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Insecticidal efficacy and safety of Phoxim and influence on hematological, biochemical, and antioxidant profiles in German Shepherd dogs

Mamdouh M. El-Maghraby[1], Ahmed E. Mahmoud[1], Dina A. Abdelkhalek[1], Nahla H. Sallam[2], Abdelfattah M. Ali[3], Aliaa A. Abd-Elaziz[3] and Essam S. Soliman[4*]

[1]*Internal Medicine Division, Department of Animal Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*
[3]*Department of Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*
[4]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*

Abstract

Background: Dogs' health and welfare enhancement can be achieved using some prophylactics and immunization go with strict hygienic and optimum biosecurity measures.

Aim: Exploration of the insecticidal action of Phoxim® for combating *Rhipicephalus sanguineus* infestation in dogs and its prophylactic influences on the blood indices, biochemistry, antioxidant enzymes, and cortisol hormone in healthy and infested dogs.

Methods: Twenty German Shepherd male dogs at 1 year old and 44.0 kg were divided randomly into four groups of five dogs in four separate Kennels with optimum biosecurity measures. The 1st group (G1) was artificially infested with *R. sanguineus* and treated with Phoxim®, the 2nd (G2) was non-infested and treated with Phoxim®, the 3rd (G3) was infested with *R. sanguineus* and not treated (positive control), and the 4th (G4) was accounted as negative control (non-infested and non-treated). A total of 160 (80 whole blood and 80 sera) samples were collected.

Results: Parasitological examination revealed prominent characteristic features of *R. sanguineus* such as a distinct anal groove, the basis capitulum is hexagonal and lateral, the palpi are short, the second segment of the palpi as long as wide and not produced laterally, and the spiracular plate is comma-shaped and consists of stigma, peritreme, and tail. The results conveyed highly significant ($p < 0.01$) enhancement in erythrocytes, leukocytes, hematohiston, hematocrit, hemoglobin centering, granulocytes, alanine aminotransferase, random blood sugars, triglycerides, and total cholesterol, and highly significant ($p < 0.01$) declines of all measured antioxidant enzymes in treated non-infested dogs.

Conclusion: Phoxim® proved efficient insecticidal activity with optimum safety and can be brought into play in the prophylactic biosecurity measures installed to eradicate external parasitism in dogs.

Keywords: Biosecurity, Dogs, Phoxim®, Prophylactic efficacy, *Rhipicephalus sanguineus*.

Introduction

Dogs have been exposed to many disease attacks even with their owner's protection (De Leeuw, 2003). The maximum safety of dogs on small and large scales is a must for maintaining their health and welfare. Many breeders are less aware of the proper management and biosecurity measures that must be taken for maintaining the prosperity of dogs (Moore, 2003). A good keeping record has to be fulfilled with all treatments, diseases control measures, and cleaning routines took. Advancement from using some prophylactics and immunization can be achieved if accompanied by strict hygienic and optimum biosecurity measures. Biosecurity is the total measures enforced to prevent exposure to disease-causing agents and it was divided into external biosecurity (bio-extinction) to limit the entrance of the infective agents (Villarroel *et al*., 2007) and internal biosecurity (biocontainment) to prevent distribution and reoccurrence of diseases (Dendoncker *et al*., 2018). The biosecurity measures to be taken in dog houses to combat external parasitism are adapting proper housing design, sealing the cracks in walls and floors with cement, optimum in-door practices, rotational positioning to discourage the larval microclimate, routine animal cleaning, and applying prophylactic/treating agent, i.e., chemical, biological, or herbal (Cochi *et al*., 1998).

Ectoparasites are organisms that populate the external body surface of other organisms contributing to extensive harmful influences (Zendehfili *et al*., 2015), great economic losses (Tong *et al*., 2019), and transmission

***Corresponding Author:** Essam S. Soliman. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Email: *soliman.essam@vet.suez.eu.eg*

of bacterial, protozoal, and viral microorganisms (Mansour *et al*., 2017). The transmission of these microorganisms is carried out by mechanical means as in *Corynebacteria*, *Staphylococcus*, or *Streptococcus* (Kwak *et al*., 2021), or by biological means as in *Babesia*, *Thileria*, *Trypanosoma*, and *Pasteurella pestis* (Apanaskevich *et al*., 2020). Ticks are hematophagous ectoparasites that have an extended host range and wide geographic habitats, they transmit protozoan, bacterial, rickettsia, and viral pathogens contributing to debilitating/fatal diseases (Adenubi *et al*., 2016). Ticks are subclassified into *Argasidae* or soft ticks and *Ixodidae* or hard ticks. Hard ticks are the most dangerous as they feed on their hosts for days to weeks depending on the growing stage, animal species, and types of ticks (Rajput *et al*., 2006). Ticks can transmit some animal infectious and human zoonotic diseases.

Insecticides are chemical, biological, or herbal substances that are designed to kill ectoparasites by interfering with the nervous system via exerting several actions such as acetylcholinesterase inhibitors, sodium channel modulators or blockers, inhibitors of mitochondrial ATP synthase, and inhibitors of chitin biosynthesis (Metcalf, 2002). Phoxim® is a spray-effective and widely used organophosphate insecticide against sorbent and sucking lice, sheep and cattle ticks, flies, ticks, and fly larvae present in wounds, and three scabies insects in a single dose of 10 ml/10 l of water (WHO, 2000). WHO committee established a dose of 0–4 µg/kg body weight from Phoxim® concerning their influence on the liver and brain. Phoxim® of a 0.1% aqueous has proven high efficiency in reducing the prevalence of fleas (Larsen *et al*., 2018) and chicken mite infestations (Meyer-Kühling *et al*., 2007).

We aimed in the current experimental design to determine the insecticidal action of Phoxim® for combating and eradicating *Rhipicephalus sanguineus* canine infestation. The study also investigated the prophylactic and treatment influences of Phoxim® on the blood indices, biochemistry, antioxidant enzymes, and cortisol hormone in healthy and infested dogs.

Material and Methods

Study location and duration

The experiment was conducted in dog kennels installed at the educational farm in the Faculty of Veterinary Medicine, Suez Canal University. The study was structured to last for 3 months from December 1st, 2021 to February 28th, 2022. The experimental duration was partitioned into three consecutive stages of four weeks, the 1st stage for dog housing, accommodation, and habituation, the 2nd stage for experimental infestation with *R. sanguineus* ticks, and the 3rd stage was assigned for insecticidal treatment, sampling, and animals' recovery under observation.

The biochemistry assessment was conveyed in the Veterinary Public Health Laboratory at the Faculty of Veterinary Medicine, Suez Canal University. Blood indices and antioxidant enzymes were assessed in the Clinical Pathology Laboratories at the University Hospital.

