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Reno protective evaluation of biopolymer encapsulated *Lactobacillus acidophilus* in canine nephropathy

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Abstract
Chronic kidney disease (CKD) in dogs is a progressive condition that affects the kidneys' ability to function properly over time. It is characterised by non-regenerative anaemia, hyperphosphatemia, hypertension and bone marrow abnormality. Multimodal approach is required to treat the patients with CKD in order to prevent the vicious cycle of CKD. This study aimed to assess the efficacy of encapsulated probiotic in canine kidney failure cases. Eighteen (18) dogs with CKD IRIS stage 2 and /or 3 were enrolled in this study and divided into different groups randomly to evaluate the effects of conventional treatment, probiotics, and encapsulated probiotics. Blood and serum samples were collected from the dogs for hematological and biochemical analysis. Urine analysis and ultrasonography were also performed. The results of the study showed that dogs with CKD had lower levels of hemoglobin and packed cell volume (PCV) compared to healthy dogs. However, on day 10 of the study, the group received encapsulated probiotics showed a significant ($p<0.05$) increase in PCV levels as compared to day 0, suggested potential improvement in anemia with probiotic supplementation. In terms of biochemical parameters, significant ($p<0.05$)improvements were observed in creatinine, blood urea nitrogen (BUN), and serum glutamic pyruvic transaminase (SGPT) levels in the group received encapsulated probiotics compared to the conventional therapy group and non-encapsulated probiotic group. The study suggests that the supplementation of encapsulated probiotics in dogs with CKD may have beneficial effects on kidney function.

Keywords: Dog, kidney failure, probiotics

Introduction

Chronic kidney disease (CKD) is associated with the development of progressive and irreversible damage to the kidneys, leading to impaired excretory, biosynthetic, and regulatory functions. While the loss of nephrons and the subsequent decline in renal function cannot be reversed, only the progression of kidney failure can be slowed down by appropriate interventions like hemodialysis and kidney transplantation (Martello *et al*., 2021) [4]. However, these interventions are expensive and not accessible to everyone. There is an urgent need to develop an alternative and effective method to halt the progression of kidney failure. Probiotics, which are beneficial bacteria and yeasts, have been studied for their potential benefits in various health conditions, including chronic kidney disease (CKD) in dogs (Fagundes *et al*., 2018) [3]. Probiotics can maintain a healthy balance of gut bacteria, which is important for overall health and immune function (Lippi *et al*., 2017) [6]. In CKD, dogs may experience alterations in gut microbial composition, which can contribute to gastrointestinal symptoms. In CKD, uremic toxins, which are waste products that accumulate in the blood due to impaired kidney function, can passively diffuse from the blood into the gastrointestinal tract. Urease-producing probiotic species are thought to hydrolyze urea, creating a concentration gradient that facilitates the diffusion of urea from the blood to the gastrointestinal tract lumen. By doing so, probiotics may aid in converting nitrogenous waste compounds into non-toxic substances (Rysz *et al*., 2021) [17]. Probiotics can potentially help in restoring the balance of gut bacteria and improve gut health. Dogs with CKD often have impaired kidney function, which can lead to the build-up of toxins in the body. CKD is associated with immune dysregulation, and probiotics may help regulate the immune system and reduce inflammation, which is often observed in CKD. This short communication deals with reno protective evaluation biopolymer encapsulated *Lactobacillus acidophilus* in dogs.

