
Promising antiprotozoal activities of bee propolis

Propolis

Propolis is a natural mixture produced by honeybees from substances collected from parts of plants, buds, and exudates.

Propolis is used by bees in the construction and repair of their hives

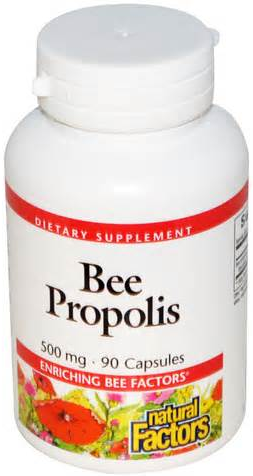
for sealing openings and cracks

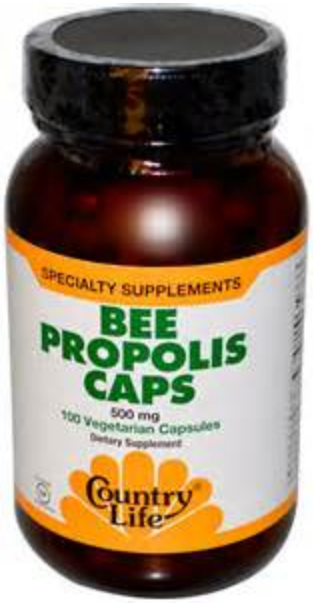
smoothing out the internal walls

a protective barrier against external invaders like snakes, lizards, and so forth, or against wind and rain.

Bees gather propolis from different plants in different temperate climatic zones.

Pharmaceutical products of propolis





Generally,

Raw propolis is composed of

around 50% resins,
30% waxes,
10% essential oils,
5% pollen,
and 5% of various organic compounds.

More than 300 constituents were identified in different samples and new ones are still being recognized during chemical characterization of new types of propolis.

Malaria

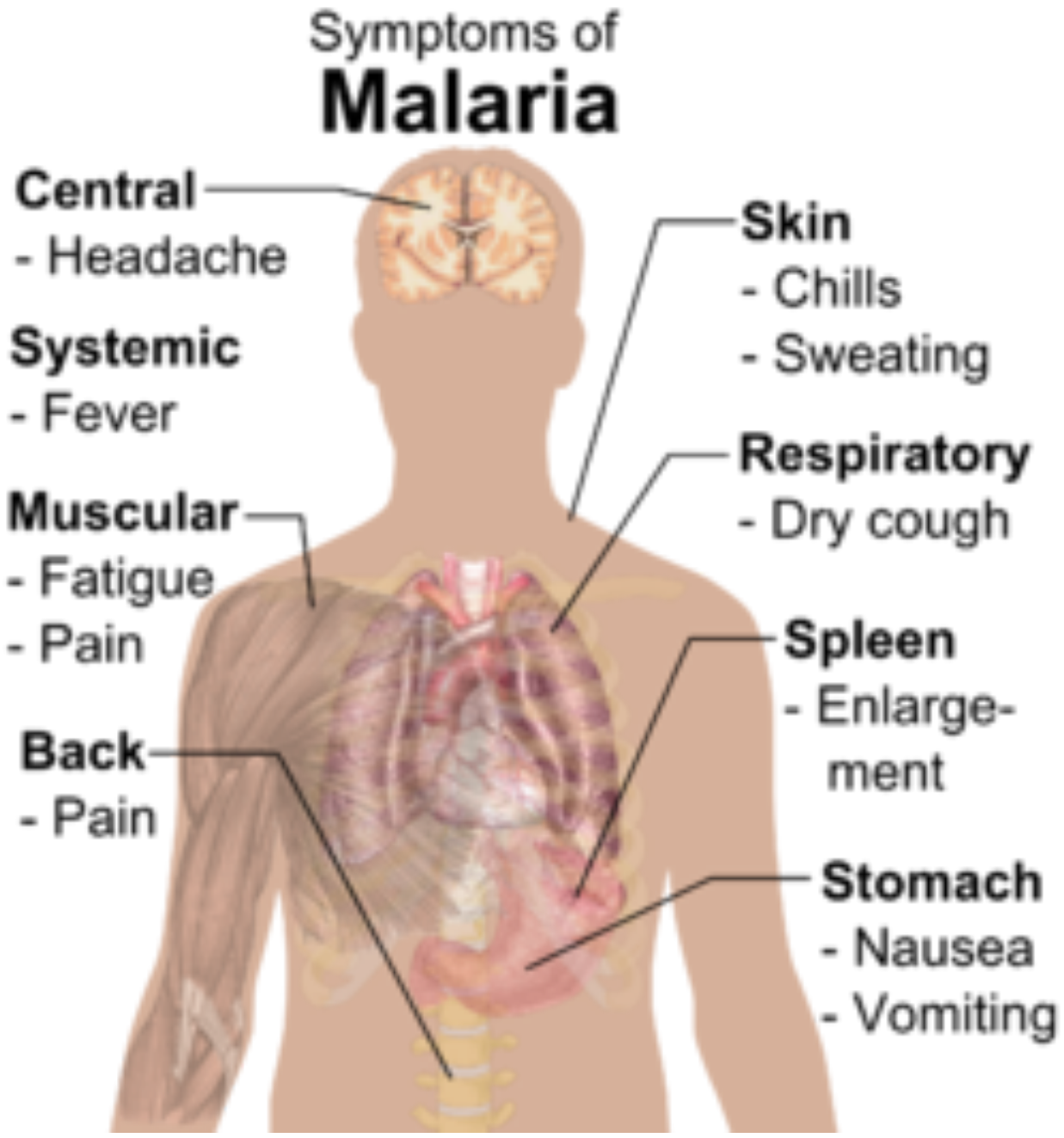
Malaria

The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator which includes much of Sub-Saharan Africa, Asia, and Latin America.

In 2015, there were 214 million cases of malaria worldwide resulting in an estimated 438,000 deaths, 90% of which occurred in Africa.

