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Acaricidal activity of *Artemisia herba-alba* and *Melia azedarach* oil nanoemulsion against *Hyalomma dromedarii* and their toxicity on Swiss albino mice

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Abstract
Biopesticides such as essential oils (EOs) are considered an improvement for integrated pest control as they appear to be less toxic to the environment than chemical acaricides. The current study aimed to evaluate the acaricidal activity of *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion as alternatives for chemical acaricides against the camel tick *Hyalomma dromedarii*, besides evaluating their toxic effect on Swiss albino mice. Transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR) were used for the characterization of loaded nano-emulsions.The immersion test was used for the bioassay of both loaded nanoemulsions on tick stages (egg, nymph, larva, and adult). Mortality percentages and LC_{50} values of each tick stage were calculated. Reproductive performance for the survived engorged females after treatment was monitored. The toxicity of both loaded nano-emulsions was evaluated on Swiss albino mice by an oral dose of 1500 mg/kg/day for five successive days. The hematological, biochemical, and histopathological changes were evaluated. TEM characterization revealed spherical droplets for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion with droplet size ranging from 62 to 69 nm and 52–91 nm, respectively. FTIR revealed the absence of extra peaks in the loaded nano-emulsions that confirmed no chemical changes existed by ultrasonication. The LC_{50} values of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion on embryonated eggs, larvae, engorged nymphs, and unfed adults were 0.3 and 1.1%, 0.7 and 1.7%, 0.3 and 0.4%, 4.4 and 22.2%, respectively. The egg productive index (EPI), egg number, and hatchability percentage were lower in the treated females compared with Butox 5% (deltamethrin) and control. The hematological picture and biochemical analysis revealed insignificant changes in the treatment group compared with the negative control group. The liver of the *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion treated group exhibited vacuolar degeneration and infiltration of lymphocytic cells. The kidney of mice treated with *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion showed hemolysis and slight degeneration of epithelial cells of tubules. It is concluded that *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion have good acaricidal activity against camel tick *H. dromedarii*.

Keywords Tick · Camel · Nano-emulsion · Acaricide · Toxicity

Introduction

In developing countries, farmers face several diseases that affect the productivity of their animals, many of which are caused by tick infestations. Tick-borne diseases such as theilerioses, babesiosis, borreliosis, and rickettsiosis are the most common diseases of large and small ruminants in Asia, Africa, and Latin America (Jongejan and Uilenberg 2004; Elhelw et al. 2021). In addition to disease transmission, ticks can cause a reduction in weight gain, losses of meat and milk production in domestic animals, severe blood loss, anemia, and damage to hides at the site of tick bites (Rajput et al. 2006). *Hyalomma dromedarii* (Acari: Ixodidae), is the predominant tick species affecting dromedary camels. This tick may have a three-host or a two-host life cycle; however, the two-host cycle is the most dominant. It was found during the year and increased in the period from March to September (Elghali and Hassan 2009).

Chemical acaricides are the most widely used intervention method for controlling ectoparasites. Overuse of these acaricides has resulted in resistance development, environmental pollution, and residues in meat and milk (Parizi et al. 2009; Chagas et al. 2012). These issues have motivated the researcher to seek an alternative such as new formulations from plant extracts to minimize the impact caused by chemical acaricides (Benelli 2016).

Artemisia herba-alba (Shih-balady) (Asteraceae) is a wild plant grown in Sinai and used in the treatment of various diseases in the Middle East. It has been used in folk medicine since ancient times as vermifuge, diuretic, tonic, and in skin troubles (Di Stasi et al. 2002). It exhibited acaricidal activity against larvae of *H. dromedarii* (Abdel-Shafy et al. 2007) and its essential oil presented a repulsive effect against *Ixodes ricinus* ticks (El-Seedi et al. 2017). *Melia azedarach* L. (Meliaceae) is naturalized in tropical and subtropical countries (Rubae 2009) and it has anti-cancer, anti-inflammatory, analgesic, and diuretic properties (Khan et al. 2018). It also possesses antiparasitic activity (Borges et al. 2003; Sousa et al. 2008, 2011; Sariosseiri et al. 2018). Biopesticides such as essential oils (EOs) may be used as an alternative for pest control (Tripathi et al. 2009; Athanassiou et al. 2013). These EOs contain mixtures of bioactive constituents, such as alcohols, aldehydes, ketones, aromatic phenols, lactones, esters as well as monoterpenes and sesquiterpenes (Regnault-Roger et al. 2012). Although, EOs have promising properties to be an alternative, some problems related to EOs have been reported such as volatility, poor solubility in water, and tendency to oxidation (Turek and Stintzing 2013). Therefore, the development of a nano-formulation system may be helpful to overcome these problems. Nano-emulsions can be formulated through the dispersion of the oil phase in an aqueous phase or an aqueous phase in the oil phase in the presence of surfactant (Solans et al. 2005). Various studies have notified the use of nano-emulsions as suitable carriers for active EOs protecting them from degradation and losses by evaporation, controlled release, and easy handling (Martin et al. 2010). In addition, encapsulation of EOs in nano-emulsions enhances their stability, utilization, and efficacy (Moghimi et al. 2016).

Nano-emulsions of EOs are promising tools for controlling arthropod vectors and infectious diseases (Wang et al. 2007). Some studies evaluated the acaricidal activity of nano-emulsion against hard ticks such as nano-structured Cinnamon oil and *Eucalyptus globulus* oil against *Rhipicephalus microplus* females (Dos Santos et al. 2017a, b; Baldissera et al. 2018), and *Pilocarpus spicatus* oil against *R. microplus* larvae (Nogueira et al. 2020). Other studies evaluated the larvicidal activity of nano-emulsion against different types of mosquito (Ghosh et al. 2013a; Duarte et al. 2015; Botas et al. 2017). To avoid negative

impacts on human health and the environment, it is necessary to assess nanoformulation toxicity on non-target species such as Swiss albino mice.

This study aimed to evaluate the acaricidal activity of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion against all the developmental stages of *H. dromedarii* and estimate their toxicity in healthy adult mice.

Materials and methods

Oil extraction from *Artemisia herba-alba* and *Melia azedarach*

Whole aerial parts of *A. herba-alba* and the ripened fruits of *M. azedarach* were kindly donated from Genetics and Cytology Department, Biotechnology Division, National Research Centre. The two plants were ground using stainless steel knife mill then subjected to Soxhlet extraction by adding 150 g powder of each plant to one litter of petroleum ether (grade 40–60 ℃) for 72 h. The solvent was evaporated using a vacuum rotary evaporator (Rotavap, China) at 40 ℃, at a rotation speed of 20 rpm.The obtained oil was transferred to dark glass bottles and stored at 4 ℃ until use (Benyacoub et al. 2019).

