# EFFECT OF ORAL SUPPLEMENTATION OF ENROFLOXACIN IN DOGS SUFFERING FROM PERIODONTAL DISEASE

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### **ABSTRACT**

The present study was conducted on 10 dogs suffering from periodontal disease of different stages. Animals were randomly divided, irrespective of age, sex and breed, into 2 groups of five animals each. All dogs had physiological parameters within normal range. Dogs suffering from systemic diseases were not included in the study. Supragingival scaling and polishing followed by tooth brushing and chlorhexidine mouthwash was recommended in all the animals of group I and II. Additionally, tablets of enrofloxacin (@5 mg/kg) orally twice a day for one week was given to dogs of group II. After recording history and general clinical examination, all the dogs were anaesthetized for full mouth examination, dental radiography and dental scaling & polishing. Blood samples were collected before treatment at 7th day, 14th day and 21st day after treatment for haemato-biochemical as well as IL-1β expression analysis. Oral supplementation of enrofloxacin in group II caused significant reduction in plasma ALT, AST, ALP, GGT, BUN, creatinine as well as IL-1β expression level in comparison to group I. It was concluded that, oral supplementation of enrofloxacin had significant therapeutic effect in the treatment of periodontal disease.

Keywords: Dental radiography, Enrofloxacin, Halitosis, IL-1β, Periodontal disease, Supragingival scaling

Periodontal Disease (PD) is the inflammatory condition initiated by bacterial plaque which affects the gingival and surrounding tissues. Plaque is a microbial biofilm on teeth surface which is formed by a well-organized community of microorganisms embedded in a matrix of polymers of bacterial and salivary origin. This biofilm leads to caries and may be accompanied with halitosis. The bacteria-host interaction initiates an immune response that includes the synthesis of cytokines, which contribute to connective tissue degradation and alveolar bone loss (Schenkein, 2006).

Unchecked PD has numerous dire consequences both locally and systemically. It is the most common cause of tooth loss in adult dogs, and associated with serious systemic health concerns (Cave *et al.*, 2012). The local consequences are oro-nasal fistulas, pathological fractures, ocular problems, osteomyelitis and increased incidence of oral tumours. Systemic effects include renal failure, hepatic disorders, pulmonary and cardiac diseases, osteoporosis, adverse pregnancy effects and diabetes mellitus (Glickman *et al.*, 2009).

The etiology of PD is multifactorial, however, dental plaque is the primary factor. Additional factors like teeth overcrowding, soft foods, absence of oral hygiene, decreased resistance to infection and metabolic diseases contribute to dental plaque accumulation (Tatakis and Kumar, 2005). The present study was planned to evaluate the periodontal disease status in dogs and evaluate the efficacy of enrofloxacin after dental scaling for treatment of PD by gross, radiographic, haematological and

biochemical analysis.

### MATERIALS AND METHODS

The present study was conducted on 10 clinical cases of dogs suffering with periodontal diseases (PD), irrespective of age, breed, sex and were free of any other systemic illness in both groups (I and II). Signalment, vaccination status, deworming status and diet given was recorded for all the dogs. After full mouth examination and anaesthesia, dental radiography was done using OraDect Pro® DDX 1500 Intra Oral RVG Sensor before scaling, after scaling and 21 day after scaling.

Blood samples were collected from cephalic or recurrent tarsal vein in EDTA as well as heparinized vials at 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of dental scaling. EDTA blood was used for haematology (MS-4 veterinary hematology analyzer, Melet Schloesing Laboratories) as well as IL-1β expression studies (using RNA isolation, reverse transcription and real-time PCR), however, blood in heparinized vials were used for harvesting plasma (after centrifugation at 2500 rpm for 15 minutes) for estimation of biochemical parameters with help kits through Transasia®–ERBAEM 200 automatic serum analyzer.

All the animals were treated by supragingival scaling and tooth brushing along with chlorhexidine lavage in both groups. Additionally, in group II enrofloxacin tablet was administered orally @ 5 mg/kg body weight twice a day for five days.

