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THE HAEMATOLOGICAL CHANGES OBSERVED IN DOGS INFESTED WITH SARCOPTES SCABIE VAR. CANIS AND TREATED WITH IVERMECTIN, CYPERMETHRIN, CITRUS AND NEEM SEED OIL.

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ABSTRACT

The haematological changes associated with anti-parasitic effects of Ivermectin, Cypermethrin, Citrus oil, Neem seed oil in Sarcoptes infested dogs is scanty in literature. Thus, this study evaluates the safety of these therapeutic interventions using changes in haematological indices. Thirty dogs were enrolled for the study out of which twenty five were infested with sarcoptes mites and the other five were sarcoptes free. Botanical preparations were made and oils were extracted for use. Infested dogs were grouped in fives and were treated with Ivermectin, Cypermethrin, Citrus oil, Neem seed oil, and Petroleum jelly (Vehicle) as recommended for six weeks. The PCV, HB, RBC, WBC, platelets, lymphocytes, neutrophils, monocytes, eosinophils, and platelet lymphocyte ratios were evaluated following various treatments. Neem seed oil and Cypermethrin pour-on significantly ($p < 0.05$) improved the PCV, Hb and RBC of infested dogs. Citrus treated dogs had significantly ($p < 0.05$) lower WBC and platelet counts. PLR was observed to be lowest in Cypermethrin treated dogs when compared with other various treatment. Treatment of canine scabies with these therapeutic mediations did not cause serious haematological derangements hence the use of any of the botanical/systemic therapy were safe to use in dogs.

Keywords: Acaricides, Haematology, Sarcoptes scabiei, Dogs, Botanical, Systemic

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INTRODUCTION

Sarcopticosis remains one of the neglected tropical disease conditions with significant economic importance in humans, livestock and companion animals. The ectoparasitic and burrowing mite; Sarcoptes scabiei is always involved in this condition with its subsequent effect birthing losses in quantity and quality of animal products (Dagleish et al., 2007). Associated with the mite as well is the characteristic itch which results from the salivary antigen of the mite produced during epidermal digestion (Hay, 2009).

Romani et al., (2014) projected a 30 million universal occurrence of scabies at the end of the 20th era. Meanwhile, The World Health Organization states that the current on-going infestation rate stands at 300 million irrespective of age and social status categories (Engelman et al., 2013). Contributory to this spread in morbidity is the shortcomings of the first-rate and golden standard of scabies therapy with the use of synthetic acaricides. Some of these shortcomings include the occurrence of parasite resistance to synthetic acaricides (Currie et al., 2004), and the inherent flaws of acaricides lacking anti-pruritic and ovicidal properties effective in averting treatment relapse (Wallengren, 2011). The subsequent disadvantage of these draw backs then includes toxicity to humans, animals and the environment from continuous acaricide exposure in order to combat treatment failures (O'Brien, 1999).

Certain botanical acaricides have been studied for its miticidal properties. Most of these plants have been found to possess glycosides, alkaloids, flavonoids, coumarin compounds, vitamins, and tannins (Altaf et al., 2019). They could also possess very negligible side effects, no side effects as well as prospective abilities in ameliorating several blood derangements associated with sarcopticosis in dogs (Khan et al., 2017).

The progressive, clinical and pathologic changes associated with sarcopticosis in dogs has earlier been documented with certain key findings of anaemia, elevated white blood cell counts and an increased platelet lymphocyte ratio as infestation progressed (Nwufoh et al., 2019). Even though systemic, topical and botanical scabies therapies possess miticidal success with improvement in certain haematological parameters (Aboelhadid et al., 2016; Behera et al., 2011), their comparative, and safety using haematological changes in treated sarcoptes infested dogs is yet to be determined. Thus, the need to embark on study was inevitable as an understanding of the effects of systemic, topical and botanical therapeutic interventions on haematological indices would serve as a tool to evaluate safety of use.

MATERIALS AND METHODS

Ethical Approval

The ethical conducts associated with infestation of animals without pains and with respects to their right to feed, shelter, management, and welfare was methodically appraised. Approval was granted (UI-ACUREC 17-0027) with the justification of animal use and benefits of study to both pet and pet owners.

Study design

Dogs were enrolled, adjusted, screened for ecto and endo-parasites, and infested as earlier described by Nwufoh et al., (2019). 30 healthy dogs were purchased while 25 were subjected

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to the same established protocols for infestation. The remaining 5 uninfected dogs were used as the negative control.

Dog grouping

Five (5) sarcoptes free dogs and twenty five (25) dogs already infested with Sarcoptes scabiei var canis were assigned to various groups. Group A had the five (5) sarcoptes free dogs. This group was assigned the control group. Groups B had five dogs (5) to be treated with Citrus oil, Group C dogs (5) were to be treated with Ivermectin Super (Ivermectin + Clorsulon-Merial-UEMOA/V/00012/2013/02/28), Group D dogs (5) were to be treated with Neem seed oil, Group E dogs (5) were to be treated with Petroleum jelly, and Group F dogs (5) were to be treated with Cypermethrin pour-on (CYPER care-Ancare, 2.5% pour-on solution).

