Efficacy Of Bee Glue Extract In The Treatment Of Some Protozoal Infection

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ABSTRACT

The effect of bee glue extract (propolis) was estimated on protozoa (*Cryptosporidia* and *Giardia sp.* isolated from newly born calves) in white albino rats experimentally infected with their oocysts, respectively. Five groups of parasite free rats (1st and 3rd rat groups consisted of 9 rats and each one sub grouped to a, b & c, 3 rats each; whereas the other 3 groups contain 4 rats in each one) were used. Four groups (1st & 2nd groups for *Cryptosporidia sp.* while 3rd & 4th ones for *Giardia sp.*) were infected with 10^6 oocysts/rat. The three sub groups (a, b & c) from each infection were treated orally with 1,2 and 3 ml 5% aqueous propolis extract /rat, respectively for 5 successive days and the 2nd & 4th groups left as +ve control (infected-non treated) while the 5th one as -ve control (non infected-non treated). All rats were sacrificed at the end of the experiment for histopathological examination. From our results, it can be concluded that, propolis was effective as an antiprotozoal agent as it reduced shedding of both parasite oocysts as well as it relieve pathological signs accompanied both infections because it inhibit both growth and viability of both parasites and the level of reduction varied according to the dose and time of exposure.

INTRODUCTION

The parasitic infections caused by intestinal protozoan specially *Cryptosporidium sp.* and *Giardia sp.* are commonly infected newly born calves causing severe diarrhea, economic losses due to high morbidity and even death. They have a significant public health threat for human and other hosts, the protozoal zoonotic diseases of mammals in which the immune status play an important role in progress of the parasites *(1,2).*

Cryptosporidiosis in children in developing countries causes persistent diarrhea and male-nutrition as well as is associated with increased mortality *(3)*; meanwhile, only in Asia, Africa and America, there are more than 200 million people infected by *Giardia sp.* in a year *(4).*

Parasitic infections caused by intestinal protozoan and helminthes affect more than 2 billion people world-wide and chemotherapy is the most commonly used therapeutic procedure *(5).*

In addition to potential production losses, *Cryptosporidium* and *Giardia* infections in food producing animals may serve as a possible source for human infection, thus necessitation to control or elimination of the infections *(6)*. Considering the problems created by parasitic infections and incorrect use of drugs, there is a new trend for using natural products for medical purposes.

Propolis, a resinous hive product collected by bees, has attracted attention as a useful and popular substances with several therapeutic activities such as anti-inflammatory; antimutagenic; antimicrobial; antiviral as well as an immune system booster *(7-11)*.

Also, propolis was proved to have insecticidal action against insect larvae *(12)* and acaricidal action against scaly legs in chickens *(13);* sheep mange *(14);* ectoparasite mite, *Varroa destructor*, *(15)* and rabbit mange, *Psoroptic & Sarcoptic (16).*

However, several investigators *(17-20);* used propolis as antiprotozoal agent against *Giardia*, *Trichomonas* and *Coccidia sp.*

Thus, the present study was aimed to assess the effect of a new broad-spectrum bee glue extract, propolis, on the growth and viability of both *Cryptosporidia* and *Giardia sp.*

MATERIAL AND METHOD

I- Collection and examination of specimen

About twenty-two faecal samples of newly born calves suffered from diarrhea were collected from different farms & houses of Sharkia governorate and transformed directly to lab. of Parasitology Dep., Fac. Vet. Med., Zag. University which were examined by modified Ziehl-Neelsen staining technique *(21)* for detection of *Cryptosporidium sp.* oocyst while for *Giardia sp* . oocyst examination was done with 1% Lugol's Iodine *(22).* Oocysts were collected by sheather's sugar solution from positive faecal samples and kept in 2.5% potassium dichromate solution at 4°C until used within one month for experimental infection of rats *(23).*

II- Experimental design

Thirty of parasite free white albino rats aging 2-3 weeks old were used in this study. They were obtained from the animal houses, Fac. Vet. Med., Zag. University. The animals were kept under hygienic conditions, fed on a balance ration.

