

ORIGINAL ARTICLES

Effect Of Propolis Extracts On Trypanosoma Evansi In Rats

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ABSTRACT

This work was conducted in the Department of Crop Protection, Faculty of Agriculture, University of Khartoum, in cooperate with the Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum. The objective of the study was to determine the effects of two extracts of propolis (water and ethanol) against *Trypanosoma evansi* on rats under laboratory conditions. Water and ethanol extracts of propolis 10% at 0.2 ml/rat were injected sub-cutaneously and intraperitoneally, parasitemia level in the rat blood was monitored up to 4 days before the next treatment. The results showed positive effect of propolis extracts against *Trypanosoma evansi*. Trypanocidal activity of water extract reached 6%, 30% and 58% when treated sub-cutaneously and 6%, 11% and 47% for those treated intraperitoneally, within three days. Trypanocidal activity of ethanol extract reached 6%, 9% and 18% when administered sub-cutaneously and 7%, 10% and 14% for those treated intraperitoneally. The results showed that water extract of propolis was more effective than ethanol extract and the effectiveness increased gradually with time; sub-cutaneously route was the best way to treat *Trypanosoma evansi*.

Key words: EFFECT, EXTRACTS, PROPOLIS, RATS, *TRYPANOSOMA EVANSI*.

Introduction

Trypanosoma evansi (Evans) is the causative agent of surra, one of the most common and widespread of the trypanosomal diseases. This trypanosome can infect most mammals, although horses and camels are the principal hosts and represent the most significant sources of economic loss. Surra is endemic in many parts of Africa, Asia, and South America where thousands of animals die during disease outbreaks each year. In the Sudan, the disease is known locally as "Gufar" (Karib, 1961). No vaccines are available for any form of the disease. Chemotherapy of this disease is still inadequate and expensive and the parasite is developing drug resistance, particularly to Suramine and Quinapyramine sulphate (Rose and Sutherland, 1996). In this context there is intense search for potential new synthetic compounds and natural products for the treatment of trypanosomosis.

Propolis is a natural brownish-green resinous product collected by honey bees, Propolis is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources, and bacterial flora of the putrefying corpses. It is a natural mixed material containing 50 - 60 % resins and balsams, 10 - 30 % wax, 8 - 10 % oils, 5 % pollen and many known and unknown material in smaller quantities (Ghisalberti *et al*, 1978). Propolis or "bee glue" possessing a variety of biological and pharmacological activities, antiseptic, antibiotic, antibacterial, antifungal, and even antiviral properties. Propolis is Nature's premiere preventive. It is so powerful in action; it is often called Russian penicillin in acknowledgement of the extensive research the Russians have mounted on this wonder worker from the bees. Propolis demonstrates strong antimicrobial properties against various bacterial and fungal infections. Propolis according to research has shown to be effective against a variety viruses and molds (Scoff, 2002). Propolis is highly recommended by modern herbalists since it displays microbicidal, anti-inflammatory, immunomodulatory and anti-ulcer properties (Paulino *et al*, 2006).

Objective of this study:

Due to an increasing interest of propolis characteristics, the present study aimed to determine the effect of its two extracts (water and ethanol) against *Trypanosoma evansi*.

Methodology:

All rats infected with *Trypanosoma* mixed blood; parasitaemia was monitored daily by taking blood drop from tail veins of infected rats, a drop of blood was placed on a clean slide and covered with cover slip before examination under x40 objective lens to detect trypanosomes.

When rats became parasitaemic, 20 rats were taken for water extract of propolis experiment; 5 rats were injected sub-cutaneously with 0.2ml per rat, other 5 rats were injected intraperitoneally with 0.2ml per rat, then the level of parasitaemia was determined daily before injection for three days by means of matching method described by Herbert and Lumsden (1976). Five rats were treated with chemical drug once (one dose on the first day); the last 5 rats were served as untreated control. Parasitaemia level was detected daily for three days for two groups.

The other 20 parasitaemic rats used in the ethanol extract of propolis experiment was followed the same procedure of the water extract of propolis experiment.