### RESULTS AND DISCUSSION

The mean age of the animals included in the study were 6 years and 11 months in group I and group II. 40% of

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dogs affected with PD belonged to small toy breeds like Pug, Spitz and Daschund. Out of 10 affected dogs, 9 were males while 1 was female. High percentage of small breeds in affected population supports the previous reports of positive correlation between age, prevalence, small toy breed predisposition and severity of PD (Kyllar and Witter, 2005). There is no report of correlation between sex predisposition and PD but owner's preference for male over female dogs due to the problem of estrus in the locality was the main reason of more number of males reported in this study.

In all the animals there was bilateral deposition of calculus on canines and 3<sup>rd</sup> & 4<sup>th</sup> premolar teeth. Halitosis was the most common sign. Out of ten animals, six had alveolar bone loss, four had tooth furcation, three had horizontal bone loss, one had vertical bone loss and six had combination of both horizontal and vertical bone loss (Table 1). Additionally, few dogs had tooth fracture, root resorption and loss of lamina dura. Dental radiography was found to be an effective tool in diagnosis and monitoring progression or response to treatment of PD in dogs. Clinical conditions and radiographs of dogs affected with PD before and after treatment are shown in Fig. 1. Dental radiography is valuable for canine dental procedures and is an important part of the medico-legal record and helps in treatment planning (Bannon, 2013). However, it can't replace the general oral examination but this aid to provide details about alveolar bone plate, tooth root and surrounding structures (Dupont and DeBowes, 2009).

There was no significant variation in mean haemoglobin, TEC and PCV of the affected dogs but these

improved non-significantly after treatment. Pathological changes and body response during periodontal diseases in dogs might have myelo-suppressive actions responsible for lower PCV during disease and improvement after treatment (Santosh *et al.*, 2016). As the disease was chronic and high production of inflammatory cytokines like IL-1, IL-6 and TNF also led to suppression of erythropoiesis (Shetty *et al.*, 2017).

TLC decreased in all dogs after treatment but the reduction was significantly higher in dogs treated with enrofloxacin than that of group I after scaling. The neutrophils also decreased significantly in both groups after scaling and polishing. This might be due to decreased bacterial load after scaling and polishing (Waghmare et al., 2013). All dogs had poor oral hygiene leading to gingivitis which progressed to periodontitis having increased microbial load and consequently more neutrophils (Rosales and Uribe Querol, 2017). The lymphocytes, monocytes and eosinophils were in normal range. There were no significant variations in the values of TEC, MCV, MCH and MCHC (Gokhale et al., 2010). The platelets count in both groups decreased significantly after treatment. Al-Rasheed (2012) also reported the increased platelets count of patients with chronic periodontitis.

There was decrease in plasma ALT, AST, GGT and ALP after treatment in PD affected dogs (Table 2). Rawlinson *et al.* (2011) reported a significant positive correlation between ALT, ALP level and PD. The reduction in the level of ALT, AST and specifically GGT suggest distressing of the liver and less toxin production from the periodontal source. There was no significant change in the

Table 1
Radiographic changes in individual dogs

AnimalNumber	Alveolar bone loss	Furcation	Horizontal bone loss	Vertical bone loss	Combination of horizontal and vertical bone loss	Any other lesion
C1			✓			Root fracture
C2	✓				✓	Root resorption
C3		✓		✓		Loss of tooth support
C4	✓					Loss of lamina dura
C5	✓				✓	
E1	✓				✓	Root fracture
E2	✓	✓			✓	Loss of lamina dura
E3	✓		✓			Calculus, loss of lamina dura
E4		✓			✓	Root resorption, loss of lamina dura
E5		✓	✓		✓	Root fracture
Total	6	4	3	1	6	

 $Table\ 2$  Effect of different treatments on plasma ALT, AST, ALP and GGT level in dogs suffering with PD (Mean  $\pm$  SE)