Ointment - Extract preparation of Azadirachta indica and Citrus sinensis

Fresh ripened seeds of Azadirachta indica were harvested from a neem tree at the Faculty of Veterinary Medicine, University of Ibadan. Seeds were authenticated by a botanist at the Department of Botany, University of Ibadan. Neem seeds were dried under room temperature after which aqueous extracts of seeds was prepared. Dried seeds were ground before 500g was boiled in a litre of water for half an hour. Ensuing mixture was filtered and new solvent was added. Procedure was carried out thrice before extract was obtained after solvent evaporation. Extract was then prepared as a 20% ointment with a Petroleum jelly vehicle (w/w) (Shahid et al., 2007).

Extraction of Citrus oil followed the procedure of Neelima et al., (2017). Fresh peels of Citrus sinensis were obtained after washing purchased fruits. Peels were obtained using laboratory knife before being subjected to hydro-distillation method. Distillation procedure involved exposing citrus peels to boiling water for the oil to be released into the water. Essential oils were then further collected by distillation. Finally, the steam and oil vapours were condensed and collected into a Florentine flask. Oil was further prepared as a 20% ointment with a Petroleum jelly vehicle (w/w).

Treatment application

Healthy animals which served as control animals were cleaned with distilled water and saline solution (0.9%) NaCl 1ml/30kg intramuscularly. Hairs around lesion areas of infested animals were shaved before application of treatment. After shaving, crusts were taken out with tepid cleaning with cotton wool and allowed to dry. Neem seed oil, Citrus oil and Petroleum jelly were then generously applied on lesion areas on alternate days till end of experiment.

Ivomec-D (Ivermectin + Clorsulon- Merial-UEMOA/V/00012/2013/02/28) was administered subcutaneously at 1ml per 50kg of body weight. Cypermethrin (CYPER care-Ancare, 2.5% pour-on solution) was administered directly to all lesion areas on the skin of the animals.

Drugs were administered on the first (1st) and fourteenth (14th) day at the neck lateral to the midline. Response to therapy was closely supervised till end of study.

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Sample collection

Blood samples for hematological analysis was obtained from selected animals in all groups A, B, C, D, E, F. Blood samples were collected under physical restraint and via the cephalic vein weekly. Blood samples for hematology were collected into 1% EDTA bottles and this was done weekly.

Hematological analysis

The packed cell volume (PCV) was determined by microhematocrit method, hemoglobin concentration (HB) was then measured by the cyanmethaemoglobin method, while red blood cell (RBC), total white blood cell, and differential white blood cell (WBC) were all determined by hemocytometer method as described by Schalm, (1986).

Statistical analysis

Data obtained from haematological analysis were expressed as mean ± Standard Error (SE) and subjected to one-way Analysis of Variance (ANOVA). Significant level of difference among treatment mean was established at 95% confidence limit. Tukey multiple-ranged test was used as post-Hoc test. All statistical analysis was accomplished using IBM SPSS 22® statistical package. The platelet, neutrophil, monocyte and eosinophil lymphocyte ratios were determined with the divisions of the absolute cell counts against the absolute lymphocyte count (Mehmet et al., 2018).

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Table 1: Heamatological response of *Sarcoptes scabiei* free dogs and infested dogs treated with Citrus oil, Ivermectin, Neem oil, Petroleum Jelly, Cypermethrin.

