EFFECT OF PROPOLIS EXTRACTS ON 
TRYPANOSOMA EVANSI IN RATS

BY

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DEDICATION

To my father who learnt me patience………..

To my mother who taught me humanitarian…

Sisters

Brothers

Colleagues

Relatives

And friends

With my love and respect.
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Firstly, I would like to thank Allah Almighty the compassionate, the merciful who helped and gave me health to complete this study.

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Lastly, I thank my father and my mother for their help and encouragement throughout the period of my studies and my life.

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, parasiteamia 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*. 

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Düşük
أجريت هذه الدراسة في جامعة الخرطوم، كلية الزراعة، قسم وقاية المحاصيل بالتعاون مع كلية الطب البيطري، قسم عالم الطفليات. بهدف تقدير تأثير مستخلصان مختلفان من صمغ النحل (مستخلص مائي ومستخلص كحول الأيثانول) ضد طفيلي Trypanosome evansi في الفئران تحت ظروف المعمل.

تم حقن مستخلصي صمغ النحل بتركيز 10% بمعدل 0.2 مللتر/ فأر تحت الجلد و في البطن و تم مراقبة مستوى الطفيلي في دم الفأر لمدة أربعة أيام قبل المعالجة التالية. أظهرت النتائج تأثيرًا إيجابيًا في مقاومة T.evansi المستخلص المائي إلى 6% و 30% و 58% عندما تم الحقن تحت الجلد و 6% و 11% و 47% عندما تم الحقن عن طريق البطن خلال الثلاثة أيام الأولى من المعالجة.

بلغ نشاط مستخلص كحول الأيثانول 6% و 9% و 18% عندما تم الحقن تحت الجلد و 7% و 10% و 14% للفئران الذين تم علاجهم عن طريق البطن. أظهرت النتائج بأن المستخلص المائي أكثر فعالية من مستخلص كحول الأيثانول و يزيد التأثير تدريجيا مع مرور زمن التجربة؛ وإن العلاج عن طريق الحقن تحت الجلد هو أفضل . T.evansi

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1. INTRODUCTION

Trypanosomosis is one of the most serious and economically important disease in domestic animals in Africa as about 50 million cattle...
and tens of million of ruminants are at risk of the disease. Direct losses in meat production, milk yield and cost of its control is estimated between 600 million and 1.2 billion dollars each year (FAO, 1994).

*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. In last decades occurred movement sometimes called “Back to Nature” which in the area of drug development was accentuated by success obtained, for examples neem tree *Azadiracha indica* Ajuss has been identified as pesticides (Schmutterer, 1995). Several reports were also observed on the activity of a variety of crude natural extracts especially form plants collected in the tropical zones, against *Leishmania* species of new world (Fourent *et al*, 1994, 1996, Akendengue *et al*, 1999, Weniger *et al*, 2001 and Fourent and Munoz, 2002).

Other researches were done on the investigation of propolis effect against pathogenic trypanosomatids, especially *Trypanosoma cruzi*, agent of chagas disease (De Castro and Higashi, 1995, Marcucci *et al*, 2002, Cunha *et al*, 2004 and Dantas *et al*, 2005, 2006).
Propolis is a natural brownish-green resinous product collected by honey bees. The word is derived from the Greek pro (before) and polis (city). Propolis is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is used for small gaps (approximately 6.35 millimeters (0.3 in or less), while larger spaces are usually filled with beeswax. Propolis was being used to make the protective shield at the entrance of Beehive. Also it has been used to fill the cracks in the hive, to attach the corners of frames to the grooves in the hive, and also to polish the cells of the honeycomb. The bodies of dead lizards, snakes and mice that have entered hives are sealed into the walls with bee glue, thereby protecting the colonies against the unpleasant odour 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*.

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2. LITERATURE REVIEW

2.1 Camels in Sudan
Sudan has the second largest camel population in the world, estimated at nearly 3,000,000 (Salih, 1988), and the country is home to some of the most well-known camel nomads, the Kababish, Shukria, Hadendowa and others. Tribal groups in Sudan breed distinctive types of camels (Mason and Maule, 1960). Well-known among these are the Anafi and Bishareen, prized for their racing and riding capacities, the Rashaidi, a sturdy transport camel with superior drought resistance, and the large whitish Lahaween, which gives high meat yields.