Phoxim® insecticide

A commercial form of Phoxim® was acquired from a clinical veterinary pharmacy in Ismailia governorate, Egypt. It is recommended to treat ectoparasitic infestations of cattle, sheep, goats, and pigs. Phoxim® is an organophosphate insecticide that is composed of Diethoxy-trio-phosphoryl-oxyimino-phenyl-acetor ($C10H15N_2O_3PS$, Fig. 1) with a molecular weight up to 298.30 and melting point of 6.1°C. It was designed to act as a cholinesterases inhibitor causing cessation of the acetylcholine at the cholinergic synapses (Wang *et al*., 2010). Phoxim® can contribute to the pooling of acetylcholine up to toxic levels contributing to the overexcitation of nerve cells, parasympathetic, and neuromuscular systems. It also might contribute to neuropathy by inhibiting target esterase and aging of the esterase enzyme. Usually, after exerting its action, it is excreted in urine and feces. Phoxim® can act as

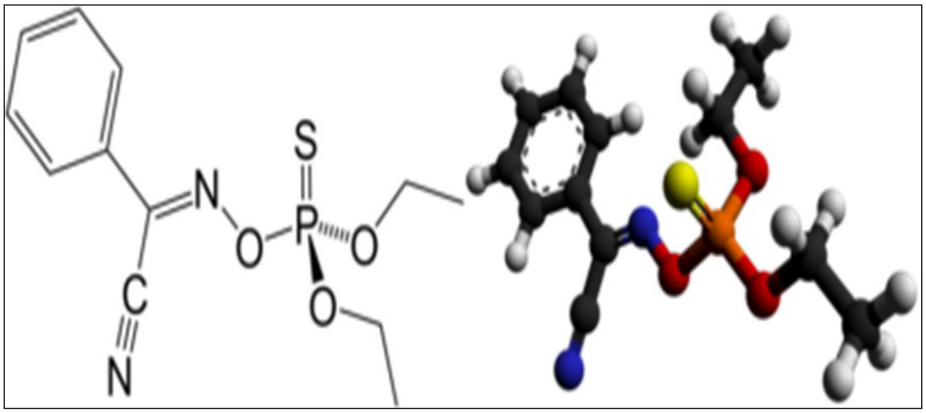

Fig. 1. The molecular structure and 3D shape of the commercial Phoxim®.

contact, stomach, and fumigant poison (Taktak *et al*., 2021).

Experimental dogs' housing microclimate

Kennels were provided with as much as biosecurity measures to control and minimize the entrance and spread of pathogens after Soliman and Abdallah (2020). The external and internal biosecurity measures were foot dips of crude carbolic acid 5%, fly-proof nets, clean feeding porcelain dishes, clean watering bowls, secured feed storage area, mechanical control of rodents using baited wire traps, and a proper drainage system. The Kennels were supplied with white LED lights following Soliman and Hassan (2019). The floors were regularly washed and disinfected in sequence with hypochlorite 3%, quaternary ammonium compounds, and glutaraldehyde. The gutter was sprinkled with slaked lime from time to time to maintain the optimum microclimatic conditions after Soliman *et al*. (2018). The Kennels were ventilated with a mixed design of natural (V-shaped side wall windows) and artificial means (ceiling fans and sidewall suction fans) to encourage cross-sectional air convection (Stack effect).

Experimental animals' management

Twenty German Shepherd male dogs at one year old and 44.0 kg body weight were picked up from a pet shop in Ismailia governorate, Egypt. All the picked-up dogs were nearly similar to overcome the individual biological variation in response to the Phoxim® treatment (Taktak et al., 2021). Dogs were divided randomly on their arrival into four groups of five dogs in four separate Kennels. The Kennels were adjusted at 37°C with oil heaters (Oil—11 blades—2,500 W heater) after Soliman *et al.* (2021).

Dogs were provided *ad libitum* access to dechlorinated tap water previously tested for residual chlorine using an orthotolidine test. Dogs were fed twice (in the morning and evening) daily to eliminate the influence of overwhelming stress. The morning meal consisted of a homogenous mix of well-done chicken, bread, liver, rice, minced meat, and pasta, while the evening meal contained ½ to ¾ kg of dry food with 23% crude protein. Dogs were dewormed on their first arrival with praziquantel and fenbendazole to ensure the complete freedom of internal parasites that might contribute to overwhelming stressful conditions. Dogs were also vaccinated subcutaneously in the back of the neck with two doses of Vanguard® Plus 5 DHLPPC viral vaccine (Zeotis® US) with an interval of 21 days between the two shots against canine adenovirus type I and II, canine distemper, parainfluenza, canine hepatitis, leptospirosis, canine parvovirus, and coronavirus, and rabies.

Study design

Experimentally challenged animals were divided in a completely randomized pattern into four groups of five dogs in four separate Kennels. The groups were assigned for treatments as (Fig. 2) as follows: the 1st group (G1) was artificially infested with *R. sanguineus* and treated with Phoxim®, the 2nd group (G2) was non-infested and treated with Phoxim®, the 3rd group (G3) was infested with *R. sanguineus* and did not receive any treatment (positive control), and the 4th group (G4) was kept non-infested and non-treated (negative control).

Experimental infestation of dogs

Rhipicephalus sanguineus ticks were aseptically handpicked from field cases admitted to veterinary clinics in Ismailia-Egypt by anticlockwise orientation technique until the capitulum was detached undamaged from the hosts with forceps into sterilized screw-capped bottles and transferred to the laboratory. The female ticks were identified by the morphological characteristics key after Walker *et al.* (2003) including scutum formation, capitulum, and leg coloration using light microscopy (Barska® AY13180 Binocular Stereo Microscope, 10x), and incubated at microclimatic temperature and humidity of up to 25°C and 75%, respectively. *Rhipicephalus sanguineus* ticks were used as prescribed by Aboelela *et al*. (2022b) to produce artificial infestation using 6 females per dog in two experimental groups (G1; infested and treated with Phoxim® and G3; infested and did not receive any treatment i.e. positive control) at the beginning of the 2nd stage. The infested dogs were regularly observed until ensuring the manifestation signs through the development of new stages on the animal's skin. The infesting ticks were sampled in sterile screw-capped bottles for light microscopical (10x) confirmation.

Parasitological examination

Ticks were collected by brushing over the different parts of the dog's body into screw-capped bottles containing an appropriate amount of 70% alcohol. Sample preparations were conveyed as recommended by Farid *et al*. (2021a,b). Ticks were subjected to installments such as being immersed into sodium hydroxide (NaOH 10%), washed in double distilled water, and dehydrated using ethyl alcohol. The processed tick samples were immersed into xylene, mounted onto the slides, and covered. The slides were dried at 40-50°C for 24 h in a hot oven (Daihan® LabTech® Hot air Oven, LDO-080N, Indonesia) and examined using a light microscope (Barska® AY13180 Binocular Stereo Microscope 10x magnification). Tick identification was carried out according to Walker *et al*. (2003).

Sampling

A total of 160 samples (4 sampling times × 20 dogs × 2 types of samples) including 80 whole blood on EDTA and 80 sera were collected by the end of the experiment. The samples were aseptically transferred to the laboratory into a dry-ice box.

Whole blood samples (n = 80) were collected on EDTA-vacutainers and used for the blood indices examinations. Sera samples (n = 80) on plain serum tubes were held at 25°C for 30 minutes in a water bath (Thermo® water bath Precision series Standard, 20°C, 30°C to 100°C, 392 mm, GP20) and centrifuged

