Acaricidal activity of Eucalyptus essential oil based nanoformulations against ticks in Goats

A.Sheeba[1], N.Rani[2], G.Ponnudurai[3], A.K.Thiruvenkadan[4] B.Puvarajan[5], P.Senthil Kumar[6], K.Dhandapani[7],K.Gopal [8]
Veterinary College and Research Institute, Namakkal
Tamil Nadu Veterinary and Animal Sciences University

Abstract

Tick infestation poses a major threat to the lifestyle of livestock. The main tick species found in domestic animals of India is Rhipicephalus [Boophilus] microplus. Several plants have the ability to inhibit the growth of the ticks in domestic animals. The present study aimed to evaluate the acaricidal activity of Acaricidal activity of Eucalyptus essential oil based nanoformulations against Ticks. Eucalyptus oil nanoemulsion was formulated in 1:1 ratio. The formulated eucalyptus oilnanoemulsion was characterized by transmission electron microscopy and dynamic light scattering, Fourier Transformed Infrared Analysis . The nanoemulsion droplets were found to have a Z-average diameter of 55.67nm and were spherical in shape. Efficacy of eucalyptus based nanoemulsion against ticks were tested based on *In vitro* and *In Vivo* test. The histopathology of tick -treated and untreated nanoemulsion was analyzed.

Keywords: Eucalyptus oil, Ticks , histopathology, Acaricidal activity,nanoemulsion,

Introduction

Ectoparasites are a major threat for the livestock with about 80% of world's cattle population infested with ticks (FAO, 2004). India accounts for a significant share of the world's livestock resources and the damage caused by ticks and tick-borne diseases (TTBDs) to livestock is considered very high. Tick control methods primarily include the use of chemical acaricides, which is the most common approach for tick management. Chemical acaricides have detrimental consequences, including the selection of resistant tick populations and adverse effects on animals, living beings, and the environment. Because of the numerous major drawbacks of chemical acaricides, the use of herbal based preparations attracted many researchers for controlling this ectoparasite due to their biodegradability, target efficiency and cost effectiveness. Essential Oils (EOs) are the plant secretion derived from various parts of the plant such as leaf, flower, bud, stem, etc. EOs are the volatile compounds, having strong aroma and are the by-products of the plant metabolism and have been used for many biological properties such as antibacterial, fungicidal, insecticidal etc. They have also been tested against ticks recently.Now a day's nanotechnology emerged as a booming field with wider application in various fields that has attracted various researchers due to unconventional physical, chemical, and biological characterstics of nanopreparations.Nanoemulsions are emulsions whose droplet size is uniform and extremely small with the size ranging from 20 to 200 nm. Nanoemulsions are metastable. It is evident from literature survey that only essential oils have been tested in recent past for their acaricidal activity however, there are only few reports exists till date on the enhanced activity of these oils through nanoformulations. Therefore, the present study would focus on the synthesis and characterization of essential oil based nanoformulations and their acaricidal activity against ticks

Materials and Methods

Plant Material

Fresh leaves of Eucalyptus(*Eucalyptus globulus*) were used in this study. Leaves were collected from Pudukkottai district.

Exraction of essential oil

In this study, the essential oil was extracted by hydrodistillation method as described by Raduz *et al*. (2002) with some modification. Approximately 100gm fresh leaves were cut into small pieces and ground in mixer. These ground material was placed in 1000 ml boiling flask and then 500ml of water was added to this. The ground leaves containing flask was placed on heating mantle and then carefully fixed with Clevenger appararatus**.** The Clevenger apparatus was then connected with tap water to ensure continuous circulation of water during extraction of oil. The contents were boiled at 70^{0} C for 2 hours. The essential oil that got separated in the graduated column was than collected in the glass vial and stored at 4^{0} C until use

Preparation of nanoemulsion

The oil-in-water nanoemulsion was formulated using eucalyptus oil, non-ionic surfactant (Tween 80) and water. Initially, coarse emulsion was prepared at the ratio of 1:1 by adding Tween 80 (2%) in distilled water and placed in magnetic stirrer for 30 minutes. *Eucalyptus* essential oil was added drop by drop in aqueous phase, after reaction it was subjected to ultrasonic emulsification using 20kHz with power output of 750W. The diameter of the droplet from coarse emulsion to nanoemulsion was converted by using sonication.