The Arab breed of camel is well suited for meat production and transportation. Camel milk is important at the subsistence level but is rarely marketed. The export of camels for slaughter mostly to Egypt, but also to the Libyan Arab Jamahiriya and other countries, is an important source of foreign currency, which is not overlooked in a country with few roads and a chronic fuel shortage.

Among the most common diseases affected livestock are trypanosomosis (Gufar), mange (jerab), pneumonia (haboob), camel pox (ghedderi), contagious skin necrosis (na'aita) and ringworm (ghoob) (Musa and Ahmed, 1991).

2.2 *Trypanosoma evansi* (Evans)
Trypanosoma evansi (Evans) is a protozoan trypanosome in the genus trypanosoma that causes one form of the surra disease in animals. It is pathogenic and rarely causes disease in humans. It is very common in India and causes acute disease in camels and horses and chronic disease in cattle and buffaloes (Powar, 2006).

According to Hoare (1972), Trypanosoma evansi was the first pathogenic trypanosome discovered by Griffith Evans in 1880 in horses and camels in India. In the Sudan, this parasite was first recognized by Balfour in camels in 1904 (Karib, 1961).

2.2.1 Classification

The systematic position of Trypanosoma among the protozoa and the revised classification of the mammalian trypanosomes cited by Levine et al, (1980) and Corliss (1994) is as follows:

Sub-kingdom: Protozoa

Phylum: Sarcomastigophra

Sub-phylum: Mastigophra

Class: Zoomastigophra

Order: Kinetoplastida

Family: Trypanosomatidae

Geuns: Trypanosoma

Species: Trypanosoma evansi
2.2.2 Description and Morphology

*Trypanosoma evansi* is a parasitic in the blood, lymph and tissues of invertebrates and vertebrates, including humans; most species live part of their life cycle in the intestines of insects and other invertebrates, the flagellate stage being found only in the vertebrate host, which is long and slender, measuring 14 to 33 µm in length and 1.5 to 2.2 µm in width. This organism is generally monomorphic, although a shorter intermediate form occasionally is observed. This hemoflagellate has a free flagellum and a circular kinetoplast which is sometimes missing in mutated wild strains or strains that have been isolated following drug treatment. Additionally, *T. evansi* is covered by a thick layer of a single glycoprotein (or variable surface antigen), that is the primary immunogenic eliciting antibody formation. Periodically, the organism alters the glycoprotein coating and evades the host’s defensive responses (Luckins, 1999).

2.2.3 Mechanical transmission

*Trypanosoma evansi* is a protozoal hemoflagellate transmitted mechanically by arthropod vectors. The most prominent arthropod vector varies geographically. For example, horseflies (*Tabanus* sp.) and stable flies (*Stomoxys* sp.) are the primary vectors in China and Indonesia, while tsetse flies are more prevalent in endemic areas of Africa. In the Sudan, camel trypanosomosis has been reported to be transmitted mechanically from camel to camel by a number of species of haematophagous biting flies including the genera *Tabanus, Stomoxys, Lyprosia* and *Haematobia* (Diptera), (Rutter, 1967; Scott, 1973). Tabanid flies play an important role
in the mechanical transmission of animal trypanosomosis (Karib, 1961). Lewis (1954) reported two species of *Stomoxys* in the Sudan namely *Stomoxys calcitrans*, and *S. nigra*. Mohamed et al. (1991) showed that the peak of *Stomoxys* spp. coincided with high trypanosomosis incidence during or after the rainy season due to the growth and proliferation of flies during this favorable season. The tsetse infested area in the Sudan was estimated at 300,000 Km\(^2\) in the south–western part of the country (Razig and Yagi, 1973).

*Trypanosoma evansi* has a limited survival time in the mouth parts of a potential vector. In contrast, *T. evansi* reproduces by binary fission in the mammalian host. In addition to mechanical transmission, *T. evansi* infection may be spread during nursing, copulation, or following ingestion of infected tissues by carnivores (Brun et al, 1998).

### 2.2.4 Distribution

Originally, the distribution of *T evansi* coincided with that of camel. It is widespread throughout India, Middle East, Far East, North Africa and Central and South America. The enzootic areas of camel trypanosomosis extend across the Sudan between latitude 13° N and 18° N (Karib, 1961)

### 2.2.5 Host range

*Trypanosoma evansi* affects a wide range of hosts including camels and horses which are commonly infected with *Trypanosoma evansi*, but other mammals such as buffalo, cattle, deer, dogs, and elephants also are susceptible. Laboratory rodents such as mice, rabbits, rats and guine-pigs are readily infected. Numerous species of wild animals have been shown
to be susceptible to *T. evansi* infection. The severity of illness depends upon the protozoal strain, concurrent infections, stress, and environmental factors (Sarah *et al*, 2006).