The rats were divided into 5 groups

Group I: Consisted of 9 rats (sub grouped to a, b & c, 3 rats each), experimentally infected with *Cryptosporidium sp.* oocysts (10^6 oocysts/rat), then treated by oral administration of propolis extract (1, 2, 3 ml/rat/sub group, respectively.).

Group II: Consisted of 4 rats used as +ve control (infected by Cryptosporidium sp. oocysts and non treated).

Group III: Consisted of 9 rats as group I, but experimentally infected with *Giardia sp.* oocysts and also treated with propolis extract at 3 doses to 3 subgroups, respectively.

Group IV: Consisted of 4 rats, experimentally infected with *Giardia sp.* oocysts-non treated as control+ve ones.

Group V: Consisted of 4 rats used as -ve control which non-infected non-treated.

III- Preparation of propolis extract

Bee glue aqueous extract was prepared *(19)* five grams of porpolis (which collected form Sharkia province) were cutted into small pieces and mixed with 25 ml deionized water and shacked at 95°C for 2 hours, then cooled to room temperature, water was added to 100 ml and the contents were centrifuged at 1500 r.p.m for 5 min. to obtain supernatant (5% aqueous propolis extract).

V- Experimental infection and treatment

Each rat in the first 4 groups (1st & 2nd for *Cryptosporidium sp.* while 3rd & 4th for *Giardia sp.*) was experimentally infected with 10^6 oocyst orally using an orogastric intubation *(23).* One week later, faecal pellets were collected per rectum from each rat of each infected groups, and faecal smears were examined by special stain according to the type of oocyst.

After confirmation of infection of all inoculated rats, the rats in. group I & III were subdivided to a, b & c sub groups (each contains 3 rats). Then, the sub groups in both infected groups were treated with propolis extract 5% by different 3 doses, respectively. (1, 2 and 3 ml/rat/group) using gastric tube. Faecal smears from each rat of all groups were examined daily after infection till the end of the experiment for counting both types of oocysts per 1 gram of faecal sample of infected rat using MC-Master technique *(24)* were suspended. Three gram of faecal sample in 42 cm^3 sheather's solution then filtrated, 0.15 cm^3 from filtrate was taken using haemocytometer pipette filled the two chamber of MC-Master slide and counting the oocysts.

$$\text{No. of oocysts/1gm of faeces} = \frac{\text{Total no. of oocysts in two chamber}}{2} \times 100$$

IV- Histopathological examination

All rats were sacrificed 5 days post-treatment and parts of their ilea were fixed in 10% formalin solution, embedded in paraffin, sectioned at 5 microns and stained with H&E stain *(25)* for histopathological examination.

Differences between faecal oocysts shedding among treated and control groups

were tested for statistical analysis by ANOVA *(26).*

RESULTS

Faecal examination of infected rats, which was carried out periodically every 2 days, revealed that *Cryptosporidium* oocyst looked spherical in shape, 4 to 5 µ in diameter (plate I, Fig. A&B), while *Giardia* oocyst was oval or elliptical contains 2 or 4 nuclei, it is 8 to 12 µ by 7 to 10 µ (plate I, Fig.C).

Data presented in Table 1 showed that the numbers of either *Cryptosporidia* or *Giardia* oocysts in the treated groups (1st & 3rd) were decreased significantly from the 2nd day of treatment until the end of the experiment (5th days post-treatment) according to the dose, whereas the highest reduction recorded with the highest dose (3ml of 5% extract) at the last day of treatment compared with +ve control groups (2nd & 4th, infected-non treated) which shed more oocysts of both parasites until the end of the experiment. Negative control group (5th, non-infected non-treated) shed no oocysts during the whole period of the experiment.

Table 1. Mean values of *Cryptosporidia* and *Giaradia sp.* oocysts shedding from infected, treated and non treated rat groups.

