In-vivo and in-vitro effectiveness of three insecticides types for eradication of the tick Rhipicephalus sanguineus in dogs

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Abstract

Background: External parasites contribute to extensive harmful impacts on their hosts which is why control and eradication of external parasites have been included in all biosecurity plans of dog houses.

Aim: To evaluate the in-vitro and in-vivo effectiveness of chemicals like Doramectin injectable and Fipronil 50 mg/ml drops and herbal mixes eco-friendly insecticides like phenylpyrazole–garlic–camphor mix spray for combating the external parasitism in dogs and their influence on the hematological, biochemical, and cortisol (CORT) profiles.

Method: The in-vitro effectiveness of the insecticides was conducted by using a total of 216 developmental stage Rhipicephalus sanguineus (72 adults, 72 larvae, and 72 eggs) designed into three replicates of petri dishes (3 plates × 8 units × 3 stages replicates); each replicate was exposed to 1 ml insecticide. The number of surviving ticks was recorded after 0, 2, 4, 8, 16, and 24 hours. Sixteen Rottweiler male dogs aged 1 year and 45.5 kg were divided into four groups. Three groups (G1, G2, and G3) were experimentally infested with R. sanguineus ticks 3–4 weeks post-dog arrival and kept under observation from zero-time of experimental infestation for 1–2 weeks. The three experimentally infested dog groups were treated with Doramectin injectable, Fipronil 50 mg/ml drops, and phenylpyrazole–garlic–camphor mix spray, respectively, and the fourth group was designed as a negative control. A total of 144 samples, including 48 ethylenediaminetetraacetic acid blood, 48 whole blood, and 48 sera samples, were collected.

Results: The in-vitro efficacy revealed highly significant (p < 0.01) 100% killing efficacy that was achieved after 8 hours in Doramectin and Fipronil 50 mg/ml and 24 hours in phenylpyrazole–garlic–camphor mix. The in-vivo trials revealed highly significant (p < 0.01) improvements of red blood cells, hematocrit, mean corpuscular hemoglobin concentrations, platelets, total and differential leukocytic counts, erythrocyte sedimentation rates in the second hour, total protein, creatinine, alanine aminotransferase, urea, glucose, triglycerides, total cholesterol, and CORT levels in 2-week (P2) and 4-week posttreatment (P4) samples in Dormectin, Fipronil 50 mg/ml, and phenylpyrazole–garlic–camphor mix spray-treated dogs with more pronounced recovery in phenylpyrazole–garlic–camphor mix spray-treated dogs.

Conclusion: The insecticides were able to provide a high level of protection against experimental infestation with concern to the different modes of application. Phenylpyrazole–garlic–camphor mix spray (eco-friendly) achieved higher insecticidal action compared to the chemicals.

Keywords: Dogs, Doramectin, Fipronil, Phenylpyrazole–garlic–camphor mix, Ticks.

Introduction

A code of practice has to specify the minimum standards of accommodation, management, and care that are appropriate to meet the physical and behavioral demands of dogs (De Leeuw, 2003). These practices include monitoring the physical score of animals, proper housing, reporting of diseases, supervision of feeding and watering (National Research Council, 1996), satisfying the nutritional requirements, routine animal examination, strict hygienic measures, routine disinfection and sanitization, proper waste disposal, vaccination act against canine distemper, infectious canine hepatitis, canine parvovirus, canine cough, and rabies (Moore, 2003). Control and eradication of external parasites in dogs have been included in every single plan for biosecurity. The control actions depend on sealing with cement all the cracks in the walls and floors of the animals building, good housing practices, and design; rotational locations to encourage the death of the larvae from starvation; depopulation, if possible; washing of the animals routinely; and application of chemical or biological treatment means on the animals (Cochi et al., 1998).

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External parasites or ectoparasites can be defined as organisms that live and survive on the outer body surface at the expense of another organism (Rasouli et al., 2011). These external parasites might contribute to extensive harmful impacts on their hosts, including cardiovascular disorders such as congenital heart failure and anemia (Zendehfili et al., 2015). External parasites must suck their blood meals, first causing wounds in the skin of the host which can act as a second gate for the entrance of other microorganisms as bacterial and viral agents, whose toxins may enter into the bloodstream and contribute to intoxication in the animal host (Mansour et al., 2017). The presence of the external parasites all over the body of their host can contribute to irritation and sometimes allergy. In light of the external parasitism, parasites suck their blood meal from the animals, and as a consequence; feeding the animals become a waste of money, as they suffer from severe bodyweight loss, reduced strength, and reduced ability to work, contributing to great economic losses (Tong et al., 2019). The greater danger of external parasitism might be caused by the mechanical transmission of enteric microorganisms like Escherichia coli species, Salmonella species, or some pyrogenic microorganisms like Corynebacteria species, Staphylococcus species, or Streptococcus species (Apanaskevich and Apanaskevich, 2016; Kwak et al., 2021). They also can biologically transmit some pathogenic blood protozoa, such as Babesia spp. (red water), Thileria spp., and Trypanosoma species; bacterial pathogens, such as Pasteurella pestis; and viral pathogens, such as yellow fever (Shirazi et al., 2013; Apanaskevich et al., 2019).

Insecticides are substances that are designed to kill external parasites and insects. They act mainly as ovicidal or larvicidal agents (Lucia and Guzmán, 2021). The insecticides can act directly on the nervous system of the external parasites through many means: as an acetylcholinesterase inhibitor, sodium channel blockers, GABA-gated chloride channel blockers, voltage-dependent sodium channel modulators, glutamate-gated chloride (GluCl) channel allosteric modulators, juvenile hormone mimics, chordotonal organ channel modulators, microbial disruptors of insect midgut membranes, inhibitors of mitochondrial adenosine triphosphate synthase, uncouplers of oxidative phosphorylation, inhibitors of chitin biosynthesis, mitochondrial complex electron transport inhibitors, and inhibitors of acetyl CoA carboxylase (Metcalf, 2002). The use of insecticides has been problematic in affecting nontarget species, runoff and percolation, pollinator decline, and bird decline. The development and spread of insecticide resistance can be considered a threat to the biosecurity, prevention, and control strategies applied to animal farms, as well as for human beings (Hafez and Abbas, 2021). The higher the resistance of the external parasites to the commercially available insecticides, the higher the chances for the spread of infectious and contagious vector-borne diseases that might contribute to epidemics in such cases (Minetti et al., 2020). A fact that deliberates a necessity for the use of some alternatives categorized as natural eco-friendly compounds or green chemistry has been investigated to reduce the use of chemical insecticides and is associated with higher and extended efficacy on animals and humans, like garlic (González-Macedo et al., 2021; Chen et al., 2022), camphor (Elbrense et al., 2022), potato extract (Khorrami and Soleyman, 2021), pomegranate (Saad et al., 2021), and clay (Oliveira et al., 2022).