### 2.2.6 Nature of damage and economic importance

Camels are highly susceptible to *Trypanosoma evansi* infection, the disease ranging from an acute or chronic course ended fatally in untreated cases. In acute form, trypanosomes are routinely present in the blood and the disease is almost fatal (Rutter, 1967). The disease, however generally takes a chronic form and the huge production losses occurs due to lower milk and meat yield in adult abortions, premature births and an inability to feed the young greatly reduce reproductive potential in affected herds (Yagil, 1982). Chronically infected animals may survive for 3-4 years; the disease in this form is characterized by anemia, emaciation, recurrent fever, disappearance of the hum atrophy of the high muscles, oedema of the dependent parts, corneal opacity, diarrhea and sexual excitement (Singh *et al*, 1980).

### 2.2.7 Treatment and control

*Tryptosoma evansi* disease currently is on the list of notifiable diseases of the OIE (World Organization for Animal Health). Reduction of the global economic impact of this disease on animal health requires dependable diagnostic tests, efficacious treatment, and diligent control programs. Control of trypanosomosis may be attempted by many approaches including:

(a) Chemotherapy and chemoprophylaxis
Historically, three drugs have been used to animals with *T. evansi* infections. These drugs include diminazene, suramin, and quinapyramine sulfate. Cymelarsan is the newest drug to be introduced in the last decade. The choice of drug, dosage, and route of administration vary by species affected, local preference, and presence or absence trypanosome drug resistance. (Brun *et al*., 1998). In one study, diminazene cleared the parasiteamia of horses following natural infection. However, parasiteamia returned in many animals within 1 week of the first treatment with diminazene or within 24 hours of the second treatment with diminazene. This suggests that diminazene affords little protective effect against repeated *T. evansi* infection or the drug is ineffective in totally eliminating the initial *T. evansi* infection (Tuntasuvan *et al*., 2003). Relapses have also been observed in horses following treatment with quinapyramine sulfate. (Monzon *et al*., 2003). In summary, there is a growing body of evidence that *T. evansi* is becoming resistant to currently available drugs and may restrict their use or retard their effectiveness in the future.

### 2.3 Propolis

Bees have been in existence for >125 million years and their evolutionary success has allowed them to become perennial species that can exploit virtually all habitats on Earth. This success is largely because of the chemistry and application of the specific products that bees manufacture:
honey, beeswax, venom, propolis, pollen and royal jelly. As the most important ‘chemical weapon’ of bees against pathogenic microorganisms, humans have used propolis as a remedy since ancient times. It is still one of the most frequently used remedies in many causes (Wollenweber et al., 1990), applied for treatment of wounds and burns, sore throat, stomach ulcer, etc.

Propolis starts as the sticky resinous sap, which seeps from the buds of certain trees and oozes from the bark of others. The bees gather this "bee glue" and carry it back to the hive where it is blended with wax flakes secreted from special glands on the underside of the bee’s abdomen. Propolis is used to line the interior of brood cells in preparation for the Queens laying of eggs. With its antiseptic properties, it provides a hospital clean environment for the rearing of brood. However, 20th century research has revealed that bees not only survive, but also thrive, with increased ventilation during the winter months throughout most temperate regions of the world.

Propolis is now believed to:

1. Reinforce the structural stability of the hive
2. Reduce vibration
3. Make the hive more defensible by sealing alternate entrances
4. Prevent diseases and parasites from entering the hive, and to inhibit bacterial growth (Walker, 2009).
5. Prevent putrefaction within the hive. Bees usually carry waste out of and away from the hive. However if a small lizard or mouse, for example, found its way into the hive and died there, bees may be
unable to carry it out through the hive entrance. In that case, they would attempt instead to seal the carcass in propolis, essentially mummifying it and making it odorless and harmless.

2.3.1 Origin

Propolis is made from substances collected by bees from the buds of trees (Willow, Poplar, Birch, Fir, Pine, Horse, Chestnut, …etc). It is prepared from pollen. Researchers stated that propolis has originated either internal or external origins.

