



## Botanicals for Treatment of Babesiosis and Theileriosis

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### Abstract

Problems of toxicity, resistance and residues of existing drugs necessitate development of newer drugs against parasitic diseases. Babesiosis and theileriosis are the economically most important haemoprotozoan diseases of domestic animals. Drugs of herbal origin are considered safe for use in human and animal medicine as they have less toxicity and are easily metabolized compared to synthetic chemicals. Potential plant extracts and phytochemicals that have antibabesial and antitheilerial activity are discussed in the present communication.

### Introduction

Ticks and Tick Borne Diseases (TTBDs) cause huge economic loss for the livestock sector. The estimated global cost associated with ticks and tick-transmitted pathogens in cattle was between US\$ 13.9 billion and US\$ 18.7 billion annually [1]. It was documented that approximately 105 *Rhipicephalus microplus* in cattle can cause a milk reduction of 23 percentage per day [2]. Moreover, nearly 40 ticks per day can lead to a loss of weight equivalent to 20 kg/year [3]. There are 904 tick species recognized in the world [4] with 198 tick species in the family Argasidae (soft ticks), 705 species in the family Ixodidae (hard ticks) and one species in the family Nuttalliellidae [5]. There are 106 tick species documented from India [5].

The annual control cost for TTBDs in livestock was US\$ 498.7 million (*Theileria annulata* - 384.3 million US\$, Babesiosis - 57.2 million US\$ and tick worry - 57.2 million US\$) [6] in India. Tick bite alone diminishes 20-30 percentage value of hide or skin [7] in the country. Babesiosis and theileriosis are the major tick borne haemoprotozoan diseases in domestic animals in the country.

In the present review, the problems of available drugs and the need for the newer drugs against the common tick-borne haemoparasitic diseases are discussed. Plant extracts or the natural products derived from medicinal plants are alternative strategies that are recommended globally [8]. Hence, various phytotherapeutic lead compounds with antibabesial and antitheilerial activities are presented here in detail.

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Received Date: 26 Jun 2017

Accepted Date: 20 Oct 2017

Published Date: 15 Nov 2017

#### Citation:

Ravindran R, Ghosh S. Botanicals for Treatment of Babesiosis and Theileriosis. *Ann Pharmacol Pharm.* 2017; 2(20): 1105.

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### Babesiosis

Babesiosis is an important tick transmitted disease caused by the intracellular apicomplexan haemoparasite *Babesia* sp. in bovines, equines and canines that results in heavy economic loss throughout the world. One major problem with babesiosis is that the animals that recover from the disease become chronic carriers which act as source of infection to other animals and ticks. *Babesia bigemina* is the predominant species of the bovines in India. However, few reports exist on the occurrence of *B. bovis* [9-11] from the country. In *B. bigemina* infections, the pathogenesis is almost entirely related to the rapid and sometimes massive intravascular haemolysis resulting in anaemia and haemoglobinuria. In *B. bovis* infections, the pathogenesis develop due to the overproduction of cytokines and pharmacologically active substances resulting in cerebral babesiosis, respiratory distress syndrome and progressive haemolytic anaemia.

In canines, two morphologically distinct forms of erythrocytic piroplasms viz., *B. canis vogeli* and *B. gibsoni* are often observed [12]. *B. canis vogeli* infection is often characterized by fever, anaemia, jaundice, inappetance, marked thirst, weakness, prostration and often death. *B. gibsoni* causes marked anaemia, remittent fever, haemoglobinuria, constipation, splenomegaly and hepatomegaly.

*B. equi* (currently *Theileria equi*) and *B. caballi* are babesial parasites of equines which cause severe disease. Equine babesiosis is often characterized by clinical symptoms like hemolytic anaemia, icterus, fever, oedema, loss of weight and poor performance leading to enormous economic loss in equine husbandry. *B. ovis* and *B. motasi* are babesial parasites of small ruminants.