We aimed in this study to evaluate the in-vitro and in-vivo effectiveness of some commercial chemical insecticides like Doramectin injectable (Dectomax®) and Fipronil 50 mg/ml drops (Bars®), and herbal-based (Eco-friendly) insecticides like phenylpyrazole–garlic–camphor mix spray (Safeline®) in the recommended dose and concentration for combating the external parasitism in dogs and for their influence on the hematological and biochemical profile, as well as on the cortisol (CORT) stress marker.

### Materials and Methods

#### Study period and location

The in-vitro study was conducted for four successive weeks from April 1, 2021 to May 1, 2021 in the Animal, Poultry, and Environmental Hygiene laboratory. The in-vivo study was conducted from June 1, 2021 to August 1, 2021 in the laboratory animal units of the Faculty of Veterinary Medicine at Suez Canal University in Ismailia, Egypt. The in-vivo study period was divided into three stages: the first 2 weeks for acclimatization of dogs; the second 2 weeks for experimental infestation; and the third stage was 1 month for zero-sampling, treatment application, and posttreatment sampling (P1 at 2 weeks and P2 at 4 weeks posttreatment). Biochemical profile was conducted in the Animal, Poultry, and Environmental Hygiene laboratory. Hematological and hormonal assays were conducted in the Clinical Pathology laboratories at Suez Canal University Hospital.

#### Commercial insecticides

Three insecticides were chosen were Dectomax® (Dramectin), Bars® (Fipronil 50 mg/ml), and Safeline® (Phenylpyrazole–garlic–camphor mix). Dectomax® (Dramectin) is an injectable solution of Doramectin (1 g) in the form of an oily excipient for the treatment. It is indicated for the treatment and control of gastrointestinal roundworms, lungworms, eye worms, grubs, sucking lice, and mange mites. It is administered as a subcutaneous or intramuscular single injection at a dosage of 200 μg of Doramectin per kg (1 ml/50 kg) body weight. Bars® (Fipronil 50 mg/ml) drops used against fleas and mites for dogs contain Fipronil 50 mg/ml, diflubenzuron 1 mg/ml, and dicarboximide (MGC-264) 5 mg/ml. It is appropriate for dogs from the age of 8 weeks for the treatment and prevention.
of entomoses (lice, fleas, and withers) and sarcoptic disease. It is applied as 1 dropper pipette (1.4 ml) per 10 kg of the animal weight. Safeline® (phenylpyrazole–garlic–camphor mix) spray (1 l) is a fast-acting, long-lasting, waterproof treatment. Herbal mix eco-friendly Safeline® spray is used for the control of fleas, ticks, and chewing lice in dogs. It is composed of phenylpyrazole (Fipronil), garlic oil, camphor oil, and vehicle.

**In-vitro effectiveness of commercial insecticides**
The in-vitro effectiveness of the three commercial insecticides was tested as recommended by Balcioğlu et al. (2015) and Kiesewetter et al. (2013) using direct exposure of *Rhipicephalus sanguineus* ticks to the recommended (by the manufacturer) dose and concentration of the tested insecticides. *Rhipicephalus sanguineus* ticks were obtained from naturally parasitized dogs and maintained in the laboratory.

A total of 216 developmental stage *R. sanguineus* (72 adults, 72 larvae, and 72 eggs) were divided into replicates of three petri dishes each (3 plates × 8 units × 3 stages/replicate). Each replicate of three plates was exposed to 1 ml of the recommended concentration of each insecticide. The different developmental stages of *R. sanguineus* ticks were recorded for survival after contact times of 0, 2, 4, 8, 16, and 24 hours. After each contact time, the number of surviving ticks and intact eggs was recorded and the killing efficiencies were calculated and expressed as a percentage (%) using the following formula:

$$\text{The initial number of used ticks} - \frac{\text{The initial number of used ticks}}{\text{X 100}}$$

**Experimental dogs’ housing microclimate**
The laboratory animal units were adjusted prior to housing the dogs to allow biosecurity measures to maintain their health and protection according to Soliman and Abdallah (2020). These biosecurity measures included foot dips supplied with commercial phenol 5% at the entrance of the units, break-back traps to discourage the entrance of rodents, clean feeding, watering bowls, fly proof nets, safe feed storage area, and proper drainage of the units for the removal of the wastes daily.

The laboratory animal units were provided with V-shaped side wall windows, ceiling fans, and sidewall suction fans to stimulate the air movement and ventilation act of the rooms that was based on cross-ventilation. Rooms were supplied with white LED lights of 18 watts to be consumed in a continuous lighting regimen following Soliman and Hassan (2019). The floor of the rooms was cleaned sufficiently using hypochlorites and glutaraldehyde, as well the drainage area was lined with slaked lime to achieve maximum cleanliness, dryness, and durability according to Soliman et al. (2018).

**Experimental animals’ management**
Sixteen Rottweiler male dogs aged 1 year and 45.5 kg body weight were purchased from a commercial pet shop in Ismailia, Egypt. The animals were handled in human manners, considering all the national and international ethical guidelines in treating the animals. The synchronization in the selected dogs was important to minimize the influences of the physiological variation between the members of each group on the responses to the insecticides used for the treatments (Taktak et al., 2021). Dogs on their arrival were double-checked for their physical conditions and fitness, as well as some random blood samples were collected in a human manner using a local anesthetic to check any signs of internal or external parasitism. Dogs were divided into four groups and placed in four separate previously prepared rooms. The rooms were optimized 24 hours a day at a microclimatic temperature of 37°C using halogen heaters according to Soliman et al. (2021).

Dogs were given *ad libitum* access to clean tap water that was previously stored for de-chlorination, as well as provided with balanced feed twice a day per dog in a routine manner to maintain a healthy lifestyle and prevent the stress that arises from starvation or underfeeding. The first meal was provided in the morning and contained a mix of bread, rice, pasta, boiled well-done chickens, well-cooked liver, and minced meat. The second meal was provided in the evening and contained three-quarter kilogram of commercial dry food with 22%–23% crude protein for each dog.