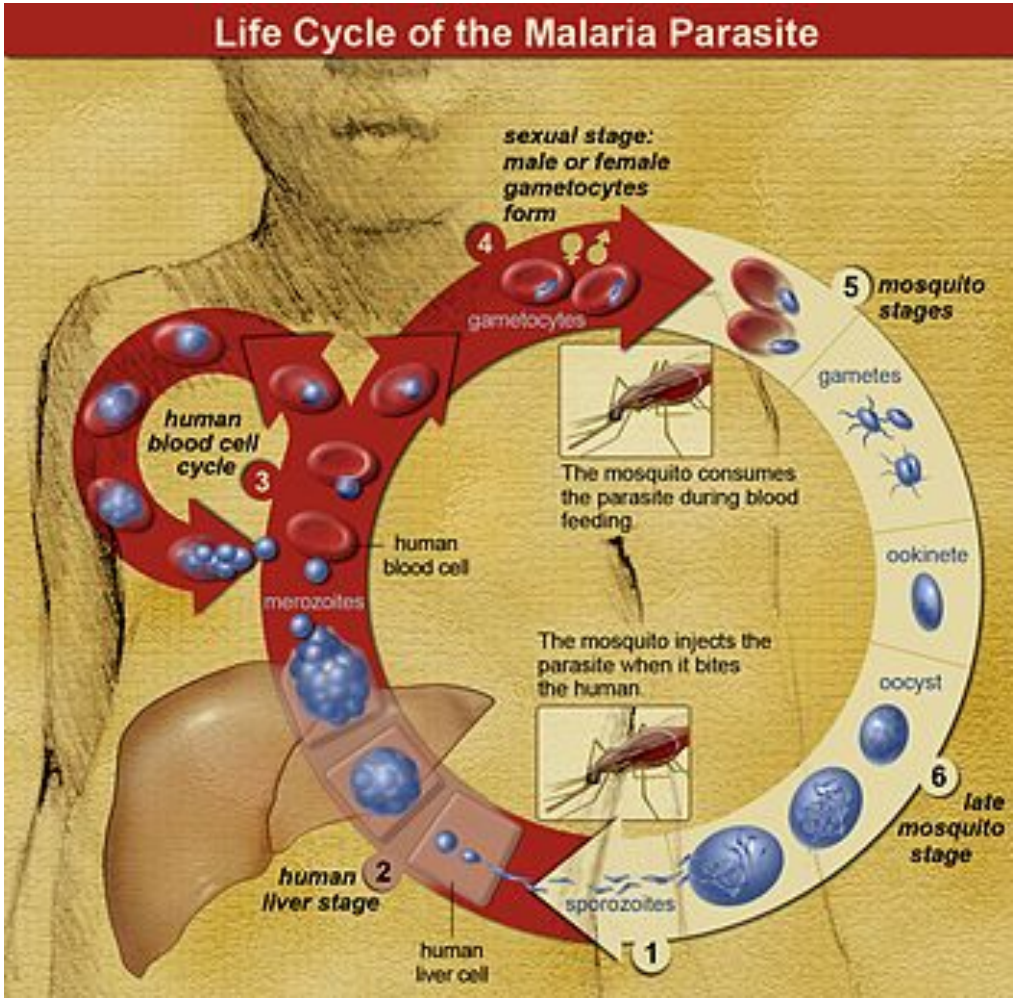
Rates of disease have decreased from 2000 to 2015 by 37%,but increased from 2014 during which there were 198 million cases.

In Africa, it is estimated to result in losses of US$12 billion a year due to increased healthcare costs, lost ability to work, and negative effects on tourism.


Symptoms of
Malaria
Central
- Headache
Systemic
- Fever
Muscular
- Fatigue
- Pain
Back
- Pain
Skin
- Chills
- Sweating
Respiratory
- Dry cough
Spleen
- Enlarge-
ment
Stomach
- Nausea
- Vomiting

Life Cycle

The life cycle of malaria parasites. A mosquito causes an infection by a bite. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells, where they multiply into merozoites, rupture the liver cells, and return to the bloodstream. The merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts that in turn produce further merozoites. Sexual forms are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.



Antimalarial effect of propolis

- **In vivo evaluation of antimalarial effect in albino mice were used**
  PE significantly suppressed the parasitaemia and showed significant efficacy in reducing anaemic conditions in *P. chabaudi*-infected mice in a dose-dependent manner(25, 50 and 100 mg PE/kg).

Antimalarial effect of propolis

- Histological investigation of the spleen tissue of treated and untreated mice further supports the antimalarial potential of PE. In addition, our study proved that Saudi PE reduced oxidative damage by decreasing the malondialdehyde (MDA) and increasing the catalase (CAT) activity and the glutathione (GSH) levels.

Antimalarial effect of propolis

- **Also, Saudi PE increased the level of some pro-inflammatory cytokines such as IFN-γ, TNF-α,.**
  In conclusion, PE showed antimalarial and antioxidant activities and provided protection against spleen tissue damage in *P. chabaudi*-infected mice.

Leishmania

Leishmaniasis **currently affects 12 million people in 98 countries. About 2 million new cases occur each year,**
21 species are known to cause disease in humans.

Leishmania

Phylum Sarcomastigophora

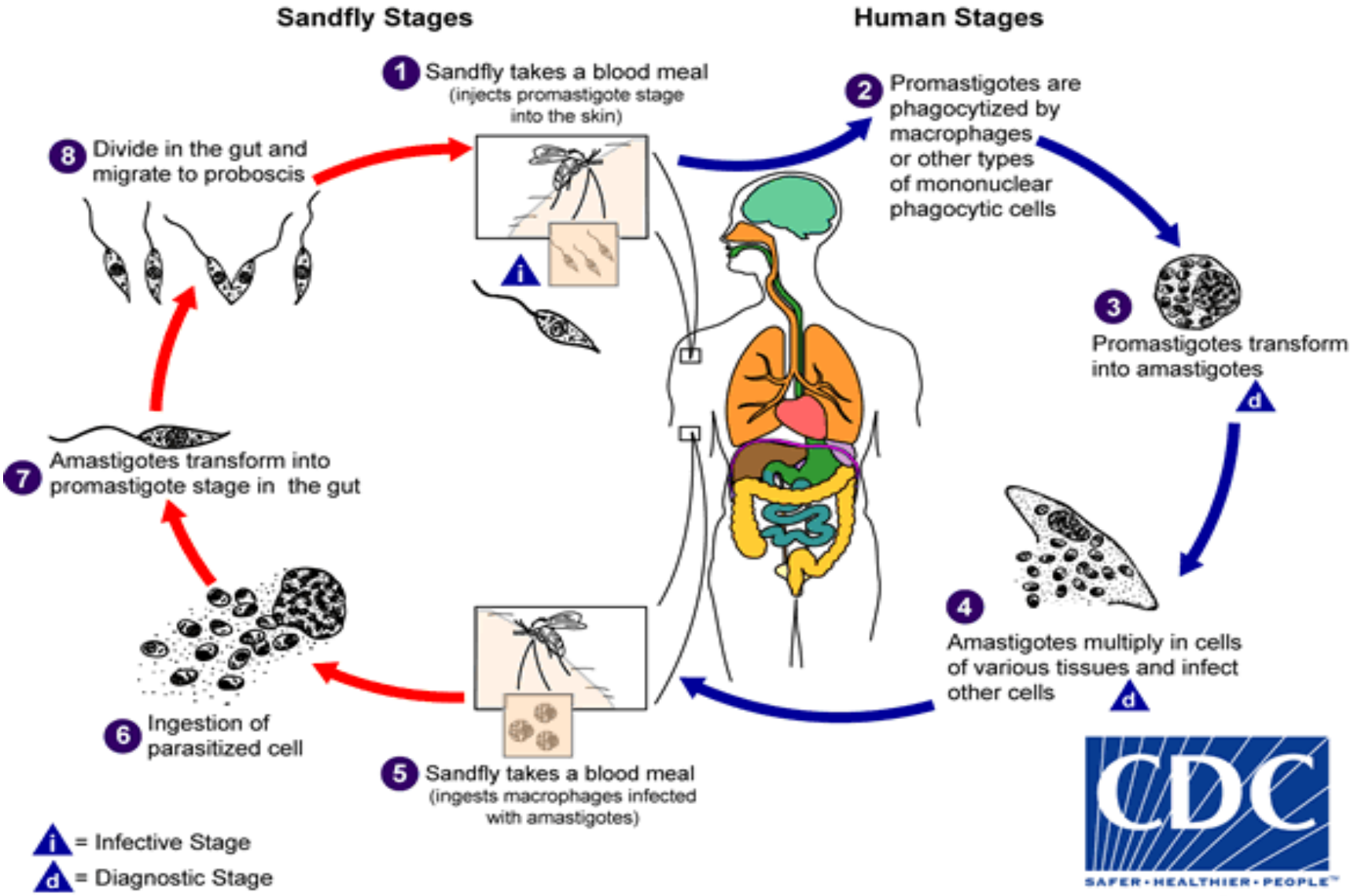
Order Kinetoplastida

Family Trypanosomatidae

Genus *Leishmania*

- Transmitted to the mammalian hosts by the bite of infected sandflies, *Phlebotomus* and *Lutzomyia*