1- An Internal origin:

Propolis might be a resin residue forming from the first phase of pollen digestion in small organ placed between the sac and lower gut. All cells and specially the newly built ones are varnished with this internal propolis before the queen lays eggs in them (Caillas, 1978).

2- An External origin:

Bee forager harvested propolis only from the buds particularly from poplar and older. It has been found however, that they harvest it also from other trees propolis has been found in hives in places where there are neither older nor poplar. It is well known by all apiarists that the hives placed in forests have more propolis in them than those on the plain (Caillas, 1978).

2.3.2 Chemical composition

Propolis collecting bees will use resins from a large variety of tree and other plant species, and these naturally will differ in their qualitative and quantitative chemical composition, Bee glue's chemical composition depends on the specificity of the local flora at the site of collection and thus
on the geographic and climatic characteristics of this site. This fact results in the striking diversity of propolis chemical composition, especially of propolis originating from tropical regions, bee gather propolis from flower (Clusia) when compares with temperate zone propolis that is gather from poplar trees only; poplar trees cannot grow in tropical and subtropical regions. For this reason, in these habitats, bees have to find other plant sources of propolis to replace their beloved poplar. As a result, propolis from tropical regions has a different chemical composition from that of poplar type propolis. Poplar propolis is rich in flavanoids and clusia propolis contains polyrenylated benzophenones (Bankova2 et al, 002)

Propolis is a very complex mixture that varies according to its sources. At least 180 different compounds have been identified so far in propolis. A broad analysis reveals approximately 55% resinous compounds and balsams, 30% beeswax, 10% ethereal and aromatic oils, and 5% bee pollen (Burduck, 1998). Many flavonoids contribute to propolis and have a great deal to do with its antibacterial qualities. Other components include cinnamic acid, cinnamyl alcohol, vanillin, caffeic acid, tectochrysin, isalpinin, pinocembrin, chrysin, galangin, and ferulic acid. Propolis contains some minerals such as aluminum, sodium, potassium, chromium, barium, cobalt, copper, calcium, manganese, stain, nickel, iron, lead, silicon, strontium, titanium, vanadium and zinc as well as some vitamins like B1, B2, B6, C and E, and a number of fatty acids. In addition, it contains some enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Hegazi, 1997).
2.3.3 Physical characteristics

The composition of propolis varies from hive to other, district to district, and from season to season. Normally it is dark brown in colour, but it can be found in green, red, black and white hues, depending on the sources of resin found in the particular hive area. Transparent propolis has even been reported. At 25° to 45°C propolis is a soft, pliable and very sticky substance. At less than 15°C, and particularly when frozen or at near freezing, it will become hard and brittle. It will remain brittle after such treatment even at higher temperatures. Above 45°C it will become increasingly sticky and gummy. Typically, propolis will become liquid at 60° to 70°C, but in some samples melting point may be as high as 100°C.

The most common solvents used for commercial extraction are ethanol ether, glycol and water. For chemical analysis, a large variety of solvents may be used in order to extract the different fractions (Arvouet et al., 1993). Propolis is a stable product, but should nevertheless be stored in airtight containers in the dark, preferably away from excessive and direct heat. Propolis does not lose much of its antibiotic activity, even when stored for 12 months or longer. Propolis and its extract function as a mild preservative due to their antioxidant and antimicrobial activities and thus may actually prolong the shelf life of some products (Bankova et al., 2002).

2.3.4 Uses

Propolis was first used as folk medicine from the day of Aristotle (which is around 350 BC). Apart from that, others believe it was first used
by Egyptian priest doctor who started using it as medicine after first utilizing it successfully for mummification.

Later on, propolis was also mentioned in Arabs, Greeks and Roman medical treatises during the late 19th century. Equivalent to today's medical journals, these treatises talk about using propolis for treating infection, skin diseases, respiratory and joint problems. The Greeks were known to use it for abscesses while the Assyrians used it to heal wounds and possibly tumors. In Europe and North Africa, propolis has been used for treating wound, caries and all forms of mouth or throat infections (Ikeno et al, 1991).

2.3.4.1 Traditional use

Propolis was mostly valued as a medicinal agent. Hippocrates prescribed propolis to help heal sores as well as ulcers, both external and internal. Propolis-making bees were also depicted on vases from ancient Egypt where the sign of the bee was often interwoven with the titles of the kings and used as the motif on ornaments presented as rewards for valor. The ancient Egyptians looked upon the bees and their propolis as the source of eternal health and life. In the 17th century, propolis was a major ingredient of healing ointments in the European pharmacopoeia.