Dogs on their arrival were dewormed as a routine monthly preventive action using praziquantel (5 mg/kg body weight) and fenbendazole (50 mg/kg body weight). Dogs were vaccinated with a booster dose of Vanguard® Plus 5 DHLPPC vaccine (Zeotis® US) against canine parvovirus, canine distemper, parainfluenza, canine adenovirus type I and II, canine hepatitis, leptospirosis, and coronavirus. Dogs were also vaccinated against rabies. The two shots were administered subcutaneously in the back of the neck.

**Experimental infestation of dogs**
Ticks were collected from naturally parasitized dogs admitted to a veterinary clinic in Ismailia, Egypt, into screw-capped bottles. The bottles were transferred to the laboratory as quickly as possible. In the laboratory, female ticks were identified morphologically and by light microscopy (LM) (10×), then maintained in an incubator at 25°C and 75% relative humidity for 1–2 weeks until they laid eggs. Hatching was encouraged by optimizing microclimatic temperature and relative humidity until the larvae were obtained. The different developmental stages were used for the *in-vitro* trials. The identified *R. sanguineus* ticks (five females per dog) were used to infest three out of the four experimental groups (G1, G2, and G3) at the beginning of the second stage of the *in-vivo* study period (3–4 weeks post-dog arrival) and the fourth group was kept as the control. The dogs of the three infested groups were kept under observation for 2 weeks until the development of ticks up to the adult stage on the animals’ skin. One or two of
the infesting ticks were harvested for more confirmation by LM (10×).

**Sampling**

A total of 144 samples (3 samples × 16 dogs × 3 types of samples) including 48 ethylenediaminetetraacetic acid (EDTA) blood, 48 whole blood, and 48 sera samples were collected. The samples were transferred to the laboratory in a dry-ice box as quickly as possible.

EDTA blood samples on EDTA vacutainers (VACUETTE® TUBE 5 ml K3EDTA 13 × 100 lavender cap-black ring, PREMIUM) were used for hematological analysis. Whole blood samples in erythrocyte sedimentation rates (ESR) tubes (ESR-Vacuum Tubes 1.2 ml for use with the ESR-Auto Plus® and ESR-10 Manual Rack) were examined for the ESR within 1 hour. Sera blood samples on plain serum tubes (BD vacutainer®, Serum tubes, 10.0 ml, 16 × 100 mm, Plastic, Additive: Clot Activator, Silicone-Coated, Red Conventional Closure, and Paper Label) were held in a water bath (Thermo® water bath Precision series Standard, 20, 30°C–100°C, 392 mm, GP20) at 25°C for 30 minutes and centrifuged at 3,500 rpm for 15 minutes. Clear sera were collected and pipetted using an automatic pipette (Fisherbrand®, variable 200:1,000 micron) into 2.5 ml capacity Eppendorf tubes and stored at −20°C until tested for biochemical and stress marker assay (Soliman et al., 2017).

**Hematological profile**

EDTA blood samples were collected (48 from 4 groups of 4 dogs) and examined for the following hematological parameters: red blood cells count (RBCs, ×10⁶/µl), hemoglobin concentrations (Hb, g/dl), hematocrit (HCT, %), mean corpuscular hemoglobin concentrations (MCHC, g/dl), platelet counts (PLT, ×10⁹/µl), white blood cells count (WBCs, ×10⁹/µl), neutrophils (N, %), lymphocytes (L, %), monocytes (M, %), eosinophil (E, %), and basophils (B, %) using Sysmex XP-300 Automated Hematology Analyzer. Whole blood samples were collected (48 from 4 groups of 4 dogs) and examined for ESR (mm/hour) using STAT™ PLUS Automated ESR Analyzer.

**Biochemical profile**

Sera samples were collected (48 from 4 groups of 4 dogs) and examined for the following biochemical parameters: total protein (TP) and expressed as g/dl, alanine aminotransferase (ALT) and expressed as IU/L, urea (UREA) and expressed as mg/dl, creatinine (CREAT) and expressed as mg/dl, glucose (GLUCO) and expressed as mg/dl, total cholesterol (TC) and expressed as mg/dl, and triglycerides (TG) and expressed as mg/dl calorimetrically using ROCHE COBAS Integra 800 chemical analyzer. CORT hormone (mcg/dl) and immunoglobulin G and M concentrations (IgG and IgM, mg/dl) were measured by using ROCHE Elesys 1010 Immunoassay Analyzer (Wu et al., 2017).

**Statistical analysis**

The statistical analysis was conducted using a statistical Statistical Package for the Social Sciences (SPSS) software version 20 (IBM Corp, 2016—IBM SPSS Statistics 20). Data were analyzed statistically using multifactorial analysis of variance to estimate the effect of insecticides treatments in infested dogs against sampling time. The influence of insecticides treatments on the prevalence of infestations and sampling times and their interactions were displayed in the results tables. The statistical model utilized the following formula:

\[ Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk} \]

where \( Y_{ijk} \) is the measurement of the dependent variables; \( \mu \) is the overall mean; \( \alpha_i \) is the fixed effect of the different treatments (insecticides), \( \beta_j \) is the fixed effect of sampling time, \( (\alpha\beta)_{ij} \) is the interactions of treatments by sampling time, and \( \epsilon_{ijk} \) is the random error. The killing efficiencies in the in-vitro trials were calculated and expressed as percentage (%). Nonparametric Kruskal–Wallis test was used to determine the significant differences between the reduction percentages. The results were expressed as highly significant at \( p \leq 0.01 \), significant at \( p \leq 0.05 \), and nonsignificant at \( p > 0.05 \).

**Ethical approval**

The study design and animal management system were approved by the Scientific Research Ethics Committee on Animal and poultry researches, Faculty of Veterinary Medicine, Suez Canal University, Egypt, with approval number (2021028). The animals in the current study were handled in a professional way to meet the national and international regulations for experimental animals’ care. Dogs were received and housed in proper housing rooms to meet their requirement with the satisfaction of all the nutritional needs according to the regulation. Infesting animals with R. sanguineus ticks was carried out with a small number of female ticks to minimize the animals’ suffering until treatment with the three appointed insecticides (Doramectin, Fipronil 50 mg/ml, and phenylpyrazole–garlic–camphor mix). The samples’ collection was carried with complete care and the use of local anesthetic to overcome pain during samples collection from animals.