Biochemical Test	Normal Range*		0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21st day
Plasma ALT (IU/L)	10-109	Group I	$24.42 \pm 6.40$	17.16±4.29	$15.88 \pm 4.86$	$13.56 \pm 1.19$
		Group II	$27.10^{\rm B}\!\pm\!6.51$	$18.22^{^{AB}}\!\pm3.64$	$14.42^{^{A}} \pm 2.11$	$12.98^{^{\mathrm{A}}}\!\pm1.72$
Plasma AST (IU/L)	13-15	Group I	$13.90^{\rm B}\!\pm\!0.25$	$12.60^{\text{A}} \pm 0.24$	$12.16^{A} \pm 0.44$	$12.56^{A} \pm 0.56$
		Group II	$13.32 \pm 0.45$	$13.08 \pm 0.15$	$12.56 \pm 0.40$	$12.60 \pm 0.30$
Plasma ALP (IU/L)	1-114	Group I	$25.20^{\rm B}\!\pm\!2.72$	$15.60^{\text{A}} \pm 2.92$	$11.60^{^{\mathrm{A}}} \pm 2.08$	$14.00^{^{\mathrm{A}}}\!\pm1.87$
		Group II	$41.00^{\rm B}\!\pm\!8.18$	$17.00^{A} \pm 2.16$	$11.80^{^{\mathrm{A}}}\!\pm1.31$	$11.80^{\text{A}} \pm 1.59$
Plasma GGT (IU/L)	1-10	Group I	$6.20^{\rm B}\!\pm\!0.58$	$3.60^{\text{A}} \pm 0.40$	$3.40^{^{A}} \pm 0.40$	$2.60^{^{\mathrm{A}}} \pm 0.40$
		Group II	$6.20^{\circ} \pm 0.37$	$4.00^{^{A}} \pm 0.31$	$3.20^{^{AB}}\!\pm\!0.37$	$2.80^{^{A}}\!\pm\!0.37$

Means with different superscripts (A, B, C: within a row) vary significantly (p<0.05); \*Reference values from Merck Veterinary Manual

Table 3 Effect of different treatments on plasma albumin, TP, A:G, BUN, creatinine, calcium, phosphorus, sodium and potassium level in dogs suffering with PD (Mean  $\pm$  SE)

		icver in dogs	suffering with 1 D	(Mean = SE)		
Biochemical Test	Normal Range*		0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
Albumin (g/dl)	2.3-3.1	Group I	$3.01 \pm 0.17$	$2.90 \pm 0.24$	$3.23 \pm 0.17$	$2.95 \pm 0.27$
		Group II	$3.00^{^{\mathrm{A}}} \pm 0.09$	$3.42^{\rm B}\!\pm\!0.12$	$3.37^{^{AB}}\!\pm\!0.18$	$3.29^{^{AB}}\!\pm\!0.11$
TP(g/dl)	5.4-7.5	Group I	$6.83^{\rm B}\!\pm\!0.27$	$6.04^{^{A}}\!\pm0.25$	$6.28^{^{AB}}\!\pm0.18$	$5.89^{A} \pm 0.19$
		Group II	$6.57\pm0.20$	$6.53 \pm 0.18$	$6.58 \pm 0.18$	$6.34 \pm 0.26$
A:G	0.70-0.85	Group I	$0.81 \pm\ 0.09$	$0.97\pm0.13$	$1.08 \pm 0.11$	$1.03\pm0.15$
		Group II	$0.86\pm0.08$	$1.14 \pm 0.14$	$1.08\pm0.13$	$1.11\pm0.11$
BUN (mg/dl)	8-28	Group I	$23.32^{\rm B}\!\pm\!2.19$	$17.86^{^{AB}} \pm 2.62$	$13.94^{^{Aa}}\!\pm1.07$	$16.30^{^{\mathrm{A}}}\!\pm1.50$
		Group II	$27.66^{^{\mathrm{B}}}\!\pm1.95$	$17.30^{^{\mathrm{A}}}\!\pm1.39$	$20.78^{\rm Ab}\!\pm\!1.57$	$18.24^{^{\rm A}}\!\pm\!0.88$
Creatinine (mg/dl)	0.5-1.7	Group I	$1.32B \pm\ 0.06$	$1.05^{^{AB}}\!\pm\!0.08$	$0.88^{^{\mathrm{A}}} \pm 0.13$	$0.86^{^{\mathrm{A}}} \pm 0.11$
		Group II	$1.44^{\circ} \pm 0.05$	$1.14^{\rm B}\!\pm\!0.04$	$0.82^{^{\mathrm{A}}}\!\pm\!0.05$	$1.00^{^{B}}\!\pm\!0.06$
Calcium (mg/dl)	9.1-11.7	Group I	$9.20^{^{\mathrm{A}}}\!\pm\!0.03$	$9.70^{\rm B}\!\pm\!0.20$	$9.92^{\rm B}\!\pm\!0.09$	$9.72^{^{\rm B}}\!\pm\!0.17$
		Group II	$9.30^{^{A}}\!\pm\!0.08$	$10.06^{\mathrm{B}} \pm 0.16$	$10.10^{\rm B}\!\pm\!0.08$	$9.88^{^{B}}\!\pm\!0.16$
Phosphorus (mg/dl)	2.9-5.3	Group I	$4.33\pm0.27$	$4.79 \pm 0.23$	$4.65 \pm 0.11$	$4.77\pm0.20$
		Group II	$4.01^{^{\mathrm{A}}}\!\pm\!0.17$	$4.84B \pm 0.17$	$4.96^{\rm B}\!\pm\!0.14$	$4.72^{\rm B}\!\pm\!0.15$
Sodium (mmol/L)	142-152	Group I	$155.25^{AB} \pm 1.05$	$157.96^{\text{Bb}} \pm 1.41$	$154.44^{AB} \pm 1.61$	153.23 <sup>A</sup> ±0.87
		Group II	$155.79. \pm 1.80$	$154.00^{\text{a}}\!\pm1.37$	$156.61 \pm 1.21$	$155.89 \pm 1.67$
Potassium (mmol/L)	3.9-5.1	Group I	$4.04\pm0.27$	$4.06 \pm 0.17$	$4.76\pm0.36$	$4.50\pm0.24$
		Group II	$3.93 \pm 0.16$	$4.58 \pm 0.30$	$3.87 \pm 0.22$	$4.06 \pm 0.19$

