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THE EFFECT OF CERTAIN ESSENTIAL OILS ON LABORATORY MICE INTESTILAN PROTOZOA

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Abstract: The research took place between October 2006 and May 2007 on a number of 30 laboratory mice; in order to determine the way in which essential oils (*Ocimum basilicum* - Basil; *Tymus vulgaris* - thyme; *Calendula officinalis* - Pot Marigold or English Marigold) administered in doss of 10µl/mouse over a period of 5 consecutive days; act on the intestinal protozoa; showed that these influenced differently the structure of the flagellate population determining certain particularities: *Ocimum basilicum* had an effect on *Giardia; Hexamita* and *Trichomonas* species*; Calendula officinalis* had an effect on *Hexamita muris; Chilomastix bettencourti; Trichomonas* spp.; while the *Tymus vulgaris* extract was effective only in case of *Chilomastix bettencourti.*

INTRODUCTION

The European Union considers that protection of the consumer is a priority objective and highlights that anti-parasitical drugs determine accumulation of residue in food (milk and meat)that represents a major risk for the consumer (Hamscherd; 2004). FAO/WHO (2004; quoted by Hamscherd; 2004) evaluating the levels of drug residues in food recommends that these levels be kept as low as possible in order to ensure food safety and the protection of the consumer. Benoit and Laignel (2002) state that in case of creating biological farms; and therefore obtaining animal produces with a low level of chemical residue is necessary to limit anti-parasitic treatment with synthetic compounds; and for preventing the sanitary risk; the alternative may be plant-based therapy.

MATERIALS AND METHODS

In order to determine the effectiveness over the intestinal micro-flora and micro-fauna of laboratory mice 3 plants were chosen for study. The extracts utilized were in form of essential oils (oil extraction). The main objective was the analysis of the way in which plant extracts administered 5 consecutive days influence the intestinal micro-fauna. 30 mice were divided in 5 experimental groups. The oily extracts of *Ocimum basilicum; Tymus vulgaris;* and *Calendula officinalis* were administered in dose of 10µ\ mouse. In order to comparatively follow the results 2 control groups were established; one given water; (negative control) and one (treated control) given orally metronidazol in dose of 0;0012 g/ mouse. The comparative investigation of the plant extracts effect was done after 24 hours from the finalization of the period of administration and consisted of harvesting samples from the duodenum and cecum in order to identify and quantify the intestinal micro-fauna. In this case determination and quantification of the protozoa population structure was done comparatively to the negative and positive controls.

RESULTS AND DISCUSSIONS

The effect over the flagellates varied in view of the protozoa specie and the utilized extract (tab.1)

Giardia muris –the highest extensivity was noted in the negative control group (50%) while in the *Ocimum basilicum* extract treated group infection whit this species was not noticed; in the other groups the extensivity was of 33;33%. Infection intesivity and its average level were in relative concordance with the values of the extensivity. In the comparative assay of these parameters with the negative control group (M[1] = 16;64 G/l; I[2] = 33;28 G/l) it was noticed that in case of the metronidazole therapy the average sum was only of 0;01 G/l with an intesivity of 0;015 G/l. Meanwhile the groups treated with *Tymus vulgaris* and calendula officinalis showed average of 1.28 G/l and an intensivity of 3;85G\l.

Hexamita muris – extenivity was of 83;33% in the negative control group; 50% in the metronidazol treated group and Calendula officinalis and 33;33% in the *Tymus vulgaris*. In the group treated with *Ocimum basilicum* this species was not noticed. Regarding the average level of infection in the metronidazole treated group (M = 12;8 G/l; I = 25;6 G/l) these values being similar to the negative control group (M= 21;77 G/l; I = 26;12 G/l). The *Calendula officinalis* extract positively influenced the level of infection with *Hexamita muris* thus the average value (1;25G/l) was approximately 20 times lower than in the negative control group and 10 times lower than the metronidazole tested group. This fact is also highlighted by the value of intensivity that in the group treated with *Calendula oficinalis* which was 10 times lower than in the control. In case of the group treated with Tymus vulgaris even thaw the extensivity was low (33;33%) the average was only 6;4 G/l; and the intensivity of 19;2G/l; both parameters being lower than those recorded in the control group.

Chilomastix bettencourti – the lowest extenivity was noticed in the group treated with metronidazole 33;33% meanwhile the other groups were of 100% (negative control; basil) and respectively 83;33% (groups treated with thyme and marigold). These results are reflected also by level of infection; the highest levels being in the negative control group (101;12 G/l) and in the basil treated group (384 G/l) the lowest level was indicated in the metronidazole treated group; with an average of 1;28 G/l and an intensivity of 3;84 G/l; followed by the thyme treated group ((M = 10;24 G/l; I= 12;29 G/l) and marigold treated group (M = 43;52 G/l; I = 52;22 G/l).

Trichomonas spp. – comparative to the negative control in which the extensivity was of 100% in the others fluctuated between 33;33% (metronidazol control group and the basil treated group) and 50% (groups treated with thyme and marigold). In correlation with these results are also the *Trichomonas* average and the infestation level. For this we mention the fact that negative control group registered the lowest level of infection; the average and intensivity being of 138;37 G/l. The best effect was noticed in the group treated with metronidazole; the infection level being represented by an average of 2;56 G/L and an intensivity of 7;68 G/l. Quite good effectiveness was noticed in the *Ocimum basilicum* tested group (M = 7;68 G/l; I = 23;04 G/l) and *Calendula officinalis* (M = 12;8 G/l; I = 25;6 G/l); in the *Thymus vulgaris* treated group the average level of infection was of 44;8 G/l; and an intesivity of 89;6 G/l.