Preparation of *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion

The oil-in-water nano-emulsion was formulated using *A. herba-alba* and *M. azedarach* oil, non-ionic surfactant (Tween 80), and distilled water. Polyethylene glycol (MW 6000, Sigma-Aldrich) was used in the PEGylation process of the nano-emulsion. The non-ionic surfactant (Tween 80, 2% v/v) was dissolved in distilled water at room temperature, then the mixture was homogenized using a magnetic stirrer (Thermo Scientific, CIMAREC) for 10 min, then 0.25% w/v SDS was added. Determining the concentration of the two oils under investigation was (10% v/v).

Artemisia herba-alba and *M. azedarach* nano-emulsions were prepared by slowly mixing the two oils to the last prepared non-ionic surfactant (in ratio 1:1) then well mixed using a magnetic stirrer for 30 min. The resulting emulsions were subjected to ultrasonic emulsification [using a 20 kHz Sonicator (Sonics, Vibra cell—Ultra sonicator, USA) with a power out put of 750 W] for 20 min that generates intensive and disruptive forces to minimize the nano-emulsion droplets (Moradi and Barati 2019). Polyethylene glycol (3% w/v; PEG) was prepared by dissolving in distilled water with the aid of a magnetic stirrer for 10 min till complete dissolving to be used in the encapsulation process according to the method of Zhang et al. (2008) with modification. The polyethylene glycol nano-capsule was made by drop-wise dispersion of diluted oil into an appropriate volume of polyethylene glycol under continuous mechanical stirring for 30 min, at ambient temperature then subjected to ultrasonication for 20 min. Avoiding temperature rising during the ultrasonication process, an ice bath was used to maintain the temperature difference (before and after sonication) less than 5 ℃. The obtained loaded nano-capsule suspension was equilibrated overnight for producing dispersed nano-capsules in an aqueous solution.

Characterization of loaded nano-emulsion

Transmission electron microscopy (TEM)

The morphology of the loaded nano-emulsion was characterized using TEM (JEOL, JEM-2100, NRC). One drop of the nano-capsule suspensions was deposited onto a carbon-coated copper grid and stained with phosphotungstic acid then examined and photographed (Sugumar et al. 2014).

Fourier transform infrared spectroscopy (FTIR)

FTIR (JASCO 6100-FTIR, NRC) spectrum of the pure oil and prepared loaded nano-emulsion were studied in the scan range of 4000–400 cm^{-1}. FTIR analysis was used to examine the chemical changes that occurred in the oil molecules as a result of exposure to ultrasonication (Carpenter and Saharan 2017).

Tick colony

For the establishment of tick colony in the laboratory, fully engorged females were collected from camels from the Birqash village (30°09′58.4″N, 31°02′13.2″E) in Giza, Egypt then identified using stereomicroscope (LEICA DM 750, Russia) according to Walker et al. (2003). For oviposition, these engorged females incubated at 25 ± 1 ℃ and 75–80% relative humidity (RH) in an incubator (Friocell, MMM, Germany) in a plastic cup. Daily collection of the laid eggs in separate cups to obtain eggs of the same age and incubated under the same previous conditions. Some of these collected eggs were used in the egg immersion test (EIT) and the others were incubated for hatching. Part of the hatched larvae was used for larval immersion test (LIT) and the other part was fed on healthy rabbits using a capsule technique (Abdel-Shafy 2008) to obtain engorged nymphs. Part of the engorged nymphs was used in the nymphal immersion test (NIT) and the other part was incubated for molting to unfed adults. A group of molted adults was used in the unfed adult immersion test and the other group was fed on healthy rabbits (equal number of unfed male and female) to obtain fully engorged females that used in the immersion test.

Effect of *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion

The effect of *A. herba-alba* and *M. azedarach* oil loadednano-emulsion on the developmental stages of *H. dromedarii* were evaluated. For the selection of suitable concentrations, a pilot test was performed for each tick stage. Butox 5% (deltamethrin, 1 ml/L) was used as reference acaricide, whereas Tween 80, SDS 0.25%, and PEG were considered as solvent control.

Egg immersion test (EIT)

To assess the effect of *A. herba-alba* and *M. azedarach* oil loadednano-emulsion on the embryonated eggs, nearly, 300 embryonated eggs were immersed in a plastic cup containing one ml of the tested concentrations 2.5, 1.25, 0.625, and 0.312% for *A. herba-alba* oil loaded nano-emulsion and 5, 2.5, 1.25, and 0.0625% for *M. azedarach* oil loaded

nano-emulsion for 1 min (Abdel-Ghany et al. 2019). Subsequently, the solutions were decanted, and the cups closed with muslin clothe then incubated for 14 days. Each concentration was replicated 3×. To calculate the mortality percentage of eggs, dead eggs, and hatched larvae were counted using a binocular dissecting microscope (LEICA DM 750, Russia).

Larval immersion test (LIT)

Approximately, 300 larvae of *H. dromedarii* larvae (7–14 day) were immersed in 1 ml of *A. herba-alba* oil loaded nano-emulsion 2.5, 1.25, 0.625, and 0.312%, *M. azedarach* oil nano-emulsion 10, 5, 2.5, 1.25% for 1 min. Each concentration was replicated 3×. Dead larvae were counted after 24 h to calculate the mortality percentage. The larvae were considered dead when failed to move after stimulation by breathing or with ataxia.

Nymphal immersion test (NIT)

To evaluate the efficacy of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion on *H. dromedarii* nymphs, 30 engorged nymphs were immersed for 1 min in 5 ml of each concentration of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion 1.25, 0.625, 0.312, and 0.156%. Each concentration was used in triplicate. The treated nymphs were incubated at 25 ± 1 ℃ and 75–80% RH. Engorged nymphs that failed to molt were counted and their mortality percentages were calculated.

Adult immersion test (AIT)

Unfed adults

In AIT, 30 unfed adults (equal number of males and females) of *H. dromedarii* (10 day old) were exposed to each concentration of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion 10, 5, 2.5, and 1.25% in 5 ml for 1 min. These concentrations were replicated 3×. After that, the treated ticks were incubated at 25 ± 1 ℃ and 75–80% RH and daily checked for 7 days to record the mortality.

Engorged females

The acaricidal efficacy of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion on *H. dromedarii* engorged females were evaluated. The fully fed females were immersed in different concentrations of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion (5, 2.5, 1.25, and 0.625%) following the method described by Drummond et al. (1973) with slight modification. Each engorged female was weighed before treatment. For each concentration, nine engorged females were divided into three replicates. These females were immersed in 10 ml of the tested concentration for 1 min. After that, the solutions were removed then the ticks were dried by filter paper and kept in the incubator in separate cups at 25 ± 1 ℃ and 75–80% RH for 15 days. The laid eggs were collected and incubated at the same conditions until the hatching occurred to record the hatching rate. The egg productive index (EPI = [egg mass (mg)/initial weight of engorged female (mg)] × 100) and hatchability (%) of laid eggs were calculated (Abuowarda et al. 2020).