Leishmania Life cycle



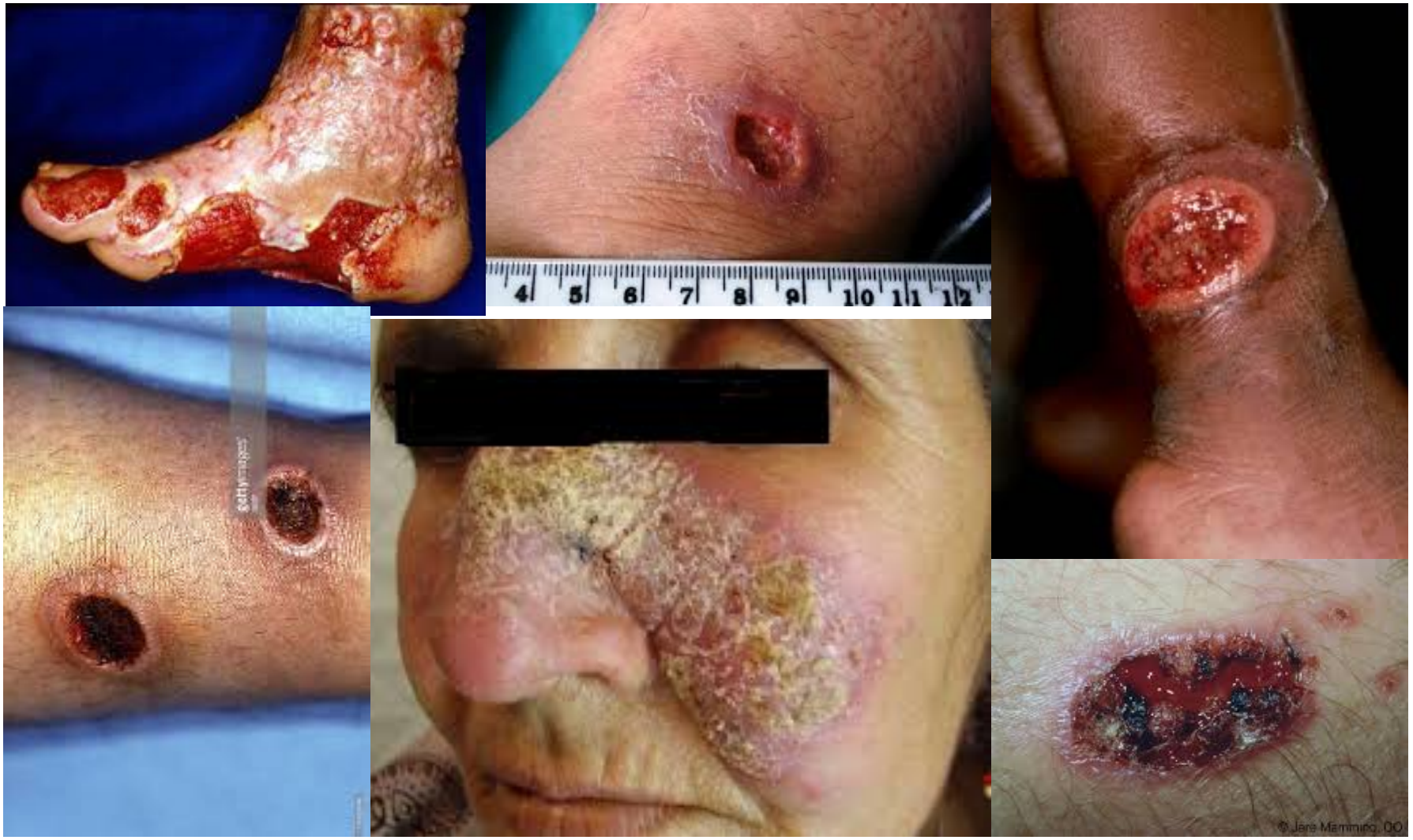
Leishmaniasis

Leishmaniasis is caused by infection with *Leishmania* parasites, which are spread by the bite of phlebotomine sand flies.

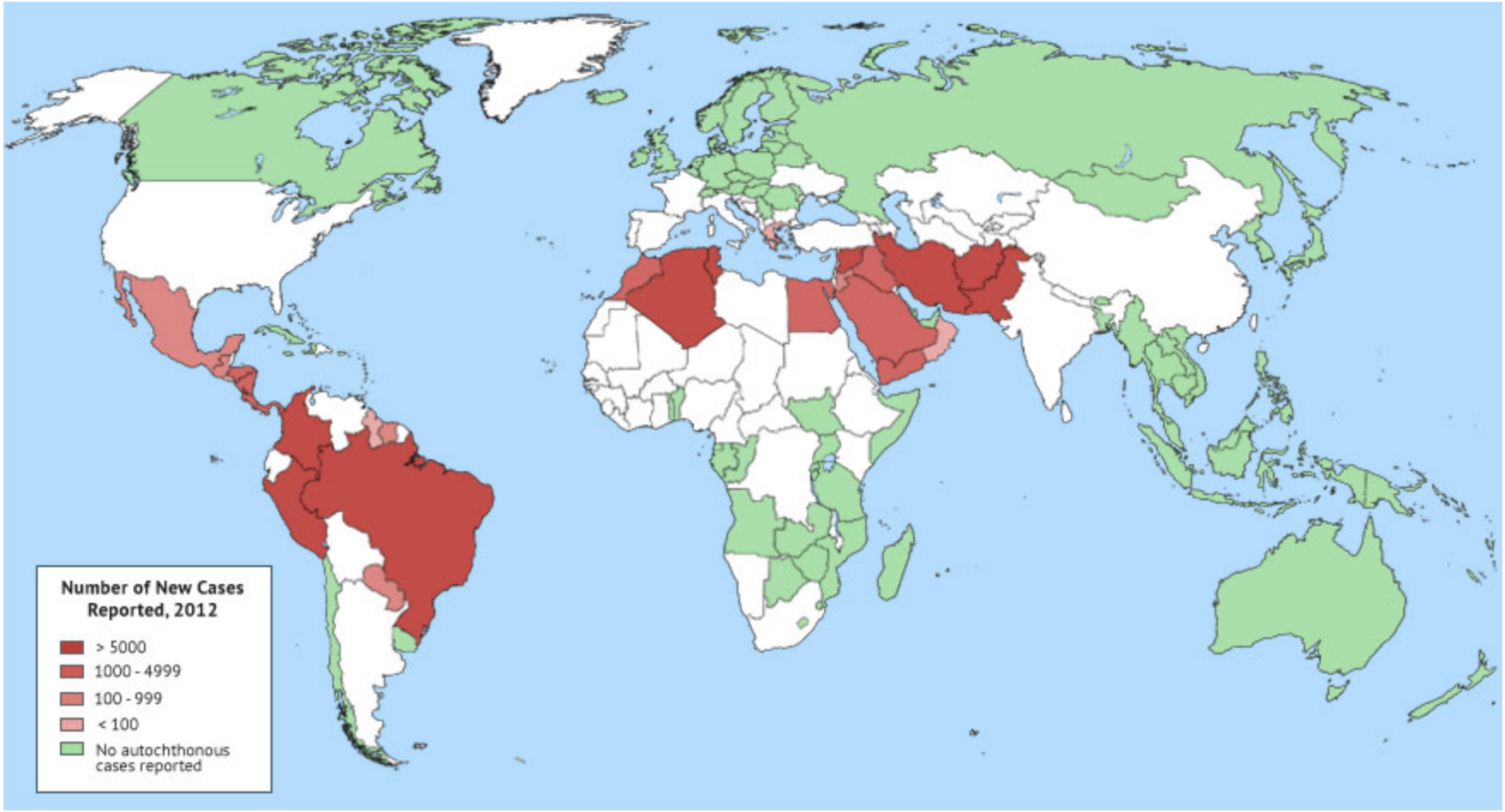
There are several different forms of leishmaniasis in people.