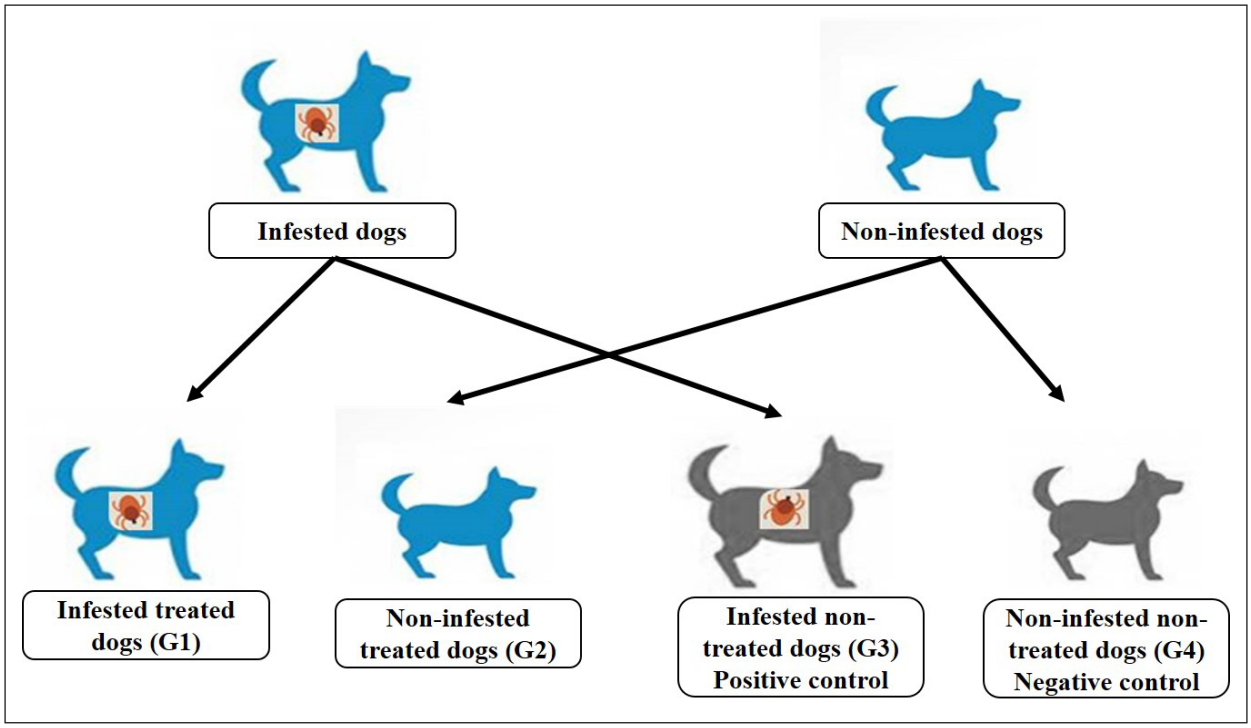

Fig. 2. Experimental design showing the grouping of the experimental dogs into dogs infested with *R. sanguineus* and received Phoxim® treatment (G1), Non-infested dogs who received Phoxim® treatment (G2), Dogs infested with *R. sanguineus* with no treatment (Positive control, G3), and Non-infested and non-treated dogs (Negative control, G4).

(Fisher®Thermo Scientific CL10 Centrifuge w/ F-G3 Rotor with a max RPM of 4000) at 3,000 rpm for 20 minutes. Clear non-hemolytic sera were pipetted using an automatic pipette (Fisherbrand®, variable 200: 1,000 micron) into 2.5 ml Eppendorf tubes and stored at −20°C for biochemical and antioxidant assessment as recommended by Soliman *et al*. (2017).

Hematological profile

Whole blood samples (n = 80) were examined for erythrocytic count (RBCs, $\times 10^6$/µl), hematohiston (Hb, g/dl), hematocrit (PCV, %), mean corpuscular volume (MCV, fl), hemoglobin centering (MCH, pg), hemoglobin consolidation (MCHC, g%), platelet counts (PLT, $\times 10^3$/µl), leukocytic count (WBCs, $\times 10^3$/µl), neutrophils (N, %), lymphocytes (L, %), monocytes (M, %), eosinophil (E, %), and basophils (B, %) using Sysmex XP-300 Automated Hematology Analyzer.

Biochemical, antioxidant, and hormonal profiles

Sera samples (n = 80) were examined for total protein (TP, g/dl), alanine aminotransferase (ALT, IU/L), glucose (GLUCO, mg/dl), total cholesterol (TC, mg/dl), triglycerides (TG, mg/dl), glutathione total (GSH, µmol/l), glutathione peroxidase (GHPx, U/ml), malondialdehyde (MDA, nmol/ml), catalase (CAT, MU/L), superoxide dismutase (SOD, U/ml), and total antioxidant capacity (TAC, U/ml) calorimetrically using H144211 ROCHE COBAS Integra 800 chemical analyzer. Cortisol hormone (CORT, mcg/dl) was measured by using ROCHE Elecsys 1010 Immunoassay Analyzer (Wu *et al*., 2017).

Statistical analysis

The statistical analysis was run using a statistical package for social sciences version 20 IBM Corp (SPSS, 2016 - IBM SPSS Statistics 20). The data were analyzed with a Two-tailed multifactorial Analysis of Variance (Two-tailed analysis of variance) to estimate the influence of Phoxim® as a treating and prophylactic agent on infested and non-infested dogs concerning different sampling times. The statistical model empathized as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \mathcal{E}_{ijk}$$

Where Y_{ijk} was the measurement of dependent variables; μ was the overall mean; α_i was the fixed effect of the Phoxim® treatment, β_j was the fixed effect of sampling time, $(\alpha\beta)_{ij}$ was the interactions of Phoxim® treatment by sampling time, and $\mathcal{E}_{ijk}$ was the random error. The results were expressed as highly significant at ($p \leq 0.01$), significant at ($p \leq 0.05$), and non-significant at ($p > 0.05$).

Ethical approval

The experimental design, study protocol, guidance on authorship, transparency, and ensuring accuracy away from potential complications were approved by the Scientific Research Ethics Committee at the Faculty of Veterinary Medicine, Suez Canal University, Egypt

with approval number (2022008). The experimental German Shepherd dogs were subjected to infestation with all the precautions required and provided the animals with human management and approach. The samples were collected with minimum pain during collection using all the necessary precautions.

Results

Parasitological examination

The parasitological examination revealed *R. sanguineus* ticks (The brown dog tick, *Rhipicephalus sanguineus* Latreille, 1806) (Nava et al., 2015) are characterized where by a distinct anal groove (Fig. 3). Male has adenal and accessory adenal shields and caudal process. The basis capitulum is hexagonal and laterally produced. Palpi are short. Mouthparts as long as basis capitulum. The second segment of the palpi is as long and wide and is not produced laterally and the festoons are present. The spiracular plate is comma-shaped and consists of a stigma, peritreme, and tail.

Hematological assessment

The erythrocyte count, hematohiston, and hematocrit disclosed (Table 1) highly significant ($p < 0.01$) increases in treated non-infested dogs (G2) and highly significant ($p < 0.01$) declines in both G1 and G3 dogs. Mean corpuscular volume revealed a highly significant ($p < 0.01$) improvement in the G1 with no significant differences between the positive control (G3) and negative control (G4). Hemoglobin centering and hemoglobin consolidation recorded (Table 1) highly significant ($p < 0.01$) declines in G1, G2, and G3 compared to the G4 concerning the G2; the treated non-infested group showed highly significant ($p < 0.01$) increases compared to other groups.

Platelet count illustrated (Table 2) highly significant ($p < 0.01$) increases in the G2 dogs compared to the other groups. Meanwhile, leukocytes (Table 2) recorded highly significant ($p < 0.01$) improvement in G2 dogs compared to the other groups. Neutrophils and basophils (Table 3) disclosed highly significant ($p < 0.01$) increases in the G2 dogs compared to other groups. Agranulocytes revealed highly significant ($p < 0.01$) declines in all groups.

The sampling time revealed no significant differences (Table 1) in erythrocytes, hematohiston, hematocrit, mean corpuscular volume, hemoglobin centering, and hemoglobin consolidation between all the treated and non-treated groups. On the other hand, platelets (Table 2) recorded highly significant ($p < 0.01$) increases on the 4th-day post-treatment and highly significant ($p < 0.01$) declines in leukocytes (Table 2) and differential counts (Table 3) post-treatment.

Biochemical assessment

Phoxim® treatment in both infested and non-infested animals (Table 4) illustrated highly significant ($p < 0.01$) improvement of all assessed biochemical parameters concerning the higher improvement of alanine aminotransferase, glucose, triglycerides, and total cholesterol in G2 (treated non-infested) dogs compared to other animals.

The time scale revealed highly significant ($p < 0.01$, Table 4) declines in alanine aminotransferase, glucose, and triglycerides on the 7th-day post-treatment and total protein and total cholesterol on the 4th-day post-treatment.