Toxicological effects of *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion on Swiss albino mice

Forty adult male mice (2–3 months, 20–25 g) were obtained and housed in the animal house, National Research Centre in a ventilated room at 26 ± 2 ℃ and 44–56% RH, light and dark cycles of 14 and 10 h, respectively. Food and water were allowed ad libitum for all control and experimental group. These mice were divided into four groups with ten mice in each group. Both *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion treated group received 1500 mg/kg/day. The negative control group received no treatment to measure basic parameters, the positive control group received (Tween 80, SDS 0.25% and PEG) for five successive days by oral gavage. Daily following of the mice for any behavior changes such as the water and food intake and the possible appearance of toxic symptoms. On day 10 the blood samples were collected from their retro-orbital plexus, some fresh blood collected in a sterile tube containing (EDTA) to determine the blood cell count (erythrocytes, hemoglobin, platelets, and total leukocytes, by an automatic hematology counter) (Medonic, NRC). The other blood samples were left at room temperature for 1 h followed by centrifugation at 3000 rpm for 15 min at 4 ℃ to obtain sera. Then, the sera were stored at −20 ℃ for clinical biochemistry. The sera were analyzed by an automatic biochemical analyzer for the determination of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatinine (CR). A small piece of liver and kidney was fixed with 10% formalin then were dehydrated and embedded in paraffin wax. Sections with 5–8 µm thickness were prepared and stained with hematoxylin–eosin stain (Biodiagnostic, Giza, Egypt), and the sections were photographed under a light microscope (Leica DM 750, Switzerland).

Statistical analysis

Data were analyzed by a one-way ANOVA test followed by Duncan's test using SPSS program v.20. The lethal concentration (LC_{50}) values were calculated by applying regression equation analysis to the probit transformed data of mortality. The dose–response data were analyzed by the probit method (Finney 1962).

Results

Characterization of loaded nano-emulsion

Transmission electron microscope (TEM)

According to TEM characterization, *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion droplets were spherical with droplet size ranged from 62 to 69 nm for *A. herba-alba* oil loaded nano-emulsion (Fig. 1a) and 52–91 nm for *M. azedarach* oil loaded nano-emulsion (Fig. 1b).

Fourier transforms infrared spectroscopy (FTIR)

FTIR analysis of loaded nano-emulsions was performed and compared with *A. herba-alba* and *M. azedarach* oils to detect the possible impact of ultrasonication to induce chemical

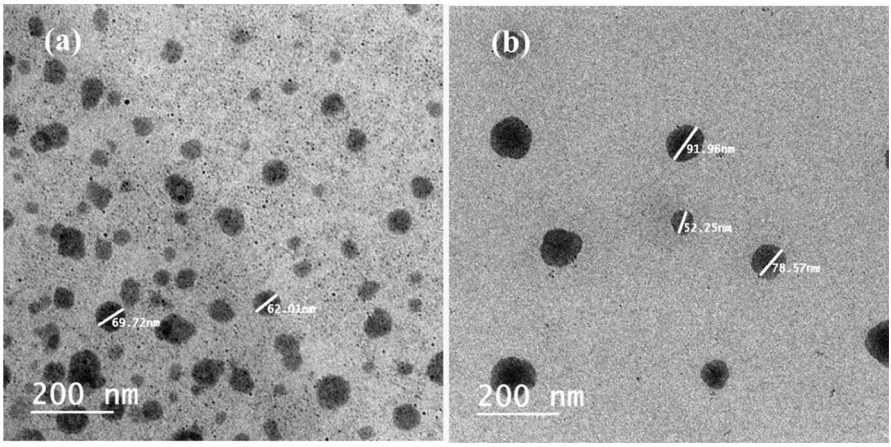

Fig. 1 Transmission electron microscopy (TEM) image of oil loaded nano-emulsions: *Artemisia herba-alba* (**a**) and *Melia azedarach* (**b**)

effects on *A. herba-alba* and *M. azedarach* oils. FTIR of *A. herba-alba* and *M. azedarach* oils and loaded nano-emulsions are presented in (Figs. 2 and 3). The FTIR spectrum of *A. herba-alba* oil (Fig. 2a) revealed characteristic sharp peaks at 3440.39 cm^{-1} (O–H), 2923 cm^{-1} (aromatic and/or vinylic C-H), 2854 cm^{-1} (aliphatic C-H). These peaks were found with slight variation even after the formation of *A. herba-alba* oil loaded nano-emulsion (Fig. 2b). The FTIR spectrum of *M. azedarach* oil (Fig. 3a) revealed high absorption bands at 3437.49 cm^{-1} (O–H stretching vibration), 2926.45 cm^{-1} (aromatic and/or vinylic C–H), 2857.02 cm^{-1} (aliphatic C–H), 1745.27 cm^{-1} (C = O carbonyl groups of esters). These peaks were found with slight variation after the formation of *M. azedarach* loaded nano-emulsion (Fig. 3b). Therefore, the absence of extra peaks confirmed that no chemical changes existed by ultrasonication.

Acaricidal efficacy of *Artemisia herba-alba* and *Melia azedarach* loaded nano-emulsions

Effect of loaded nano-emulsions on embryonated eggs

Table 1 shows the acaricidal efficacy of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions on *H. dromedarii* embryonated eggs. Both formulations showed ovicidal activity higher than Butox 5% and control. At the highest concentrations of both *A. herba-alba* (2.5%) and *M. azedarach* (5%) oil loaded nano-emulsions, mortality was 100%. The calculated LC_{50} values of oil loaded nano-emulsion against embryonated eggs were 0.29 and 1.10% for *A. herba-alba* and *M. azedarach*, respectively (Table 1).

Effect of loaded nano-emulsionsons on larvae

The acaricidal activity of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions on *H. dromedarii* larvae are shown in Table 1. There was a significant effect of both formulations compared with reference acaricide (Butox 5%) and control. *Artemisia herba-alba* oil loaded nano-emulsion showed higher larvicidal activity especially at the highest

concentration of 2.5%, where the mortality reached 100%, this result was similar with reference acaricide (Butox 5%). The larvicidal activity of *M. azedarach* oil loaded nano-emulsion was lower compared with *A. herba-alba* oil loaded nano-emulsion especially at the highest concentration of 10% the mortality rate was 90.4%. The calculated LC_{50} values of oil loaded nano-emulsion against larvae were 0.72 and 1.7% for *A. herba-alba* and *M. azedarach*, respectively (Table 1). The calculated LC_{50} confirmed that *A. herba-alba* oil loaded nano-emulsion had a higher effect against larvae than *M. azedarach* oil nano-emulsion (Table 1).

