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EFFECT OF THE PROPOLIS COMPARED WITH METRONIDAZOLE ON (*GIARDIA LAMBLIA*) AND MEASUREMENT OF SECRETORY IMMUNOGLOBULIN A (SIGA) IN INTESTINAL TISSUE FOR EXPERIMENTAL MICE

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Abstract

Propolis (bee glue) is one of the natural products that use in medicine, such as anti-bacterial, anti-oxidant, anti-cancer and anti-parasitic. Giardiasis is one of the most spread diseases all over the world, and the treatments used to treat *Giardia* have many side effects, most of which are expensive. The first aspect of study involved testing the effect of propolis suspension on the *Giardia* parasite in white mice infected with the parasite. the infected mice were inoculated with propolis suspension at a concentration of (500 µg/ ml) for 12 days, during this period the stool was examined daily and the parasite numbers were calculated after being dipped with the propolis. The results showed that the propolis suspension was effective in reducing the number of parasites shedding in the feces of mice gradually from the first day where it was (31 cells/ml) and until it became (zero) on the ninth day of the experiment, while metronidazole was more effective in reducing the parasite numbers that was shedding in mice feces from the first day (32 cells/ml) and continued until become (zero) on the eighth day of experiment. When measuring the therapeutic efficacy, the results showed an approximation of the therapeutic efficacy between propolis and metronidazole (51% and 55%, respectively). As for the second aspect, the study included the effect of propolis suspension on the level of secretory immunoglobulin (SIgA) in the intestinal tissue of infected mice and comparing it with metronidazole, the results showed a significant increase in the level of secretory immunoglobulin and was after 4, 7 and 12 days (0.619 ± 0.14, 0.790 ± 0.09, 0.666 ± 0.08) ng / ml respectively in propolis while metronidazole there were decreasing and it was (0.713 ± 0.20, 0.468 ± 0.08, 0.447 ± 0.04) ng / ml respectively, compared with the positive control group wich have higher level (0.766 ± 0.15, 0.863 ± 0.13, 0.907 ± 0.02) ng / ml respectively.

Keywords: Propolis, Giardia, SIgA, Mice

Table 1.1 : Numbers of *G. lamblia* parasite in treated groups with time (mean ± SD) × 10^2

Groups	Day after treatment												LSD value
	1	2	3	4	5	6	7	8	9	10	11	12	
Control Positive	27.5 ± 9.38 A	29.5 ± 4.90 A	28.5 ± 9.05 A	23.7 ± 2.58 A	25.1 ±7.25 A	20.8 ± 3.97 A	28.3 ± 7.39 A	32.0 ± 4.89 A	31.40 ± 9.07 A	35.0 ± 10.7 A	30.6 ± 8.11 A	30.8 ± 3.27 A	8.11 *
Metronidazole	32.6 ± 4.92 A	28 ± 7.93 A	21.3 ± 2.78 B	15.22 ± 2.10 B	9.33 ± 1.86 B	7.83 ± 1.16 B	2.50 ± 1.04 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 B	3.98 *
Propolis	31.8 ± 4.42 A	25.8 ± 4.07 A	19.1 ± 3.75 B	17.11 ± 2.97 B	14.33 ± 2.42 B	10.33 ± 2.87 B	9.83 ± 2.78 B	5.20 ± 1.30 B	0.00 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 B	3.37 *
LSD value	6.45 NS	5.71 NS	5.72 *	2.51 *	5.58 *	3.58 *	5.66 *	4.03 *	7.21 *	8.56 *	6.45 *	2.60 *	---

*: Significant (P<0.05), NS: Non-Significant.
The different letters in the same row means there are significant differences.

Table 1.2 : The percentage sufficient treatment of treated groups.

Type of treatment	Sufficient treatment %
Metronidazole	55 %
Propolis	51 %
Chi-Square (χ^2)	0.782 NS
NS: Non-Significant	

Metronidazole is widely used to treat a variety of infections such as giardiasis, trichomoniasis and amoebiasis due to its high efficacy compared with others drugs (Löfmark *et al*., 2010), Despite their efficacy, treatment with this drug is associated with several adverse effects (Coutinho, 2012). However, cross-resistance between 5-nitro antimicrobials exists, and treatment failures occur in up to 20% of cases (Upcroft and Upcroft, 2001; Tejman-Yarden *et al*., 2011).