Antioxidant profile and hormonal stress marker

The glutathione total, glutathione peroxidase, malondialdehyde, catalase, superoxide dismutase, and total antioxidant capacity (Table 5) recorded highly

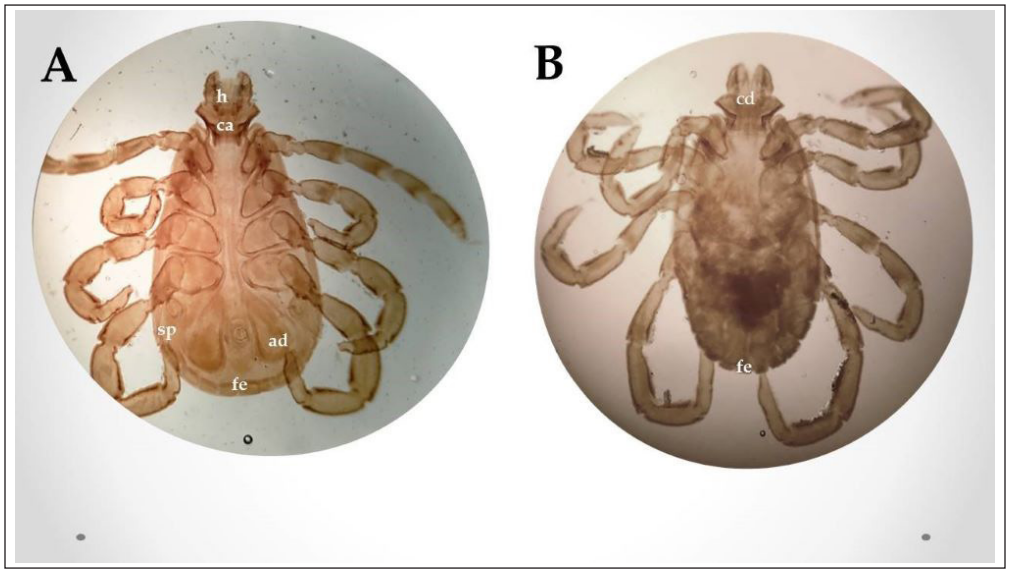

Fig. 3. *Rhipicephalus sanguineus*, **(A)** ventral view; **(B)** dorsal view. (Sp): Spicular plate, (fe): festoon, (ca): capituli, (ad): adanal shield, (h): hypostome.

Table 1. Corpuscular indices (Mean ±SE) of Phoxim® treated and untreated dog groups.

Groups	Time (days)	RBCs (×10⁶/µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g%)
Overall means concerning the experimented insecticides							
G1		3.8^{d}±0.08	8.1^{c}±0.01	29.6^{c}±0.03	76.5^{a}±0.06	21.1^{b}±0.03	27.6^{c}±0.04
G2		7.3^{a}±0.14	13.4^{a}±0.15	44.3^{a}±0.02	60.9^{b}±0.04	18.4^{c}±0.02	30.2^{b}±0.02
G3		4.3^{c}±0.17	8.5^{c}±0.01	30.4^{c}±0.02	71.1^{a}±0.02	20.0^{bc}±0.09	28.2^{c}±0.03
G4		4.9^{b}±0.14	11.9^{b}±0.04	35.0^{b}±0.01	71.3^{a}±0.08	24.2^{a}±0.07	33.0^{a}±0.02
p-value		0.000	0.000	0.001	0.002	0.000	0.000
Overall means concerning the sampling time							
Zero		5.1^{a}±0.37	10.5^{a}±0.06	34.7^{a}±0.01	69.4^{a}±0.02	20.8^{a}±0.08	29.8^{a}±0.06
2 days		5.1^{a}±0.43	10.6^{a}±0.04	34.8^{a}±0.07	69.9^{a}±0.03	21.0^{a}±0.09	29.8^{a}±0.07
4 days		5.0^{a}±0.42	10.4^{a}±0.08	34.9^{a}±0.07	71.3^{a}±0.02	21.3^{a}±0.08	29.7^{a}±0.07
7 days		5.2^{a}±0.44	10.8^{a}±0.06	34.8^{a}±0.07	69.2^{a}±0.07	20.6^{a}±0.04	29.6^{a}±0.08
p-value		0.892	0.994	0.987	0.905	0.919	0.982
Experimented insecticides by sampling time interactions							
G1	Zero	4.1^{a}±0.03	8.4^{a}±0.01	29.9^{a}±0.05	71.6^{a}±0.09	20.1^{a}±0.08	28.1^{a}±0.02
	2 days	3.9^{a}±0.14	8.3^{a}±0.02	29.8^{a}±0.03	75.5^{a}±0.12	20.9^{a}±0.02	27.9^{a}±0.03
	4 days	3.7^{ab}±0.15	8.1^{a}±0.06	29.5^{a}±0.08	79.2^{a}±0.08	21.8^{a}±0.08	27.5^{a}±0.06
	7 days	3.6^{b}±0.16	7.8^{a}±0.02	29.2^{a}±0.09	79.7^{a}±0.01	21.4^{a}±0.02	26.9^{a}±0.06
G2	Zero	7.1^{a}±0.18	13.4^{a}±0.03	44.2^{a}±0.03	62.0^{a}±0.12	18.8^{a}±0.02	30.3^{a}±0.06
	2 days	7.3^{a}±0.33	13.3^{a}±0.08	44.3^{a}±0.08	60.5^{a}±0.06	18.1^{a}±0.02	30.0^{a}±0.04
	4 days	7.2^{a}±0.43	13.5^{a}±0.09	44.4^{a}±0.03	61.8^{a}±0.03	18.7^{a}±0.06	30.4^{a}±0.01
	7 days	7.5^{a}±0.28	13.3^{a}±0.03	44.3^{a}±0.06	59.1^{a}±0.17	17.7^{a}±0.077	30.0^{a}±0.05
G3	Zero	4.2^{a}±0.32	8.3^{a}±0.17	29.9^{a}±0.05	71.5^{a}±0.04	20.0^{a}±0.02	28.0^{a}±0.01
	2 days	4.2^{a}±0.35	8.5^{a}±0.13	30.2^{a}±0.08	73.4^{a}±0.03	20.7^{a}±0.08	28.2^{a}±0.02
	4 days	4.3^{a}±0.33	8.6^{a}±0.03	30.7^{a}±0.06	71.8^{a}±0.02	20.2^{a}±0.08	28.1^{a}±0.05
	7 days	4.6^{a}±0.31	8.7^{a}±0.05	30.8^{a}±0.04	67.6^{a}±0.05	19.2^{a}±0.02	28.3^{a}±0.04
G4	Zero	4.8^{a}±0.27	11.8^{a}±0.03	35.0^{a}±0.04	72.3^{a}±0.03	24.5^{a}±0.05	32.9^{a}±0.04
	2 days	5.0^{a}±0.38	11.9^{a}±0.12	35.0^{a}±0.01	70.2^{a}±0.04	24.0^{a}±0.09	33.2^{a}±0.06
	4 days	4.8^{a}±0.27	11.8^{a}±0.03	34.9^{a}±0.04	72.3^{a}±0.04	24.5^{a}±0.05	32.9^{a}±0.04
	7 days	5.0^{a}±0.38	11.9^{a}±0.12	35.3^{a}±0.02	70.2^{a}±0.07	24.0^{a}±0.09	33.2^{a}±0.06
p-value		0.953	0.936	0.983	0.954	0.997	0.995

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). (G1): Dogs infested with *R. sanguineus* and received Phoxim® treatment; (G2): No-infested dogs received Phoxim® treatment; (G3): Dogs infested with *R. sanguineus* with no treatment (positive control); (G4): Non-infested and non-treated dogs (Negative control); (RBCs): Red blood cells; (HB): Hematohiston; (PCV): Packed cell volume; (MCV): Mean corpuscular volume; (MCH): Hemoglobin centering; (MCHC): Hemoglobin consolidation; (SE): Standard error.

significant ($p < 0.01$) declines in the G2 dogs compared to G1 and G3 animals.

