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Amelioration of haematological alterations in dogs with generalised demodicosis by adjunct administration of a novel polyherbal formulation

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Abstract
The ameliorative potential of a polyherbal formulation on haematology of dogs with generalised demodicosis was evaluated in terms of alterations in the both leukograms and haemograms. The dogs diagnosed with generalised demodicosis were allocated into two groups Group 1(n=09) and Group 2(n=10) out of which Group 2 dogs were supplemented with polyherbal supplementation. Proper blood samples were collected on day 0, 30 and 60 respectively to evaluate the amelioration of haematological alterations of diseased dogs by adjunct administration of a polyherbal formulation. The diseased dogs supplemented with polyherbal formulation revealed remarkable amelioration in the leukograms e.g., reduction in absolute granulocytes counts and increment in lymphocytes percentage at days 30 and 60 post-therapy along with improvement in Haemograms.

Keywords: Demodicosis, leukograms, haemograms, polyherbal formulation

1. Introduction
Dermatological ailment accounts for sizeable percentage of the small animal sufferer [2]. The management of canine demodicosis remains one of the main challenges in veterinary Dermatology. Owing to wide prevalence of canine demodicosis, increasing incidence of drug resistance and detrimental side effects with most of the allopathic drugs, there is quest for the safe alternative adjunctive medicines. Beside this traditional medicines hold a great promise as a source of easily available effective therapy for skin diseases to the people, particularly in tropical developing countries, including India. It is globally well established fact that herbal drugs/indigenous drugs exhibited multiple immunomodulatory actions including modulation of cytokine secretion, histamine release, immunoglobulin production, immunoglobulin class switching, cellular co-receptor expression, lymphocyte proliferation and phagocytosis promotion [6], thus the resultant effect of all these changes hasten the clinical recovery of the sufferers. Under the light of these facts the present study was chalked out to evaluate the effects of a polyherbal formulation on clinical recovery of dogs with generalised demodicosis.

2. Materials and Methods
2.1 Selection criteria of animals and design of the study
Client-owned dogs presented with dermatological ailments at Teaching Veterinary Clinical Complex (Kothari Hospital) of the University for clinical and dermatological examination were examined and diagnosis of demodicosis was made by detection of mature and immature Demodex canis mites in scrapings and/or hair pluck samples from lesional skin. The dogs were diagnosed with generalized demodicosis when they have either minimum of five affected areas (>10 cm^2 each) or have a single-affected body region (>100cm^2) or have at least one affected paw (pododemodicosis) [3]. The dogs must be older than 7 weeks of age and weighing >4kg.

2.2 Animal inclusion criteria
Dogs with generalised demodicosis that have not been treated with ectoparasiticides or steroidal anti-inflammatory drugs in the last 30 days prior to clinical examination were included in this study. Diseased dogs have history of regular routine deworming and were free of any other concurrent diseases. The participated demodicosed dogs were also free of other ecto-parasites infestations, except for *D. canis* mites. Dogs were also found negative for haemoprotozoa on thin blood smears examination and have physiological parameters like body temperature, respiratory-rate and heart-rate within the normal reference range.

2.3 Treatment plan
The dogs diagnosed with generalised demodicosis were allocated into Group 1 (n= 09) and Group 2 (n=10). The demodicosed dogs of Group 1 and Group 2 both were treated with 0.0375% solution v/v of amitraz rinse at weekly intervals for 8 weeks. While Group 2 dogs were additionally supplemented orally with one capsule (250 mg) twice in a day of a polyherbal formulation for 8 weeks. Demodicosed dogs of both of the groups were bathed thoroughly with 2.5% benzoyl peroxide shampoo and towel dried before each application of amitraz rinse.

2.4 Blood sample collection
With the informed verbal consent of the pet owners, approximately 2 mL blood samples were obtained into vials containing EDTA from each of the diseased dog on every examination during the period of the therapy and were subjected for routine haematology. Blood samples were collected on day 0(start of the therapy); day 30 post-therapy and day 60 post-therapy.

2.5 Haematology
CBC (Complete blood count) of all the collected blood samples were carried out by using fully automated haematology analyzer (BS-2800 Vet Haematology analyzer, Mindray Electronic Co. Ltd).
Total erythrocyte counts (TEC), haemoglobin (Hb), haematocrit (HCT), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC), differential leukocyte counts, granulocytes, monocytes, lymphocytes, eosinophils counts were measured for each collected blood samples.