[1] M = average
[2] I = intensivity

Table 1

The influence of oily extracts on the intestinal flagellate population in laboratory mice (administrated 5 consecutive days)

No. crt.	*Giardia muris*	*Hexamita muris*	*Chilomastix bettencourti*	*Trichomonas* spp.
GROUP 1 –negative control group (distilled water)				
1.	76;800	30;720	15;360	276;480
2.	7;680	0;025	23;040	53;760
3.	0	0;040	330;240	8;440
4.	0	0	15;360	130;560
5.	0	7;680	176;640	353;280
6.	15;360	92;160	46;080	7;680
Average	***16;64***	***21;77***	***101;12***	***138;37***
Extensivity (%)	***50***	***83;33***	***100***	***100***
Intensivity (G/l)	***33;28***	***26;125***	***101;12***	***138;367***
GROUP 2 – positive control (Metronidazole)				
1.	0	0	0	15;360
2.	0	0	0	0
3.	0	7;680	7;680	0
4.	0	53;760	0	0;015
5.	0;025	15;360	0	0
6.	0;005	0	0;005	0
Average	***0;01***	***12;80***	***1;28***	***2;56***
Extensivity (%)	***33;33***	***50***	***33;33***	***33;33***
Intensivity (G/l)	***0;015***	***25;6***	***3;8425***	***7;6875***
GROUP 3 – *Ocimum basilicum*				
1.	0	0	161;280	0
2.	0	0	437;760	0
3.	0	0	552;960	23;040
4.	-	-	-	-
5.	-	-	-	-
6.	-	-	-	-
Average	***0***	***0***	***384***	***7;68***
Extensivity (%)	***0***	***0***	***100***	***33;33***
Intensivity (G/l)	***0***	***0***	***384***	***23;04***
GROUP 4 – *Tymus vulgaris*				
1.	0	0	23;040	0
2.	0	0	23;040	7;680
3.	0	38;400	7;680	53;760
4.	0	0;005	7;680	0
5.	7;680	0	0;005	0
6.	0.005	0	0	207;360
Average	***1;28***	***6;40***	***10;24***	***44;80***
Extensivity (%)	***33;33***	***33;33***	***83;33***	***50***
Intensivity (G/l)	***3;84***	***19;20***	***12;29***	***89;60***
GROUP 5 – *Calendula officinalis*				
1.	0	0;005	61;440	0
2.	0	0;025	46;080	0
3.	0	0	122;880	0
4.	7;680	7;680	0	23;040
5.	0	0	23;040	53;760
6.	0;025	0	7.680	0;005
Average	***1;28***	***1;29***	***43;52***	***12;80***
Extensivity (%)	***33;33***	***50***	***83;33***	***50***
Intensivity (G/l)	***3;85***	***2;57***	***52;22***	***25;60***

In the global analysis of the effectiveness of the oily plant extracts it has been noticed that these influenced differently the structure of the intestinal flagellate population distinguishing certain particularities. The effectiveness registered is due to the fact that the essential oils are mixtures of substances that belong to different chemical groups; most representative of these being the threnodies. It seams that this group of substances is the one that influences the parasites and therefore on the protozoa; however the mechanisms by which the interaction is realized are not fully understood. The obtained results we consider as being in line with the research in the field of parasitical phitotherapy. In this context we reiterate the excellent therapeutic results over *Giardia lamblia* and *Entamoeba hystolitica* of *Artemisia ludoviciana*; *Zanthoxylum liebmannianum* extracts (Arrieta şi colab.; 2001); *Helianthemum glomeratum* (Meckeset all.; 1999); *Piper longum* (Tripathi et all.; 1999); *Geranium mexicanum; Teloxys graveolens* (Calzada et all.; 2003). Some plants had an effect only on the trophozoites of *Giardia* spp.: *Justicia spiciera*; *Lipia beriandieri* (oregano); *Psidium guajava* (guava); *Punica granatus*; *Magnifera indica* (mango); *Plantago major* (Greater Plantain or Common Plantain); *Cupressus sempervirens* (Mediterranean Cypress); *Castella tormentosa*; *Hematoxilon campechanum*; *Persea americana* (avocado); *Capsicum annum Oriza sativa* (rice) (Ponce-Macetola et all.; 1994); *Yucca schidigera* (McAllister et all; 2001).

CONCLUSIONS

Establishing the therapeutic effectiveness of essential oils administered for 5 consecutive days (10µl/mouse/day) has shown that essential oils influenced differently the structure of the intestinal flagellate population:

- *Ocimum basilicum* had an effect on species from the Giardia genus (0G/l); Hexamita (0 G/l) and Trichomonas (7;68G/l)
- *Calendula officinalis* had an effect on *Hexamita muris* (1;29 G/l); *Chilomastix bettencourti* (43;52 G/l); *Trichomonas* spp. (12;80 G/l).
- *Tymus vulgaris* had aa good effect only on *Chilomastix bettencourti* (10;24 G/l).

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