Propolis is used in anti-inflammatory properties, and for wound healing. It has been used internally and externally, and is believed to possess antibacterial, antiviral and antifungal (Khayyal et al, 1993).
2.3.4.2 Medicinal uses

Propolis has been used by man since early times, for various purposes, and especially as a medicine because of its antimicrobial properties. Ancient Greek texts refer to the substance as a "cure for bruises and suppurating sore", and in Rome propolis was used by physicians in making poultices. The Hebrew word for propolis is tzori, and the therapeutic properties of tzori are mentioned throughout the Old Testament. Records from 12th century Europe describe medical preparations using propolis for the treatment of mouth and throat infections, and dental caries. Old beekeepers recommend a piece of propolis kept in the mouth as a remedy for a sore throat. Propolis is a subject of recent dentistry research, since there is some evidence that propolis may actively protect against caries and other forms of oral disease, due to its antimicrobial properties (Samet et al, 2007 and Park et al, 1998). Propolis can also be used to treat canker sores, its use in canal debridement for endodontic procedures has been explored in Brazil.

Propolis is also believed to promote heart health, strengthen the immune system and reduce the chances of cataracts (Orhan et al, 1999). Propolis also exhibits immunomodulatory effects. It has been shown to stimulate an immune response in mice. More recently, Japanese researchers have shown an extract of propolis to produce a macrophage activation phenomenon related to the immune function in humans (Ansorge et al, 2003). Propolis activates immune cells, which produce cytokines. The results help to explain the anti-tumor effect produced by propolis. Propolis has been shown to suppress HIV-1 replication and modulate in vitro immune responses, and, according to the authors, "May constitute a non-toxic natural product with both anti-HIV-1, and immunoregulatory effects"
Propolis use in inhibiting tumorigenesis has been studied in mice in Japan, ethanol extracts of propolis have been found to transform human hepatic and uterine carcinoma cells in vitro, and to inhibit their growth. Substances isolated in propolis, which produce this cytotoxic effect, are quercetin, caffeic acid, and clerodane diterpendoid. Clerodane diterpendoid shows a selective toxicity to tumor cells (Sugimoto et al., 2003).

Propolis has been shown to reduce blood pressure, produce a sedative effect, and maintain serum glucose. Dihydroflavonoids, as contained in propolis, have been shown to strengthen capillaries, and produce antihyperlipidemic activity. Propolis has also been shown to protect the liver against alcohol (ethanol) and tetrachloride in rats (Banskota et al., 2001).

Propolis is marketed by health food stores as a traditional medicine, and for its claimed beneficial effect on human health. Natural medicine practitioners use propolis for the relief of various conditions, including inflamations, viral diseases, ulcers, superficial burns or scalds. Propolis has been shown to stimulate various enzyme systems, cell metabolism, circulation and collagen formation, as well as improve the healing of burn wounds. These effects have been shown to be the result of the presence of arginine in propolis. Propolis was found to be superior to standard wound treatment products in trials on mice (Marcucci, 1995).

Propolis lozenges and tinctures can be bought in many countries. Though claims have been made for its use in treating allergies, propolis may cause severe allergic reactions if the user is sensitive to bees or bee products (Brovko and Kravchuk, 1970).
2.3.4.3 Commercial uses

Raw propolis is collected by beekeepers and sold in bulk to companies that refine the product and turn it into usable extracts. Most commercial uses of propolis are based on preparations made up from these extracts. Methods include ethanol extraction (EEP), glycol extraction (GEP), aqueous (water) extraction (AEP), oil extraction (OEP), and water-soluble derivatives (WSD). Where solvents are used, reduction or elimination of the solvent is necessary, either by freeze-drying, vacuum distillation, or evaporation. Extraction is used to remove the beeswax, which is mixed with the propolis by the bees during use in the hive, as well as other non-active components such as resinous-balsam substances.

Main commercial uses of propolis are as a dietary supplement and therapeutic. Propolis is sold in tablets (singularly, or in combination with other substances such as pollen, royal jelly and non-hive products), and tinctures, and as an ingredient in lozenges, skin creams, shampoos, lipsticks, toothpastes and mouthwashes. Tinctures and lozenges are popular treatment for sore throats, and tinctures are often used to treat cuts, mouth sores and skin rashes. The antioxidant, antimicrobial and antifungal activities of propolis also offer opportunities in food technology. In Japan, the use of propolis is permitted as a preservative in frozen fish (Paulino et al., 2006).