The most common forms are cutaneous leishmaniasis, which causes skin sores, and visceral leishmaniasis, which affects several internal organs (usually spleen, liver, and bone marrow).

Cutaneous Leishmaniasis

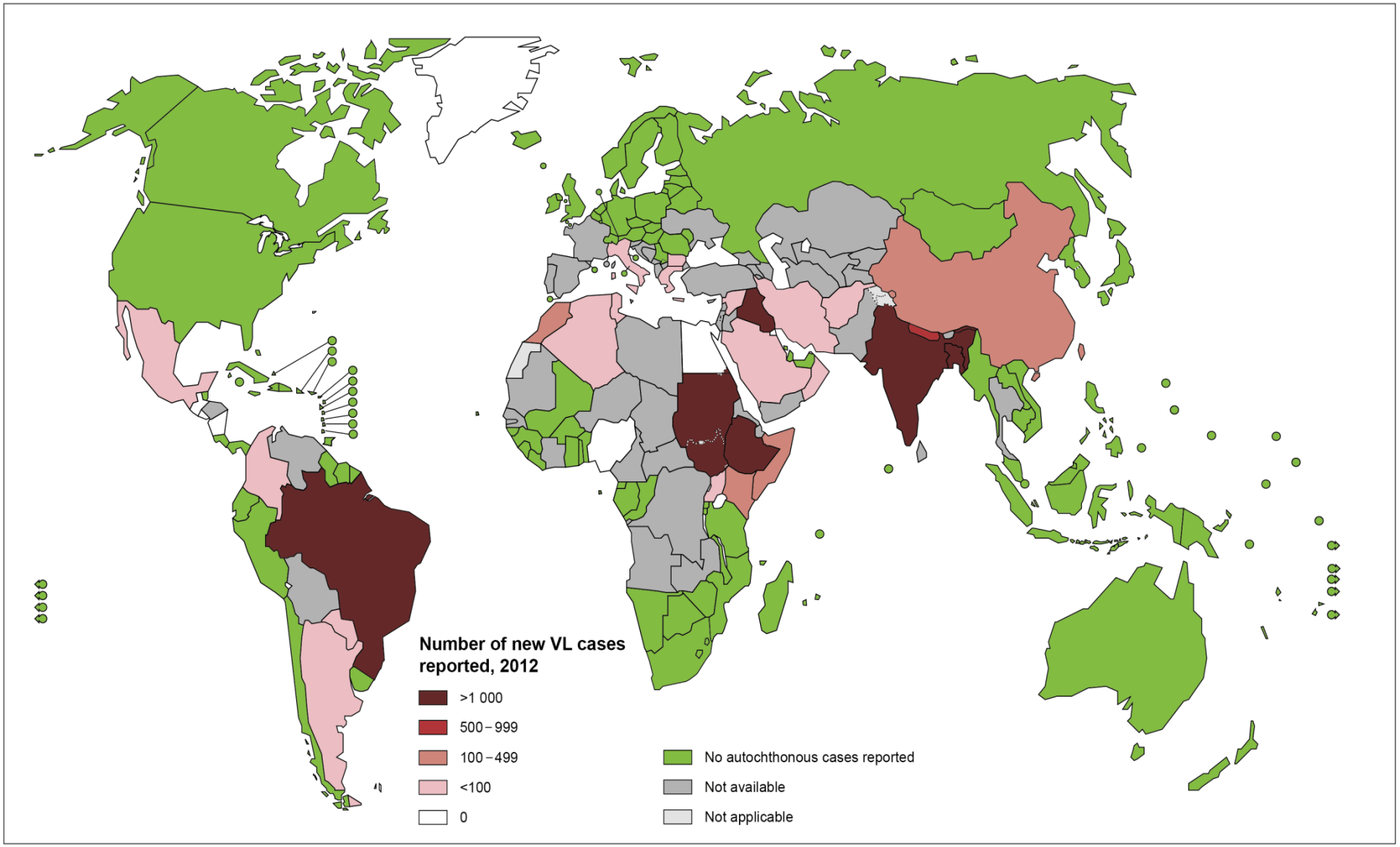


Epidemiology of cutaneous Leishmaniasis



Visceral Leishmaniasis

Status of endemicity of visceral leishmaniasis, worldwide, 2012



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Anti leishmania activities of propolis