Group	I			II	III			IV	V
Days post treatment	Infected with *Crypto.* oocyst-treated rat*			Infected with *Crypto.* oocyst non treated rat	Infected with *Giardia* oocyst-treated rat*			Infected with *Giardia* oocyst non treated rat	Non infected non treated rat
	1 ml	2 ml	3ml		1 ml	2 ml	3 ml		
D_1	1905	1800	1020	8810	1002	905	508	9256	-
D_2	101	80	9	7965	80	15	5	8751	-
D_3	80	30	1	6165	30	3	-	7603	-
D_4	29	10	-	3200	2	-	-	2800	-
D_5	10	5	-	1010	-	-	-	998	-

* Dose of 5% propolis

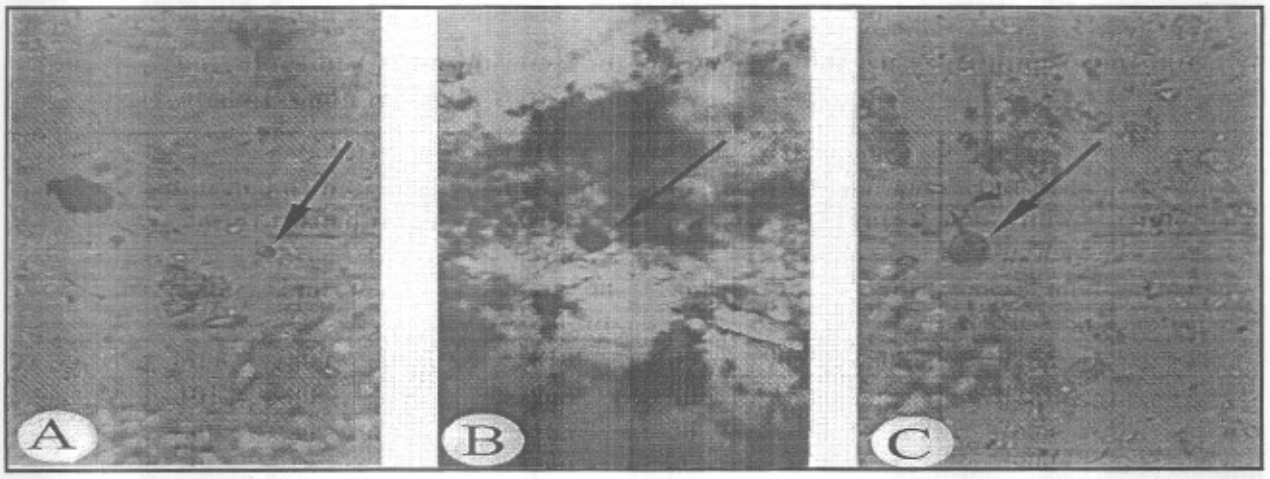

Plate I.

Fig.A. *Cryptosporidium* oocyst in fresh faecal smear from infected rat (X20).

Fig.B. *Cryptosporidium* oocyst stained with modified Ziehl-Neelsen in fresh prepared faecal smear from infected rat. (X40).

Fig.C. *Giardia* oocyst in fresh faecal smear from infected rat (X40).

ileum, in addition to shortening, atrophy and fusion of ileal villi leading to blunting and desquamation of most villi (Plate II, Fig. A&B); while treated rats in both parasitic infection groups in addition to –ve control had normal ileal villi without any atrophy or fusion and also with neither growth nor adherence of trophozoites of both parasites with complete reduction of clinical signs.

Clinical signs and histopathological findings

From pathological examination, it was noticed that both infected rat groups showed nausea, loss of appetite followed by loose watery diarrhea, and emaciation in both infections. At the end of the experiment, the sarificed rats in the +ve control groups (2nd & 4th) had numerous endogenous developmental stages of both parasites in the brush border of