Propolis is a natural, non-toxic resinous substance with strong antiparasitic activity and can act on numerous parasite. Some authors have studied the antiparasite properties of propolis against some parasites such as *Trypanosoma brucei* (Omar *et al*., 2016), *Trichomonas vaginalis* (Fidalgo *et al*., 2011), *Entamoeba histolytica* (Ardalan, 2011) and *Leishmania braziliensis* (Da Silva, 2013).

Propolis activity on *in vivo Giardia* proliferation has been reported in previous studies. Our results corroborate these findings, since we demonstrated a growth inhibition of *Giardia* by crude propolis, using propolis concentrations with inhibitory effect similar to those previously reported (Freitas *et al*., 2006; Alday-Provencio *et al.*, 2015). Many studies were carried out aiming to evaluate the effect of propolis on the growth and adherence of *Giardia lamblia* trophozoites. Freitas *et al*. (2006) studied *in vitro* activity of propolis crude extract against *Giardia*, They observed that 90% trophozoite growth inhibition by using 500 µg/ml concentrations.

Sonoran Propolis from Mexico and Some of its Chemical Constituents show Inhibition on *in vitro* Growth of *Giardia lamblia* Trophozoites (Alday-Provencio *et al.*, 2015). (Abdel-Fattah and Nada, 2007) study on experimental animal infected with *G. lamblia* showed that Combined therapy of metronidazole and propolis was more effective in reducing the parasite count than by each drug alone.

Concentration of secretory immunoglobulin A (SIgA) in intestinal tissue

The concentration of SIgA in the intestine tissue was determined by using ELISA-kit. Mice inoculated with propolis showed slight increasing in concentration of SIgA through the experiment days 4,7,12 were (0.619 ± 0.14, 0.790 ± 0.09, 0.666 ± 0.08 ng/ml) respectively, while the metronidazole group SIgA concentration decreasing gradually through the experiment days (0.713 ± 0.20, 0.468 ± 0.08, 0.447 ± 0.04 ng/ml) respectively. in positive groups the SIgA concentration continued increasing along with experiment days (0.766 ± 0.15, 0.863 ± 0.13, 0.907 ± 0.02 ng/ml) respectively, There were significant deference ($p<0.05$) in SIgA concentration between control positive group and others groups propolis and metronidazole in day seventh and eleventh of experiment as shown in table (1.3).

Table 1.3 : The level of SIgA concentration in intestinal tissue of treated and control groups (mean ± SD) ng/ml

Groups	Time (day)			LSD value
	4	7	12	
Control negative	0.554 ± 0.15 A	0.501 ± 0.08 B	0.576 ± 0.20 BC	0.216NS
Control positive	0.766± 0.15 A	0.863 ± 0.13 A	0.907 ± 0.02 A	0.168NS
Metronidazole	0.713 ± 0.20 A	0.468 ± 0.08 B	0.447 ± 0.04 C	0.181*
Propolis	0.619 ± 0.14 A	0.790 ± 0.09 A	0.666 ± 0.08 B	0.151*
LSD value	0.224 NS	0.141 *	0.151 *	---

*: Significant (P<0.05), NS: Non-Significant.
The different letters in the same row means there are significant differences.

Metronidazole is used as the drug of choice to treatment giardiasis by prevents the parasite from forming new DNA (Mudry *et al.,* 2001; Sonja and Carl, 2010), but also causes damage to proteins in the body cell and decreasing in SIgA concentration (Eisenstein and Schaechter, 2007; Powel, 2009).

When propolis was given orally this leads to enhance the immune response against parasitic infection. These results combined with those reported by other researchers lead to suggest that propolis enhance immune response against *G. lamblia*. (Abdel-Fattah and Nada, 2007) study on experimental animal infected with *G. lamblia* and treated with propolis, showed that propolis, as a prophylaxis resulted in a significant decrease in the intensity of infection and a reversed CD4+: CD8+ T-lymphocyte ratio resulting in a strong immune enhancing effect, which lead to an adverse increase in inflammatory response at the intestinal level. Also It has been demonstrated that propolis increases the ratio of CD4+/CD8+ cells, which are the main producer of these cytokines, and an increase in their ratio is in favour of immune enhancement (Brätter *et al*., 1999). The present sample of propolis can be described as being an immune stimulating agent in the investigated mice. Such view is enhanced by the findings of other investigators who demonstrated that propolis of different regions around the world can enhance the functions of the immune system (Orsatti and Sforcin, 2012)

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