounts of oocysts (Frenkel & Ruiz 1980). Domiciliar rodents, principal source of infection for cats, take the oocyst from the soil and get the parasite. We have found rats and mice infected with *T. gondii* in Costa Rica (Chinchilla 1978 and Frenkel & Ruiz 1981) and Mairena *et al.* (1986) have reported the presence of oocyst in several soil samples, thus it was convenient to determine if laboratory mice infected with oocysts could be cured by using the Sd seeds combination. In fact, as shown in table 3 (underlined numbers), not only does the combination induce animal cure but also treatment with watermelon or cantaloupe seeds alone induced an important cure percentage, as compared with non—treated animals. This findings could be very important since it has been suggested that in Costa Rica *T. gondii* is

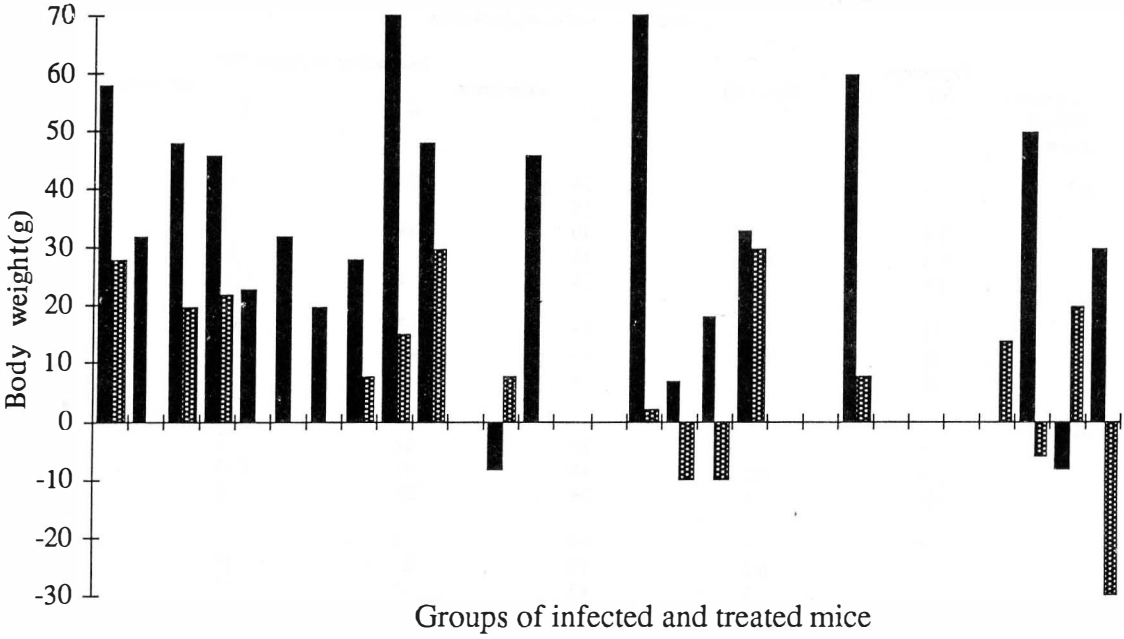

Fig. 5. Body weight increase or decrease (%) in mice after 30 days of infection and treatment with watermelon or cantaloupe seeds.

Studies to determine the anti—Toxoplasma active component present in these seeds are in progress.

Results in challenge and CIA test confirmed cure and chronic infection showing that this last serological method is useful in mice studies as has been demonstrated for cats (Pakes & Lai 1985) and other animals (Uggla & Hjort 1984).

RESUMEN

Mediante la utilización de ratones infectados experimentalmente con *T. gondii*, se estudió el efecto curativo de sulfadiazina ($Sd_{7.5}$ y Sd_{15}) combinado con semillas de sandía y de melón administrado por vía oral. La mezcla de cada una de las semillas en el alimento con la droga en el agua aumentó la supervivencia de ratones infectados con taquizotos. El efecto fue inducido igualmente por semillas completas o desprovistas de cáscara y los mejores resultados fueron obtenidos cuando se usó la concentración $Sd_{7.5}$ de la droga y un inóculo de 10^3 taquizotos. Un porcentaje importante de ratones infectados con ooquistes se curó completamente no sólo con la mezcla sino también con las semillas solas de sandía (75%) y de melón (37.5 y 50%) agregadas al alimento. Las variaciones en peso no indicaron diferencia significativa entre los animales tratados con sólo la droga y los tratados además con las semillas de sandía o de melón.

ACKNOWLEDGMENTS

This work was supported in part by the Vicerrectora de Investigación of the Universidad de Costa Rica and the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT).

The authors thanks Fabio Camacho and Zayda Umaña for the animal care and secretarial assistance, respectivaly.

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