Effect of loaded nano-emulsions on engorged nymphs

Table 1 shows the effect of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions on the molting of *H. dromedarii* engorged nymphs compared with Butox 5% and control. The effect of *A. herba-alba* oil loaded nano-emulsion was slightly higher than *M. azedarach* oil loaded nano-emulsion. The mortality rate was 100 and 90% at the concentration of 1.25% for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion, respectively. The calculated LC_{50} values were 0.33 and 0.38% for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion, respectively (Table 1).

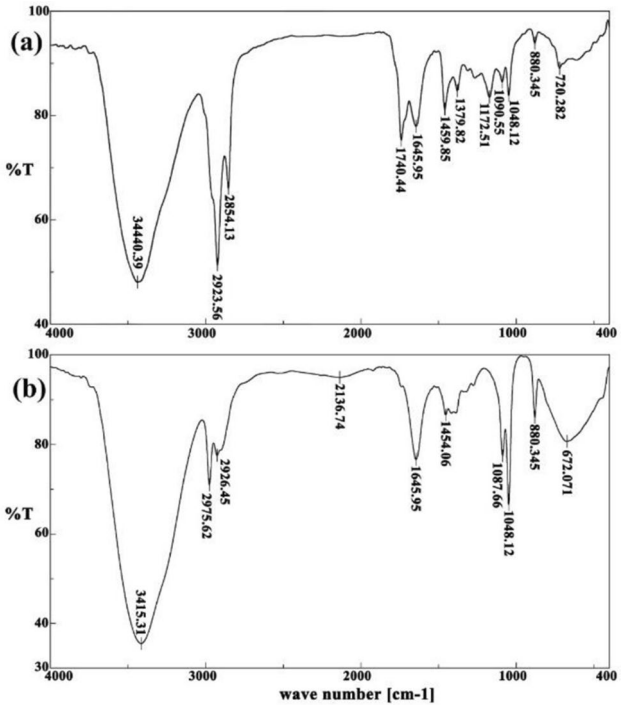

Fig. 2 Fourier transforms infrared spectroscopy (FTIR) spectrum of *Artemisia herba-alba*: oil (**a**) and oil loaded nano-emulsion (**b**)

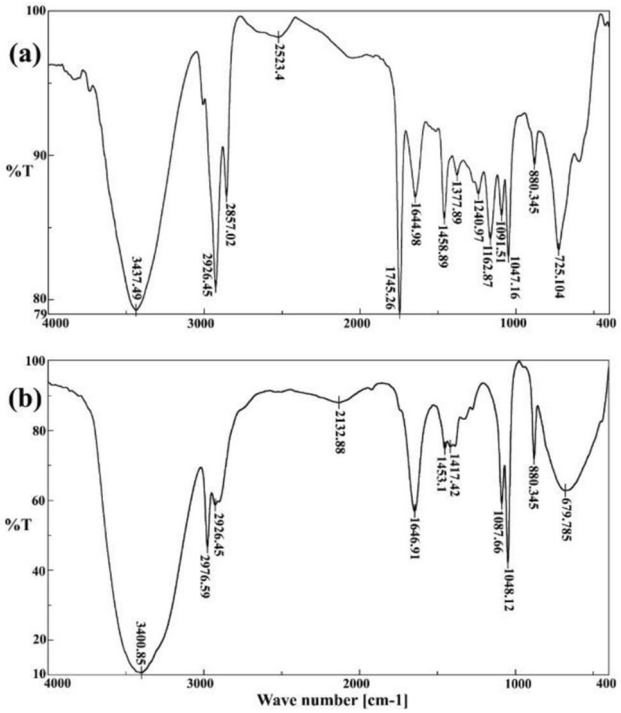

Fig. 3 Fourier transforms infrared spectroscopy (FTIR) spectrum of *Melia azedarach*: oil (**a**) and oil loaded nano-emulsion (**b**)

Effect of loaded nano-emulsions on unfed adults

The acaricidal efficacy of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions against unfed adults of *H. dromedarii* are presented in Table 1. The assessment of both formulations was recorded 3 days post-treatment where some of the adults dead on the first day and the others were with the slow movement then died on the third day. The unfed adults were more sensitive to *A. herba-alba* oil loaded nano-emulsion than *M. azedarach* oil loaded nano-emulsion. At the highest concentration of 10%, the mortality was 86.6% for *A. herba-alba* oil loaded nano-emulsion which was insignificantly lower than that recorded for Butox 5% treatment, whereas *M. azedarach* loaded nano-emulsion recorded mortality 33.3% at the highest concentration (10%) which was significantly lower than recorded in Butox 5%. No mortality was recorded in the control group. The calculated LC_{50} values were 4.4 and 22.2% for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion, respectively (Table 1).

Effect of loaded nano-emulsions on the reproductive performance of engorged females

After treatment of *H. dromedarii* engorged females with *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions, they were followed to evaluate their reproductive performance including egg productive index (EPI), egg number, and hatchability as shown in

Table 1 Mean (±SE) mortality (%) of *Hyalomma dromedarii* embryonated eggs, larvae, engorged nymphs and unfed adults treated with *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion, deltamethrin (Butox 5%) as toxic control, or Tween 80, SDS 0.25%, and PEG as solvent control

Treatment	Tick stage	Concentration (%)	Mortality (%)	$F_{5,12}$	P	LC_{50} (%)	LC_{90} (%)	Slope ± SE
A. herba-alba	Embryonated eggs	2.5	100 ± 0.00^{d}					
		1.25	91.81 ± 0.910^{d}					
		0.625	82.31 ± 3.70^{cd}	34.408	< 0.001	0.29	1.05	2.85 ± 0.26
		0.312	55.2 ± 12.67^{b}					
Deltamethrin		1 ml/L	71.6 ± 4.4^{bc}					
Control			7.56 ± 1.25^{a}					
M. azedarach		5	100 ± 0.00^{e}					
		2.5	81.37 ± 1.37^{d}					
		1.25	56.38 ± 2.34^{c}	97.616	< 0.001	1.10	3.12	2.85 ± 0.26
		0.625	32.86 ± 6.43^{b}					
Deltamethrin		1 ml/L	71.6 ± 4.4^{d}					
Control			7.56 ± 1.25^{a}					
A. herba-alba	Larvae	2.5	100 ± 0.00^{e}					
		1.25	72.50 ± 5.20^{d}	59.829	< 0.001	0.718	2.54	2.33 ± 0.24
		0.625	44.64 ± 11.26^{c}					
		0.312	19.28 ± 4.64^{b}					
Deltamethrin		1 ml/L	100 ± 0.00^{e}					
Control			0.00 ± 0.00^{a}					
M. azedarach		10	90.42 ± 1.56^{d}					
		5	68.64 ± 5.82^{c}	56.149	< 0.001	1.72	14.33	1.39 ± 0.20
		2.5	53.80 ± 9.11^{b}					
		1.25	47.55 ± 4.14^{b}					
Deltamethrin		1 ml/L	100 ± 0.00^{d}					
Control			0.00 ± 0.00^{a}					