SD (week)		Control	Citrus Oil	Ivermectin	Neem Oil	Petroleum Jelly	Cypermethrin
	PCV	29.33 ± 2.96	23.66 ± 2.72	22.00 ± 1.52	29.00 ± 1.53	25.66 ± 0.33	23.00 ± 1.15
	HB	9.73 ± 0.94	7.70 ± 0.76	7.73 ± 0.52	9.43 ± 0.53	8.70 ± 0.05	7.13 ± 0.06
1	RBC	4.80 ± 0.40^{a}	3.72 ± 0.41^{ab}	3.94 ± 0.31^{ab}	4.64 ± 0.33^{ab}	4.51 ± 0.03^{ab}	3.33 ± 0.01^{b}
	WBC	9.97 ± 2.37	6.90 ± 0.80	7.11 ± 1.07	10.70 ± 0.37	7.15 ± 1.07	8.23 ± 1.71
	PLT	13.80 ± 5.50^{d}	19.93 ± 1.08^{bc}	23.16 ± 2.40^{ab}	24.06 ± 9.27^{a}	22.30 ± 6.11^{ab}	18.23 ± 3.75^{c}
	PCV	35.66 ± 0.88^{a}	24.33 ± 3.92^{b}	24.33 ± 1.85^{b}	28.33 ± 1.85^{ab}	25.33 ± 0.33^{b}	29.66 ± 1.20^{ab}
	HB	11.83 ± 0.28^{a}	7.96 ± 1.29^{b}	7.96 ± 0.47^{b}	8.46 ± 1.00^{ab}	8.66 ± 0.03^{ab}	9.36 ± 0.47^{ab}
2	RBC	5.56 ± 0.34	3.89 ± 0.66	3.85 ± 0.44	4.24 ± 0.53	4.55 ± 0.02	4.69 ± 0.31
	WBC	7.35 ± 1.57	7.78 ± 1.04	6.90 ± 1.17	8.53 ± 0.92	6.61 ± 0.81	9.70 ± 0.27
	PLT	16.10 ± 1.94^{b}	19.03 ± 0.68^{ab}	23.96 ± 1.25^{a}	19.46 ± 0.55^{ab}	21.00 ± 0.90^{ab}	21.83 ± 0.20^{a}
	PCV	36.66 ± 0.33^{a}	28.33 ± 1.66^{bc}	22.33 ± 0.88^{c}	31.66 ± 1.20^{ab}	28.33 ± 0.88^{bc}	30.00 ± 2.30^{b}
	HB	12.26 ± 0.17^{a}	9.86 ± 0.58^{ab}	7.66 ± 0.41^{b}	10.93 ± 0.43^{a}	9.73 ± 0.33^{ab}	9.43 ± 1.14^{ab}
3	RBC	6.24 ± 0.18^{a}	4.83 ± 0.17^{bc}	3.89 ± 0.14^{c}	5.30 ± 0.12^{ab}	4.70 ± 0.21^{bc}	4.79 ± 0.36^{bc}
	WBC	8.73 ± 1.68	11.70 ± 0.27	7.43 ± 1.47	11.56 ± 0.79	7.26 ± 0.58	10.40 ± 0.61
	PLT	20.73 ± 0.68	21.86 ± 0.67	24.26 ± 1.79	22.36 ± 0.37	23.63 ± 0.49	24.23 ± 0.17
	PCV	40.00 ± 1.52^{a}	30.00 ± 2.08^{bc}	24.33 ± 0.88^{c}	33.33 ± 1.66^{ab}	28.66 ± 0.88^{bc}	34.66 ± 1.85^{ab}
	HB	12.86 ± 1.20^{a}	10.30 ± 0.76^{ab}	8.00 ± 0.36^{b}	11.26 ± 0.33^{ab}	9.66 ± 0.37^{ab}	11.96 ± 0.68^{a}
4	RBC	6.46 ± 0.82^{a}	5.52 ± 0.37^{ab}	3.96 ± 0.24^{b}	5.54 ± 0.22^{ab}	4.66 ± 0.27^{ab}	6.03 ± 0.34^{a}
	WBC	9.46 ± 2.37	13.06 ± 1.95	8.01 ± 0.89	10.35 ± 0.45	8.50 ± 0.32	9.46 ± 0.38
	PLT	22.60 ± 1.50	24.53 ± 0.82	24.06 ± 0.32	23.80 ± 1.02	24.40 ± 0.81	25.93 ± 1.04

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SD (week)		Control	Citrus Oil	Ivermectin	Neem Oil	Petroleum Jelly	Cypermethrin
5	PCV	37.66 ± 2.18^{a}	22.66 ± 0.88^{c}	23.66 ± 0.66^{bc}	28.00 ± 1.52^{bc}	29.33 ± 1.20^{b}	27.00 ± 1.00^{bc}
	HB	12.36 ± 1.14^{a}	7.70 ± 0.36^{b}	8.40 ± 1.10^{b}	8.90 ± 1.02^{ab}	10.16 ± 0.33^{ab}	9.43 ± 0.03^{ab}
	RBC	6.79 ± 0.77^{a}	3.48 ± 0.36^{b}	4.38 ± 0.63^{b}	4.70 ± 0.32^{ab}	4.99 ± 0.33^{ab}	4.31 ± 0.05^{b}
	WBC	8.21 ± 0.77^{b}	12.80 ± 1.04^{a}	10.43 ± 0.23^{ab}	9.56 ± 0.29^{ab}	10.08 ± 0.55^{ab}	7.95 ± 1.01^{b}
	PLT	17.60 ± 1.05^{b}	23.10 ± 1.15^{a}	25.70 ± 0.78^{a}	23.06 ± 0.52^{a}	24.90 ± 0.95^{a}	23.26 ± 1.01^{a}
6	PCV	33.00 ± 0.57^{a}	23.66 ± 2.02^{b}	25.00 ± 0.57^{b}	26.66 ± 1.76^{b}	29.00 ± 0.57^{ab}	27.00 ± 1.00^{b}
	HB	11.10 ± 0.10^{a}	7.00 ± 0.94^{b}	8.36 ± 1.01^{ab}	7.86 ± 1.33^{ab}	9.93 ± 0.12^{ab}	7.40 ± 0.58^{ab}
	RBC	5.90 ± 0.49^{a}	3.38 ± 0.52^{b}	4.63 ± 0.54^{ab}	3.93 ± 0.71^{ab}	5.09 ± 0.31^{ab}	3.71 ± 0.30^{ab}
	WBC	5.86 ± 0.95^{b}	5.90 ± 1.35^{ab}	10.70 ± 0.30^{a}	6.03 ± 1.52^{ab}	10.26 ± 0.62^{ab}	7.01 ± 0.78^{ab}
	PLT	20.23 ± 0.24^{d}	20.83 ± 0.63^{cd}	24.10 ± 0.41^{ab}	22.00 ± 0.42^{bcd}	26.50 ± 0.70^{a}	22.93 ± 0.55^{bc}