**Results**

**In-vitro efficacy of chemical and herbal insecticides**

The in-vitro efficacy of the three tested insecticides in Figure 1 show highly significant \( (p < 0.01) \) increases in the killing efficiencies as Doramectin (Dectomax®) achieved up to 95.8% after 2 hours and 100% killing efficacy after 8 hours. Fipronil 50 mg/ml (Bars®) achieved up to 91.0% after 2 hours and 100% killing efficacy after 8 hours. Phenylpyrazole–
garlic–camphor mix (Safeline®) achieved up to 58.3% after 2 hours and 100% killing efficacy after 24 hours.

**Experimental infestation of dogs and clinical examinations**

Rottweiler dogs are shown in Figure 2 during the second stage of the *in-vivo* experiment (third to fourth week) with high infestation rates with ticks (*R. sanguineus*). The successful infestation was impacted by the development of a large number of adult ticks on the animal’s skin in the three challenged experimental groups. The LM examination shows in Figure 3 the characterized shape of *R. sanguineus* (Fig. 3A), the hexagonal shape of the basis capituli (Fig. 3B), and the comma-shaped spiracular plate (Fig. 3C). The clinical examinations of the experimentally infested dogs revealed irritation, lethargy, loss of appetite, fever, swollen lymph nodes, swollen legs, paralysis, shifting leg lameness, and the presence of different developmental stages of ticks on the dogs’ skin.

**Hematological profile of dogs**

The hematological analysis reveals in Table 1 highly significant (*p* < 0.01) reductions in the overall means of RBCs, HCT, MCHC, and PLT in all groups treated with Doramectin (Dectomax®), Fipronil 50 mg/ml (Bars®), and phenylpyrazole–garlic–camphor mix (Safeline®) compared to the control with more concern toward the phenylpyrazole–garlic–camphor mix (Safeline®). The overall means concerning sampling time reveal in Table 1 highly significant (*p* < 0.01) reductions in all the measured hematological parameters in the 2-week (*P*₁) and 4-week posttreatment (*P*₂) samples compared to the zero-time samples with no significant differences between the two sampling times (*P*₁ and *P*₂) with

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**Fig. 1.** *In-vitro* killing efficiency (% mean ± SE) of the tested insecticides on ticks.

**Fig. 2.** Photographs of the experimental dogs after experimental infestations. Red arrows point to the ticks.

**Fig. 3.** LM pictures of (A) *R. sanguineus*, (B) hexagonal shape of the basis capituli, and (C) comma-shaped spiracular plate.
more pronounced improvement in the hematological parameters in the 4-week posttreatment samples.

RBCs and HCT interactions in infested dogs treated with Doramectin (Dectomax®) shown in Table 1 reveal highly significant ($p < 0.01$) increases through all the posttreatment sampling times, while Hb, MCHC, and PLT interactions reveal highly significant ($p < 0.01$) increases in $P_1$ and $P_2$ with no significant between
the two sampling times. Fipronil 50 mg/ml (Bars*) and phenylpyrazole–garlic–camphor mix (Safeline*) reveal highly significant \((p < 0.01)\) increases in the hematological parameters with no significant difference between \(P_1\) and \(P_2\) samples of HCT, MCHC, and PLT in Bars* and of RBCs, HCT, and MCHC in Safeline*-treated dogs.

The total and differential leukocytic counts in Table 2 reveal highly significant \((p < 0.01)\) increases in the overall means of WBCs and N in all groups treated with Doramectin (Dectomax*), Fipronil 50 mg/ml (Bars*), and phenylpyrazole–garlic–camphor mix (Safeline*) compared to the control. Lymphocytes, M, E, and B reveal no significant differences in all dog groups treated with Doramectin (Dectomax*), Fipronil 50 mg/ml (Bars*), and phenylpyrazole–garlic–camphor mix (Safeline*) compared to the control. The overall means concerning sampling time in Table 2 reveal highly significant \((p < 0.01)\) reductions in WBCs, E, and B in the 2-week \((P_2)\) and 4-week posttreatment \((P_4)\) samples compared to the zero-time samples with no significant differences between the two sampling times \((P_1\) and \(P_2)\). Lymphocytes and M reveal no significant differences between the three sampling times. Doramectin (Dectomax*), Fipronil 50 mg/ml (Bars*), and phenylpyrazole–garlic–camphor mix (Safeline*) treatment interactions in Table 2 reveal highly significant \((p < 0.01)\) reductions in WBCs, E, and B, as well as highly significant increases in N and L, and no significant differences in M.

**Erythrocyte sedimentation rate**

ESR (in the first hour) in Figure 4A show no significant differences between the groups, while highly significant \((p < 0.01)\) increases were recorded in ESR in the second hour with no significant differences between the three dog groups compared to the control. The overall means concerning sampling time in Figure 4B show highly significant \((p < 0.01)\) reductions in ESR in the first hour and ESR in the second hour in the 2-week \((P_2)\) and 4-week posttreatment \((P_4)\) samples compared to the zero-time samples with no significant differences between the two sampling times \((P_1\) and \(P_2)\). The ESR first- and second-hour interactions in Figure 4C show highly significant \((p < 0.01)\) reductions in Doramectin (Dectomax*), Fipronil 50 mg/ml (Bars*), and phenylpyrazole–garlic–camphor mix (Safeline*)-treated dogs, especially concerning the strong action of phenylpyrazole–garlic–camphor mix (Safeline*) that reveals highly significant \((p < 0.01)\) reductions between the first- and second-hour ESR.

**Biochemical profile of dogs**

The overall mean shown in Table 3 indicates highly significant \((p < 0.01)\) increases in TP and CREAT in phenylpyrazole–garlic–camphor mix (Safeline*)-treated dogs and ALT and UREA in Fipronil 50 mg/ml (Bars*)-treated dogs compared to the other treated groups and control. The overall means concerning sampling time in Table 3 shows highly significant \((p < 0.01)\) reductions in TP, ALT, UREA, and CREAT in the 2-week \((P_2)\) and 4-week posttreatment \((P_4)\) samples compared to the zero-time samples with no significant differences between the two sampling times \((P_1\) and \(P_2)\) in TP, UREA, and CREAT.

The results indicate highly significant \((p < 0.01)\) reductions in TP with no significant differences between the two sampling times \((P_1\) and \(P_2)\); ALT and UREA with no significant differences between the two sampling times \((P_1\) and \(P_2)\); and CREAT with no significant differences between the two sampling times \((P_1\) and \(P_2)\).