2.6 Statistical analysis
All data were expressed as mean ± S.E.M. Statistical analysis were conducted to determine the difference between the groups by using one-way ANOVA, post-hoc Tukey's test with general linear models in SPSS 16. While, the comparison among the values within the same group at different time intervals were analyzed by the Repeated Measures approach using ANOVA with mixed linear models in SPSS 16. The level of statistical significance for all the comparisons were at $P<0.05$.

3. Results and Discussion
The ameliorative potential of a polyherbal formulation on haematology of dogs with generalised demodicosis was evaluated in terms of alterations in the both leukograms (Table-1) and haemograms (Table-2). The results of the present study evidently indicate the marked alterations in haematological panels of dogs with generalised demodicosis and need of amelioration for holistic management of diseased animal health. The demodicosed dogs adjunctly supplemented with polyherbal formulation found to have remarkably ameliorated leukograms, mainly TLC and granulocytes at days 30 and 60 post-therapy. Moreover at days 30 and 60 post-therapy, the dogs of this group also found to have significantly ($P<0.01$) lower values of TLC, granulocytes and lymphocytes as compared with the same days post-therapy values of non-supplemented dogs i.e. (Group 1).Remarkably elevated TLC and granulocytes at day 60 post-therapy in the control group could be resulted from the dog's immune response to ameliorate the mite's proliferation. In agreement to our observation, Reddy *et al.* [4] demonstrated an elevated total TLC and absolute number of granulocytes in dogs with demodicosis. The diseased dogs supplemented with polyherbal formulation revealed remarkable amelioration in the leukograms e.g., reduction in absolute ($P<0.01$) granulocytes counts and increment in absolute ($P<0.05$) lymphocytes percentage at days 30 and 60 post-therapy. The absence of mites and/or remarkably reduced mites at days 30 and 60 post-therapy in the dogs of this group might responsible for amelioration of leukograms. These finding validates the potential immuno-restorative activities of the polyherabal formulation. The demodicosed dogs of the control group that were only treated with the miticide have not revealed remarkable amelioration in the leukograms at days 30 and 60 post-therapy.
Moreover at days 30 and 60 post-therapy, remarkable improvements in Haemograms were not revealed by the demodicosed dogs of control group. Whereas, at the same days of therapy; the demodicosed dogs supplemented with polyherbal formulation revealed remarkable amelioration in the haemograms. Reddy *et al.* [4] demonstrated an elevated remarkably lower haemograms including TEC, Hb and HCT in dogs with generalised demodicosis. Remarkably shoddier haemograms of dogs with generalised demodicosis is attributed to the loss of skin protein [1]. Free radical mediated damage and/or production of erythrocytes may be attributed to alterations in the haemograms of dogs with generalised demodicosis. This hypothesis is also supported by the findings of the present study that the dogs of control group revealed remarkable clinical recovery at days 30 and 60 post-therapy but still they found to have markedly high levels of inflammatory cell mainly granulocytes. Whereas, the dogs supplemented with the polyherbal formulation revealed marked improvement in haemograms. Singh *et al.* [5] demonstrated the antioxidant augmenting potential of Withania somnifera extract (WSE) in dogs with demodicosis [5]. Therefore, it is quite possible that polyherbal formulation supplementation might has lucratively ameliorated the altered leukograms of dogs with generalised demodicosis to augment the haemograms at days 30 and 60 post-therapy.

Table 1: Effects of Polyherbal supplementation on Leukogram of dogs with generalised demodicosis