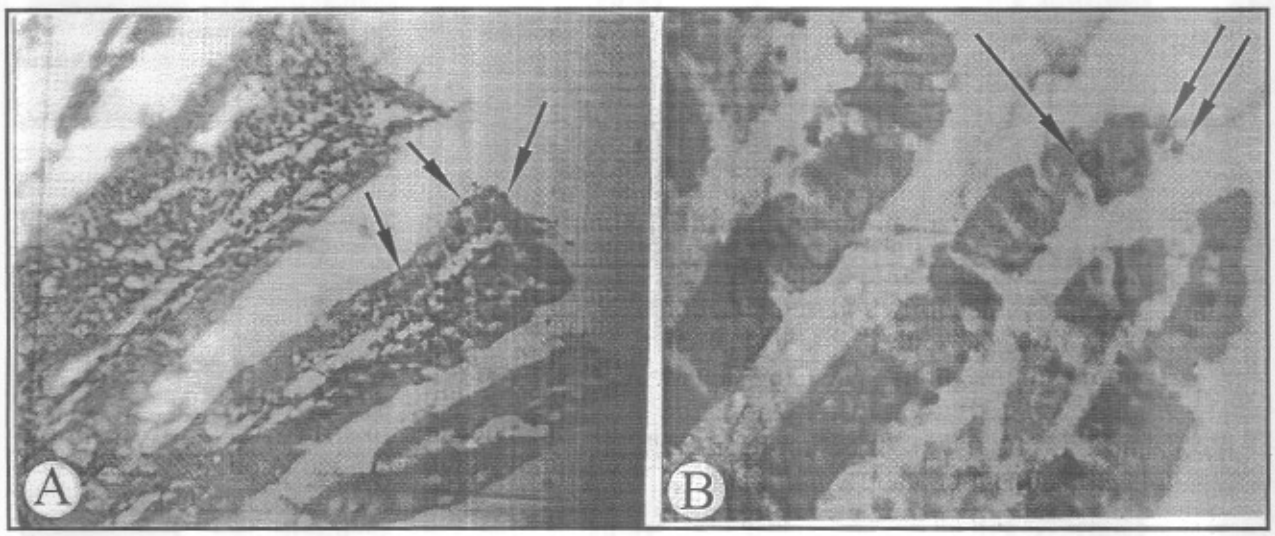

Plate II.

Fig. A. Cross section of ileum from rat of infected non-treated control group II showing *Cryptosporidium* cyst at brush border with degeneration, atrophy and desquamation of villi. H&E (X10).

Fig. B. Cross section of ileum from rat of infected non-treated control group (IV) showing trophozoites of *Giardia sp.* with heavy destruction, desquamation of villi. H&E (X40).

DISCUSSION

Cryptosporidia and *Giardia sp.* are the most zoonotic enteric protozoan parasites located on the brush border of small intestine, especially the ileum of all mammals ***(27,28).***

Propolis was reported to have an immune-stimulant and contained several ingredients with potential value which was proved to have different therapeutic effects ***(20).***

The obtained data shown in Table 1 postulated that propolis in different doses (1, 2 and 3ml 5% aqueous extract) induced significant decrease in both *Cryptosporidium sp.* and *Giardia sp.* oocysts numbers in all treated rats from the 2nd day post treatment till the end of the experiment (5 d.p.t.) with reduction of clinical signs whereas the maximum reduction occurred with the higher dose used at the last day. However, histopathological findings revealed that the appearance of small intestines specially ileum returned to its normal appearance without any growth or adherence of trophozoites of both parasites in all treated groups. Our results are in harmony with those which stated that propolis was effective against giardiosis in both children and adults patients with no side effects ***(17)***; and also which demonstrated that propolis inhibited both growth and adherence

of *Giardia* trophozoites and also promoted the detachment of trophozoites as a result of changes of the pear-shaped aspect of the cell and reduction of flagellar beating frequency in the great part of the trophozoites ***(20)***. In the meantime, propolis proved to have antiprotozoal activities on *Trichomonas* ***(18)*** and *Coccidia* ***(19).***

So, it was concluded that administration of bee glue aqueous extract proved to be efficient therapeutically in the treatment of both cryptosporidiosis and giardiosis. Being zoonotic diseases, such results could be adopted for human being having similar infection .

REFERENCES

1.Soulsby, E. J. L. (1982): Helminths, arthropods and protozoa of domenticated animals. 7th edition. The English Language Book Society and Bailliere Tinall. London.

2.Unger, B. L. (1990) : Cryptosporidiosis in humans (Homo sapiens). In cryptosporidiosis of man and animals. C. R. C. Press, Boca Raton, Florida.

3.Amadi, B.; Mwiya, M.; Musuku, J.; Watuka, A.; Sianongo, S.; Ayoub, A. and Kelly, P. (2002): Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled. Trial. Lancet, Nov.2,360 (9343) : 1375-1380.