^{a,b,c} Means of different superscript along the row within each group of SD are significantly different ($P < 0.05$). SD – Study Duration, PCV – Packed Cell Volume (%), HB – Haemoglobin concentration (g/dl). RBC – Red Blood Cell ($\times 10^{6}$/µl), WBC – White Blood Cell ($\times 10^{3}$/µl), PLT – Platelets ($\times 10^{4}$/µl).

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The response of Packed Cell Volume (PCV), Hemoglobin (HB), Red blood Cells (RBC), White Blood Cells (WBC) and Platelets (PLT) to synthetic and botanical therapies in sarcoptes infested dogs is demonstrated in Table 1. The first week after treatment showed that infested dogs treated with Neem seed oil had a numerically high PCV and Hemoglobin response. However, differences in PCV and Hb values of all dogs treated and the control group did not significantly vary ($p < 0.05$). The highest significant ($p < 0.05$) RBC was recorded in the control group while the lowest was recorded in the Cypermethrin group. However, the RBC of other treatment groups were not significant ($p > 0.05$) to the control group. The WBC of dogs was not significantly influenced ($p > 0.05$) by the treatment agents after the first week of treatment. However, the lowest white blood cell count amidst the various treatments was observed in Citrus oil treated dogs. The platelets were significantly influenced ($P < 0.05$) as infection persisted. The lowest significant ($p < 0.05$) platelet count was recorded in the control group while among the various therapies, Cypermethrin treated dogs had the lowest platelet count.

At the second week of treatment (Week 2), the RBC and WBC counts in the dogs among the treatment groups were not significantly influenced ($p > 0.05$) by the treatment agents. However, the PCV and Hb of dogs in the control group were significantly higher ($p > 0.05$) than the other treatment groups. The PCV of the dogs treated with Cypermethrin and Neem oil were not significantly inferior ($p < 0.05$) to the control group. The Hb values in dogs treated with Citrus oil and Ivermectin were significantly lower ($p < 0.05$) than the control group while Citrus and Neem oil treated dogs had the lowest platelet count among all treatments.

The WBC and Platelet counts in the dogs did not vary significantly ($p > 0.05$) among the treatment groups at the third week of treatment. Nevertheless, Neem oil treated dogs had significant improvement ($p < 0.05$) in the PCV, Hb and RBC counts. Similar trends (WBC and PLT) was observed at the fourth week of treatment as the PCV, Hb and RBC count in Cypermethrin and Neem oil treated dog were not significantly reduced ($p < 0.05$) to the control group.

The results at the fifth week of study duration showed that Petroleum jellied dogs showed significant improvement ($p > 0.05$) in the PCV, Hb and RBC count. Conversely, WBC and the Platelet count in treated dogs showed a significant increase ($p > 0.05$). Citrus oil, Ivermectin, Neem oil, Petroleum jelly and Cypermethrin treatment did not significantly influence ($p < 0.05$) the WBC and Platelet count in dog.

Similar to the results obtained at week 5 of study, there was also a significant increase in PCV, Hb and RBC count in the Petroleum jelly treated dogs at week six of treatment. The WBC and the Platelet count in treated dogs also showed a significant increase ($p > 0.05$) relative to the control group while Citrus oil treated dogs had decreased WBC and Platelet counts.

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Table 2: Differential white blood cell count response of *Sarcoptes scabiei* free dogs and infested dogs treated with Citrus oil, Ivermectin, Neem oil, Petroleum Jelly, Cypermethrin.