## Theileriosis

*Theileria annulata*, transmitted by *Hyalomma anatolicum anatolicum* is the major theilerial parasite of cattle and domestic buffaloes, which causes bovine tropical theileriosis in India. Even though, *T. orientalis* was considered to be a benign parasite previously, recent reports on outbreaks of oriental theileriosis indicate that the organism is also pathogenic one [13-16].

*T. lestoquardi* (*T. hirci*) transmitted by *Hyalomma anatolicum anatolicum* which causes acute and malignant theileriosis of sheep [17,18]. *Theileria ovis* is considered as a benign parasite.

Recent reviews on the bovine babesiosis [19], Canine babesiosis [20,21] and equine babesiosis [22] and malignant ovine theileriosis [23] provide excellent descriptions of these topics.

## Need for newer drugs

**Antibabesial drugs:** Most of the babesicidal drugs available today in the market have problems due to toxicity and development of resistant parasites [24]. Currently, diminazene aceturate and imidocarb dipropionate (imidocarb) are the most widely used drugs for treatment of babesiosis. Chemotherapy is generally effective against bovine babesiosis. Diminazene at the dose rate of 3.5 mg / kg (I/M) and imidocarb at the dose of 1.2 mg / kg (S/C) are well tolerated and can protect cattle from *B. bovis* and *B. bigemina* infections. However, in canines, diminazene aceturate can cause toxicity to kidney, brain and liver thereby leading to side effects such as weakness, irritability, paralysis, non-responsiveness to stimuli and fatal central nervous system haemorrhage [26-28]. Due to these side effects, diminazene aceturate is not approved by Food and Drug Administration (FDA) in U. S. A [29]. In many countries like Japan, the production of diminazene itself is stopped [30]. Imidocarb dipropionate is also not widely used in dogs. This is because of the high cost and systemic side effects of the drug such as acute hepatic and renal failures especially in debilitated animals [31]. Administration of imidocarb dipropionate is associated with pain at the injection site and cholinergic signs, mainly salivation, vomiting, and diarrhea. Diminazene (@11mg/kg I/M on each of two consecutive days) and imidocarb (two doses @ 2.2mg/kg I/M repeated 24 hour interval) are used for treatment of *B. caballi* infections. *B. equi* is relatively resistant to therapy and repeated treatments are needed for control of the disease. Diminazene is not at all effective against *B. equi*, while even higher doses of imidocarb (@ 4mg/kg 72 hours apart, four treatments) are not successful in eliminating *B. equi* from infected horses [32].

**Antitheilerial drugs:** Currently, the treatment of theileriosis is performed mainly with the use of synthetic chemical drugs, parvoquone and buparvaquone. The recovery rate of animals treated with parvaquone was 60.7 per cent while buparvaquone showed 88.7 per cent [33] recovery rate. Chemotherapy with buparvaquone is more effective in mild (early) stage of the disease compared to moderate and severe forms (late) of the disease [34]. These drugs infiltrate in the muscles of cattle and are not easily eliminated from body of animals [34]. They are highly expensive drugs too. So there is a need for alternative therapeutic strategies or drug formulations for the treatment and control of theileriosis.

## Herbal extracts for babesiosis

The efficacy of treatment of natural [35] and experimental [36] haemosporidan infections of cattle with total alkaloid of *Peganum harmala* was reported in literature. Out of 82 cattle naturally infected with haemosporidians, the cure rate was above 85 per cent.

In experimental infections, treatment was effective in animals with infections due to either *B. bigemina* or *T. sergenti* alone. However, lesser effects were observed in animals with mixed infections.

*Babeisa gibsoni* culture system was utilized by many researchers to identify the antibabesial activities of many extracts. [30] studied the inhibitory effect of 45 plant extracts selected from Central Kalimantan, Indonesia. Antibabesial activity was observed with *Arcangelisia flava*, *Curcuma zedoaria*, *Garcinia benthamiana*, *Lansium domesticum* and *Peronema canescens*. In another study, it was observed that aqueous extracts of *Achillea millefolium*, *Baeckea frutescens*, *Brucea javanica*, *C. xanthorrhiza*, *Strychnos lucida* and *Swietenia macrophylla* were significantly anti-babesial [37,38] identified the fruit extract of *B. javanica* as most potent anti-babesial extract against *B. gibsoni* after screening the boiled extracts from twenty eight Indonesian medicinal plants. Moreover, [39] isolated the protoberberine alkaloids (palmatine, berberine, jatrorrhizine, dihydroberberine) from the boiled extract of *A. flava* and detected the growth inhibiting effects against *B. gibsoni* in culture, at concentration from 100 to 1 µg/mL which was similar in activity as that of diminazene [40], using the *B. caballi* culture assay, identified the acetone extract of *Elephantorrhiza elephantina* rhizome as the effective extract.