Panels	Controls (Group 1; n=09)			Pyodermacare-G supplemented (Group 2; n=10)		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
TLC ($10^3/\mu L$)	27.26±2.64	24.10±2.9^{A}	20.21±1.85^{A,B}	24.40±1.9	11.57±0.64^{a}	7.67±0.37^{a,B}
Lymphocytes ($10^3/\mu L$)	5.26±0.41	4.45±0.32^{A}	4.54±0.40^{A,B}	5.01±0.52	2.48±0.21^{a}	2.03±0.10^{a,B}
Monocytes ($10^3/\mu L$)	0.97±0.14	0.68±0.07^{A}	0.63±0.08^{A,B}	1.06±0.16	0.68±0.14^{A}	0.45±0.03^{a,B,‡}
Granulocytes ($10^3/\mu L$)	20.77±2.40	18.33±3.01^{A}	14.66±1.52^{A,B}	18.34±1.70	7.96±0.47^{a}	5.04±0.26^{a,B}
Lymphocytes (%)	20.07±2.02	20.36±1.60^{A}	22.83±1.42^{A,B}	20.71±1.71	23.08±1.41^{A}	26.64±0.65^{a,B,‡}
Monocytes (%)	3.71±0.45	2.98±0.26^{A}	3.36±021^{A,B}	4.37±0.61	5.09±0.32^{A}	5.79±0.19^{b,B}
Neutrophils (%)	72.60±2.08	73.68±1.82^{A}	70.81±1.57^{A,B}	71.77±2.53	68.86±1.17^{A,‡}	65.73±0.51^{b,c}
Eosinophils (%)	3.36±0.57	3.18±0.52^{A}	3.87±0.71^{A,B}	3.05±0.68	2.05±0.46^{A}	1.89±0.24^{A,B}

^{A}Non-significant difference, when compared with day 0 values of the same group; ^{B}Non-significant difference, when compared with day 30 values of the same group; ^{a}Significant ($P<0.01$) difference, when compared with day 0 values of the same group; ^{b}Significant ($P<0.05$) difference, when compared with day 0 values of the same group; ^{c}Significant ($P<0.05$) difference, when compared with day 30 values of the same group; ; ‡Significant ($P<0.05$) difference, when compared with same day post-treatment values of Group 1

Table 2: Effects of Polyherbal supplementation on Haemogram of dogs with generalised demodicosis

Panels	Controls (Group 1; n=09)			Pyodermacare-G supplemented (Group 2; n=10)		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
RBC (10^6/µL)	5.12±0.5	5.65±0.36^{A,}	6.20±0.29^{A,B}	4.88±0.37	6.37±0.13^{a}	7.33±0.16^{a}
HB (gm/dL)	9.25±0.96	9.94±0.82^{A}	11.02±0.48^{A,B}	8.7±0.60	12.14±0.15^{a}	13.79±0.24^{a}
HCT (%)	38.58±3.4	39.30±2.39^{A}	42.90±2.20^{A,B}	35.99±2.5	47.34±0.84^{a}	52.78±1.93^{a}
MCV (fL)	71.93±1.5	70.26±1.3^{A}	69.15±1.4^{A,B}	75.58±1.92	75.51±2.34^{A}	71.99±1.32^{A,B}
MCH (pg)	19.6±1.18	16.74±0.75^{A}	17.43±0.34^{A,B}	17.37±0.5	19.08±0.41^{a}	18.79±0.23^{b}
MCHC (g/dL)	24.85±0.86	24.35±1.0^{A}	25.76±0.46^{A,B}	24.01±0.29	25.62±0.27^{b}	26.26±0.66^{a}
Platelets (10^3/µL)	442±91	276±22^{A}	316±27^{A,B}	317±40	269±17^{A}	288±21^{A,B}

^{A}Non-significant difference, when compared with day 0 values of the same group; ^{B}Non-significant difference, when compared with day 30 values of the same group; ^{a}Significant (P<0.01) difference, when compared with day 0 values of the same group; ^{b}Significant (P<0.05) difference, when compared with day 0 values of the same group.

4. Conclusion

The ameliorative potential of the polyherbal formulation on haematology of dogs with generalised demodicosis was evaluated in terms of alterations in the both leukograms and haemograms. The polyherbal formulation revealed marked improvement in haemograms. Therefore, it is quite possible that the polyherbal formulation supplementation might had lucratively ameliorated the altered leukograms of dogs with generalised demodicosis to augment the haemograms at days 30 and 60 post-therapy. Therefore, it can be concluded that miticidal therapeutic regimens of canine generalised demodicosis warrants supplementary medicines having immunomodulatory potential for the holistic management and to get rid of the wretched clinical condition. The polyherbal formulation could be promising candidate for the holistic managements of immuno-clinico-pathological anarchies of canine generalised demodicosis.

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