The GLUCO and lipid profile overall means of the treated dogs shown in Table 4 indicate highly significant \((p < 0.01)\) increases in Doramectin (Dectomax*), Fipronil 50 mg/ml (Bars*), and phenylpyrazole–garlic–camphor mix (Safeline*)-treated dogs compared to the control. The overall means concerning sampling time shown in Table 4 indicate highly significant \((p < 0.01)\) reductions in GLUCO, TG, and TC in the 2-week \((P_2)\) and 4-week posttreatment \((P_4)\) samples compared to the zero-time samples with no significant differences between the two sampling times \((P_1\) and \(P_2)\). The data shown in Table 4 reveal highly significant \((p < 0.01)\) reductions in GLUCO (Table 4) with no significant differences between the two sampling times \((P_1\) and \(P_2)\).

**Stress marker**

The CORT overall means shown in Figure 5A reveal highly significant \((p < 0.01)\) increases in Doramectin (Dectomax*), Fipronil 50 mg/ml (Bars*), and phenylpyrazole–garlic–camphor mix (Safeline*)-treated dogs compared to control with no significant differences between the three groups. The overall means concerning sampling time shown in Figure 5B indicate highly significant \((p < 0.01)\) reductions in CORT levels in the 2-week \((P_2)\) and 4-week posttreatment \((P_4)\) samples compared to the zero-time samples with no significant differences between the two sampling times \((P_1\) and \(P_2)\). The data shown in Figure 5C reveal highly significant \((p < 0.01)\) reductions of CORT levels with no significant differences between the two sampling times \((P_1\) and \(P_2)\) in Doramectin (Dectomax*) and Fipronil 50 mg/ml (Bars*)-treated dogs.

**Discussion**

Dog–human interrelationships provide opportunities for enhancing exercise, minimizing stressful events, and developing empathy. The infestation of dogs with external parasites like ticks can be considered a threat to both companions for their abilities to be transmitted easily, as well as for carrying many zoonotic diseases. The control of the external parasites infesting companion animals like dogs and cats is critical for their wellbeing, as control and eradication regime of
Table 2. Total and differential leukocytic counts (mean ± SE) of infested dogs treated with different experimented insecticides.

<table>
<thead>
<tr>
<th>Insecticides / time</th>
<th>Time</th>
<th>WBCs ×10^3/µl</th>
<th>N %</th>
<th>L %</th>
<th>M %</th>
<th>E %</th>
<th>B %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall means concerning the experimented insecticides</strong></td>
<td></td>
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<tr>
<td>Dectomax®</td>
<td></td>
<td>15.3 ± 0.13</td>
<td>38± 0.37</td>
<td>47± 0.07</td>
<td>16± 0.14</td>
<td>2± 0.35</td>
<td>0.3± 0.04</td>
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<tr>
<td>Bars®</td>
<td></td>
<td>14.8 ± 0.12</td>
<td>33± 0.42</td>
<td>52± 0.35</td>
<td>16± 0.18</td>
<td>2± 0.26</td>
<td>0.2± 0.03</td>
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<tr>
<td>Safeline®</td>
<td></td>
<td>15.1 ± 0.12</td>
<td>37± 0.00</td>
<td>46± 0.29</td>
<td>15± 0.04</td>
<td>2± 0.06</td>
<td>0.4± 0.01</td>
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<tr>
<td>Control</td>
<td></td>
<td>11.2 ± 0.15</td>
<td>35± 0.57</td>
<td>48± 0.37</td>
<td>13± 0.13</td>
<td>2± 0.11</td>
<td>0.1± 0.01</td>
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<td>0.863</td>
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<td>0.165</td>
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<tr>
<td><strong>Overall means concerning the sampling time</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td></td>
<td>18.2 ± 0.10</td>
<td>31± 0.25</td>
<td>48± 0.19</td>
<td>17± 0.14</td>
<td>3± 0.30</td>
<td>0.6± 0.01</td>
</tr>
<tr>
<td>P₁</td>
<td></td>
<td>12.1 ± 0.30</td>
<td>40± 0.36</td>
<td>47± 0.07</td>
<td>13± 0.18</td>
<td>2± 0.06</td>
<td>0.1± 0.08</td>
</tr>
<tr>
<td>P₂</td>
<td></td>
<td>12.1 ± 0.27</td>
<td>37± 0.38</td>
<td>50± 0.33</td>
<td>15± 0.15</td>
<td>2± 0.02</td>
<td>0.1± 0.08</td>
</tr>
<tr>
<td>p-value</td>
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<td>0.002</td>
<td>0.223</td>
<td>0.662</td>
<td>0.328</td>
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</tr>
<tr>
<td><strong>Experimented insecticides by sampling time interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dectomax®</td>
<td>Zero</td>
<td>20.3 ± 0.22</td>
<td>29± 0.24</td>
<td>38± 0.17</td>
<td>19± 0.16</td>
<td>4± 0.47</td>
<td>0.7± 0.25</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>12.9 ± 0.70</td>
<td>44± 0.33</td>
<td>59± 0.10</td>
<td>13± 0.34</td>
<td>2± 0.00</td>
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</tr>
<tr>
<td></td>
<td>P₂</td>
<td>12.8 ± 0.14</td>
<td>42± 0.11</td>
<td>54± 0.80</td>
<td>17± 0.17</td>
<td>2± 0.00</td>
<td>0.0± 0.00</td>
</tr>
<tr>
<td>Bars®</td>
<td>Zero</td>
<td>20.2 ± 0.13</td>
<td>30± 0.33</td>
<td>37± 0.47</td>
<td>19± 0.26</td>
<td>3± 0.62</td>
<td>0.7± 0.12</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>13.0 ± 0.30</td>
<td>44± 0.33</td>
<td>54± 0.08</td>
<td>14± 0.37</td>
<td>2± 0.00</td>
<td>0.0± 0.00</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>11.3 ± 0.18</td>
<td>47± 0.02</td>
<td>54± 0.27</td>
<td>16± 0.38</td>
<td>2± 0.00</td>
<td>0.0± 0.00</td>
</tr>
<tr>
<td>Safeline®</td>
<td>Zero</td>
<td>20.7 ± 0.09</td>
<td>29± 0.14</td>
<td>28± 0.11</td>
<td>18± 0.18</td>
<td>3± 0.62</td>
<td>1.0± 0.00</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>11.3 ± 0.24</td>
<td>39± 0.42</td>
<td>45± 0.56</td>
<td>13± 0.49</td>
<td>2± 0.00</td>
<td>0.0± 0.00</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>13.3 ± 0.30</td>
<td>45± 0.03</td>
<td>46± 0.28</td>
<td>13± 0.34</td>
<td>2± 0.00</td>
<td>0.0± 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>Zero</td>
<td>11.5 ± 0.14</td>
<td>37± 0.25</td>
<td>47± 0.64</td>
<td>13± 0.46</td>
<td>2± 0.00</td>
<td>0.2± 0.00</td>
</tr>
<tr>
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<td>P₁</td>
<td>11.0 ± 0.31</td>
<td>35± 0.79</td>
<td>48± 0.43</td>
<td>13± 0.37</td>
<td>2± 0.00</td>
<td>0.2± 0.02</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>11.0 ± 0.31</td>
<td>35± 0.79</td>
<td>48± 0.43</td>
<td>13± 0.37</td>
<td>2± 0.00</td>
<td>0.2± 0.05</td>
</tr>
<tr>
<td>p-value</td>
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<td>0.000</td>
<td>0.017</td>
<td>0.019</td>
<td>0.456</td>
<td>0.006</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same column are significantly different at p ≤ 0.05 or highly significantly different at p < 0.01. Means with the same superscripts in the same column are nonsignificantly different at p > 0.05.