Characterisation of nanoemulsion

The synthesised nanoemulsion was characterised by Transmission electron microscope (TEM), Polydispersity index (PDI) and Fourier Transformed Infrared Analysis (FTIR

Transmission Electron Microscope(TEM)

The synthesised eucalyptus nanoemulsion was submitted to Centre for Agricultural Nanotechnology, Tamil Nadu Agricultural University, Coimbatore to determine the surface morphology using transmission electron microscope.

Dynamic Light Scattering

The synthesised eucalyptus nanoemulsion was submitted to Centre for Advanced Research in Indian system of Medicine (CARISM), SASTRA, Deemed University, Thanjavur to determine the polydispersity index, zeta potential. Polydispersity of nanoparticles in suspension was performed by dynamic light scattering using the Zetasizer. The zeta potential was obtained using the technique of electrophoretic mobility Zetasizer®, Malvern Nano-ZS instrument. The samples were pre-diluted at 500 times (v/v) in 10 mM sodium chloride and filtered through a membrane with 0.45 micrometers. The results were expressed in millivolts (mV).

Fourier Transformed Infrared Analysis(FTIR)

The synthesised eucalyptus nanoemulsion was submitted to Centre for Agricultural Nanotechnology,Tamil Nadu Agricultural University, Coimbatore for identification of functional groups using Fourier transformed Infrared Analysis (FTIR). The nanoemulsion was freeze dried to obtain powder. Finally, the nano particles were analysed by FTIR Nicolet Avatar 660 (Nicolet, USA)

Efficacy of eucalyptus based nanoemulsion against ticks

***In vitro* bioassays**

Engorged ticks were collected from naturally infested animals of both large and small ruminants. Collected engorged ticks were weighed individually and transferred to individual glass vial. In this assay, the efficacy of the different concentrations of eucalyptus nanoemulsion *viz*., 1,2 and 3per cent were tested in 3 replicates each, besides water and Tween 80 (surfactant) control were maintained. Engorged ticks were immersed in test solution for one minute, after that they were transferred to individual glass vial, then covered with gauze cloth and kept in tray containing moist sand. The ticks were monitored regularly to record preoviposition and oviposition period in treatment and control groups. Egg mass weight and hatchability were also recorded

***In vivo* trial**

A trial was carried out on goats naturally infested with ticks in puthurasapatti village, Pudukkottai district to assess the acaricidal activity of eucalyptus nanoemulsion against ruminant ticks. Six animals divided into three groups(G1,G2&G3) with two animals each. Pre treatment tick intensity was recorded and the animals belonged to G1 &G2 were sprayed with 2 and 3 per cent nanoemulsion using hand sprayer respectively, while G3 served as control group. Post treatment tick intensity was recorded on 3,7&14 day post treatment

Pathological study

The gross and histopathological changes in ticks from treatment and control groups were studied as per standard procedure.

Results and discussion

Yield of essential oil

In the present study, 100gm of eucalyptus leaves yielded 1.5 ml to 2ml of essential oil. This finding is in agreement with the observation of Rani (2013) who recorded the yield of 1.5 ml to 2ml of essential oil from 100gm of eucalyptus leaves.

Synthesis of nanoparticle

Eucalyptus oil, non-ionic surfactant (Tween 80) and water were used for preparation of nanoemulsion. The emulsion showed no phase separation at 1:1 ratio, indicating that the nanoemulsion is found to be stable. This finding is in agreement with Anuratha and Shabad (2022) who observed the stability of *Cinnamomum camphora* (*F. Lauraceae*) essential oil nanoemulsion at a ratio of 1:1.