4.Ponce-Macotela, M.; Rufino-Gonzalez, Y.; Gonzalez-Maciel, A., Reynoso-Robles, R. (2006): Oregano (*Lippia spp.*) kills *Giardia intestinalis* trophozoites in vitro: antigiardiosic activity and ultra-structural damage. Parasitol. Res., 20 : 1-4.

5.Morrone, F. B.; Carneiro, J. A.; Dos Reis, C. ; Cardozo, C. M.; Ubal, C. and De Carli, G. A. (2004): Study of enteroparasites infection frequency and chemotherapeutic agents used in pediatric patients in a community living in Porto Alegre, RS, Brazil. Rev. Inst. Med. Trop. Soo Pauls, 46 (2) : 77-80.

6.Huetin, R. E.; Van der Giessen, J. W.; Noordhuizen, J. P. and Ploeger, H. W. (2002):Epidemiology of *Cryptosporidium sp.* and *Giardia duodenalis* on a dairy farm. Vet. Parasitol. 3, 102 (1-2) : 53-67.

7.Khayyal, M. T.; El-Gazaly, M. A. and El-Khatib, A. S. (1993): Mechanisms involved in the anti-inflammatory effect of propolis extract. Drugs Exp. Clin. Res., 19 : 197-203.

8.Varando, E. A.; Monti, R. and Tavares, D. C. (1999): Inhibitory effect of propolis and bee venom on mutagenicity of some direct and indirect acting mutagens. Teratog. Carcinogen. Mutagen. 19 : 403-413.

9.Koo, H.; Gomes, B. P. ; Rosalen, P. L.; Ambrosano, G. M.; Park, Y. K. and Cury, J. A. (2000) : In vitro antimicrobial activities of propolis and Amica Montana against oral pathogens. Arch. Oral Biol., 45 : 141-148.

10.Velikova, M.; Bankova, V.; Tsvetkova, I.; Kubumgiev, A. and Marcucci, M. (2000): Antibacterial entkaurene from Brazilian propolis of native stingless bees. Fitoterapia, 11 : 693-696.

11.Murad, J. M.; Calvi, S. A.; Soares, A. M.; Bankova, V. and sforin, J. (2002): Effect of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against paracoccidiosis brasiliensis. J. Ethnopharm., 79 : 331-334.

12.Konig, D. and Dustmann, J. H. (1988): Baumharze, bienen und antivirale chemotherapie. Nature Wissen. Rundschan, 2 : 42-53.

13.Raef A. M.; Fayek; S. A. and Gaffar, N. (1998): Some studies on cnemidocoptes mutans (Acari, Sarcoptoidea) sarcoptic mange on chickens. 4th Vet. Med. Zagazig Congress, 26-28 August, PP 388-393.

14.Kandil, N. S. A. (2000): Some ectoparasites of goats. M. V. Sc., Faculty of Vet. Med., Zagazig Univ.

15.Garedew, A. (2003): Microcolorimetric and microbiological in vitro investigations on

the acaricidal, inseticidal and antimicrobial effects of propolis. Ph. D. thesis, Fu. Berlin.

16.Amer, O. H. and Abd EL-Aal, A. M. I. (2005): Effect of propolis on rabbit mange. EVMSP J., 11 (1) : 293-304.

17.Miyares, C.; Hollands, I.; Castaneda, C.; Gonzalez, I.; Fragoso, I.; Curras, R. and Soria, C. (1988): Clinical trial with a preparation based on propolis "propolisina" in human giardiasis. Acta Gastroenterol. Latinoam, 18 (3): 195-201.

18.Santana Perez, E.; Lugones Botell, M.; Perez Stuart, O. and Castilllo Brito, B. (1995): Vaginal parasites and acute cervicitis: Local treatment with propolis. Preliminary Report. Rev. Cubana Enferm., 11 : 51-56

19.El-Akabawy, L. M.; Zayan, K. A.; Tantawy, A. A. and Omar, R. E. M. (2004): Anticoccidial efficacy of propolis and toltrazuil against *Eimeria stiedae* in New Zealand white rabbits. Zagazig vet. J., 32 (1) : 129-145.