Means with different superscripts (a, b, c: within a column and A, B, C: within a row) vary significantly (p<0.05) \*Reference values from Merck Veterinary Manual

plasma total protein, albumin and globulin suggesting no systemic involvement of liver (Table 3).

Plasma BUN concentration decreased in both groups significantly (p<0.05) after treatment. This indicates a decrease in catabolic activity on proteins due to less production of microbial toxin and decrease in self generated immune reactions in response to PD after treatment. Even prophylactic cleaning of teeth also markedly

decreases plasma BUN concentration (Rawlinson *et al.*, 2011). Also, there was significant reduction in the plasma creatinine in both groups after treatment (Table 3). The dogs with systemic diseases were not included and none of the dogs exhibited any signs of renal failure. The plasma calcium level in both groups improved significantly (p<0.05) after treatment. It has been reported that low calcium level and PD are positively correlated (Henrikson,



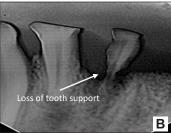






Fig. 1. Clinical conditions and radiograph of dog affected with PD before and after treatment: A) Before dental scaling and polishing; B) Radiographic before dental scaling and polishing; C) After dental scaling and polishing; D) Radiograph after 21 day of dental scaling and polishing

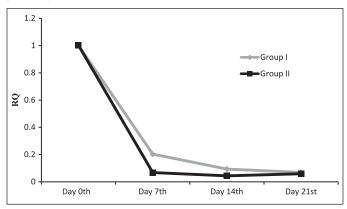


Fig. 2. Effect of different treatments on fold change in expression (RQ) in dogs suffering with PD (Mean ± SE)

1968). However, some claimed that animals with PD had more serum calcium levels, similar to those seen in people (Sah *et al.*, 2012). There was no significant variation in the level of plasma phosphorus between the groups, however, phosphorus level improved to maintain the ratio with calcium.

The IL-1 $\beta$  transcryptome analysis through real time qPCR in blood revealed significant decrease in the proinflammatory cytokinine IL-1 $\beta$  expression level in both the groups but the reduction was significantly greater in group II than group I which is shown in Fig. 2.

It is concluded that oral administration of enrofloxacin, after dental scaling-polishing along with tooth brushing and chlorhexidine lavage is more effective than treatment without antibiotic supplementation in dogs with periodontal disease.

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