Material and Methods

The study was conducted at Division of Medicine, ICAR-Indian Veterinary Research Institute (IVRI) from January 2023-April 2023. Dogs diagnosed with IRIS stage II and/or III kidney failure were enrolled in this study. Dogs were randomly divided into three groups viz., conventional treatment, blank *L. acidophilus* and encapsulated *L. acidophilus.* Treatment details are presented in the table no. 1. Encapsulated probiotic was used as adjunct therapy wherein *Lactobacillus acidophilus* was encapsulated using alginate and pectin biopolymers by modified extrusion method with some modifications (Pedroso and Fleitas, 2020) [13]. During the clinical study for adjunct therapy against chronic kidney disease in canines, the dose obtained in the rat model has been converted by the formula of Nair and Jacob (2016) [10], with slight modifications which revealed calculated dose of dog was 13 × 10^{8-10} CFU BID per orally.Blood and serum samples were collected from kidney failure dogs for haemato-biochemical analysis. Haematology was done by auto analyser. Biochemical parameters were estimated by using semi-automatic biochemical analyser and commercially available biochemical kits. Urine analysis was also done in the affected dogs. Dogs were also subjected to ultrasonography to study the extent of renal damage.

Table 1: Therapeutic details in dogs

Groups	Treatment (n-6)	Therapeutic trial in nephropathy	Duration
Gr. I	Healthy control	No therapy	-
Gr. II	Standard Therapy	Disease specific therapy + Symptomatic therapy with standard dose and duration	10 days therapy followed by correlation of clinico-diagnostic tools with canine reference values (*INFORMED CLIENT CONSENT TRIAL*)
Gr. III	Adjunct Therapy	Disease specific therapy + non-encapsulated probiotic + Symptomatic therapy with standard dose and duration	
Gr. IV	Adjunct Therapy	Disease specific therapy + encapsulated probiotic + Symptomatictherapy with standard dose and duration	

*Disease specific therapy: Fluids (NS, DNS), Syp. Neeri®, Tab. Renodyl®
#Symptomatic therapy: Antibiotics, Inj. Ondansetron, Inj. Rantitidine

Statistical analysis

Data was analysed by using two-way ANOVA test using JMP software and they are significant at $p<0.05$.

Results

Study with encapsulated probiotic revealed that non-significant improvement was observed in various haematological values. However, there was significant ($p<0.05$) increase in levels of PCV on day 10 of NEP group. There was significant ($p<0.05$) decrease on day 10 of WBC values and RBC values of conventional therapy. The haematological results of dog trial have been tabulated in table no. 2 and Fig. no.1.Regarding biochemical parameters, significant ($p<0.05$) improvement was noticed in the values of creatinine, BUN, SGPT of dogs received encapsulated probiotic (EP) group depicted its beneficial effect over and above of conventional therapy as well as non-encapsulated probiotic (NEP) group by the end of therapy. However, there was significant ($p<0.05$) decrement in the level of creatinine in conventional therapy. Same trend was observed in the level of BUN in NEP group. Regarding other biochemical parameters no such significant change was observed as depicted in table no. 3 and Fig. no 2.

Table 2: Pre and post haematological changes of dogs received different adjunct therapy

Parameters	Groups	Day 0	Day 10
Hb (g/dl)	HC	13.65±0.75^a	13.65±0.75^a
	CT	11.88±2.31^a	11.52±1.81^a
	NEP	7.23±1.21^a	8.10±1.13^a
	EP	9.92±0.75^a	11.23±0.68^a
PCV (%)	HC	40.60±2.20^a	40.60±2.20^a
	CT	31.10±3.45^a	34.95±4.91^a
	NEP	22.77±3.55^a	27.32±3.33^b
	EP	31.10±2.65^a	35.10±1.74^a
WBC (10^3/µl)	HC	14.42±1.40^a	14.42±1.40^a
	CT	36.55±12.35^a	13.58±1.91^b
	NEP	18.95±3.20^a	14.03±0.57^a
	EP	24.97±7.46^a	11.00±0.88^a
RBC (10^6/µl)	HC	6.33±0.47^a	6.33±0.47^a
	CT	5.33±1.05^a	4.30±0.44^b
	NEP	3.02±0.48^a	3.40±0.43^a
	EP	4.60±0.59^a	4.82±0.51^a
PLT (10^3/µl)	HC	412.50±36.70^a	412.50±36.70^a
	CT	216.33±38.04^a	259±31.07^a
	NEP	320.83±48.82^a	367.7±40.47^a
	EP	205.83±26.03^a	285.33±30.58^a

Values within same in same row (small letter) bearing similar superscript do not differ at $p<0.05$. HC: Healthy control, CT: Conventional therapy, NEP: Non-encapsulated probiotic. EP: Encapsulated probiotic.