Characterisation of nano emulsion

Transmission electron microscope

The data obtained in TEM showed distinct shape and size of nanoparticles. The nanoparticles were spherical with average diameter of 55.67 nm without agglomerisation. The TEM analysis confirmed the presence of eucalyptus nanoemulsion with size and shape (Plate 4.3 to 4.4). This finding of the present study is not in accordance with Galli *et al.(*2018). The difference in size of the nanoemulsion may be due to difference in the species of the plants, age of the leaves and season as opined by Brooker and Kleinig (2006) (Fig1).

Dynamic light scattering

The polydispersity index (PDI) of the nanoemulsions was 0.297 which shows that it is uniform with Zeta potential of nano emulsion at -22mV, which provides stability for the synthesised nanoemulsion. This finding of the present study is in accordance with Galli *et al*.(2018) who reported that polydispersity of nanoemulsion eucalyputus essential oil was 0.20 with zeta potential of -21 mV.

FTIR

The FTIR spectrum of the Eucalyptus nanoemulsion was recorded at 4000–400 $cm.^{-1}$ Range is presented in (Fig 1)**.** Peak at 3341 cm−1 reveals N–H strength of amine. The peak at 2971 cm−1 indicates the –C–H strength of alky group. The peak at 2216 indicates the nitrite group. The peak at 1636 is attributed to –C–C strength of alkene group. The peak at 1457 is attributed to mehyl group. The peak at 549 is attributed to halogen group. These findings are in accordance with Anuradha *et al.* (2023) Fig(2).

Efficacy of eucalyptus based nanoemulsion against ticks

In vitro Assay(Table-1)

A mean engorgement weight(gm) of ticks allotted to different treatment group was 0.142 ± 0.030(1%), 0.09 ± 0.003(2%),0.101±0.004(3%),0.096 ± 0.004 (water control) and 098 ±0.005 (Tween 80 control). The preoviposition period observed (in days) as 9.714 ± 0.837(1%),10.375 ± 0.323(2%),11.70±0.558(3%),6.833±0.282 (water control) and 6.555 ± 0.771 (Tween 80 control). The results of the present study concur with Galli *et al*. (2022).

In this study, preoviposition period was found to be higher in 3 per cent followed by 2 per cent and 1 per cent. These findings are in consonance with Galli *et al*. (2022) who reported that preoviposition period had extended in treatment group than control group.

In the present study, there was a low egg mass weight (gm) 0.040±0.014 in 3 per cent followed by 0.043± 0.008 and 0.054±.0.110 in 2 and 1 per cent respectively, while 0.073±0.013 & 0.079±0.093 in water and Tween 80 control group respectively. This finding concurs with the findings of Spirali and Silwa (2011).

In the present study, percentage of hatchability had increased in control group than treatment group. A high reduction in hatchability was observed in (3%) followed by (2%) and (1%). This finding also akins to the findings of Galli *et al*.(2018) who reported that ticks from treated animals laid eggs with lower fertility

A maximum reduction in tick intensity of 77.01 per cent was observed in 3 per cent followed by 66.04 per cent in 2 per cent, 13.12 per cent in 1 per cent. These finding are in concurrence with Galli *et al.*(2018) who reported 62 per cent mortality of ticks in treated groups with cinnamon nanoemulsion of 2 per cent. This slight variation in mortality pattern between the present study and earlier workers might be due to the method adopted for synthesis of nanoparticles and concentration of synthesised nanoparticles.

In vivo trial

The result of the present study (Table 2), revealed a highest mortality in (3%) followed by (2%). The results showed variation with results of Daiane *et al.(*2017) who reported that cinnamon oil showed 90.5 per cent efficacy against *Rhipicephalus microplus* ticks in cattle. The variation in efficacy may due to variation in composition of nanoemulsion particle of eucalyptus and cinnamon oil.