Sampling times (Table 5) illustrated highly significant ($p < 0.01$) declines of glutathione total and total antioxidant capacity at the zero-time; glutathione peroxidase, catalase, and superoxide dismutase on the 2nd day; and malondialdehyde at the 7th-day post-treatment.

Cortisol stress markers (Fig. 4) illustrated highly significant ($p < 0.01$) declines in both G1 and G3 groups concerning the G1 compared to other groups. Sampling times showed no significant differences in all treated and non-treated groups.

Discussion

Ectoparasitic infestations in dogs are usually influenced and associated with numerous associations and

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Table 2. White blood cells and platelet counts (Mean ±SE) of Phoxim® treated and untreated dog groups.

Groups	*Time (days)*	*PLT (×10³/µl)*	*WBCs (×10³/µl)*
Overall means concerning the experimented insecticides			
G1		213.9^{c}±0.41	20.8^{a}±0.03
G2		286.0^{b}±0.47	12.1^{c}±0.08
G3		219.7^{c}±0.26	16.3^{b}±0.08
G4		419.0^{a}±0.46	11.2^{d}±0.01
p-value		0.001	0.000
Overall means concerning the sampling time			
Zero		282.0^{b}±0.41	15.8^{a}±0.02
2 days		285.7^{ab}±0.92	15.1^{ab}±0.01
4 days		283.6^{b}±0.09	15.0^{ab}±0.01
7 days		287.2^{a}±0.50	14.5^{b}±0.03
p-value		0.048	0.026
Experimented insecticides by sampling time interactions			
G1	Zero	215.0^{a}±0.00	19.6^{d}±0.08
	2 days	214.0^{a}±0.57	20.5^{c}±0.03
	4 days	213.6^{a}±0.33	21.1^{b}±0.05
	7 days	213.0^{a}±0.15	22.0^{a}±0.08
G2	Zero	285.0^{a}±0.15	12.2^{a}±0.02
	2 days	287.0^{a}±0.00	12.1^{a}±0.01
	4 days	286.3^{a}±0.88	12.3^{a}±0.01
	7 days	285.6^{a}±0.82	12.1^{a}±0.01
G3	Zero	215.0^{c}±0.15	19.6^{a}±0.01
	2 days	217.0^{c}±0.57	17.2^{b}±0.05
	4 days	221.6^{b}±0.88	15.2^{c}±0.05
	7 days	225.3^{a}±0.33	13.1^{d}±0.01
G4	Zero	413.0^{b}±0.00	11.6^{a}±0.01
	2 days	425.0^{a}±0.12	10.8^{b}±0.02
	4 days	413.0^{b}±0.00	11.6^{a}±0.01
	7 days	425.0^{a}±0.14	10.8^{b}±0.02
p-value		0.697	0.000

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). (G1): Dogs infested with *R. sanguineus* and received Phoxim® treatment; (G2): No-infested dogs received Phoxim® treatment; (G3): Dogs infested with *R. sanguineus* with no treatment (positive control); (G4): Non-infested and non-treated dogs (Negative control); (WBCs): White blood cells; (PLT): Platelets; (SE): Standard error.

determinants such as animal associates including breed, sex, age, body configurations, and coat color as revealed by Földvári *et al*. (2016); Kumsa *et al*. (2019); Aboelela *et al*. (2022a), macro and microclimatic associates such as geographical locations, seasonal variations, housing system, living pattern, and type of food as revealed by Kruse and Schuz (2016); Hasib *et al.* (2020); Minabaji *et al*. (2020), and agent associates including the type and characteristics of infesting parasites as revealed by Moravvej *et al*. (2015). Thus, control, elimination, and eradication strategies and plans for external parasites have been included in all biosecurity programs on pet animal houses, kennels, and shelters (Alho *et al*., 2017, 2018).

Pesticides are conventional in the control of agricultural pests and insects contributing to extensive ecosystem

Table 3. Differential leukocytic counts (mean ±SE) of Phoxim® treated and untreated dog groups.

Groups	Time (days)	N (%)	L (%)	M (%)	E (%)	B (%)
Overall means concerning the experimented insecticides						
G1		56.5^{c}±0.03	27.7^{b}±0.03	7.5^{c}±0.03	7.4^{a}±0.02	0.5^{ab}±0.01
G2		60.3^{a}±0.03	28.7^{b}±0.03	8.6^{b}±0.02	1.6^{d}±0.01	0.6^{a}±0.01
G3		58.1^{b}±0.05	28.7^{b}±0.02	8.4^{b}±0.02	4.2^{b}±0.05	0.6^{a}±0.00
G4		35.8^{d}±0.07	48.3^{a}±0.07	13.5^{a}±0.01	2.1^{c}±0.00	0.1^{b}±0.00
p-value		0.000	0.000	0.004	0.000	0.098
Overall means concerning the sampling time						
Zero		54.7^{a}±0.03	32.1^{a}±0.06	8.9^{b}±0.01	4.1^{a}±0.07	0.5^{a}±0.01
2 days		50.6^{b}±0.06	34.5^{a}±0.07	10.2^{a}±0.03	4.0^{a}±0.06	0.6^{a}±0.00
4 days		54.8^{a}±0.03	32.2^{a}±0.05	8.7^{b}±0.02	3.6^{b}±0.07	0.4^{a}±0.01
7 days		50.7^{b}±0.04	34.6^{a}±0.06	10.3^{a}±0.01	3.6^{b}±0.05	0.5^{a}±0.00
p-value		**0.017**	**0.721**	**0.036**	**0.016**	**0.902**
Experimented insecticides by sampling time interactions						
G1	Zero	57.1^{a}±0.06	28.0^{a}±0.05	8.2^{a}±0.05	6.5^{c}±0.08	0.6^{a}±0.01
	2 days	56.7^{a}±0.09	27.8^{a}±0.05	7.4^{b}±0.09	7.2^{b}±0.02	0.6^{a}±0.01
	4 days	56.6^{a}±0.06	27.8^{a}±0.04	7.3^{b}±0.03	7.8^{b}±0.02	0.3^{b}±0.00
	7 days	55.7^{a}±0.05	27.3^{a}±0.03	7.1^{b}±0.04	8.4^{a}±0.03	0.3^{b}±0.01
G2	Zero	60.5^{a}±0.02	28.5^{a}±0.06	8.7^{a}±0.04	1.6^{a}±0.02	0.6^{a}±0.01
	2 days	60.4^{a}±0.01	28.6^{a}±0.05	8.6^{a}±0.06	1.6^{a}±0.06	0.6^{a}±0.00
	4 days	60.3^{a}±0.03	28.7^{a}±0.07	8.6^{a}±0.08	1.6^{a}±0.03	0.6^{a}±0.00
	7 days	60.3^{a}±0.07	28.8^{a}±0.02	8.5^{a}±0.01	1.6^{a}±0.03	0.6^{a}±0.01
G3	Zero	57.3^{ab}±0.03	28.2^{a}±0.05	8.2^{a}±0.06	6.5^{a}±0.05	0.6^{a}±0.01
	2 days	57.6^{ab}±0.01	28.5^{a}±0.08	8.0^{a}±0.06	5.0^{b}±0.01	0.6^{a}±0.00
	4 days	58.3^{a}±0.06	28.9^{a}±0.04	8.5^{a}±0.03	3.2^{c}±0.02	0.6^{a}±0.00
	7 days	59.0^{a}±0.03	29.4^{a}±0.03	8.8^{a}±0.05	2.0^{d}±0.02	0.6^{a}±0.01
G4	Zero	44.0^{b}±0.00	43.6^{b}±0.06	10.3^{b}±0.03	2.0^{a}±0.00	0.0^{b}±0.00
	2 days	47.6^{a}±0.02	53.0^{a}±0.06	16.6^{a}±0.08	2.3^{a}±0.03	0.3^{a}±0.00
	4 days	44.0^{a}±0.00	43.6^{b}±0.06	10.3^{b}±0.05	2.0^{a}±0.00	0.0^{b}±0.00
	7 days	47.6^{b}±0.02	53.0^{a}±0.02	16.6^{a}±0.08	2.1^{a}±0.00	0.3^{a}±0.00
p-value		0.788	0.910	0.711	0.000	0.994