Study Duration (week)	Parameter	Control	Citrus Oil	Ivermectin	Neem Oil	Petroleum Jelly	Cypermethrin
1	LYM	33.66 ± 1.85	20.66 ± 1.33	26.66 ± 2.40	28.00 ± 6.08	31.33 ± 3.52	26.66 ± 2.18
	NEU	62.00 ± 1.52	74.00 ± 0.57	66.00 ± 5.29	66.33 ± 6.69	64.66 ± 3.17	65.66 ± 2.33
	MON	2.00 ± 0.00	3.33 ± 0.33	2.00 ± 0.57	2.66 ± 0.33	2.00 ± 0.52	3.66 ± 0.88
	EOS	3.00 ± 0.57	2.33 ± 0.33	2.00 ± 0.57	3.00 ± 1.15	2.00 ± 0.00	2.00 ± 0.57
2	LYM	32.33 ± 2.84	36.66 ± 9.02	34.66 ± 1.66	35.00 ± 2.88	32.33 ± 3.52	41.00 ± 2.08
	NEU	59.66 ± 3.66	57.00 ± 9.07	59.66 ± 1.85	57.00 ± 3.21	63.66 ± 2.84	53.66 ± 1.45
	MON	3.00 ± 0.00^{ab}	4.66 ± 0.33^{a}	3.00 ± 0.57^{ab}	4.66 ± 0.88^{a}	2.00 ± 0.57^{b}	4.00 ± 0.00^{ab}
	EOS	2.00 ± 0.57	1.66 ± 0.33	2.66 ± 0.33	3.33 ± 0.88	2.00 ± 1.15	1.33 ± 0.66
3	LYM	22.66 ± 1.33	31.33 ± 0.88	27.66 ± 2.96	29.33 ± 4.48	30.33 ± 4.25	27.00 ± 0.57
	NEU	70.00 ± 4.72	63.66 ± 1.20	66.33 ± 2.84	67.33 ± 4.25	64.33 ± 4.84	66.00 ± 2.00
	MON	2.00 ± 0.57^{b}	2.66 ± 0.66^{ab}	3.33 ± 0.33^{ab}	2.33 ± 0.33^{ab}	4.33 ± 0.33^{a}	2.00 ± 0.57^{b}
	EOS	2.33 ± 0.33	2.33 ± 0.33	2.66 ± 0.33	1.33 ± 0.33	2.00 ± 0.57	2.33 ± 0.33
4	LYM	30.33 ± 3.84	30.66 ± 7.44	31.00 ± 4.93	38.66 ± 0.88	30.00 ± 5.50	24.66 ± 0.88
	NEU	64.00 ± 3.05	63.33 ± 6.88	64.00 ± 4.93	55.33 ± 0.88	64.33 ± 5.17	69.33 ± 0.88
	MON	3.00 ± 1.00	3.00 ± 0.57	3.00 ± 0.57	4.00 ± 0.57	3.33 ± 0.33	3.66 ± 0.33
	EOS	2.66 ± 0.88	3.00 ± 0.00	2.00 ± 0.57	2.00 ± 0.57	2.33 ± 0.88	3.00 ± 1.15

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Study Duration (week)	Parameter	Control	Citrus Oil	Ivermectin	Neem Oil	Petroleum Jelly	Cypermethrin
5	LYM	25.66 ± 1.33^{ab}	24.66 ± 2.18^{ab}	23.33 ± 2.02^{b}	36.66 ± 1.66^{a}	28.66 ± 1.85^{ab}	28.66 ± 5.36^{ab}
	NEU	66.33 ± 0.66	68.33 ± 6.33	71.66 ± 1.76	56.33 ± 2.33	67.00 ± 2.08	65.00 ± 4.58
	MON	3.66 ± 0.66^{ab}	5.00 ± 0.57^{a}	2.33 ± 0.33^{bc}	4.00 ± 0.57^{ab}	1.33 ± 0.33^{c}	3.66 ± 0.33^{ab}
	EOS	3.00 ± 0.00	2.00 ± 0.57	2.66 ± 0.33	3.00 ± 0.57	3.00 ± 0.00	3.00 ± 0.57
6	LYM	22.33 ± 0.88	36.00 ± 4.04	22.33 ± 0.66	34.00 ± 3.21	30.00 ± 3.60	29.00 ± 5.50
	NEU	68.00 ± 2.64^{a}	56.66 ± 2.90^{b}	69.66 ± 0.88^{a}	57.00 ± 1.15^{b}	64.00 ± 3.00^{ab}	61.66 ± 0.66^{ab}
	MON	2.00 ± 0.00	4.00 ± 0.57	1.66 ± 0.33	3.33 ± 0.88	1.33 ± 0.33	3.33 ± 0.88
	EOS	1.33 ± 0.33	2.66 ± 0.66	3.00 ± 0.00	1.66 ± 0.33	2.00 ± 0.57	1.66 ± 0.33

^{a,b,c} Means of different superscript along the row within each group of SD are significantly different ($P < 0.05$). SD – Study Duration, LYM- Lymphocyte ($\times 10^3/\mu l$), NEU- Neutrophil ($\times 10^3/\mu l$), MON- Monocyte ($\times 10^3/\mu l$), EOS- Eosinophil ($\times 10^3/\mu l$).

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The response of the differential white blood cell counts to synthetic and botanical therapies in infested dogs is demonstrated in Table 2. The first week of treatment (Week 1) showed no significant alteration in lymphocyte, neutrophil, monocyte and eosinophil counts. However, Citrus oil treated dogs had lower lymphocytes, while Petroleum jelly treated dogs had lower neutrophils, monocytes and eosinophils.

The second week of treatment (Week 2) also had no significant changes in the lymphocyte, neutrophil and eosinophil amounts. Despite this, lymphocytes were lowest in Petroleum jellied dogs, while neutrophil and eosinophils were lowest in Cypermethrin treated dogs. Monocyte values were significantly lowered in infested dogs treated with Petroleum jelly.