Pathological study

Gross pathological changes in ticks

Gross pathological changes of shrunken and shrivelled with blackened scutum was observed in dead tick from 2 and 3 per cent treatment groups in *in vitro* assay. These morphological changes might be due to penetration of drug into deep lying tissue

Histopathological changes in ticks (Fig 3)

Histopathological study revealed intact cuticle with mild degenerative changes in the internal organs of ticks from 1 per cent group as against damaged cuticle with degenerative changes in the internal organ in 2 per cent and damaged cuticle with size reduction, degenerative and necrotic changes in the internal organ of ticks in 3 per cent. In the control groups, intact cuticle, with absence of changes in the internal organss were recorded. This finding is in concurrence with findings of Remedio *et al.* (2014) who observed the use of neem extract had damaged the cuticle and necrosis of the internal organs of the ticks **.**

Conclusion

Eucalyptus oil being a plant-based component is eco friendly and has natural pesticide activity. The nanoemulsion formulation of eucalyptus oil showed 77 per cent acaricidal efficacy agent against ruminant ticks. Based on the study, it could be concluded that eucalyptus oil nanoemulsion can be used as a safe and effective alternative for control of ticks

Table 1

Parameters	Treatment group (Conc. of nanoemulsion)			Control group	
	1%	2%	3%	Water Control	Tween 80 Control
Preovipositon Weight (gm)	0.142±0.030	0.09±0.003	0.101± 0.004	0.096±0.004	0.098±0.005
Preoviposition Period (Days)	9.714±0.837	10.375± 0.323	11.70± 0.558	6.833±0.282	6.555±0.771
Egg mass(gm)	0.054±.0.110	0.043± 0.008	0.040±0.014	0.073±0.013	0.079±0.093
Hatchability of larvae(%)	38%	11%	6%	100%	100%
Moratality percentage (%)	13.12%	66.04%	77.01%	-	-

Table 2

***In vivo* trial**

Conc.of nanoemulsion	Pre treatment tick intensity	Post treatment reduction in tick intensity (Mean number±SE)		
		3rd day	7th day	14th day
2%	34.5±13.78	11 ± 0.35	12±0.30	12±0.30
3%	51±.13.435	16 ± 0.35	15±0.30	15±0.30

Fig1

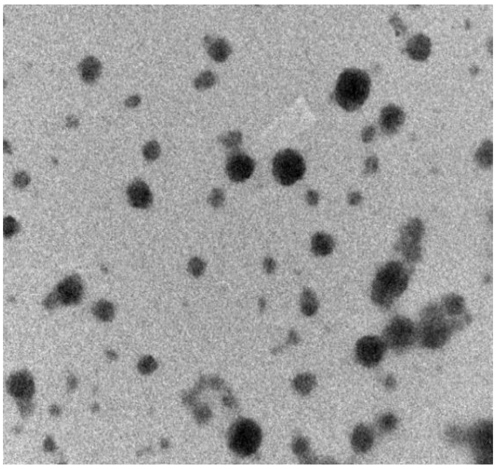
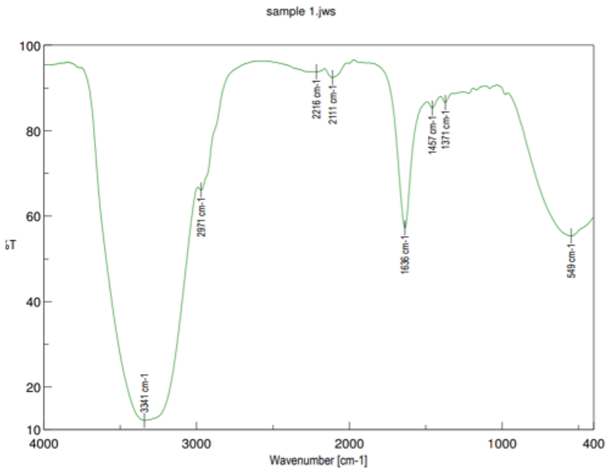


Fig2


sample 1.jws
100
80
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Wavenumber [cm-1]
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2971 cm-1
2216 cm-1
2111 cm-1
1636 cm-1
1457 cm-1
1371 cm-1
549 cm-1