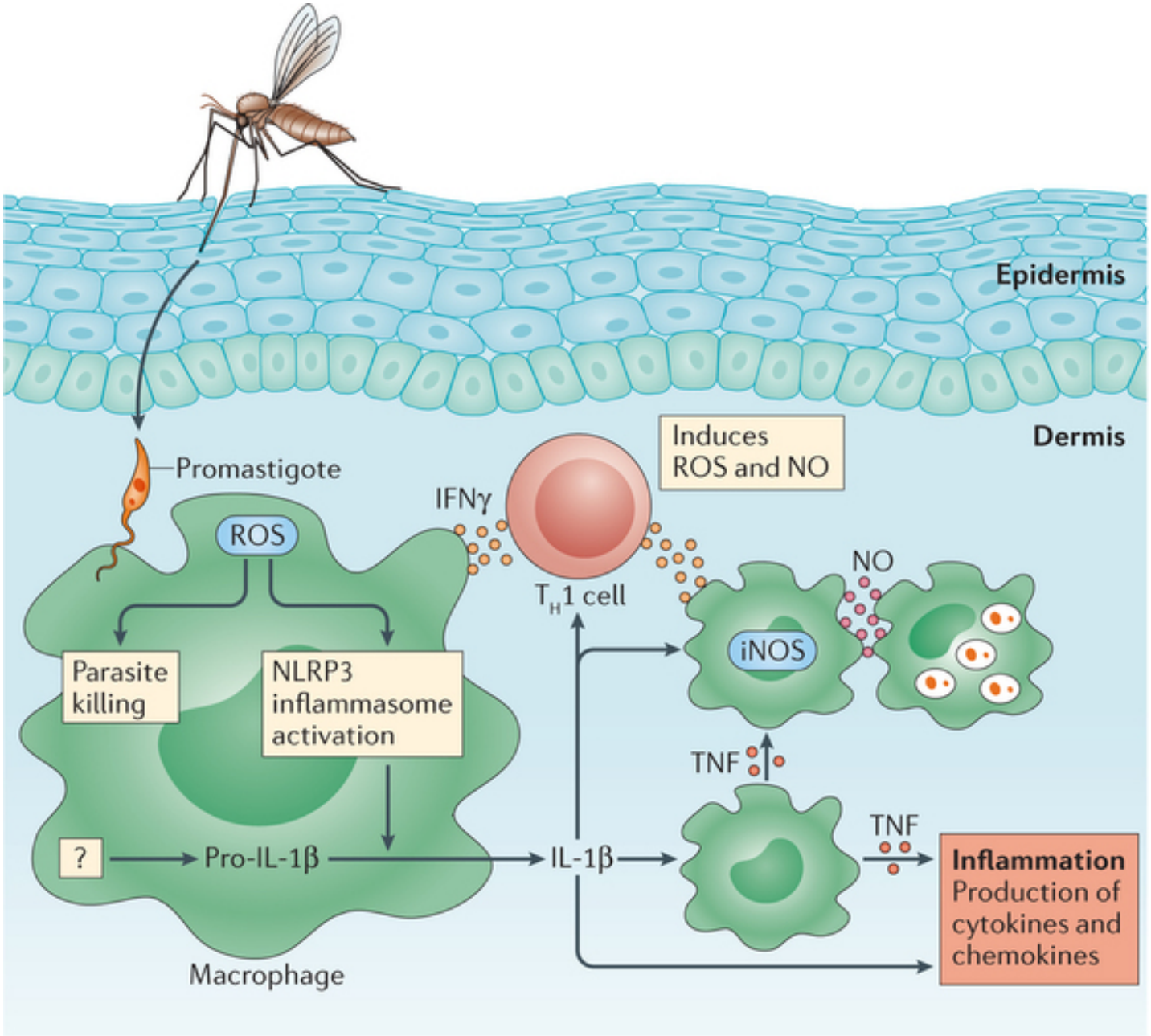
- Effect of four Brazilian propolis ethanolic extracts on Leishmania amazonensis performing assays with promastigote forms,
- extracellular amastigotes,
- infected peritoneal macrophages.

Anti leishmania activities of propolis

- reduce parasite load as monitored by the percentage of infected macrophages and the number of intracellular parasites.
- Red propolis contains high concentration of prenylated and benzophenones compounds, was the most active extract against L. amazonensis.
- The anti-Leishmania effect of red propolis was increased in a concentration and time dependent manner.
- Red propolis treatment proved to be non-toxic to macrophage cultures.
-

Anti leishmania activities of propolis

- **25 µg/ml Red propolis extract:**
- **reduced the parasite load of macrophages**
- **presented no direct toxic to promastigotes**
- **presented no direct toxic to extracellular amastigotes**
- **It was suggested that constituents of propolis intensify the mechanism of macrophage activation leading to killing of L. amazonensis.**


Epidermis
Dermis
Promastigote
ROS
Parasite killing
NLRP3 inflammasome activation
?
Pro-IL-1β
Macrophage
IFNγ
T_H1 cell
Induces ROS and NO
iNOS
NO
TNF
IL-1β
TNF
Inflammation
Production of cytokines and chemokines

Human trypanosomiasis

- Human trypanosomiasis affects as many as 66 million people in sub-Saharan Africa. Trypanosomes are also found in the Americas in the form of ***Trypanosoma cruzi***, which causes American human trypanosomiasis, or Chagas' disease.
- This disease is found in humans in two forms: as an **amastigote** in the cells, and as a **trymastigote** in the blood.

Trypanosomiasis

- Trypanosomes infect a variety of hosts and cause various diseases, including the fatal human diseases sleepin sickness, caused by Trypanosoma brucei, and Chagas disease, caused by Trypanosoma cruzei