Infested dogs treated with Cypermethrin oil at the third week (Week 3) had lower lymphocytes while Citrus oil treated dogs had lower neutrophils. It is worthy of note that both lymphocyte and neutrophil values in all treated dogs did not differ significantly. Monocyte amounts were significantly lower in Cypermethrin treated dogs while Neem seed oil treated dogs had lower eosinophils.

The fourth week of treatment (Week 4) had no significant alteration in lymphocytes, neutrophils, monocytes and eosinophils of sarcoptes treated dogs. That nonetheless, Cypermethrin treated dogs had lower lymphocytes, Neem oil treated dogs had lower neutrophils, Citrus oil and Ivermectin treated dogs had lower monocytes while Ivermectin and Neem oil treated dogs had lower eosinophils.

The fifth week of treatment (Week 5) revealed a significant reduction in lymphocytes in Ivermectin treated dogs. Neutrophil amounts differed insignificantly with Neem treated dogs having lower neutrophils. Monocyte amounts were significantly lower in Petroleum jellied dogs while eosinophil amounts were reduced in Citrus oil treated dogs.

At the sixth week of treatment (Week 6), values of lymphocytes, monocytes and eosinophils differed insignificantly. Nevertheless, Ivermectin treated dogs had the lowest lymphocytes, Citrus oil treated dogs had the lowest neutrophils significantly, Petroleum jelly treated dogs had the lowest monocytes while Cypermethrin and Neem oil treated dogs had the lowest eosinophil counts.

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Table 3: The Ratio of Differential White Blood Cell Count of *Sarcoptes scabiei* free dogs and infested dogs treated with Citrus oil, Ivermectin, Neem oil, Petroleum Jelly, and Cypermethrin

SD (week)		Control	Citrus Oil	Ivermectin	Neem Oil	Petroleum Jelly	Cypermethrin
1	PLR	4.12 ± 0.25[b]	9.67 ± 0.40[a]	8.82 ± 0.76[ab]	9.51 ± 2.13[a]	7.29 ± 0.85[ab]	6.90 ± 0.43[ab]
	NLR	1.85 ± 0.14	3.61 ± 0.27	2.55 ± 0.43	2.76 ± 0.92	2.13 ± 0.32	2.50 ± 0.25
	MLR	0.05 ± 0.01	0.16 ± 0.02	0.07 ± 0.01	0.10 ± 0.03	0.06 ± 0.02	0.14 ± 0.04
	ELR	0.09 ± 0.02	0.11 ± 0.02	0.08 ± 0.03	0.11 ± 0.04	0.06 ± 0.01	0.07 ± 0.01
2	PLR	5.12 ± 0.95	5.95 ± 1.61	6.91 ± 0.14	5.62 ± 0.39	6.69 ± 0.95	5.35 ± 0.29
	NLR	1.87 ± 0.22	1.97 ± 0.87	1.73 ± 0.13	1.66 ± 0.22	2.03 ± 0.28	1.31 ± 0.09
	MLR	0.09 ± 0.01	0.15 ± 0.05	0.08 ± 0.01	0.13 ± 0.01	0.06 ± 0.02	0.09 ± 0.01
	ELR	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.09 ± 0.03	0.06 ± 0.04	0.03 ± 0.01
3	PLR	9.19 ± 0.46	6.99 ± 0.33	9.81 ± 1.39	7.99 ± 1.22	8.15 ± 1.33	8.98 ± 0.18
	NLR	3.13 ± 0.38	2.03 ± 0.08	2.48 ± 0.40	2.43 ± 0.47	2.27 ± 0.54	2.44 ± 0.03
	MLR	0.09 ± 0.03	0.08 ± 0.02	0.12 ± 0.01	0.08 ± 0.02	0.14 ± 0.02	0.07 ± 0.02
	ELR	0.10 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.04 ± 0.01	0.07 ± 0.03	0.08 ± 0.01
4	PLR	7.83 ± 1.58	8.80 ± 1.69	8.24 ± 1.54	6.16 ± 0.34	8.59 ± 1.27	10.55 ± 0.70
	NLR	2.16 ± 0.22	2.41 ± 0.73	2.24 ± 0.56	1.43 ± 0.05	2.33 ± 0.50	2.82 ± 0.13
	MLR	0.09 ± 0.02	0.11 ± 0.04	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.14 ± 0.01
	ELR	0.09 ± 0.03	0.10 ± 0.02	0.07 ± 0.03	0.05 ± 0.01	0.08 ± 0.04	0.12 ± 0.05