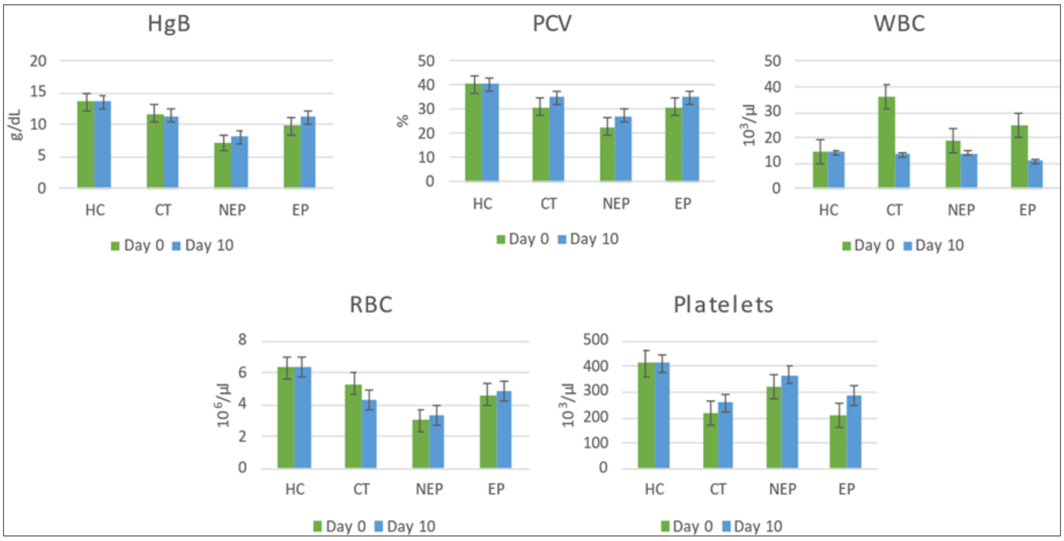

Fig 1: Pre and post haematological changes of dogs received different adjunct therapy

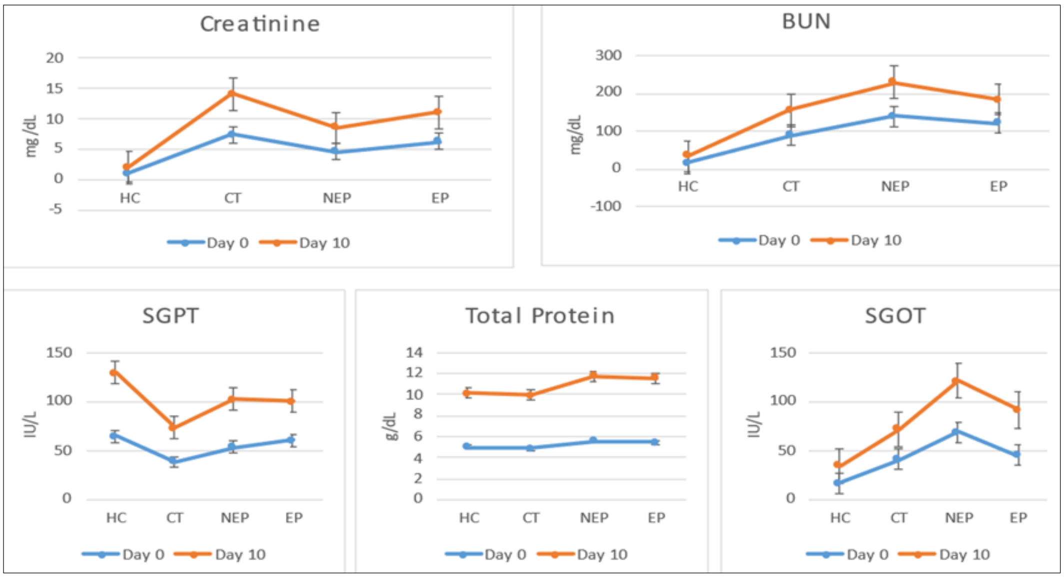

Fig 2: Pre and post biochemical changes of dogs received different adjunct therapy

Table 3: Pre and post biochemical changes of dogs received different adjunct therapy.