WBCs: White blood cells; N: Neutrophils; L: Lymphocyte; M: Monocytes; E: Eosinophils; B: Basophils; SE: Standard error.

Infesting external parasites in companion animals like dogs protects not only dogs but also humans from many infectious and zoonotic diseases that can be transmitted by these organisms (Barker and Wolen, 2008; Berg et al., 2021). Ticks like *R. sanguineus* widely infest dogs and their kennels (Medlock et al., 2013; Mahai et al., 2021). Ticks are known to be abundant ectoparasite that can infest a wide range of animals including humans. Once infestation takes place, the deterioration actions...
The current results showed a significant in-vitro efficiency of the three tested insecticides against R. sanguineus with 100% killing efficiency achieved after 8 hours in Doramectin (Dectomax®) and Fipronil 50 mg/ml (Bars®) and 24 hours in phenylpyrazole–garlic–camphor mix (Safeline®). The high insecticidal actions of the three tested insecticides are related to their deep structure and broad-spectrum neurological action on the insect. The same results were recorded by Taenzler et al. (2018), Becskei et al. (2016), Beugnet et al. (2016), Cherni et al. (2016), and Six et al. (2016) who determined the broad-spectrum action of many chemically based insecticides on R. sanguineus ticks, fleas of Ctenocephalides species, mites of Demodex species, sarcotic mange from Sarcoptes scabiei, and ear mites from Otodectes cynotis. Padula et al. (2020) reported the importance of using effective antiseria against ticks for obtaining full protection against their infestations in companion animals like dogs. Poché et al. (2017) recorded that insecticides containing Fipronil 50 mg/kg were able to reduce ectoparasitic infestations up to 95% from their original load after a single time treatment. Dolan et al. (2017) also recorded the ability of Fipronil in reducing ticks up to 76% following 9–17 weeks of treatment.

At the chemical structure level, Doramectin (Dectomax®) is a macrocyclic lactone that acts as reported by Page (2018) by modulating chloride ion channel activity in the nervous system of nematodes and arthropods. Macrocyclic lactones bind to receptors that increase membrane permeability to chloride ions. This inhibits the electrical activity of nerve cells in nematodes and muscle cells in arthropods and causes paralysis and the death of the parasites. Fipronil 50 mg/ml (Bars®) is based on its chemical structure of Fipronil, diflubenzuron, and dicarboximide. Fipronil, according to Bonmatin et al. (2015), causes blocking of the GABA-dependent receptors of ectoparasites, impairing the transmission of nerve impulses, which leads to paralysis and death of ectoparasites. Diflubenzuron, as reported by Sankar and Kumar (2021), acts by inhibiting the synthesis of chitin in parasites and disrupting the molting, egg-laying, and hatchery process, which leads to an end to the replenishment of the population. Dicarboximide is a synergist and is used with insecticides to increase their activity. Dicarboximide, as reported by d’Errico et al. (2017), stops microsomal detoxification of the insecticide, increasing its toxicity for the parasite.

The extensive use of chemical insecticides lead to resistance against these compounds in many of the arthropods and external parasites, and that is why new lines of insecticides had to be developed. This resistance is multifactorial and usually can be developed by several means through behavioral, biochemical, and metabolic ways (Cossío-Bayúgar et al., 2018; Thangam and Kathiresan, 2021). That is why molecular markers have been developed to target the nucleotide sequence and genes responsible for this resistance as sodium channels, acetylcholinesterase,
carboxylesterase, β-adrenergic octopamine receptor, and octopamine–tyramine in the insect as reported by Kumar (2019) and Aguilar et al. (2018).

Herbal insecticides and acaricides include pyrethroids, neem, and various essential oils, as well as newly based insecticides, such as phenylpyrazole–garlic–camphor mix (Safeline®) that was used in our experiment. Although the herbal insecticide delayed in achieving 100% killing efficacy compared to the other two chemical insecticides, its safety is higher compared