Symptoms of sleeping sickness Disease

changes of behaviour,

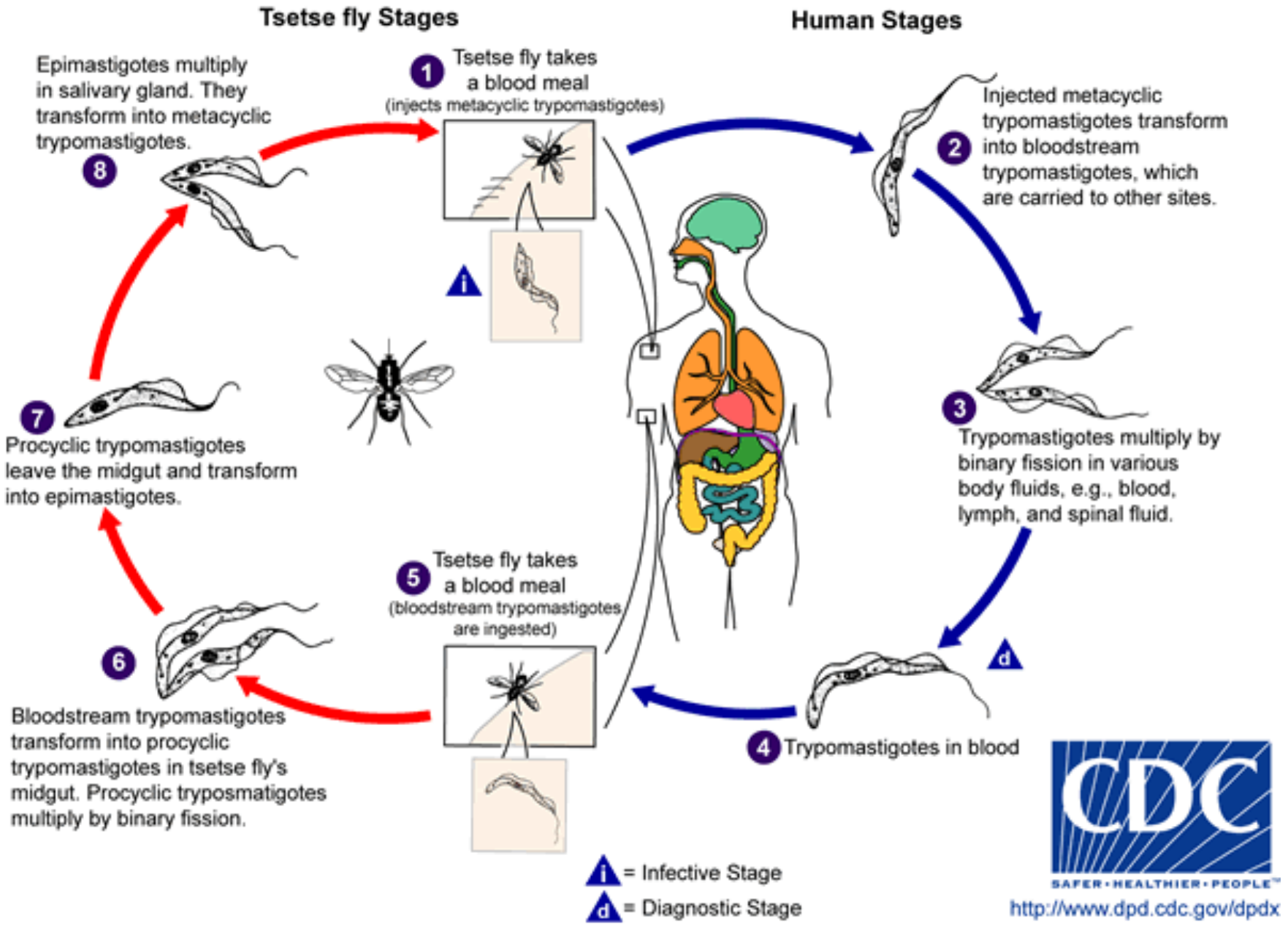
confusion,

sensory disturbances

poor coordination.

Disturbance of the sleep cycle

Life cycle


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
i
2 Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
3 Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
d
4 Trypomastigotes in blood
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6 Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
7 Procyclic trypomastigotes leave the midgut and transform into epimastigotes.
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx

Anti trypanosoma activities of propolis

Brazilian green propolis ethanolic extract targets T. cruzi and its effect on experimental infection of mice.

The IC_{50}/4 days for inhibition of amastigote proliferation was 8.5 ± 1.8 µg mL^{-1}, with no damage to the host cells.

In epimastigotes, Brazilian green propolis ethanolic extract induced alterations in reservosomes, Golgi complex and mitochondrion.

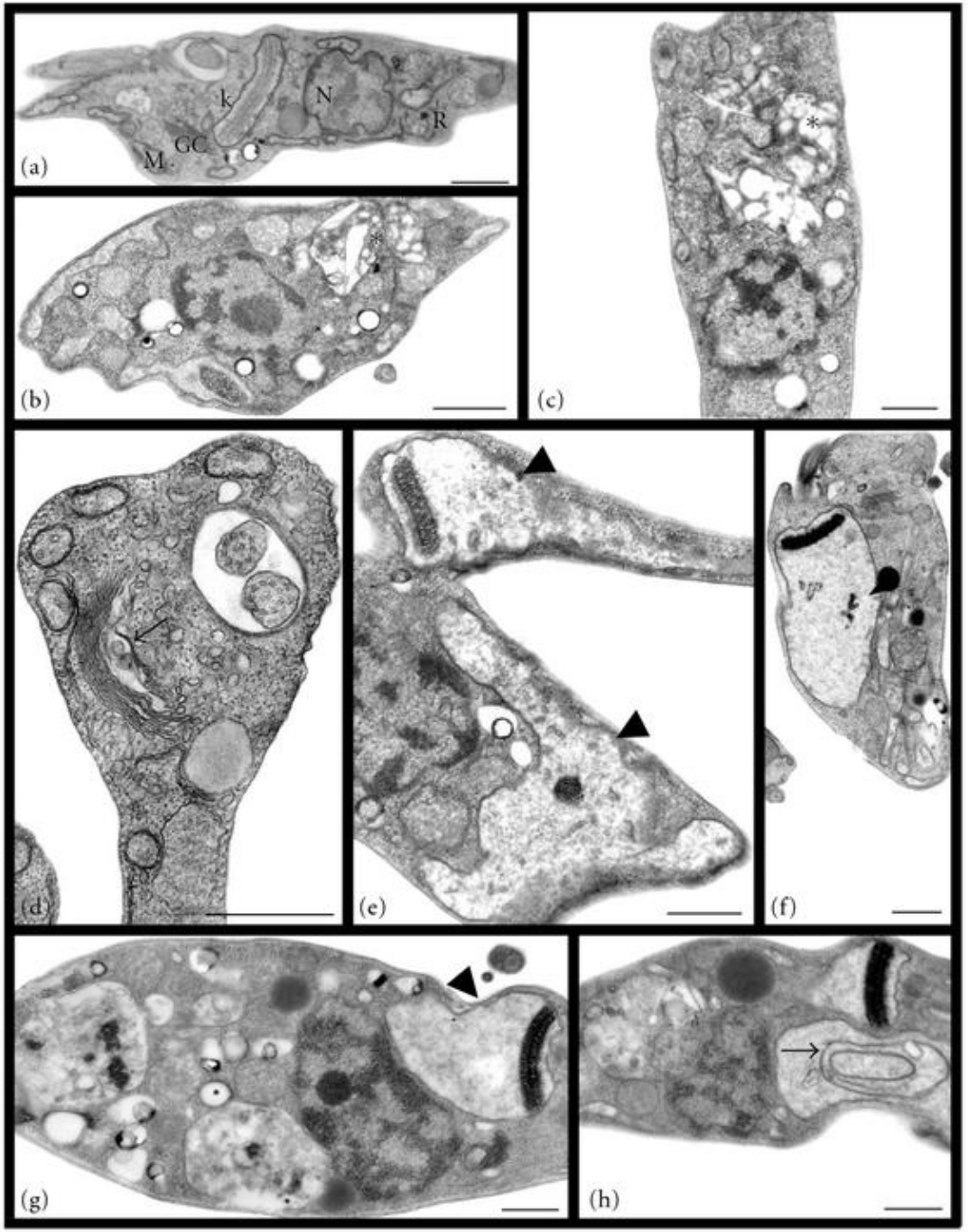
In trypomastigotes, Brazilian green propolis ethanolic extract led to the loss of plasma membrane integrity.

The in vitro studies indicate that Brazilian green propolis ethanolic extract interferes in the functionality of the plasma membrane in trypomastigotes and of reservosomes and mitochondrion in epimastigotes.