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$); (G1): Dogs infested with *R. sanguineus* and received Phoxim® treatment; (G2): No-infested dogs received Phoxim® treatment; (G3): Dogs infested with *R. sanguineus* with no treatment (positive control); (G4): Non-infested and non-treated dogs (Negative control); (N): Neutrophils; (L): Lymphocyte; (M): Monocytes; (E): Eosinophils; (B): Basophils; (SE): Standard error.

damage and public health concerns with toxicity that occurs with direct exposure to insecticides or the indirect consumption of contaminated feed and water (Hussein *et al*., 2012). Organophosphorus pesticides like Phoxim® recorded by Kalender *et al.* (2006) to suppress the activity of acetylcholinesterase and pseudocholinesterase with partial influences on immunity (Handy *et al*., 2002), liver (Kalender *et al*., 2005), and blood indices (Kalender *et al*., 2006). Chemical insecticides were and unfortunately still are the predominating tools for the eradication of insects and external parasites (Singh, 2010). The use of chemical insecticides has been subjected to evaluation for their numerous disadvantages such as non-specific targets, resistance, and residual actions (Saeed *et al*., 2016).

Organophosphorus pesticide toxicity is usually associated with their chemical composition (oxygen, sulfur, or phosphorus atoms) and the replacement of the sulfur with oxygen during the detoxication process

Table 4. Biochemical profile (Mean ±SE) of Phoxim® treated and untreated dog groups.

Groups	Time (days)	TP (g/dl)	ALT (IU/l)	GLUCO (mg/dl)	TG (mg/dl)	TC (mg/dl)
Overall means concerning the experimented insecticides						
G1		3.7^{c}±0.05	17.5^{c}±0.05	63.8^{b}±0.81	105.3^{c}±0.63	94.6^{c}±0.70
G2		3.3^{c}±0.06	19.0^{b}±0.08	74.2^{b}±0.17	118.5^{b}±0.15	102.7^{b}±0.82
G3		15.0^{a}±0.09	52.5^{a}±0.06	858.1^{a}±0.18	246.8^{a}±0.98	270.3^{a}±0.42
G4		5.8^{b}±0.06	17.6^{c}±0.01	64.2^{b}±0.04	104.1^{c}±0.81	94.4^{c}±0.74
p-value		0.000	0.001	0.000	0.000	0.001
Overall means concerning the sampling time						
Zero		6.8^{ab}±0.02	27.1^{a}±0.01	294.0^{a}±0.19	147.6^{a}±0.54	137.5^{b}±0.02
2 days		5.8^{ab}±0.05	28.2^{a}±0.01	288.0^{a}±0.09	149.7^{a}±0.85	144.1^{a}±0.47
4 days		5.6^{b}±0.02	27.4^{a}±0.02	256.1^{b}±0.15	141.4^{a}±0.52	138.5^{b}±0.58
7 days		7.5^{a}±0.05	27.0^{a}±0.07	222.2^{c}±0.63	136.0^{b}±0.54	141.8^{a}±0.28
p-value		0.035	0.288	0.017	0.014	0.025
Experimented insecticides by sampling time interactions						
G1	Zero	3.9^{a}±0.07	16.5^{b}±0.01	65.4^{b}±0.51	101.7^{b}±0.34	94.7^{b}±0.37
	2 d	1.9^{b}±0.01	18.8^{a}±0.04	67.9^{b}±0.17	120.6^{a}±0.81	96.8^{a}±0.90
	4 d	4.8^{a}±0.04	18.2^{a}±0.05	74.2^{a}±0.74	104.8^{b}±0.83	97.3^{a}±0.33
	7 d	4.2^{a}±0.02	16.5^{b}±0.06	47.8^{c}±0.26	94.1^{c}±0.51	89.5^{c}±0.37
G2	Zero	3.0^{b}±0.07	20.0^{b}±0.04	77.9^{b}±0.32	118.0^{b}±0.34	110.4^{a}±.37
	2 d	2.1^{b}±0.08	21.6^{a}±0.05	81.7^{a}±0.65	126.2^{a}±0.01	109.8^{a}±0.33
	4 d	2.1^{b}±0.08	19.0^{c}±0.03	72.9^{b}±0.80	120.1^{b}±0.34	101.5^{b}±0.68
	7 d	5.9^{a}±0.02	15.5^{d}±0.06	64.1^{c}±0.17	109.9^{c}±0.33	89.0^{c}±0.90
G3	Zero	18.0^{a}±0.05	50.8^{d}±0.06	969.8^{a}±0.87	267.1^{a}±0.38	250.0^{d}±0.56
	2 days	13.5^{c}±0.03	52.0^{b}±0.06	938.3^{a}±0.75	249.3^{b}±0.96	276.0^{b}±0.46
	4 days	12.6^{c}±0.06	51.2^{c}±0.01	810.0^{b}±0.36	235.1^{c}±0.68	259.8^{c}±0.53
	7 days	15.8^{b}±0.02	56.0^{a}±0.04	714.4^{c}±0.45	235.6^{c}±0.17	295.3^{a}±0.42
G4	Zero	6.5^{a}±0.02	17.0^{b}±0.03	62.8^{a}±0.53	103.3^{a}±0.52	95.1^{a}±0.40
	2 days	5.5^{b}±0.02	15.2^{c}±0.01	64.2^{a}±0.95	102.8^{a}±0.56	93.9^{a}±0.81
	4 days	6.0^{a}±0.07	16.9^{b}±0.03	67.5^{a}±0.51	105.8^{a}±0.88	95.4^{a}±0.08
	7 days	5.3^{b}±0.05	18.2^{a}±0.01	62.5^{a}±0.13	104.4^{a}±0.94	93.4^{a}±0.80
p-value		0.052	0.000	0.035	0.027	0.009

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($P < 0.05$); (G1): Dogs infested with *R. sanguineus* and received Phoxim® treatment; (G2): No-infested dogs received Phoxim® treatment; (G3): Dogs infested with *R. sanguineus* with no treatment (positive control); (G4): Non-infested and non-treated dogs (Negative control); (TP): Total protein; (ALT): Alanine aminotransferase; (GLUCO): Glucose; (TG): Triglycerides; (TC): Total cholesterol; (SE): Standard error.

causing further activation of the compound. The toxic influence usually arises from their binding affinity to some amino acids concerning serine and prevents their catalytic activity as recorded by Mahananda and Mohanty (2012). The continuous and repetitive use of insecticides is contraindicated for the development of multifactorial resistance build-up against these compounds as recorded by Aguilar *et al*. (2018); Cossío-Bayúgar *et al*. (2018); and Kumar (2019).