### Table 3. Liver and kidney functions (mean ± SE) of infested dogs treated with different experimented insecticides.

<table>
<thead>
<tr>
<th>Insecticides /time</th>
<th>TP g/dl</th>
<th>ALT IU/l</th>
<th>UREA mg/dl</th>
<th>CREAT Mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall means concerning the experimented insecticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dectomax®</td>
<td>7.9ab ± 0.192</td>
<td>29.3± 0.464</td>
<td>32.6± 0.451</td>
<td>1.32ab ± 0.053</td>
</tr>
<tr>
<td>Bars®</td>
<td>5.9± 0.168</td>
<td>30.9± 0.004</td>
<td>31.4± 0.054</td>
<td>0.64ab ± 0.015</td>
</tr>
<tr>
<td>Safeline®</td>
<td>8.1± 0.169</td>
<td>28.8± 0.055</td>
<td>21.0± 0.314</td>
<td>1.77± 0.071</td>
</tr>
<tr>
<td>Control</td>
<td>3.8± 0.657</td>
<td>20.6± 0.168</td>
<td>16.4± 0.219</td>
<td>0.37± 0.026</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.011</td>
</tr>
<tr>
<td>Overall means concerning the sampling time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>12.2± 0.148</td>
<td>44.6± 0.579</td>
<td>38.4± 0.073</td>
<td>2.41± 0.056</td>
</tr>
<tr>
<td>P₁</td>
<td>3.3± 0.476</td>
<td>19.5± 0.067</td>
<td>19.6± 0.117</td>
<td>0.35± 0.023</td>
</tr>
<tr>
<td>P₂</td>
<td>3.8± 0.040</td>
<td>18.2± 0.053</td>
<td>18.1± 0.132</td>
<td>0.32± 0.024</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Experimented insecticides by sampling time interactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dectomax®</td>
<td>Zero</td>
<td>16.4± 0.195</td>
<td>50.8± 0.039</td>
<td>53.6± 0.015</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>3.3± 0.059</td>
<td>20.6± 0.070</td>
<td>23.3± 0.002</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>4.0± 0.051</td>
<td>16.4± 0.002</td>
<td>20.9± 0.190</td>
</tr>
<tr>
<td>Bars®</td>
<td>Zero</td>
<td>13.1± 0.159</td>
<td>51.8± 0.043</td>
<td>53.2± 0.365</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>3.8± 0.070</td>
<td>21.2± 0.023</td>
<td>19.2± 0.248</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>4.9± 0.125</td>
<td>19.8± 0.309</td>
<td>21.7± 0.364</td>
</tr>
<tr>
<td>Safeline®</td>
<td>Zero</td>
<td>15.4± 0.161</td>
<td>54.8± 0.219</td>
<td>40.4± 0.370</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>4.7± 0.136</td>
<td>15.2± 0.048</td>
<td>13.9± 0.036</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>4.9± 0.085</td>
<td>16.4± 0.044</td>
<td>8.9± 0.040</td>
</tr>
<tr>
<td>Control</td>
<td>Zero</td>
<td>4.0± 0.020</td>
<td>20.8± 0.034</td>
<td>19.3± 0.171</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
<td>3.3± 0.059</td>
<td>20.8± 0.048</td>
<td>21.9± 0.042</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
<td>4.0± 0.051</td>
<td>20.3± 0.092</td>
<td>21.0± 0.038</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same column are significantly different at $p \leq 0.05$ or highly significantly different at $p < 0.01$. Means with the same superscripts in the same column are non-significantly different at $p < 0.05$.

TP: Total protein; ALT: Alanine aminotransferase; CREAT: Creatinine; SE: Standard error.
to other insecticides. Khare et al. (2019) revealed that the phenylpyrazole–garlic–camphor mix (Safeline®) disrupts the insect central nervous system by blocking GABA-gated chloride channels and GluCl channels. This causes hyperexcitation of contaminated insects' nerves and muscles. Garlic and camphor oils reported by Krishnananda et al. (2017) and Shaji et al. (2017) inhibit the release of acetylcholinesterase which is essential for insects for their activity and synaptic transmission and act on octopamine (circulating neuromodulator) causing disruption and a complete breakdown of the nervous system in insects, and they are hydrophobic and cause water stress in insects by blocking the spiracles resulting in suffocation and distressing the cuticular waxes. Doramectin (Dectomax®) is a semisynthetic avermectin, and when administered repetitively, it can diffuse to tissues and contribute to mild toxicity, hepatic toxicity, renal toxicity, and central nervous system depression (Zhang et al., 2016). Usually, it can be deposited in adipose tissue, interfere with hepatocellular activity causing lipid peroxidation and degradation, and suppress the glutathione enzymatic system activity, contributing to the suppression of proliferating cell nuclear antigen (Venkateswarlu et al., 2017). That is why the use of such synthetic insecticides should not be repeated without cause. Care also should be given to the dose and concentration used, whether it is used for prophylaxis or treatment. Doramectin (Dectomax®) is usually recommended in monthly doses for prophylaxis and one dose for treatment as reported by Foy et al. (2019). It is also recommended to use avermectins by injection route to avoid stomach upset and vomiting (Barrett et al., 2016). The in-vivo-tested insecticides were applied as recommended by the manufacturer to the infested

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Table 4. GLUCO and lipid profile overall means (mean ± SE) of infested dogs treated with different experimented insecticides.

<table>
<thead>
<tr>
<th>Insecticides /time</th>
<th>Time</th>
<th>GLUCO mg/dl</th>
<th>TG mg/dl</th>
<th>TC mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall means concerning the experimented insecticides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dectomax®</td>
<td>154.9 ± 0.551</td>
<td>357.2 ± 0.066</td>
<td>159.4 ± 0.787</td>
<td></td>
</tr>
<tr>
<td>Bars®</td>
<td>162.2 ± 0.050</td>
<td>337.7 ± 0.247</td>
<td>162.3 ± 0.760</td>
<td></td>
</tr>
<tr>
<td>Safeline®</td>
<td>150.5 ± 0.021</td>
<td>301.2 ± 0.089</td>
<td>148.9 ± 0.601</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>116.1 ± 0.420</td>
<td>70.1 ± 0.166</td>
<td>121.6 ± 0.050</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td><strong>Overall means concerning the sampling time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>230.2 ± 0.097</td>
<td>659.1 ± 0.876</td>
<td>216.5 ± 0.046</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>105.7 ± 0.065</td>
<td>74.5 ± 0.543</td>
<td>118.4 ± 0.195</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>101.8 ± 0.046</td>
<td>66.0 ± 0.393</td>
<td>109.3 ± 0.498</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
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<td>0.000</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Experimented insecticides by sampling time interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dectomax®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>258.2 ± 0.082</td>
<td>930.1 ± 0.479</td>
<td>257.2 ± 0.056</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>110.9 ± 0.105</td>
<td>76.4 ± 0.830</td>
<td>118.3 ± 0.098</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>95.7 ± 0.035</td>
<td>65.0 ± 0.805</td>
<td>102.6 ± 0.200</td>
<td></td>
</tr>
<tr>
<td>Bars®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>279.6 ± 0.029</td>
<td>851.8 ± 0.820</td>
<td>243.5 ± 0.402</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>107.4 ± 0.074</td>
<td>83.9 ± 0.183</td>
<td>125.5 ± 0.049</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>99.6 ± 0.087</td>
<td>77.3 ± 0.492</td>
<td>117.9 ± 0.072</td>
<td></td>
</tr>
<tr>
<td>Safeline®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>273.0 ± 0.092</td>
<td>792.4 ± 0.691</td>
<td>239.6 ± 0.061</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>89.8 ± 0.010</td>
<td>62.2 ± 0.435</td>
<td>111.8 ± 0.052</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>88.6 ± 0.034</td>
<td>49.0 ± 0.541</td>
<td>95.4 ± 0.023</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>110.1 ± 0.061</td>
<td>62.2 ± 0.269</td>
<td>125.5 ± 0.094</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>114.8 ± 0.010</td>
<td>75.4 ± 0.076</td>
<td>117.9 ± 0.074</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>123.4 ± 0.076</td>
<td>72.6 ± 0.121</td>
<td>121.3 ± 0.059</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Means with different superscripts in the same column are significantly different at (p ≤ 0.05) or highly significantly different at p < 0.01. Means with the same superscripts in the same column are non-significantly different at p < 0.05.