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5	PLR	6.88 ± 0.48^{b}	9.51 ± 1.00^{ab}	11.14 ± 0.84^{a}	6.31 ± 0.29^{b}	8.80 ± 0.95^{ab}	8.57 ± 1.24^{ab}
	NLR	2.60 ± 0.15^{ab}	2.79 ± 0.27^{ab}	3.13 ± 0.34^{a}	1.54 ± 0.12^{b}	2.36 ± 0.23^{ab}	2.47 ± 0.56^{ab}
	MLR	0.14 ± 0.02^{ab}	0.20 ± 0.03^{a}	0.10 ± 0.01^{ab}	0.10 ± 0.01^{ab}	0.04 ± 0.01^{b}	0.14 ± 0.03^{ab}
	ELR	0.11 ± 0.01	0.07 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.02
6	PLR	9.08 ± 0.35^{ab}	5.93 ± 0.65^{b}	10.81 ± 0.48^{a}	6.56 ± 0.48^{b}	9.10 ± 1.14^{ab}	8.41 ± 1.36^{ab}
	NLR	3.04 ± 0.04^{a}	1.63 ± 0.28^{b}	3.12 ± 0.11^{a}	1.70 ± 0.17^{b}	2.23 ± 0.40^{ab}	2.26 ± 0.37^{ab}
	MLR	0.08 ± 0.01	0.11 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	0.04 ± 0.01	0.11 ± 0.01
	ELR	0.06 ± 0.01^{b}	0.07 ± 0.01^{ab}	0.13 ± 0.01^{a}	0.05 ± 0.01^{b}	0.06 ± 0.01^{ab}	0.05 ± 0.01^{b}

^{a,b,c} Means of different superscript along the row within each group of SEM are significantly different ($P < 0.05$). SD – Study Duration, PLR – Platelet Lymphocyte Ratio, NLR – Neutrophil Lymphocyte Ratio, MLR – Monocyte Lymphocyte Ratio, ELR – Eosinophil Lymphocyte Ratio.

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The Platelet Lymphocyte Ratio (PLR) of the sarcoptes free dogs at the first week of treatment was significantly lower ($p < 0.05$) than the other treatment groups. Amongst the treatments, dogs treated with Cypermethrin oil had the lowest platelet lymphocyte ratios for the first week of treatment. The Neutrophil Lymphocyte Ratio (NLR), Monocyte Lymphocyte Ratio (MLR), and Eosinophil Lymphocyte Ratio (ELR) were not significantly different ($p > 0.05$) among all the treatment groups at the first week of treatment.

There was no significant variation in the PLR, NLR, MLR and ELR among the treatment groups during the second, third and fourth weeks of treatment. The PLR, MLR, NLR and ELR at the fifth and sixth week of treatment were significantly influenced ($p < 0.05$) by the treatment agents. At the fifth week of treatment (Week 5), the highest significant ($p < 0.05$) PLR was recorded in infected dog treated with Ivermectin while the lowest recorded PLR was observed in the sarcoptes free and Neem oil treated dogs. Also at the fifth week of treatment, the highest and the lowest significant MLR ratio ($p < 0.05$) were recorded in infected dog treated with Citrus oil and Petroleum jelly, respectively. For the NLR ratio, Ivermectin treated dogs had significant higher ratios while Neem oil treated dogs had the lowest ratios. The highest significant PLR, NLR and ELR ratios ($p < 0.05$) were recorded in sarcoptes infected dog treated with Ivermectin at the sixth week of treatment while the lowest PLR was observed in dogs treated with Citrus oil.

However, there was no significant variation ($p < 0.05$) in the PLR and ELR in sarcoptes infected dog treated with other treatment agents relative to the control group.

DISCUSSION

The safety of anti-parasitic effects of Ivermectin, Cypermethrin, Citrus oil and Neem seed oil on dogs infested with *Sarcoptes scabie* var. *canis* was evaluated using the haematological indices because progressive clinical and pathologic changes associated with sarcopticosis in dogs has earlier shown anaemia, elevated white blood cell counts and an increased platelet lymphocyte ratio as key changes as infestation progressed (Nwufoh *et al.,* 2019).

Comparatively, Neem seed oil and Cypermethrin pour-on had a better effect on the PCV, Hb and RBC of treated dogs especially when compared with the effect of the conventional Ivermectin drug choice. Noteworthy is the rapid improvement in the established anaemic condition of infested dogs immediately after the first week of Neem oil treatment. The continuous use of Neem oil and Cypermethrin over the next three weeks sustained the amelioration of the mite induced anaemia in dogs. Contributory to the success of Neem seed oil in eliminating mites especially after its first application is the much studied and very effective insecticidal constituent; Azadirachtin (Isman *et al.,* 1990). Schmutterer, (1990) opined that this insecticide (Azadirachtin) possesses the ability to hinder oviposition, feeding, development, fertility and activity of insects. Undoubtedly, study findings of improved PCV, RBC and Hb must have resulted from the mortalities of mites following activities of the potent Azadirachtin. Accordingly, the fatalities of these mites would thus bring an end to the fluid sucking activity of the mite and hence improve blood picture.