20.Frietas, S. F.; Shinohara, L.; Sforcin, J. M. and Guimaraes, S. (2006): In vitro effects of propolis on *Giardia duodenalis* trophozoites. Phytomedicne, 13 (3):170-175.

21.Henricksen, S.A. and Pohlenz, J. F. L. (1981): Staining of *Cryptosporidia* by modified Zeihl-Neelsen technique. Acta Vet. Scand., 22 : 594-596.

22.Smith, J. W. and Barlett, M.S. (1982): Diagnostic parasitology : Introduction and methods in "Manual of clinical microbiology" (F. H. Lennette; A.. Ballows, W. J. Hauster and H. J. Shadomy, eds), pp.595-611. Am. Soc. Microbiol., Washington D.C.

23.Kathleen, R.; Mark, C.; Liping, C. and Shiguang, Y. (1995): Effect of dehdro-epiandrosterone in immunosuppressed adult mice infected with *Cryptosporidium parvum*. J. Parasitol., 81 (3) : 429-433.

24.Kao, T. Z. and Ungar, B. L. P. (1994): Comparison of sequential random and haemocytometer methods for counting *Cryptosporidium* oocyst. J. parasitol., 8 : 816-819.

25.Harris, H. E. (1898): Cited by Carleton, M. A.;R. A. Drury; E. A. Wallington and H. Camerson.1907. Carleton histological technique, 4th ed., Oxford Univ. Press.

26.Snedecor, G. W. and Cochran, W. G. (1989): Statistical methods. 8 th ed. Iowa Univ. Press, Iowa,U.S.A.

27.Koudella, B. and Jiri, V. (1997): Experimental cryptosporidiosis in kids. Vet. Parasitol., 71: 273-281.

28.Barutzki, D.; Schimmel, A. and Scaper, R. (2002) Efficacy of pyrantel Ernbonate febantel and praziquantel against *Giardia sp.* In naturally infected dogs. In *Giardia* the cosmopolitan parasite CABS Int., PP. 177-185.

الملخص العربـــى

مدى كفاءة مستخلص صمغ النحل فى علاج العدوى ببعض الطفيليات الأولية

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لقد تم تقييم تأثير مستخلص صمغ النحل (البروبوليز) على نوعين من الطفيليات الأولية (الكريبتوسيبوريديا والجياردیا المعزولين من العجول حديثة الولادة) في فئران بيضاء تم إصابتها تجريبياً بحويصلات كل طفيل على حده. تم استخدام خمسة مجموعات من الفئران الخالية من أي إصابة طفيلية (المجموعات : الأولى والثالثة تحتوى كل منهما على ٩ فئران تقسم كل مجموعة إلى ٣ تحت مجموعات (أ، ب وجـ) تشمل ٣ فئران/تحت مجموعة بينما المجموعات الثلاث الأخرى تشتمل على ٤فئران/مجموعة). أربعة مجموعات (الأولى والثانية لطفيل الكريبتوسبوريديا والثالثة والرابعة لطفيل الجياردیا على التوالي) تم إصابتها بمقدار 10^{6} حويصلة/فأر. تم علاج تحت المجموعات الثلاث (أ، ب و جـ) في المجموعتين الأولى والثالثة بجرعات ١ ، ٢ ، ٣ ملليلتر ٥% بروبوليز/فأر/مجموعة على التوالي لمدة ٥ أيام متتالية . تركت المجموعات : الثانية والرابعة كمجموعات ضابطة موجبة (مصابة وغير معالجة) بينما المجموعة الخامسة كمجموعة ضابطة سالبة (غير مصابة وغير معالجة) وفى نهاية التجربة تم ذبح كل الفئران للفحص الهستوباثولوجى. تم تلخيص النتائج على أن: البروبوليز كان فعالاً كعامل مضاد للطفيليات الأولية حيث يقلل من تكون الحويصلات كما أنه يخفف الأعراض الباثولوجية المصاحبة للإصابة لأنه يثبط كل من النمو والحيوية لكلا الطفيليين ومستوى الانخفاض يختلف باختلاف الجرعة ومدة التعرض للمستخلص.