Table 1 (continued)

Treatment	Tick stage	Concentration (%)	Mortality (%)	$F_{5,12}$	P	LC_{50} (%)	LC_{90} (%)	Slope ± SE
A. herba-alba	Engorged nymphs	1.25	100 ± 0.00^{d}					
		0.625	83.33 ± 8.81^{cd}	39.550	< 0.001	0.325	0.77	3.37 ± 0.28
		0.321	46.66 ± 3.33^{b}					
		0.156	26.66 ± 6.66^{a}					
Deltamethrin		1 ml/L	66.66 ± 6.66^{c}					
Control			10.00 ± 0.00^{a}					
M. azedarach		1.25	90.00 ± 5.77^{e}					
		0.625	76.66 ± 3.33^{d}	52.720	< 0.001	0.38	1.20	2.57 ± 0.23
		0.312	46.66 ± 3.33^{c}					
		0.156	23.33 ± 3.33^{b}					
Deltamethrin		1 ml/L	66.66 ± 6.66^{d}					
Control			10.00 ± 0.00^{a}					
A. herba-alba	Unfed adults	10	86.6 ± 3.3^{c}					
		5	63.3 ± 6.6^{b}					
		2.5	13.3 ± 3.3^{a}	103.620	< 0.001	4.38	10.95	3.22 ± 0.27
		1.25	6.6 ± 6.6^{a}					
Deltamethrin		1 ml/L	100 ± 0.00^{d}					
Control			0.00 ± 0.00^{a}					
M. azedarach		10	33.33 ± 8.81^{c}					
		5	23.33 ± 8.81^{bc}					
		2.5	13.33 ± 3.33^{ab}	45.063	< 0.001	22.24	273.5	1.17 ± 0.23
		1.25	6.66 ± 3.33^{ab}					
Deltamethrin		1 ml/L	100 ± 0.00^{d}					
Control			0.00 ± 0.00^{a}					

Means within a column and within an oil type/tick stage combination followed by different letters are significantly different (Duncan's test: $P < 0.05$)

Table 2 Reproductive performance (mean ± SE) of *Hyalomma dromedarii* females treated with *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion, deltamethrin (Butox 5%) as toxic control, or Tween 80, SDS 0.25% and PEG as solvent control

Treatment	Conc. (%)	EPI	No. eggs	Hatchability (%)
A. herba-alba	5	0.397 ± 0.03^{b}	2830.7 ± 263.0^{b}	55.10 ± 9.51^{ab}
	2.5	0.420 ± 0.032^{b}	3497.3 ± 359.1^{b}	70.98 ± 8.95^{abc}
	1.25	0.505 ± 0.027^{c}	4664.5 ± 416.3^{c}	78.78 ± 7.59^{bc}
	0.625	0.543 ± 0.014^{c}	4615.8 ± 233.3^{c}	91.76 ± 4.47^{c}
Deltamethrin (1 ml/L)		0.106 ± 0.023^{a}	415.8 ± 91.5^{a}	43.94 ± 11.7^{a}
Control		0.568 ± 0.008^{c}	5354.4 ± 242.4^{c}	97.22 ± 0.51^{c}
$F_{5,54}$		57.768	58.118	5.544
P		< 0.001	< 0.001	< 0.001
M. azedarach	5	0.373 ± 0.049^{b}	3277.2 ± 432.9^{c}	44.12 ± 10.67^{a}
	2.5	$0.370 \pm .019^{b}$	3455.0 ± 180.5^{c}	69.97 ± 7.05^{ab}
	1.25	0.407 ± 0.032^{b}	2459.2 ± 237.1^{b}	68.81 ± 6.61^{ab}
	0.625	0.338 ± 0.043^{b}	2010.6 ± 262.4^{b}	73.42 ± 8.91^{ab}
Deltamethrin (1 cm/L)		0.106 ± 0.023^{a}	415.8 ± 91.5^{a}	43.94 ± 11.7^{a}
Control		0.568 ± 0.008^{c}	5354.4 ± 242.4^{d}	97.22 ± 0.51^{b}
$F_{5,54}$		27.430	53.870	4.579
P		< 0.001	< 0.001	0.001

Means within a column and within an oil type followed by different letters are significantly different (Duncan's test: $P < 0.05$)

EPI egg productive index

Table 2. As the concentrations of both formulations decreased, the EPI increased. The EPI recorded in the treatment of *A. herba-alba* oil loaded nano-emulsion ranged from 0.397 ± 0.03 to 0.543 ± 0.014 compared with Butox 5% treatment (0.106 ± 0.023) and control 0.568 ± 0.008. For *M. azedarach* oil loaded nano-emulsion treatment, the EPI ranged from 0.373 ± 0.049 to 0.338 ± 0.043 compared with Butox 5% treatment and control. Egg numbers ranged from 2830.7 ± 263.0 to 4615.8 ± 233.3 and from 3277.2 ± 432.9 to 2010.6 ± 262.4 for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion, respectively, compared with 415.8 ± 91.5 in Butox 5% treatment and 5354.4 ± 242.4 in control. The hatchability percentage for both formulations was lower compared with the control. The eggs laid by females exposed to *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion exhibited lower hatchability, ranged from 55.1 to 91.7% and from 44.1 to 73%, respectively.

Toxicity of *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsions on Swiss albino mice

Hematological changes

The influence of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions was evaluated on various hematological parameters (Table 3). After treatment of mice with *A. herba-alba* oil loaded nano-emulsion, hematological parameters were close to those of the negative control group with a slight increase or decrease in some parameters which were not

Table 3 Hematological data (mean ± SE) of mice administered for five consecutive days with *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion, no treatment (negative control), or Tween 80, SDS 0.25% and PEG (positive control)

Treatment	WBC (10^9/L)	RBC (10^{12}/L)	HCT (%)	MCH (pg)	MCHC (g/dL)	PLT (10^9/L)	Hb (g/dL)
A. herba-alba	6.63 ± 0.29	7.57 ± 0.72	33.96 ± 3.64	15.93 ± 0.81	35.80 ± 2.62	1073.3 ± 2.84	11.96 ± 0.58
M. azedarach	6.0 ± 1.80	7.54 ± 0.51	37.73 ± 1.83	16.80 ± 0.34	32.9 ± 0.43	872.0 ± 182.1	9.73 ± 3.17
Negative control	5.13 ± 1.68	7.45 ± 0.40	36.46 ± 1.32	16.03 ± 0.31	32.80 ± 0.41	1112.0 ± 197.4	11.93 ± 0.48
Positive control	6.31 ± 0.6	9.0 ± 0.48	40.53 ± 1.73	15.00 ± 0.30	33.53 ± 0.26	1061.66 ± 66.3	13.56 ± 0.46
$F_{3,8}$	0.25	2.0	1.39	2.24	1.0	0.60	0.91
P	0.85	0.19	0.31	0.16	0.42	0.63	0.47