Our results illustrated highly significant increases in erythrocytes, hematohiston, hematocrit, platelets, neutrophils, and basophils in treated non-infested animals (G3) compared to both infested treated and infested non-treated dogs. Mean corpuscular volume revealed highly significant improvements in the infested treated dogs (G1). Hemoglobin centering, hemoglobin consolidation, and agranulocytes recorded highly significant declines in all groups concerning the non-infested treated group (G3) which showed highly significant increases. Leukocytes illustrated highly significant improvements in treated non-infested dogs (G3). The results were in contrast to those obtained

Table 5. Antioxidant profile (Mean ±SE) of Phoxim® treated and untreated dog groups.

Groups	Time (days)	GSH (µmol/l)	GHPx (U/ml)	MDA (nmol/ml)	CAT (MU/l)	SOD (U/ml)	TAC (mM/l)
Overall means concerning the experimented insecticides							
G1		132.3^{b}±0.06	4.9^{a}±0.03	8.1^{b}±0.08	8.8^{a}±0.03	129^{b}±0.08	16.2^{b}±0.06
G2		121.3^{c}±0.07	2.9^{b}±0.00	6.8^{c}±0.01	3.8^{c}±0.06	127^{c}±0.02	15.1^{c}±0.06
G3		148.9^{a}±0.07	5.1^{a}±0.01	8.9^{a}±0.01	5.3^{b}±0.01	148^{a}±0.06	29.7^{a}±0.04
G4		109.5^{d}±0.01	0.9^{c}±0.00	5.7^{d}±0.08	3.0^{d}±0.01	95^{d}±0.08	8.8^{d}±0.02
p-value		0.001	0.019	0.000	0.006	0.000	0.000
Overall means concerning the sampling time							
Zero		119.0^{d}±0.09	2.3^{c}±0.05	8.3^{a}±0.09	6.6^{a}±0.05	120^{d}±0.08	17.0^{b}±0.03
2 days		121.9^{c}±0.03	2.3^{c}±0.04	7.2^{b}±0.03	4.5^{c}±0.03	123^{c}±0.09	17.6^{a}±0.05
4 days		131.0^{b}±0.06	5.7^{a}±0.06	7.1^{c}±0.03	4.8^{b}±0.05	133^{a}±0.03	17.6^{a}±0.09
7 days		140.0^{a}±0.01	3.4^{b}±0.05	7.0^{d}±0.04	4.9^{b}±0.06	124^{b}±0.01	17.5^{a}±0.06
p-value		0.000	0.005	0.000	0.019	0.000	0.044
Experimented insecticides by sampling time interactions							
G1	Zero	99.0^{d}±0.05	0.36^{d}±0.00	13.0^{a}±0.05	13.6^{a}±0.01	109^{d}±0.05	11.6^{d}±0.01
	2 d	128.3^{c}±0.03	1.1^{c}±0.00	8.0^{b}±0.05	5.5^{c}±0.06	130^{c}±0.03	14.3^{c}±0.00
	4 d	142.6^{b}±0.02	13.99^{a}±0.51	6.2^{c}±0.03	7.8^{b}±0.03	138^{b}±0.01	18.3^{b}±0.00
	7 d	159.3^{a}±0.03	4.1^{b}±0.00	5.4^{d}±0.03	8.1^{b}±0.06	141^{a}±0.01	20.6^{a}±0.01
G2	Zero	120.3^{c}±0.03	3.0^{a}±0.00	6.0^{d}±0.03	4.1^{a}±0.03	122^{d}±0.01	17.6^{a}±0.00
	2 d	118.3^{d}±0.02	2.8^{a}±0.00	6.8^{c}±0.03	4.0^{a}±0.00	132^{a}±0.05	16.3^{b}±0.01
	4 d	121.6^{b}±0.01	3.0^{a}±0.03	7.0^{b}±0.01	3.8^{ab}±0.03	129^{b}±0.01	15.0^{c}±0.00
	7 d	125.0^{a}±0.05	2.8^{a}±0.00	7.4^{a}±0.01	3.5^{b}±0.03	123^{c}±0.01	11.6^{d}±0.01
G3	Zero	150.3^{b}±0.03	5.0^{b}±0.00	9.0^{b}±0.01	5.5^{b}±0.01	150^{b}±0.00	29.3^{c}±0.01
	2 d	135.3^{d}±0.01	4.4^{c}±0.00	8.4^{c}±0.00	6.0^{a}±0.00	135^{d}±0.01	31.6^{a}±0.00
	4 d	149.0^{c}±0.05	5.0^{b}±0.00	9.0^{b}±0.01	4.6^{d}±0.01	167^{a}±0.00	27.6^{d}±0.00
	7 d	161.0^{a}±0.04	6.0^{a}±0.00	9.4^{a}±0.00	5.0^{c}±0.00	141^{c}±0.00	30.3^{b}±0.01
G4	Zero	106.6^{c}±0.08	1.0^{a}±0.0	5.4^{c}±0.00	3.1^{a}±0.01	98^{a}±0.01	9.6^{a}±0.01
	2 d	105.6^{c}±0.03	0.8^{b}±0.00	5.4^{c}±0.03	2.8^{b}±0.01	94^{b}±0.01	8.3^{b}±0.00
	4 d	111.0^{b}±0.05	1.0^{a}±0.00	6.0^{a}±0.06	3.0^{a}±0.00	98^{a}±0.03	9.6^{a}±0.02
	7 d	115.0^{a}±0.07	0.7^{b}±0.00	5.8^{b}±0.00	3.0^{a}±0.01	91^{c}±0.01	7.6^{c}±0.00
p-value		0.000	0.019	0.000	0.008	0.001	0.000

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$); (G1): Dogs infested with *R. sanguineus* and received Phoxim® treatment; (G2): No-infested dogs received Phoxim® treatment; (G3): Dogs infested with *R. sanguineus* with no treatment (positive control); (G4): Non-infested and non-treated dogs (Negative control); (GSH): Glutathione total; (GHPx): Glutathione perioxidase; (MDA): Malondialdehyde; (CAT): Catalase; (SOD): Superoxide dismutase; (TAC): Total antioxidant capacity; (SE): Standard error.

by Mossa and Abbassy (2012) and Holy *et al*. (2015) who recorded significant declines in erythrocytes, hematohiston, and hematocrit of male rats treated with phoxim at a rate of 2,007.13 mg/kg body weight. Meanwhile, Aboelela *et al*. (2022b) in agreement with our study recorded significant improvements in hematological profile and erythrocyte sedimentation rates.

The significant increase in the total leukocytic count might be attributed to the regeneration and relocations of leukocytes from marginal granulocytes affected by the high background of adrenaline. The recorded significant increase in the total leukocytic count was consistent with those reported by Al-Sahhaf (2006) and Yassa and Girgis (2018) after using the organophosphorus compound as an insecticidal agent in rats. Meanwhile, Mossa and Abbassy (2012) recorded a significant decline in total leukocytic count in chlorpyrifos-treated rats. The recorded significant increase in the inflammatory agranulocytes (lymphocytes and monocytes) can be considered a typical picture that developed usually in any animal under stress, and in this case, the treatment