Parameters	Groups	Day 0	Day 10
Creatinine (mg/dl)	HC	1.00±0.11^{a}	1.00±0.11^{a}
	CT	7.41±1.82	6.71±1.61^{b}
	NEP	4.57±0.53^{a}	4.03±0.47^{a}
	EP	6.22±0.80^{a}	4.87±0.65^{b}
BUN (mg/dl)	HC	17.82±2.54^{a}	17.82±2.54^{a}
	CT	91.33±23.13^{a}	65.80±10.25^{a}
	NEP	141.91±24.75^{a}	89.63±10.20^{b}
	EP	122.69±26.45^{a}	62.10±8.00^{b}
SGPT (IU/L)	HC	64.48±9.51^{a}	64.48±9.51^{a}
	CT	38.69±9.56^{a}	34.47±8.08^{a}
	NEP	54.02±14.71^{a}	48.21±13.32^{a}
	EP	60.34±9.47^{a}	40.22±3.54^{b}
SGOT (IU/L)	HC	17.50±1.96^{a}	17.50±1.96^{a}
	CT	41.41±6.79^{a}	30.50±7.35^{a}
	NEP	68.84±25.32^{a}	52.15±7.53^{a}
	EP	45.74±6.06^{a}	45.60±4.93^{a}
Total Protein (g/dl)	HC	5.04±0.52^{a}	5.04±0.52^{a}
	CT	4.90±0.65^{a}	5.04±0.52^{a}
	NEP	5.62±0.65^{a}	6.16±0.43^{a}
	EP	5.46±0.52^{a}	6.05±0.27^{a}

Values within same row (small letter) bearing similar superscript do not differ at $p<0.05$. HC: Healthy control, CT: Conventional therapy, NEP: Non-encapsulated probiotic. EP: Encapsulated probiotic.

In the present study, 18 dogs were also subjected for ultrasonographic examination. Increased cortical echogenicity, glomerulonephritis (Fig. 3) was the most common abnormality in CKD dogs followed by hyper echogenicity of cortex and decreased demarcation between cortex and medulla (Fig. 4).

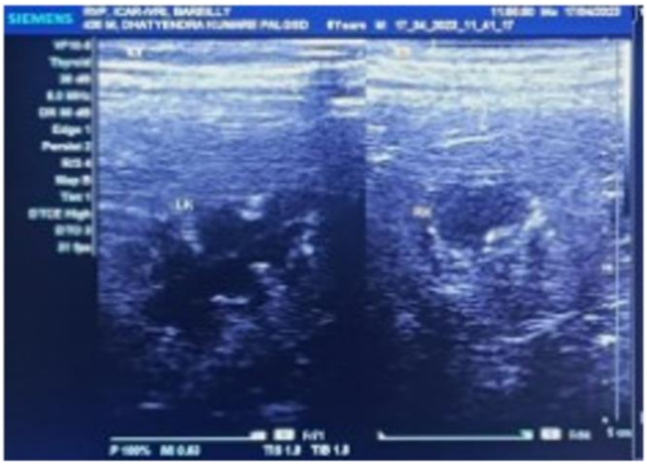

Fig 3: Glomerulonep hritis

Fig 4: Decreased corticomedullary differentiation

Discussion

Anaemia is considered as a common finding in dogs with chronic kidney disease (CKD), typically as normochromic, normocytic, and non-regenerative type reflected by marked reduction in haemoglobin levels in conventional therapy, encapsulated and non-encapsulated probiotic treated groups as compared to healthy dogs in our study. It was in accordance with findings of Lippi *et al.* (2021) [7]. The primary mechanism related to anaemia in CKD dogs is decreased renal production of erythropoietin (EPO). EPO is produced in renal peritubular fibroblast-like type-1 interstitial cells in response to cellular hypoxia (Polzin, 2011) [15]. There was significant (p<0.05) increase in levels of PCV on day 10 of NEP group. There was significant (p<0.05) decrease in the WBC and RBC values on day 10 in conventional therapy treated dogs. Probiotic supplementation helped to combat the ongoing crisis and encapsulated probiotic group revealed better efficacy because of its increased bioavailability to the host (Jo *et al*., 2014) [5].