2.3.4.4 Other uses

Propolis is used by certain music instrument makers to enhance the appearance of the wood grain. Propolis is used by some chewing gum manufacturers to make propolis gum. Propolis is also used in Sub Saharaby
the African natives. It is used to water-proof their containers and also as a form of adhesive. However, the main use of propolis is still as a traditional medicine. In other parts of the world, propolis is also used as preservatives for living and dead things. From Stradivarius violins to fish, propolis offers great protection as it does to beehives.
3. MATERIALS AND METHODS

3.1 Parasite source

*Trypanosoma evansi* was obtained from the Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, in Shambat

3.2.1 Preparation of water extract of propolis:

Six grams of propolis powder were mixed with 60 ml of distilled water in the conical flask with well covered and put in the heater at boiling point for well dissolving, then it was placed in the shaker apparatus for 48hrs to well shaking. After that the solution was filtrated by filter paper to discrete water extract from the other components of propolis. The supernatant was evaporated and the residue was adjusted with distilled water to make extract of propolis (AEP10%) which was then ready to use in the experiment.

3.2.2 Preparation of ethanol extract of propolis:

Six grams of propolis powder were mixed with 60 ml of ethanol (70% conc.) in the conical flask with well covered and put in the heater at boiling point for well dissolving, then it was placed in the shaker apparatus for 48hrs to well shaking. After that, the mixture was filtrated by filter paper to separate ethanol extract from the other components of propolis solution. The supernatant was evaporated and the residue was adjusted with ethanol (70% conc.) to make extract of propolis (EEP10%) which was then ready to use in the experiment.
3.3.1 Bioassay:

Prepared water and ethanol extracts of propolis were diluted with distilled water and ethanol (70% conc.) respectively for 5% and 1% concentrations which were used with (10% conc.) as preliminary pilot experiment.

Fourteen rats were used in preliminary pilot experiment, all rats infected with Trypanosoma mixed blood; parasiteamia 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, 6 rats were taken for water extract of propolis experiment (1%, 5% and 10%); 3 rats were injected subcutaneously with 0.2ml per rat, one rat for each concentration. Other 3 rats were injected intraperitoneally 0.2ml per rat, and then the level of parasiteamia was determined daily before injection for four days. The other 6 parasiteamic rats were used in the ethanol extract of propolis experiment was followed the same procedure of the water extract of propolis experiment. The last two rats were used as control; one was treated with 0.1 ml chemical drug (Quinapyramine sulphate) and the other one without treatment.

3.3.2 Experimental animals (Rats):

Forty rats were purchased from the Institute of Medicinal and Aromatic plants, National Centre for Research, Khartoum.
Rats were divided into two groups (20 rats in each group), the first group was used in the water extract of propolis experiment and the second group was used in the ethanol extract of propolis experiment.

### 3.3.3 Water extract of propolis experiment:

The experiment was carried out to study the effect of water extract of propolis on *Trypanosoma evansi* by treating 20 rats which were divided into four groups (5 rats in each group as replicates), group one was treated sub-cutanously (SC) with 0.2 ml (water extract 10%conc) per rat, group two treated intraperitoneally (IP) with 0.2 ml (water extract 10%conc) per rat, group three was treated with chemical drug (Quinapyramine sulphate) 0.1 ml per rat and the last group was served as untreated control.

### 3.3.4 Ethanol extract of propolis experiment:

The experiment was carried out to study the effect of ethanol extract of propolis on *Trypanosoma evansi* by treating 20 rats which were divided into four groups (5 rats in each group as replicates), group one was treated sub-cutanously (SC) with 0.2 ml (ethanol extract 10%conc) per rat, group two treated intraperitoneally (IP) with 0.2 ml (ethanol extract 10%conc) per rat, group three was treated with chemical drug (Quinapyramine sulphate) 0.1 ml per rat and the last group was served as untreated control.

Rats kept in the animal facilities, Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum.
3.4 Testing of Trypanocidal activity of propolis extracts on *Trypanosoma evansi*:

All rats infected with *Trypanosoma* mixed blood; parasiteamia 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 parasiteamia 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. Parasiteamia level was detected daily for three days for two groups.