## Antibabesial phytochemicals

Many phytochemicals isolated from plants were tested for their antibabesial activity, using *Babeisa gibsoni* culture system. The compound, 1-O-galloyl-6-O-luteoyl- $\alpha$ -D glucose was isolated [41] from the boiled extract of *Phyllanthus niruri*. The anti-babesial activity of the compound (IC<sub>50</sub> value= 4.7±0.8 µg/mL) was detected in comparison to diminazene (IC<sub>50</sub>= 0.6±0.04 µg/mL). In another study, [42] identified the antibabesial activities of four compounds viz, 3'-demethoxy cyclocurcumin from *Curcuma xanthorrhiza*, *p*-hydroxybenzaldehyde and cleomiscosin A from *B. javanica* and (+) - epiloliolide from *Excoecaria cochichinensis* in comparison with diminazene. The IC<sub>50</sub> values of 3'-demethoxycyclocurcumin, *p*-hydroxybenzaldehyde, cleomiscosin A and (+) - epiloliolide were 16.6, 7.6, 15.6 and 10 µg/mL respectively in comparison to diminazene aceturate (IC<sub>50</sub> = 0.6 µg/mL). The quassinoids from the plant, *B. javanica*, brucein A (IC<sub>50</sub>=7.7nM) and bruceantinol (IC<sub>50</sub>=19.8nM) were identified [37] as more potent anti-babesial compounds compared to diminazene aceturate (IC<sub>50</sub>= 172.6 nM). Bruceine A at a concentration of 25nM, killed the babesial parasites within 24 hours [43]. Oral administration of bruceine A at 6.4 mg/kg /day for 6 days could effectively treat two parasite infected dogs without any serious side effects [44]. Described the inhibitory effect of nerolidol, a sesquiterpene present in the essential oils of many plants on the growth of *Babesia* parasites. *In vitro* inhibition assay for nerolidol using parasites grown in Micro-Aerophilous Stationary Phase (MASP) cultures identified the IC<sub>50</sub> values as 21±1, 29.6±3, 26.9±2 and 23.1±1µM against *B. bovis*, *B. bigemina*, *B. ovata* and *B. caballi* respectively. It is speculated that nerolidol interferes with isoprenoid biosynthetic pathway in *B. bovis* apicoplast. However, the *in vivo* effect of the drug against *B. microti* in infected mice was not promising even though toxic effects were not observed on host. Hence, the drug could not be used for the treatment of babesiosis. The *in vitro* growth inhibitory effect of (-) - Epigallocatechin-3-gallate, the major tea catechin, on bovine *Babesia* parasites was assessed [45]. The IC<sub>50</sub> values of 18 and 25 µM for *B. bovis* and *B. bigemina* were observed based on *in vitro* experiments. The compound also revealed inhibition of growth of *B. microti in vivo* in infected mice at dose of 5 and 10 mg/kg body weight. The parasites were cleared by day 14 and

16 post-inoculation. Similarly, the *in vitro* growth inhibitory assays of the same compound on equine *Babesia* [46] demonstrated IC<sub>50</sub> values as 4.42 and 3.64 µM against *B. caballi* and *B. equi* respectively. The drug at 25, 50, and 100 µM concentrations did not allow re-growth of parasites for bovine *Babesia*, *B. caballi* and *B. equi* respectively. Thus the compound, (-) - Epigallocatechin-3-gallate can be considered as a potential candidate for chemotherapy of *Babesia* infections.