A remarkable significant (p<0.05) improvement was noticed in the values of creatinine, BUN, SGPT of dogs received encapsulated probiotic (EP) group depicted its beneficial effect over and above of conventional therapy as well as non-encapsulated probiotic (NEP) group by the end of therapy. Creatinine levels significantly (p<0.05) decreased in both probiotic treated groups as compared to the conventional therapy group. Our findings are in agreement with Martello *et al.* (2021) [9] who conducted a double-blind case-control study that evaluated the efficacy of a dietary supplement containing calcium carbonate, calcium lactate-gluconate, chitosan, sodium bicarbonate, *Lactobacillus acidophilus* D2/CSL, *Olea europaea* L. extract, and fructooligosaccharides, in dogs in advanced CKD stage. BUN values increased in the affected animals in our study which is also in agreement with the findings of Dunaevich *et al.* (2020) and Karunanithy *et al.* (2021) [2]. Although there was a significant (p<0.05) decrease in BUN levels of both probiotic groups on day 10 compared to day 0, encapsulated probiotics performed better than non-encapsulated probiotics group.

Increased cortical echogenicity was the most commonly observed ultrasound finding in this study. Increased cortical echogenicity may be reported in cases of glomerulonephritis, amyloidosis, interstitial nephritis, acute tubular necrosis or nephrosis, end stage renal disease (ESRD) and nephrocalcinosis (Bragato *et al.*, 2017) [1]. In addition to that, renal pelvis and ureter dilatation were also found in recored in dogs which are in agreement with Slike and George, (2011) and Neuwirth *et al.* (1993) [18, 12]. Dogs with IRIS stage 2 had fewer abnormalities as compared to dogs with IRIS stage 3 kidney failure. Similar findings were also reported by Perondi *et al.* (2020) [14]. The prognosis and recovery of patients suffering from kidney failure was directly dependent on age of animal (Dunaevich *et al.*, 2020). Lippi *et al.* (2017) [2, 6] supported the important role of probiotic supplementation in reducing the progression of CKD in dogs and the relationship between intestinal dysbiosis and kidney injury called the "gut-kidney axis" in human medicine (Nallu *et al.*, 2017) [11]. A larger study should be conducted with more dogs with each of these abnormalities to assess their association with survival (Rudinsky *et al.*, 2018). Meineri *et al.* (2021) [9] also opined that the prebiotic, probiotic and antioxidant supplement corrected the nutritional status and improve blood and kidney parameters in dogs with advanced stage of CKD, in accordance with the findings of our study.

In our study 78% of dogs were geriatric making it challenging for better prognosis. The other limitations included different age group, different breeds, different baseline values of creatinine and different dietary habits and different environmental conditions the dogs in which they were maintained. Multiple causes have been implicated in the pathogenesis of CKD, including glomerular diseases, infections (e.g., chronic pyelonephritis), repeated ischemic events, nephrotoxicity, neoplasia, previous AKI or urinary obstruction (Dunaevich *et al.*, 2020) [2].

Conclusion

The encapsulated probiotic group exhibited significant improvements in hematological and biochemical parameters related to kidney function. However, the study be conducted in large number of naturally occurring canine kidney failure cases with multiple etiological factors to assess its efficacy in detail. Biopolymer-encapsulated *Lactobacillus acidophilus* may be recommended either alone or in combination with specific interventions like hemodialysis to halter the progression of kidney failure.

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