WBC white blood cells, *RBC* red blood cells, *HCT* hematocrit, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *PLT* platelets, *Hb* hemoglobin

different from the negative control group. The level of WBCs (6.63×10^9/L) was slightly increased compared with the negative control (5.13×10^9/L), whereas the level of HCT (34%) was slightly decreased compared with the negative control (36.5%). Furthermore, the *M. azedarach* oil loaded nano-emulsion treated group showed normal WBCs, RBCs, HCT, and a slight decrease in the platelet level (872.0×10^9/L) and Hb (9.73 g/dL) compared with the negative control, whereas platelet and Hb levels were (1112.0×10^9/L) and (11.93 g/dL), respectively.

Biochemical changes

The influence of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions on some hepatic and renal parameters such as alanine aminotransferase (ALT) and alkaline phosphatase (ALP) for liver function and creatinine (CR) for kidney function was studied (Table 4). Concerning *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion treatment, respectively, the level of ALP (72.6 and 70.6 U/L) and ALT (49.0 and 52.3U/L) were not different from the negative control group for ALP (85 U/L) or ALT (67 U/L). For renal parameter, the creatinine level was 0.40 mg/dl for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion which was not different from the negative control group (0.43 mg/dl).

Histopathological examination of the liver and kidney of mice

The histopathological changes of the liver and kidney after oral uptake of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion are shown in Figs. 4 and 5. The liver of the *A. herba-alba* oil nano-emulsion treated group exhibited vacuolar degeneration (black arrow) with an aggregation of lymphocytic cells (black arrow head) (Fig. 4c). In addition to vacuolar degeneration in the hepatic parenchyma, the central vein was congested (Fig. 4d). The liver of the *M. azedarach* oil loaded nano-emulsion treated group exhibited dilated central vein with inflammatory cells, hepatic parenchyma showed mild to moderate degenerative changes including cloudy swelling and vacuolar degeneration (black arrow head) in addition to mild infiltration of lymphocytes (Fig. 4e). Furthermore, individual cell necrosis with activation of Kupffer cells (white arrow) also occurred (Fig. 4f). The liver of the positive control group showed mainly activation of Kupffer cells (white arrow) (Fig. 4b).

The kidney of mice treated with *A. herba-alba* oil loaded nano-emulsion showed an extensive area of hemolysis, slight degeneration of epithelial cells of tubules as well as lymphocytic aggregation in the kidney tissue (white arrow head) (Fig. 5c). Furthermore,

Table 4 Biochemical analysis (mean ± SE) of the mice administered for five consecutive days with *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion, no treatment (negative control), or Tween 80, SDS 0.25% and PEG (positive control)

Treatment	ALP (U/L)	ALT (U/L)	Creatinine (mg/dL)
A. herba-alba	72.6 ± 7.05	49.0 ± 2.00	0.40 ± 0.00
M. azedarach	70.6 ± 9.17	52.3 ± 6.43	0.40 ± 0.00
Negative control	85 ± 5.7	67.0 ± 9.60	0.43 ± 0.033
Positive control	51.3 ± 9.06	48.3 ± 1.85	0.33 ± 0.033
$F_{3,8}$	3.10	2.16	2.6
P	0.089	0.17	0.12

ALP alkaline phosphatase, *ALT* alanine aminotransferase

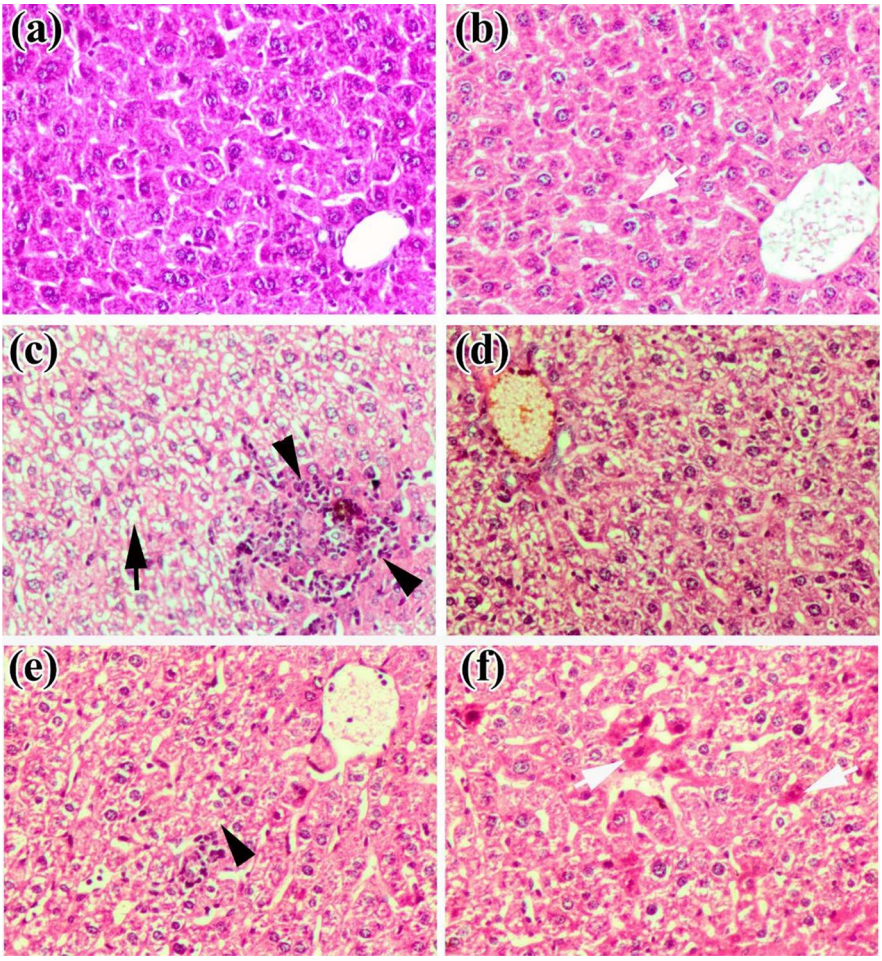

Fig. 4 Histopathological changes in the liver of mice treated with *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion. (**a**) Negative control group showing the normal architecture of liver. (**b**) Positive control group showing activation of Kupffer cells (white arrow). (**c**) *Artemisia herba-alba* oil loaded nano-emulsion treated group showing vacuolar degeneration (black arrow) with an aggregation of lymphocytic cells (blackhead arrow). (**d**) Central vein congested with vacuolar degeneration. (**e**) *Melia azedarach* oil loaded nano-emulsion treated group showing dilated central vein, degenerative changes cloudy swelling, and vacuolar degeneration (black arrow head). (**f**) Individual cell necrosis (white arrow) with activation of Kupffer cells. These micrographs were captured at a magnification of 200×

the kidney tissue of *M. azedarach* oil nano-emulsion showed mainly hemolysis (Fig. 5d). The positive control group showed slight degeneration of epithelium of tubules as well as infiltration of lymphocytes in the kidney tissue (white arrow) (Fig. 5b).