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

3.5 Statistical analysis:

The design used was completely randomized design (CRD) and data were subjected to analysis of variance (ANOVA), then least significant difference test (LSD) was used to differentiate between means.
4. RESULTS

4.1 The effect of propolis extracts on the *Trypanosoma evansi* (Evans)

4.1.1 Parasiteamia level of *Trypanosoma evansi* affected with the water extract of propolis

The results of the effect of the water extract of propolis on *Trypanosoma evansi* are depicted in table (1) and figure (1).

After 24hrs, parasiteamia level of *Trypanosoma evansi* 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 parasiteamia 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.

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 parasiteamia 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.

After 72hrs, a completely different pattern in the level of parasiteamia observed, substantial reduction of parasiteamia level obtained in rats treated sub-cutaneously or intraperitoneally when compared with untreated control. Significant differences detected among the treatments.
Mean parasiteamia level of *Trypanosoma evansi* was 3.37 for rats treated subcutaneously, 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 parasiteamia count; a level, which was 53% of 8.03, obtained in the untreated control. On the other hand, parasiteamia level was 0.0 for those animals, which were given chemical drug.
Table (1): Parasitemia means of *Trypanosoma evansi* infected rats treated with water extract of propolis

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-cutanously (SC)</td>
<td>7.35</td>
<td>5.5</td>
<td>3.37</td>
</tr>
<tr>
<td></td>
<td>(94)</td>
<td>(70)</td>
<td>(42)</td>
</tr>
<tr>
<td>Intraperitoneally (IP)</td>
<td>7.37</td>
<td>7.0</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td>(94)</td>
<td>(89)</td>
<td>(53)</td>
</tr>
<tr>
<td>Chemical drug</td>
<td>7.73</td>
<td>6.9</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>(99)</td>
<td>(88)</td>
<td>(0)</td>
</tr>
<tr>
<td>Control</td>
<td>7.83</td>
<td>7.88</td>
<td>8.03</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>1.4</td>
<td>5.5</td>
<td>3.94</td>
</tr>
</tbody>
</table>

Figures between brackets indicate the value as percentage of control. Means followed with the different letters on the same column shows a significant level.
Figure 1: Parasitemia curves of *Trypanosoma evansi* infected rats treated with water extract of propolis.
4.1.2 Parasiteamia level of *Trypanosoma evansi* affected with ethanol extract of propolis

Generally, in comparison with the results obtained for water extract of propolis, a completely different pattern of effect expressed by ethanol extract of propolis, as the results displaced on the table 2 and figure 2.

After 24hrs, parasiteamia level of *Trypanosoma evansi* reported slight reduction in rats treated sub-cutanously 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 parasiteamia 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.

After 48hrs, inconsequential change occurred in the pattern of parasiteamia level, when compared with the first day. In addition, differences among treatments were highly significant. The level of parasiteamia 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.

After 72hrs, reasonable different patterns in the level of parasiteamia were observed. As well, differences among the treatments were highly significant. Means parasiteamia 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 parasiteamia was7.38 for those imparted ethanol extract intraperitoneally, which represent 86% of that recorded for untreated control. In contrast, chemical drug produced a drastic reduction parasiteamia level until the third day.
Table (2): Mean parasiteamia count of \textit{Trypanosoma evansi} infected rats treated with ethanol extract of propolis

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day\textsubscript{1}</th>
<th>Day\textsubscript{2}</th>
<th>Day\textsubscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-cutaneously (SC)</td>
<td>7.95 (94)</td>
<td>7.72 (91)</td>
<td>7.0 (82)</td>
</tr>
<tr>
<td>Intraperitoneally (IP)</td>
<td>7.88 (93)</td>
<td>7.66 (90)</td>
<td>7.38 (86)</td>
</tr>
<tr>
<td>Chemical drug</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Control</td>
<td>8.46 (100)</td>
<td>8.52 (100)</td>
<td>8.56 (100)</td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
</tr>
</tbody>
</table>

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.
Figure 2: Parasiteemia curves of *Trypanosoma evansi* infected rats treated with ethanol extract of propolis
5. DISCUSSION

Propolis, a product from bees, is extensively used in folk medicine for a wide spectrum of diseases. This resinous material is collected from different plant exudates and thus presents a complex composition depending on the plant sources available. Over the last few decades, an increasing number of studies have been published on the chemical composition, biological and pharmacological activities and therapeutic uses of propolis. Different animal models have been used to investigate propolis as an anti-inflammatory (Sylb et al., 2006), cariostatic (Sabir et al., 2005), and anti-parasitic agent (Starzyk et al., 1977) and its protective role in models of carcinogenesis and hepatotoxicity (Padmavathi et al., 2006).