TP: Total protein; ALT: Alanine aminotransferase; Creat: Creatinine; SE: Standard error.
dogs using different means of applications: injection in Doramectin (Dectomax®), pour-on in Fipronil 50 mg/ml (Bars®), and spraying in phenylpyrazole–garlic–camphor mix (Safeline®). Despite the differences in applications between the tested insecticides, they were able to act efficiently and maintain the physiological functions of the treated dogs instead of causing physiological alterations and disturbances. The problem with using the insecticides, in general, was their low impacts on the environmental components (Al-Awthan et al., 2012). Abbassy et al. (2014) and Ali et al. (2017) stated that many synthetic insecticides such as Doramectin (Dectomax®) and pour-on in Fipronil 50 mg/ml (Bars®) contributed to extensive influences and accumulation in some environmental components, as well biochemical and hormonal alterations in animals (Nasr et al., 2016). That is why the synthetic insecticides were recommended to be used once as in the current experiment despite infestation. Desai et al. (2016) recommended that prophylactic applications are not recommended by using chemical and organic insecticides. On the other hand, Mossa et al. (2017) reported that natural insecticides such as phenylpyrazole–garlic–camphor mix (Safeline®) in the current study are much recommended for animal treatment for maintaining animal physiological functions and their low impact on the environmental components.

The in-vivo results reveal significant improvements of hematological profile (RBCs, HCT, MCHC, PLT, and total and differential leukocytic counts), ESR in the second hour, biochemical profile (TP, CREAT, ALT, UREA, GLUCO, TG, and TC), and CORT levels of the 2-week (P1) and 4-week posttreatment (P2) samples in the infested dogs, considering that the evaluation was carried out for a month after the treatments. These results are in agreement with those of El-Naggar et al. (2017) who reported no adverse effects of the experimented bio-insecticides on the histopathological and physiological functions of liver, kidney, and cerebellum in male Albino mice. Marchiondo et al. (2013, 2019) also reported the necessity to assess the long-term effectiveness of the tested insecticide with intervals of 7–31 days. Segev et al. (2018) also advocate spot-on and injectable doramectin protocols as effective in treating 10 dogs from infestation with some internal and external parasites. Benelli et al. (2017b) recorded that doramectin was efficient and able to enforce good residual action for 63 days during which no external parasitism was noticed on animals. Wanji et al. (2017) reported a significant reduction in microfilaria count and improvement in hematological and biochemical parameters following treatment of external parasitic infestations using ivermectin. Benelli et al. (2016) reported the ability of some chemical insecticides as doramectin to treat animals infested with ticks with significant improvement of the hematological and biochemical profiles of the animals. Benelli et al. (2017a) and Nicoletti et al. (2016) experimented with neem, and Pavela and Benelli (2016) experimented with essential oils as a herbal eco-friendly insecticide related to the phenylpyrazole–garlic–camphor mix (Safeline®) used in our study and they found its actions to be superior to the ideal insecticides in eradication programs of tick infestations. The maintained levels of ALT posttreatment in the current experiment excluded any sign of the development of stress conditions in line with Ahmed et al. (2020) and Khalil and Samrah (2018) who recorded the possible prophylactic effect of insecticides like ivermectin when used in a single dose. They also recommended the use of some herbal eco-friendly alternatives to avoid the alterations in the physiological functions that might occur after treatment.

Conclusion

The study revealed the significant in-vitro efficacy of chemicals like Doramectin (Dectomax®) and Fipronil 50 mg/ml (Bars®), and herbal eco-friendly insecticides like the phenylpyrazole–garlic–camphor mix (Safeline®) against R. sanguineus with 100% killing efficacy that was achieved after 8 hours in both Doramectin (Dectomax®) and Fipronil 50 mg/ml (Bars®) and 24 hours in phenylpyrazole–garlic–camphor mix (Safeline®).
The insecticidal treatments for infested dogs achieved significant improvements in hematological parameters like RBCs, HCT, MCHC, total and differential leukocytic counts, and ESR, as well biochemical and hormonal profiles, like TP, CREAT, ALT, UREA, GLUCO, TG, TC, and CORT levels in the 2-week (P.) and 4-week posttreatment (P.) samples in Doramectin (Dectomax\textsuperscript{®}), Fipronil 50 mg/ml (Bars\textsuperscript{®}), and phenylpyrazole–garlic–camphor mix (Safeline\textsuperscript{®})-treated dogs with more pronounced recovery in phenylpyrazole–garlic–camphor mix (Safeline\textsuperscript{®})-treated dogs.

Herbal insecticides like phenylpyrazole–garlic–camphor mix (Safeline\textsuperscript{®}) that are based on camphor oil and garlic and according to the current results might be recommended with a higher incidence for prophylaxis and treatment against external parasites’ infestations for their natural contents that provide extended actions, safe use, lower impact on the environment, effective actions, and lower toxicity to the animals compared to the chemical insecticides like Doramectin (Dectomax\textsuperscript{®}), Macroyclic lactones and Fipronil 50 mg/ml (Bars\textsuperscript{®}), a mix of Fipronil, diflubenzuron, and dicarboximide.

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Conflict of interest

The authors declare that they have no financial or personal conflicts which may have inappropriately influenced them in writing this manuscript.

Authors’ contributions

ESS designed the in-vitro and in-vivo experimental designs, executed the experiment, conducted biochemical analysis, and took a part in writing the manuscript. EMA participated in conducting the in-vivo experiment, conducted hematological analysis, and took a part in writing the manuscript. EMA supervised the tick collection used for both in-vitro and in-vivo experiments, induced the experimental infestation of dogs, and took a part in writing the manuscript. MAS directed the team and group work, supervised the experiments, and took a part in writing the manuscript.

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