Results:

After 24hrs, parasitaemia level of *Trypanosoma evansi* affected with water extract showed slight reduction in rats treated sub-cutaneously or intraperitoneally when compared with chemical drug and untreated control. However, differences among the treatments were non-significant. Mean parasitaemia level of *Trypanosoma evansi* was 7.35 (94%) for rats treated sub-cutaneously with water extract of propolis, 7.37 (94%) for those treated intraperitoneally, 7.73 (99%) for chemical drug and 7.83 (100%) for untreated control (table1).

After 48hrs, similar pattern, as that observed in the first day also obtained in the second day. Differences among treatments were non-significant. The level of parasitaemia mean was 5.5 (70%) for rats treated sub-cutaneously with water extract of propolis, 7.0 (89%) for those treated intraperitoneally, 6.9 (88%) for chemical drug and 7.88 (100%) for untreated control (table1).

After 72hrs, a completely different pattern in the level of parasitaemia observed, substantial reduction of parasitaemia level obtained in rats treated sub-cutaneously or intraperitoneally when compared with untreated control. Significant differences detected among the treatments. Mean parasitaemia level of *Trypanosoma evansi* was 3.37 for rats treated sub-cutaneously, this value represents 42% of that 8.03 recorded for untreated control on the third day. The intraperitoneally injected water extract had 4.23 mean of parasitaemia count; a level, which was 53% of 8.03, obtained in the untreated control. On the other hand, parasitaemia level was 0.0 for those animals, which were given chemical drug (table1).

Generally, in comparison with the results obtained for water extract of propolis, a completely different pattern of effect expressed by ethanol extract. After 24hrs, parasitaemia level of *Trypanosoma evansi* reported slight reduction in rats treated sub-cutaneously or intraperitoneally with ethanol extract of propolis when compared with untreated control while chemical drug was recorded a drastic reduction. Therefore, differences among the treatments were highly significant. Means parasitaemia level was 7.95(94 %) for the rats treated sub-cutaneously, 7.88(93 %) for those treated intraperitoneally, 0.0 for chemical drug and 8.46 (100%) for untreated control (table2).

After 48hrs, inconsequential change occurred in the pattern of parasitaemia level, when compared with the first day. In addition, differences among treatments were highly significant. The level of parasitaemia mean was 7.72 (91%) for rats treated sub-cutaneously with ethanol extract of propolis, 7.66 (90%) for those treated intraperitoneally and 8.52 (100%) for untreated control (table2).

After 72hrs, reasonable different patterns in the level of parasitaemia were observed. As well, differences among the treatments were highly significant. Means parasitaemia level of *Trypanosoma evansi* was 7.0 for the rats treated sub-cutaneously, this value denoted 82 % of that 8.56 registered for untreated control. The level of parasitaemia was 7.38 for those imparted ethanol extract intraperitoneally, which represent 86% of that recorded for untreated control. In contrast, chemical drug produced a drastic reduction parasitaemia level until the third day (table2).

Discussion:

The results obtained in this work revealed that propolis extracts (water & ethanol) have trypanocidal activity against *Trypanosoma evansi* by different percentage of parasitaemia level in two various routes to apply treatments.

Water extract is more effective against *Trypanosoma evansi* which exhibited high decreasing in parasitaemia level which were 6%, 30% and 58% for sub-cutaneously treated rats and 6%, 11% and 47% for those treated intraperitoneally when compared with control which was treated with chemical drug (100%) within three days.

Ethanol extract was less effective than water extract, although it has trypanocidal activity against *Trypanosoma evansi* to decreasing level of parasitaemia to 6%, 9% and 18% for rats treated sub-cutaneously and 7%, 10% and 14% for those treated intraperitoneally.

Similar findings were reached by Kelly *et al* (2007) through using propolis extract against different microorganisms including *Trypanosoma cruzi* which is belong to the same family of *Trypanosoma evansi* (Trypanosomatidae). They showed that propolis has trypanocidal activity against *Trypanosoma cruzi*, they found from chemical composition analysis of propolis there was positive correlation between trypanocidal activity of propolis and some phenolic acids and prenylated derivatives which were 3,5-diprenyl-4-hydroxycinnamic acid derivative 4 (DHCA4) and 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran (DCBEN). These compounds were considered as the main bioactive ingredient against trypanosomosis.