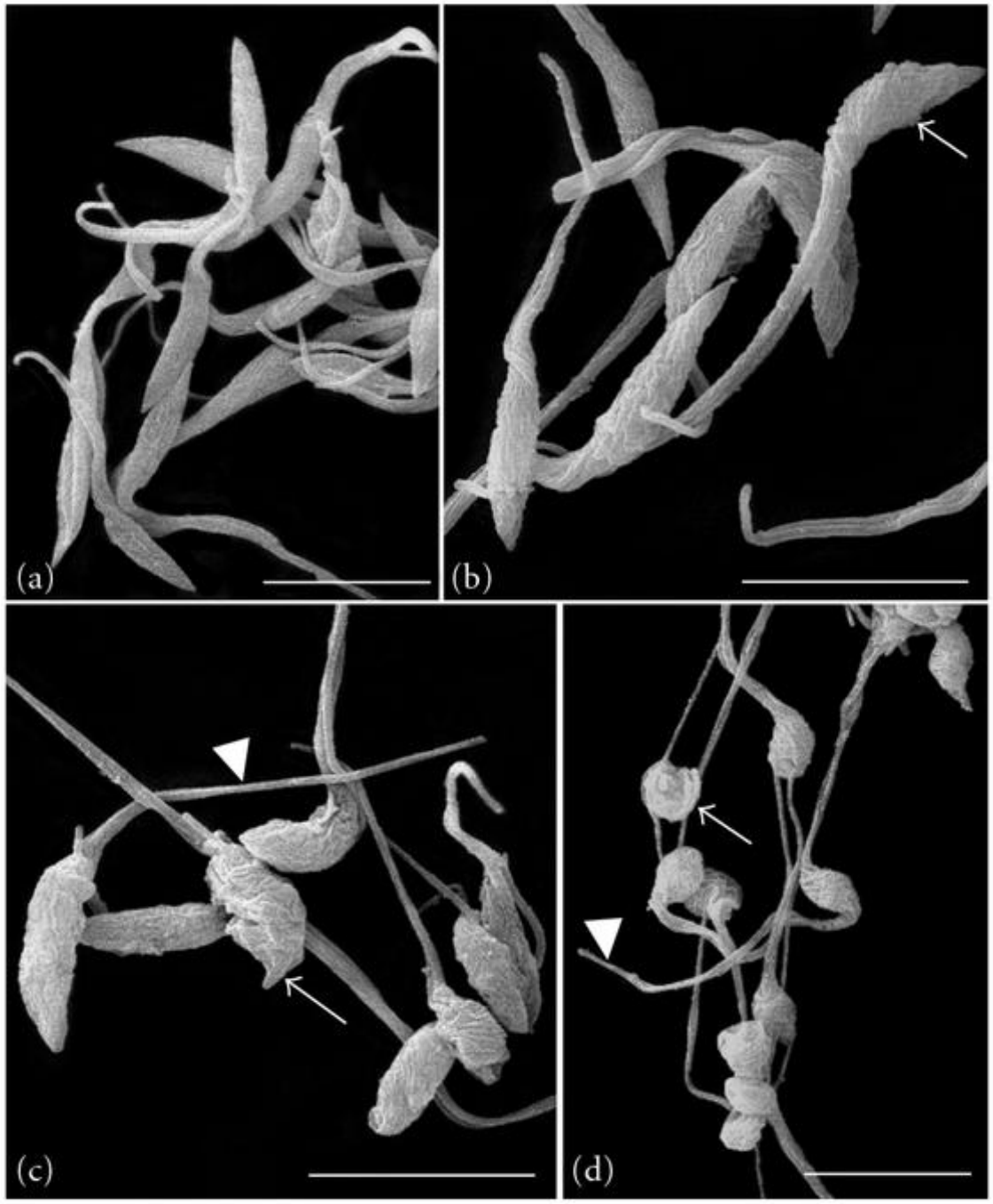
Anti trypanosoma activities of propolis

- **Acutely infected mice were treated orally with Brazilian green propolis ethanolic extract and the parasitemia, mortality and GPT, GOT and urea levels were monitored.**
- **The extract (25–300 mg kg^{-1} body weight/day for 10 days) reduced the parasitemia, increased the survival of the animals and did not induce any hepatic, muscular lesion or renal toxicity.**

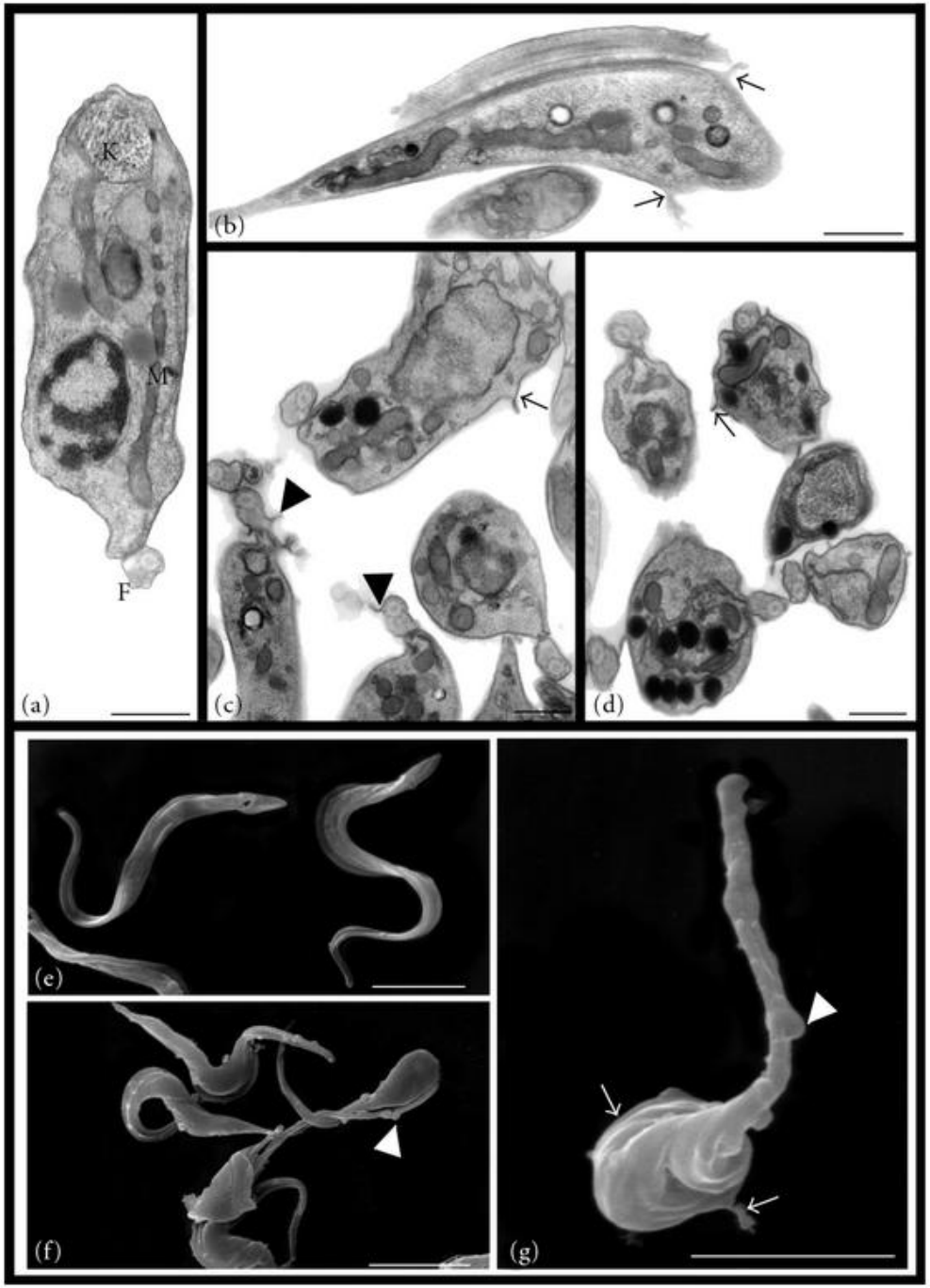
Transmission electron microscopy of T. cruzi epimastigotes treated with Et-Bra for 24 h. (a) Control parasite showing the typical elongated body and normal morphology of mitochondrion (M), Golgi complex (GC), nucleus (N) reservosomes (R), and kinetoplast (k); (b) 50 µg mL−1 and (c) 100 µg mL−1 induced alterations in the morphology of reservosomes (asterisks), with increase of the organelle volume and the number of lipid inclusions and decrease of proteic matrix and vacuolization; (d) 100 µg mL−1 induced dilatation of the Golgi complex cisternae (arrow); (e) 100 µg mL−1, (f) 250 µg mL−1 and (g, h) 300 µg mL−1 caused swelling of the mitochondrion with scarcity of the matrix and mitochondrial cristae (arrowheads), with formation of membrane structures inside the organelle (arrow). Bars: 0.5 µm.Anti trypanosoma