Similarly, Cypermethrin demonstrated good hematologic response in mangy dogs as its

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actions of disturbing the sodium channel current of the mite always results in delayed repolarization (Irfan *et al.,* 2003). The resultant effect of the delayed repolarization is the paralysis and death of the mite. Additionally, it is quite interesting to mention that the actions of Cypermethrin are not just limited to the adult parasites but remain effective against eggs, protonymphs and tritonymphs of mites (Usha and Gopalakrishnan, 2000). Bearing all these actions in mind, it can thus be suggested that the hematologic response of dogs treated with Cypermethrin improved as a result of the complete elimination of all mite parasitic stages.

Ivermectin possess quite similar actions with Cypermethrin in relation to mite paralysis and death. However, dogs treated with Ivermectin had lower hematologic response when compared with dogs treated with Cypermethrin. This finding supports Usha (2001) on recent developments of resistance to Ivermectin therapy in horses, goats and sheep. Mechanism of resistance was thought to be due to the alterations of the chloride channel receptor hence a decreased mite response to therapy. On the other hand, the anti-parasitic activity of Ivermectin has been reported strong and active but only against at least one stage of the life cycle of the parasites (Usha, 2001). In the light of this, the 14 day recommended interval for the administration of the next shot of Ivermectin would open the window for further development of the immature stages of the mite. This development of the immature mites to the next stage would involve continuous blood and fluid sucking activities of the mite hence, a continuous imbalance in the haematology parameters of infested dogs.

Citrus oil was able to achieve a different grade of success as oil possessed both anti-microbial and anti-parasitic properties from study findings. Fisher and Philips (2008) further strengthened this outcome with their attribution of anti-microbial success of Citrus to the presence of volatile substances in their essential oils. The resultant anti-parasitic effect of Citrus oil has been credited to the presence of its integral component; d-limonene which functions to interrupt the normal respiratory functions of mites (Sook and Cho-Ho, 2006). Once respiratory functions are impaired in mites, respiratory failure would follows suit which ultimately ends with mite fatalities. Following the death of the mites, improved hematologic parameters becomes expected as mite fatality would bring an end to mite activities and account for the gradual changes observed in the haematogical parameters over time.

Petroleum jellied dogs had improved PCV, Hb, and RBC at the fifth and sixth week of treatment. It is worthy to note that Petroleum jelly has no anti-parasitic background but a principle of mite suffocation. Application of jelly to infested sites especially around hair follicles traps out oxygen from the hair follicle and burrowed tunnels. These areas are typical of mite's activities such as feeding, breeding, and continuous burrowing. Once oxygen is trapped out, the adult mites therein suffocates to death (Karen *et al.,* 2012). Following the suffocation of the mites, the secretion of the antigenic saliva from the mites ceases thereby birthing less irritation and eosinophilic influx. This known mechanism of suffocation could account for the improvement of erythrocytic indices at week 5 and 6.

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The elevation of the platelet lymphocyte ratio of sarcoptes infested dogs was earlier established as a diagnostic indicator in canine sarcopticosis (Nwufoh *et al.,* 2019). From the present study, PLR was observed to be low in Cypermethrin treated dogs as against other treatments. Even though differences were insignificant at the second week of therapy, Cypermethrin treated dogs still had the lowest PLR values. Of the botanical preparations, either of Citrus or Neem oil can be used as an anti-sarcoptic agent as differences in their PLR values were insignificant.

The swift hematologic (PCV, HB, RBC) response to Neem oil and Cypermethrin positions both therapies as fit for use especially in the face of the ongoing anemia. Consequently, both therapies can be instituted in the management of canine sarcopticosis without the use of hematinic agent while Citrus oil and Ivermectin can be used alongside blood promoters. Petroleum jelly treated dogs fared better from the fifth week of treatment as the trapping and suffocation of mites could have triggered the release of histamine and eosinophils thereby reducing improvement in red blood cell counts. In the light of this, petroleum jelly can be used with hematinic especially within the first four weeks of treatment.

The elevated PLR, MLR, NLR and ELR at the fifth and sixth week of treatment indicated a chronic on-going infection that could have resulted from several means of contamination especially during the teeth itch on the body of the dogs. Bearing in mind that dogs used were initially infested for six weeks before initiation of treatment, the duration of infestation and contamination may perhaps have contributed to persistence of infection. Accordingly, it becomes vital to submit that anti-microbial agents be administered alongside any of the anti-parasitic agent.

The model and perfect Acaricide should be toxic to every life cycle stage of mites and should prevent re-infestation of parasites for complete wholeness of the animal. Asides these basic ingredients, it should also come with safety and less toxicity to pets and an increase in susceptibility of parasites. Since this model Acaricide is yet in place, therapeutic protocols for canine sarcopticosis should be synergistic in approach.

Conclusively, the readily available and affordable Neem seed oil positions itself as a choice miticide in the face of its swift improvement of parasite induced blood loss while Cypermethrin stands ideal as it also improved the anemic condition and lowered the platelet lymphocyte ratio of infested dogs which had earlier been documented a diagnostic biomarker

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