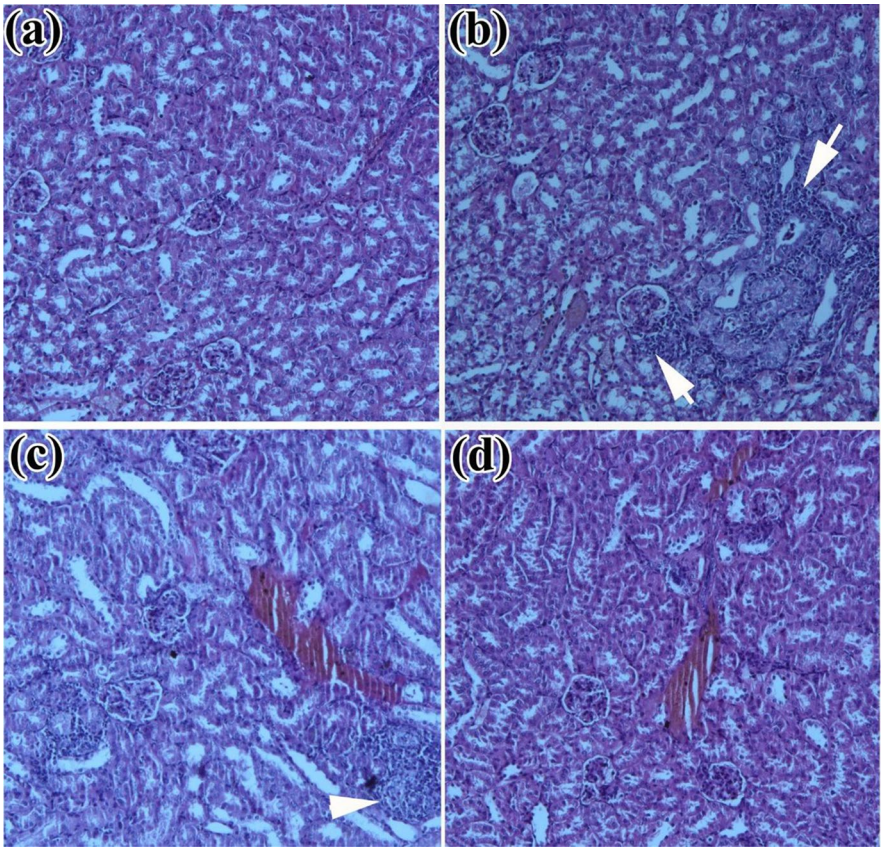

Fig. 5 Histopathological changes in the kidney of mice treated with *Artemisia herba-alba* and *Melia azedarach* oil loaded nano-emulsion. (**a**) Negative control group showing normal features of the kidney. (**b**) Positive control group showing degeneration of epithelium of tubules as well as of lymphocytic infiltration (white arrow). (**c**) *Artemisia herba-alba* oil loaded nano-emulsion treated group showing an extensive area of hemolysis, lymphocytic infiltration (white arrow head), and degeneration of epithelial cells of tubules. (**d**) *Melia azedarach* oil loaded nano-emulsion treated group showing hemolysis and mild degeneration of epithelial cells of tubules. These micrographs were captured at a magnification of 100×

Discussion

Tick control mostly depends on the use of chemical acaricides. Unfortunately, their continuous use results in resistance development and environmental pollution. Biopesticides such as EOs, are considered a more friendly way for integrated pest control as they appeared to be less toxic to the environment than chemical acaricides (Talbert and Wall 2012; Athanassiou et al. 2013). Nano formulation of these EOs is considered a promising tool for controlling parasites which may return to the physicochemical properties of the nanometric emulsion system. The efficacy of nano-emulsion was greater than bulk pesticide as their nano-size enhances specificity and delivery target (Wang et al. 2009). To decrease the losses resulted from tick infestations, it is necessary to make interventions during different developmental stages of their life cycle. The current study is targeting all developmental stages of *H.dromedarii* using *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions.

Few studies were conducted on the acaricidal activity of nano-emulsions against ixodid ticks. In terms of the acaricidal activity of *A. herba-alba* and *M. azedarach* oil nanoemulsions against arthropods, no previous studies were found. However, other species from the Meliaceae and Asteraceae families have been used: *Carapa guianensis* (Meliaceae) oil nano-emulsion exhibited high larvicidal activity against *Aedes aegypti* (Jesus et al. 2017), and *Ayapana triplinervis* (Asteraceae) oil nano-emulsion also revealed strong acaricidal activity against *Ae. aegypti* (Rodrigues et al. 2020).

The morphology and structure of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion were investigated by TEM. The droplets of both nano-emulsions were spherical and in a good dispersion. These findings were in agreement with other studies such as cinnamon oil nano-emulsion (Ghosh et al. 2013b) and neem oil nano-emulsion (Anjali et al. 2012). The droplet size *A. herba-alba* oil nano-emulsion ranged from 62 to 69 nm and from 52 to 91 nm for *M. azedarach* oil nano-emulsion. It has been reported that droplets of nano-emulsion have a size ranging from 20 to 200 nm (Sugumar et al. 2014). A smaller droplet of nano-emulsion can be achieved when the hydrophilic-lipophilic balance (HLB) of the surfactant is synchronized with the HLB of the oil (Fernandes et al. 2014).

FTIR analysis of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion were performed and compared with their pure oil. The FTIR spectrum of *A. herba-alba* oil and *M. azedarach* oil showed characteristic peaks that were appeared after the formation of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion with slight variation. These findings were similar to the study of (Carpenter and Saharan 2017) who found similar results for the preparation of mustard oil nano-emulsion using ultrasonication. All obtained peaks were the same as in pure mustard oil which confirmed no chemical changes induced by ultrasonication.