Scanning electron microscopy of T. cruzi epimastigotes treated with Et-Bra for 24 h: (a) control parasite with normal morphology; (b) 100 µg mL−1, (c) 250 µg mL−1 and (d) 400 µg mL−1 led to gradual alteration on the morphology, from body shortening to rounded parasites (arrow) with preservation of the flagellum (arrowheads). Bars: 4 µm.



Transmission electron microscopy (a–d) and scanning electron microscopy (e–g) of T. cruzi trypomastigotes treated with Et-Bra for 24 h: (a) control parasite with the normal morphology of mitochondrion (M), kinetoplast (k) and flagellum (f); (b) 30 µg mL −1 and (c, d) 60 µg mL−1 induced the formation of blebs on the body (arrow) and flagellar (arrowheads) membranes. Bars: 0.5 µm. (e) control parasite with the normal morphology; (f, g) 60 µg mL−1 leading formation of blebs on the body (arrow) and flagellar (arrowheads) membranes and some rounded parasites (small arrow). Bars: 4 µm.



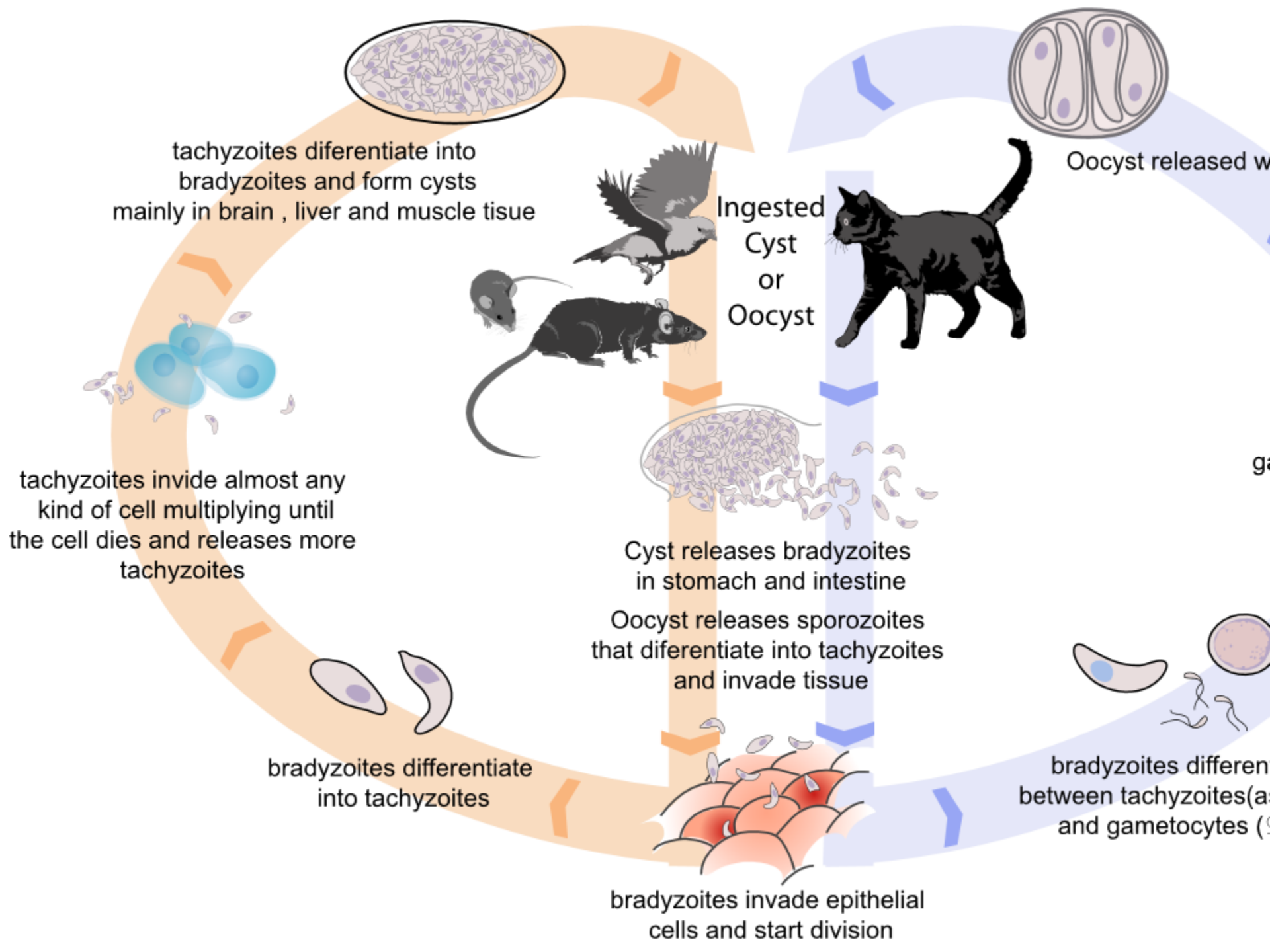
T. gondii is capable of infecting virtually all worm blooded animals, but felids such as domestic cats are the only known definitive hosts in which the parasite can undergo sexual reproduction

In humans, *T. gondii* is one of the most common parasites in developed countries

previous estimates have shown the highest prevalence of persons infected to be in France, at 84%.

Although mild, flu-like symptoms occasionally occur during the first few weeks following exposure, infection with *T. gondii* produces no readily observable symptoms in healthy human adults

This asymptomatic state of infection is referred to as a latent infection and has recently been associated with numerous subtle adverse or pathological behavioral alterations in humans.


tachyzoites diferentiate into
bradyzoites and form cysts
mainly in brain , liver and muscle tisue
Ingested
Cyst
or
Oocyst
Oocyst released w
tachyzoites invide almost any
kind of cell multiplying until
the cell dies and releases more
tachyzoites
Cyst releases bradyzoites
in stomach and intestine
Oocyst releases sporozoites
that diferentiate into tachyzoites
and invade tissue
bradyzoites differentiate
into tachyzoites
bradyzoites invade epithelial
cells and start division

Anti toxoplasma

- **Antiprotozoal activity is evaluated by an in vitro growth inhibitory effect on a culture of parasites after incubation in the presence of different concentrations of propolis**

Concluded remarks

- Propolis proved significant anti bacterial, anti viral, antifungal, antitumer and antioxidant activities
- Its anti protozoal properties are currently investigated against serious human diseases
- In near future, we expect that propolis will be formulated as noval therapeutic drug to control protozoal infections


Thank you for attention