### Herbal extracts for theileriosis

Recently, [47] reviewed the topic anti-theilerial herbal medicines extensively.

The efficacy of treatment of natural [35] and experimental [36] haemosporidan infections of cattle with total alkaloid of *Peganum harmala* was reported in literature. Out of 82 cattle naturally infected with haemosporidians, the cure rate was above 85 per cent. In experimental infections, treatment was effective in animals with infections due to *B. bigemina* or *T. sergenti* alone. However, lesser effects were observed in animals with mixed infections. [48] achieved 100 per cent therapeutic efficacy with the *P. harmala* extract in five sheep that were experimentally infected with *T. lestoquardi*. However, [49] observed that after 12-20 days of administration of chloroform extract of the same plant (I/M @ 5mg/kg body mass once daily for 5 days) in 100 sheep that were suffering from natural malignant theileriosis, 65 recovered from the disease, while 35 showed severe form of the disease and died. Later, [50] also got similar results in a clinical trial using the extract of the plant containing alkaloids (I/M @ 5mg/kg body mass once daily for 5 days) in 50 cattle naturally infected with *T. annulata*. The recovery was observed in 39 animals (78 per cent) while the rest died. The pathological and parasitological features in experimental ovine malignant theileriosis after treatment with the plant extract was studied [51]. They observed that the clinical signs and parasites in the lymphnode smears of treated and recovered animals disappeared while characteristic lesions of ovine malignant theileriosis were observed in untreated animals.

In a Thailand study [52], compared the inhibitory effect of five crude extracts using the short term *in vitro* culture of benign *Theileria* Thai isolate among which, *Vernonia cineria* revealed only slight inhibitory effect on development of parasites (IC<sub>50</sub> at 888.61 µg / mL). [53] conducted a clinical trial using 80 experimentally induced theileriosis (*T. annulata*) cases in cross-bred cattle in Pakistan by administering the homogenized buds and flowers of *Calotropis procera* (8 doses of 0.3 mg/kg orally on alternate days) and compared its efficacy with buparvaquone (2.5 mg/kg on alternative days). Chemotherapy with buparvaquone cured only 7/10 and 3/10 while *C. procera* cured 9/10 and 8/10 of moderate and severe cases of experimental theileriosis respectively. There were no signs of toxicity to the animals which were administered the herbal formulation except diarrhea which recovered spontaneously.

The aqueous extracts of *Gardenia ternifolia* (fruits), *Tinospora bakis* (roots) and *Sonchus cornutus* (whole plant) were evaluated by [54-56] for *in vitro* antitheilerial activity against *T. lestoquardi* using lymphocyte cell culture system. *G. ternifolia* and *T. bakis* produced 60 and 30 per cent death of macroschizont infected cells at 10,000 ppm respectively while no activity was found with *S. cornutus*.

### Conclusion

In the backdrop of toxicity and side effects of the presently available chemotherapeutic agents such as diminazene, imidocarb

and buparvaquone, there is a need for development of new less toxic, less costly drugs with minimum side effects and lesser residual effects. *In vitro* culture system is an essential requirement for drug development against haemoprotozoan organisms. *In vitro* culture systems for *B. gibsoni* and *T. lestoquardi* were mainly used by most of the researchers. The natural products like, total alkaloids (from *Peganum harmala*), 1-O-galloyl-6-O-luteoyl- $\alpha$ -D glucose (from *Phyllanthus niruri*), protoberberine alkaloids from *Arcangelisia flava*, brucein A and bruceantinol (quassinoids from *Brucea javanica*), (-) - Epigallocatechin-3-gallate (the major tea catechin) were identified as anti-babesial compounds.

### Acknowledgements

This research was supported by grants from Kerala State Council for Science, Technology and Environment (022/YIPB/KBC/2013/CSTE dt 11-11-2013 and 010-14/SARD/13/CSTE dt 27-11-2013), Indian Council of Agricultural Research (NAIP/Comp-4/C2066/2007-08 and NFBFARA/BSA-4004/2013-14) and Department of Animal Husbandry, Kerala (B2.8401/08/Plg. Dt 19-8-2008).

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