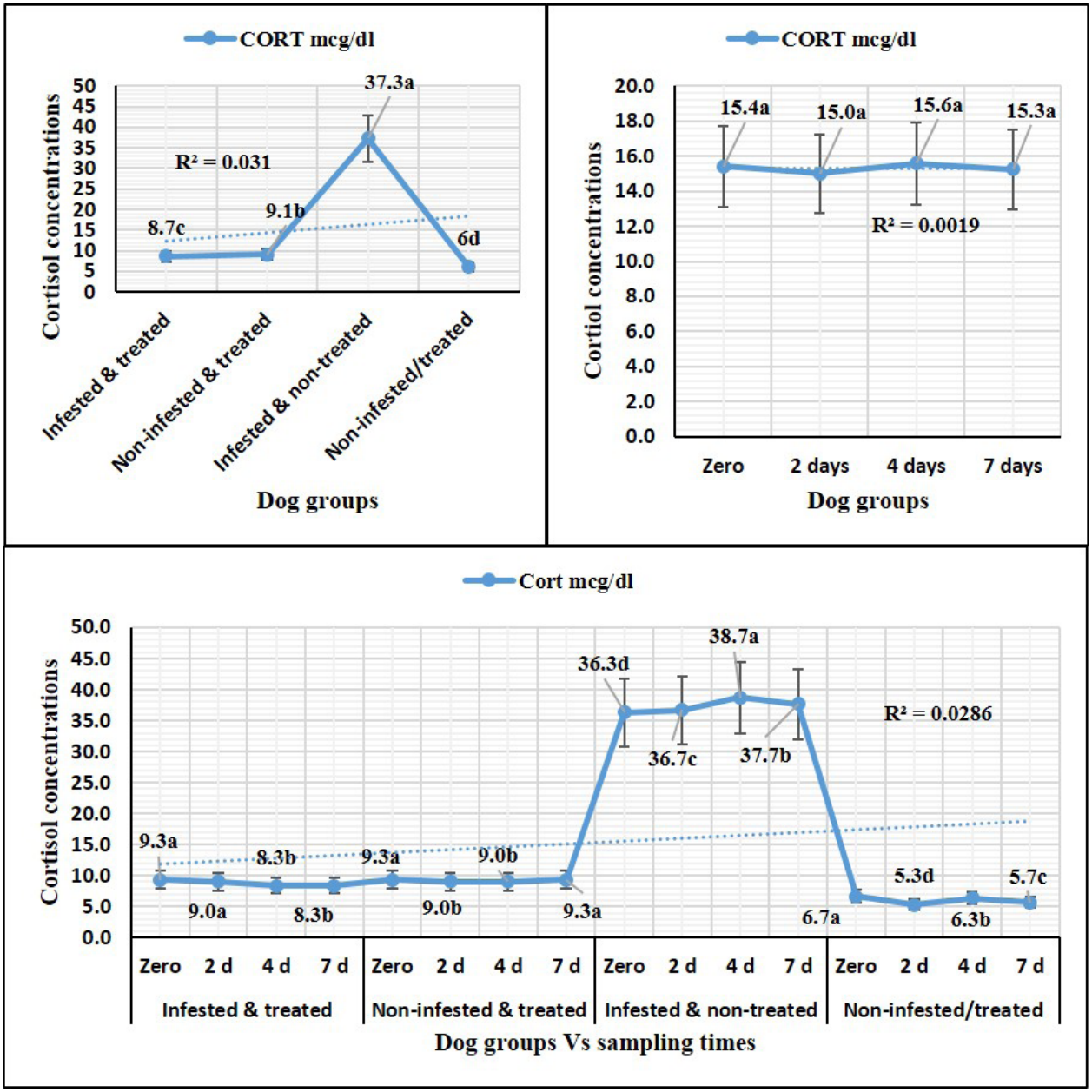

Fig. 4. Cortisol stress marker (Mean ±SE) of Phoxim® treated and untreated dog groups. (**A)** CORT overall means concerning experimental groups. (**B)** CORT overall means concerning sampling times. (**C)** CORT overall means concerning experimental groups by time interactions.

itself might be considered a stress factor on the animals that might shift the blood picture to a little extent.

Phoxim® treatment in both infested and non-infested animals showed highly significant improvement of all measured biochemical parameters concerning higher improvements in the treated noninfested dogs (G3) of alanine aminotransferase, glucose, triglycerides, and total cholesterol compared to the infested treated animals (G1). Yassa and Girgis (2018) were consistent with our results and recorded nonsignificant modifications in total protein and albumin in Phoxim®-treated rats. Meanwhile, they recorded a significant increase in liver enzymes contrary to the current results. On the contrary, Abo El-Soud *et al*. (2015) recorded a significant decrease in total protein and significant increases in liver enzyme concentrations of rats treated with profenofos 65 days post-treatment. Al-Sahhaf (2006) recorded a significant decrease in total protein concentrations of rats treated with oral doses of sumithiom. The significant improvement of the biochemical profiles concerning proteinogram and liver enzymes might be attributed to the adaptation

abilities of the animal to the treatment stress which contributed significant decline of these biochemical indicator levels in the treated and non-infested animals compared to the infested non-treated animals and infested treated animals.

Al-Sahhaf (2006) and Abo El-Soud *et al.* (2015) reported in contrast to the current results significant increase in serum creatinine and they contributed this elevation to the lower glomerular filtration capacity. Yassa and Girgis (2018) also reported coagulative necrosis of the epithelial lining of the glomeruli in Phoxim®-treated rates with marked hypercellularity and this occurred using an acute toxic dose of Phoxim.

Antioxidant enzymes are excited in certain circumstances such as shortage or cessation of the oxidative conditions of some nutrients such as lipids and proteins. Oxidative stress is a condition during which the reactive oxygen overwhelms the antioxidative defense mechanism of the body causing shifting in the macromolecules, cellular injuries, and apoptosis (Trevisan *et al*., 2001). The current results revealed that glutathione total, glutathione peroxidase, malondialdehyde, catalase, superoxide dismutase, total antioxidant capacity, and cortisol stress marker showed highly significant declines in the treated non-infested dogs compared to other animal groups. The results were consistent with those reported by Aboelela *et al*. (2022b) who recorded significant improvements in the biochemical profile and cortisol hormone of the two-week and four-week post-treatment samples. Also, El-Naggar *et al*. (2017) reported neutral influences of their tested insecticides on the tissue architecture and operational conditions of the brain, liver, and kidney in male Albino mice.

Herbal and eco-friendly insecticides that are based on a plant extract like peppermint, citrolina, garlic, onions, pomegranate, camphor, and clove might vanquish all the negative impacts contributed by the usage of some synthesized insecticides after Abd-Ella (2014, 2016); Gil et al. (2015); Nettles et al. (2016); and Aboelela *et al*. (2022b). They act by disrupting the insect's central nervous system by calcium channel blockage or direct enteric nervous system effect causing hyperexcitation of contaminated insects' nerves and muscles, a repellent that masks the scent that is attractive to insects and it would be difficult for an insect to locate their target, and interrupt the action potential which is essential for insects' activity and synaptic transmission resulting in suffocation and distressing of the cuticular waxes.

Conclusion

Phoxim® as an organophosphorus insecticidal agent contributed to significant increases and improvements in erythrocytes, hematohiston, hematocrit, hemoglobin consolidation, total leukocytic count, granulocytes, alanine aminotransferase, glucose, triglycerides, and total cholesterol, as well as significant declines of glutathione total, glutathione peroxidase, malondialdehyde, catalase, superoxide dismutase, total antioxidant capacity, and cortisol stress marker in non-infested treated dogs rather than infested treated, infested non-treated, and negative control.

Phoxim® was able to prove a significant prophylactic rather than treating influences to be included in the prophylactic schedule of the biosecurity programs for eradication of external parasites concerning *R. sanguineus* ticks in dogs. Garlic, onions, pomegranate, camphor, and clove herbal insecticides are much more recommended in the prophylaxis and treatment of external parasitism because of their lower toxicity, lower ecological impact, higher efficacy, and specific actions.

Authors' Contributions

MME, AEM, and DAA participated in the clinical investigation of the animals (infested and non-infested), supervised the Phoxim® treatment, performed the animals sampling at the scheduled intervals, conducted the antioxidant assay, and participated in manuscript writing. NHS performed the parasitological examination and participated in manuscript writing. AAA. Performed the Phoxim® treatment, conducted the hematological assessment, and participated in manuscript writing. ESS planned and illustrated the experimental design, carried out the biochemical and hormonal analysis, participated in manuscript writing, and reviewed the final edit of the manuscript.

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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.

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