Results of the present study revealed good acaricidal efficacy of both loaded nano-emulsions against all developmental stages of *H. dromedarii*. *Artemisia herba-alba* oil loaded nano-emulsion had a slightly higher effect than *M. azedarach* oil loaded nano-emulsion. *Artemisia herba-alba* (2.5%) and *M. azedarach* (5%) oil loaded nano-emulsions exhibited ovicidal activity against *H. dromedarii* embryonated eggs reached 100% mortality that was higher than reference acaricide Butox 5%. Furthermore, *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion revealed a high effect against *H. dromedarii* larvae that was similar to Butox 5%. Our results were following the study of Nogueira et al. (2020) who evaluated the repellent effect of *Pilocarpus spicatus* oil nano-emulsion against *Rhipicephalus microplus* larvae whereas the repellency at 50 mg/ml was greater than 97% at 6 and 10 h after treatment. Considering engorged nymphs, they were the most responded stage to both nano-emulsions at concentrations lower than the embryonated eggs, larvae, unfed adults, and engorged females. The calculated LC_{50} value of engorged nymphs treated with *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions confirmed these results. The LC_{50} values for *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion were 0.29 and 1.10%, 0.72 and 1.72%, 0.33 and 0.38%, and 4.38 and 22.24% for embryonated eggs, larvae, engorged nymphs, and unfed adults, respectively.

Artemisia herba-alba and *M. azedarach* oil loaded nano-emulsions exhibited a good effect on the reproductive performance of the fully engorged females whereas the EPI, egg number, and hatchability percentage were lower than those in the control group. These results contrasted with the result of Baldisser et al. (2018) who evaluated the acaricidal activity of *Eucalyptus globulus* oil in pure formand the nanostructured form (oil nano-emulsion and oil nano-capsule) against fully engorged females of *R. microplus*. The pure oil had a higher effect than the nanostructured forms.The pure oil at a concentration of 5 and 10% exhibited 85 and 97.8% efficacy whereas nano-emulsion and nano-capsule

displayed 61.2 and 50% efficacy at 5%, respectively. An in vitro study conducted by Santos et al. (2017a, b) on the reproductive performance of *R. microplus* engorged females using nano-capsule and nano-emulsion containing 5% cinnamon oil revealed 95 and 97% efficacy, respectively. Moreover, an in vivo study using 0.5% of these formulations was conducted on infested cattle, where the animals were free from ticks at 20 days post-treatment. Nano-formulation containing essential oils can affect insects through a diverse mode of action, as deregulation of growth hormones and inhibition of enzymes which resulted in insect death (Hazra 2017; Mishra et al. 2018). It is necessary to assert that nanotechnology uses small-sized particles, facilitating oil penetration into ticks, so enhancing the biological activity of natural products. Therefore, improvement in nanotechnology may be advantageous in veterinary parasitology. This facilitates the use of plant materials to break the life cycle of the parasite and consequently prevent disease transmission (Chagas and Rabelo 2012).

A toxicity experiment was conducted to evaluate the possible toxic effect of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion on the mice. The toxic effect of these formulations was investigated concerning some hematological and biochemical parameters as well as histopathological changes in the liver and kidney. Assessment of hematological parameters is valuable in determining the toxic effect of the drugs, as it gives information about the reaction of the body toward injury, deprivation, and stress (Raza et al. 2002; Rahman et al. 2001)**.** The effect of *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion on all hematological parameters (WBC, RBC, HCT, Hb, and platelet) was insignificant compared with the negative control group. These results were in the same line with the study of Ribeiro et al. (2015) who evaluated theoral administration of *Eucalyptus staigeriana* oil nano-emulsion for 30 days and did not exhibit changes in the hematological parameters. Another study conducted by Milhomem-Paixao et al. (2017) evaluating the possible toxicity of *Carapa guianensis* nano-emulsion after oral administration of (0.5, 1, and 2 g/kg) for 14 successive days which resulted insignificant alteration in the blood parameters with no genotoxicity or cytotoxicity.

The liver is the initial target organ responsible for the detoxification of pesticides and toxic compounds. The serum level of ALP, ALT, and AST is considered a bioindicator to evaluate the pesticide toxicity in humans and animals (Abbassy et al. 2014)**.** Increased levels of liver enzymes or nitrogenous wastes excreted by the kidney might be an indicator for their spillage into circulation as a result of tissue necrosis (Prakash and Manavalan 2011)**.**

In this study, the effect of *A. herba-alba* and *M. azedarach* oil nano-emulsion on serum biochemical parameters (ALP, ALT, and CR) also was insignificant compared with the negative control group. The liver of the *A. herba-alba* oil loaded nano-emulsion treated group exhibited vacuolar degeneration with congestion in the central vein. Furthermore, the liver of the *M. azedarach* oil nano-emulsion treated group presented dilated central vein with inflammatory cells and degenerative changes in the hepatic parenchyma. Despite abnormal changes appeared in the hepatocytes in the treated mice, the biochemical indicators not exhibited significant changes. These results may denote that these histopathological changes were minor or focal so, a functional reserve of the liver masks the clinical importance of early liver injury (Kumar et al. 2002). The study of Ragavan et al. (2017) evaluated the in vivo toxicity of garlic oil nano-emulsion (GNE) in Wistar rats after daily oral administration of 250, 100, and 50 mg for 45 successive days. Treated animals exhibited a significant change in the ALP, ALT, and AST levels compared with the control group. Furthermore, renal markers and hematological parameters showed insignificant changes compared with the negative control group. The liver exhibited inflammation with

significant lymphocytic infiltration. The kidney presented mild acute tubular necrosis and brownish pigment in the glomerular regions.

Conclusion

Artemisia herba-alba and *M. azedarach* oil loaded nano-emulsions revealed high acaricidal activity against all the developmental stages of *H. dromedarii*. The calculated LC_{50} values showed that *A. herba-alba* and *M. azedarach* oil loaded nano-emulsion were more toxic for engorged nymphs followed by embryonated eggs, larvae, and unfed adults. The toxicity study of both loaded nano-emulsions revealed insignificant changes in the hematological and some biochemical parameters. This study concluded that *A. herba-alba* and *M. azedarach* oil loaded nano-emulsions may be considered as an alternative for controlling the camel ticks *H. dromedarii.*

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Author Contributions MMF, SA, MA, RME, and HSMA designed the experiments. EMH and HSMA participated in the preparation of *A. herba-alba* and *M. azedarach* oil nano-emulsions. MMF, SA, MA, RME, and HSMA shared in the bioassay of the *A. herba-alba* and *M. azedarach* oil nano-emulsion against different developmental stages of *H. dromedarii* and evaluated the toxic effect of *A. herba-alba* and *M. azedarach* oil nano-emulsion on Swiss albino mice. SA and HSMA analyzed and tabulated the data. MMF, SA, MA, and HSMA wrote the draft of the manuscript. All authors revised and approved the final version of the manuscript.

Declarations

Conflict of Interest The authors declare that there is no conflict of interest.

Ethical Approval This study was approved by Ethical Committee for Medical and Veterinary Research at the National Research Centre (NRC), Egypt following local laws and regulations (approval protocol No 20148). Consent was obtained from the owners of camels included in this study.

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