Conclusion And Recommendations:

The over all conclusion indicated that propolis extracts (water& ethanol) had an evident trypanocidal active ingredients that reduce parasitemia level on the tested parasite *Trypanosoma evansi*. Water extract was more effective than ethanol extract and sub-cutaneously route was better to apply treatment on parasite than intraperitoneally route.

This work point to possibility of using propolis extracts as antitrypanosomal instead of chemical drug, which is inadequate and expensive, and the parasite is developing drug resistance.

Literature on the effect of propolis in *Trypanosoma evansi* is not available. This study is therefore representing a preliminary report on this subject.

Further studies must be carried out to determine which concentration (more than 10%) of propolis extract can eliminate trypanosoma from animal blood totally.

Other researches must be done to exert propolis on antibody response in *Trypanosoma evansi* infected animal.

Other measures besides trypanocidal drug administration such as minimizing exposure to fly populations may help control surra. Stables with suitable netting can be used to exclude fly populations. Smudge fires may also be used to repel flies.

Table 1: Parasitemia means of *Trypanosoma evansi* infected rats treated with water extract of propolis

Treatments	Day ₁	Day ₂	Day ₃
Sub-cutaneously(SC)	^a 7.35(94)	^a 5.5(70)	^b 3.37(42)
Intraperitoneally (IP)	^a 7.37(94)	^a 7.0(89)	^a 4.23(53)
Chemical drug	^a 7.73 (99)	^a 6.9 (88)	^c 0.0 (0)
Control	^a 7.83 (100)	^a 7.88 (100)	^a 8.03 (100)
LSD at 0.05	1.4	5.5	3.94

Figures between brackets indicate the value as percentage of control.

Means followed with the different letters on the same column shows a significant level.

Table 2: Mean parasitemia count of *Trypanosoma evansi* infected rats treated with ethanol extract of propolis

Treatments	Day ₁	Day ₂	Day ₃
Sub-cutaneously(SC)	^b 7.95(94)	^b 7.72(91)	^b 7.0(82)
Intraperitoneally(IP)	^b 7.88(93)	^b 7.66(90)	^b 7.38(86)
Chemical drug	^c 0.0(0)	^c 0.0(0)	^c 0.0(0)
Control	^a 8.46(100)	^a 8.52(100)	^a 8.56 (100)
LSD at 0.05	0.47	0.47	0.47

Figures between brackets indicate the value as percentage of control.

Means followed with the different letters on the same column shows a highly significant level.

References

- Ghisalberti, E.L., P.R. Jefferies, R. Lanteri and J. Matison, 1978. Constituents of propolis. *Experientia*; 34(2): 157-158.
- Herbert, W.J. and W.H.R. Lumsden, 1976. *Trypanosoma brucei*: a rapid matching method for estimating the host's parasitaemia. *Expt.Parasit.*, 40: 427-43.
- Karib, E.A., 1961. Trypanosomiasis in Sudan. *Sudan. J. Vet. Sc.*, 2: 39-46.
- Kelly, S., R.S. Paulo, L.C. Pereira, M.B. Cintia, H.C. Pedro, C.M. Maria and L.D. Solange, 2007. Brazilian propolis correlation between chemical composition and antimicrobial activity. *Compl. And Alt. Medicine.*, 5(3): 317-324.
- Paulino, N., C. Teixeira, R. Martins, F.M. Scremin, A. Scremin and V.M. Dirsch, 2006. Evaluation of the analgesic and anti-inflammatory effects of Brazilian green propolis. *Planta Med.*, 72: 899-906.
- Scoff, R., 2002. Comparison of Silver Sulfadiazine to Propolis in Second-Degree Burn Treatment. *J. Altern. Complement. Med.*, 8: 77-83.