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

Water extract is more effective against *Trypanosoma evansi* which exhibited high decreasing in parasiteamia 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 parasiteamia 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.
6. CONCLUSION AND RECOMMENDATIONS

The over all conclusion indicated that propolis extracts (water& ethanol) had an evident trypanocidal active ingredients that reduce parasiteamia level on the tested parasite *Trypanosoma evansi*.

Water extract was more effective than ethanol extract and subcutaneously 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.
REFERENCES


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APPENDICES

Appendix (1)

ANOVA table of Parasiteamia level of *Trypanosoma evansi* affected with water extract of propolis assessed on rats administered subcutaneously and intraperitoneally on first day

<table>
<thead>
<tr>
<th></th>
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<th>SS</th>
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<th>F. ratio</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cal</td>
</tr>
<tr>
<td>Treatment(t-1)</td>
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<td>0.63</td>
<td>0.21</td>
<td>n.s</td>
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<tr>
<td>Error(r-1)t</td>
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<td>0.22</td>
<td>0.96</td>
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<tr>
<td>Total</td>
<td>13</td>
<td>2.41</td>
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<td>n.s</td>
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</table>

n.s non-significant

Appendix (2)

ANOVA table of Parasiteamia level of *Trypanosoma evansi* affected with water extract of propolis assessed on rats administered subcutaneously and intraperitoneally on second day

<table>
<thead>
<tr>
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<th>F. ratio</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cal</td>
</tr>
<tr>
<td>Treatment(t-1)</td>
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<td>11.51</td>
<td>3.84</td>
<td>n.s</td>
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<tr>
<td>Error(r-1)t</td>
<td>8</td>
<td>41.73</td>
<td>5.22</td>
<td>0.74</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>53.24</td>
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<td>n.s</td>
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</table>

n.s non-significant
Appendix (3)

ANOVA table of Parasiteamia level of *Trypanosoma evansi* affected with water extract of propolis assessed on rats administered subcutaneously and intraperitoneally on third day

<table>
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<tr>
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<th>F. ratio</th>
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<td></td>
<td>Cal</td>
</tr>
<tr>
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<td>74.89</td>
<td>37.44</td>
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<tr>
<td>Error(r-1)t</td>
<td>8</td>
<td>46.74</td>
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<tr>
<td>Total</td>
<td>10</td>
<td>121.63</td>
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* Significant

Appendix (4)

ANOVA table of Parasiteamia level of *Trypanosoma evansi* affected with ethanol extract of propolis assessed on rats administered subcutaneously and intraperitoneally on first day

<table>
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<tr>
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<th>F. ratio</th>
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</thead>
<tbody>
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<td></td>
<td></td>
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<td></td>
<td>Cal</td>
</tr>
<tr>
<td>Treatment(t-1)</td>
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<td>243.13</td>
<td>81.04</td>
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<tr>
<td>Error(r-1)t</td>
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<td>0.35</td>
<td>0.023</td>
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<tr>
<td>Total</td>
<td>18</td>
<td>243.48</td>
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** Highly significant
Appendix (5)

ANOVA table of Parasiteamia level of *Trypanosoma evansi* affected with ethanol extract of propolis assessed on rats administered subcutaneously and intraperitoneally on second day

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<td>79.06</td>
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<td>5% 3.29</td>
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<tr>
<td>Error(r-1)t</td>
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<td>0.85</td>
<td>0.057</td>
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<td>Total</td>
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<td>238.02</td>
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</table>

** Highly significant**

Appendix (6)

ANOVA table of Parasiteamia level of *Trypanosoma evansi* affected with ethanol extract of propolis assessed on rats administered subcutaneously and intraperitoneally on third day

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<th>F. ratio</th>
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<tr>
<td>Treatment(t-1)</td>
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<td>224.2</td>
<td>74.73</td>
<td>**</td>
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<td>5% 3.29</td>
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<td>Error(r-1)t</td>
<td>15</td>
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<td>Total</td>
<td>18</td>
<td>227.04</td>
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</table>

** Highly significant**