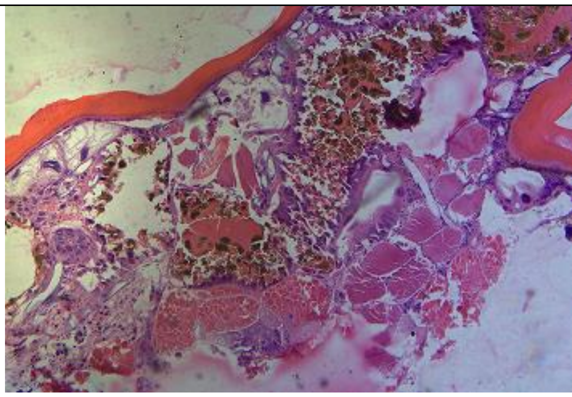
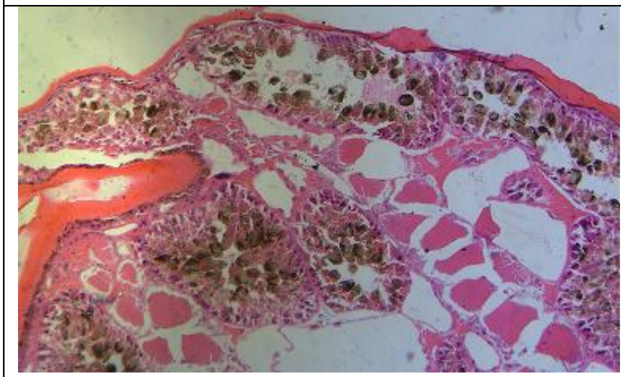
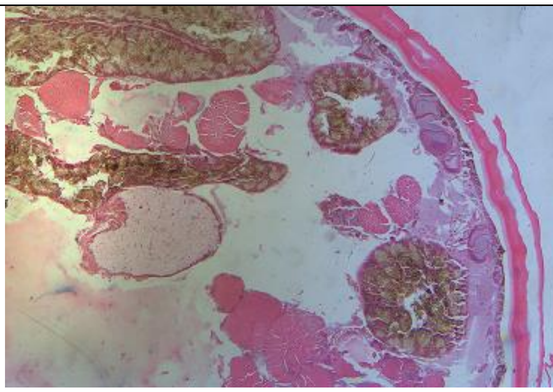
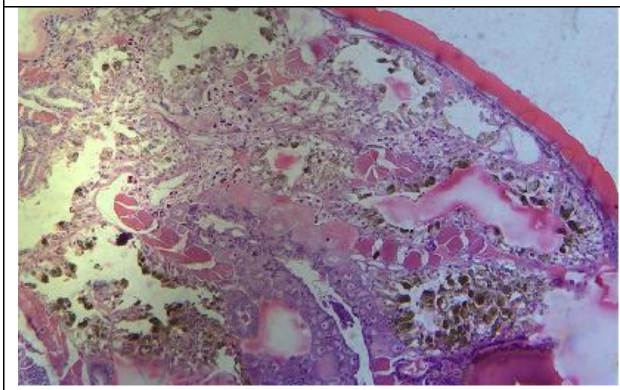
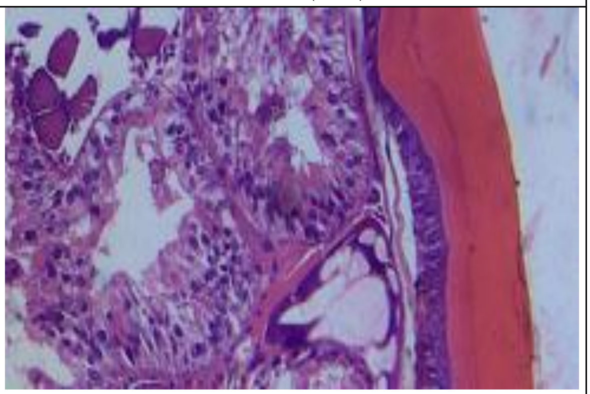
	
8.1 Gross pathogical changes	**8.2** Intact cuticle with mild degenerative changes in the internal organs **(1%)**
	
8.3 Damaged cuticle with degenerative changes in the internal organ **(2%)**	**8.4** Damaged cuticle with size reduction, degenerative and necrotic changes in the internal.**(3%)**
	
8.5 Intact cuticle **- Tween 80 control**	**8.6** Intact cuticle **- water control**

Fig 3

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