RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.1.138-143

Anti-oxidant effect of N-acetyl cysteine in dogs with chronic kidney disease

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Citation: Balakrishnan,N., Madhavan Unny,N., Ambily,V.R., Usha,N.P and Uma,R. 2023. Antioxidant effect of N-acetyl cysteine in dogs with chronic kidney disease. *J. Vet. Anim. Sci.* **54**(1):138-143
DOI: https://doi.org/10.51966/jvas.2023.54.1.138-143

Received: 27.10.2022 *Accepted: 20.12.2022* *Published: 31.03.2023*

Abstract

The present study was carried out with the objectives of assessing oxidative stress in dogs with chronic kidney disease (CKD) and evaluating response to treatment with N-acetyl cysteine (NAC). Dogs diagnosed with stage III CKD as per the guidelines of the International Renal Interest Society (IRIS) were included in the study. The animals were divided into two groups. Animals of one group were given standard therapy for CKD and the animals of the second group were administered NAC along with standard therapy. Oxidative stress parameters such as total antioxidant status (TAS), serum malondialdehyde (MDA) level and plasma glutathione peroxidase (GSH-Px) activity were studied. On the day of presentation, a significant increase in the mean values of serum MDA and TAS were observed in diseased animals compared to healthy animals, whereas a significant decline was noted in plasma GSH-Px activity. After treatment, a significant decline in serum MDA and TAS were recorded in animals of group II receiving NAC therapy. A significant increase in plasma glutathione GSH-Px activity was recorded in this group. N-acetyl cysteine therapy was found to be effective in the management of oxidative stress in dogs with chronic kidney disease.

Keywords: *Chronic kidney disease, N-acetyl cysteine, oxidative stress*

Chronic kidney disease (CKD), often considered an irreversible and progressive disease, could be defined as any structural or functional loss of one or both kidneys that have been present for three months or longer (Bartges, 2012). Although the prevalence of CKD is highest in geriatric

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dogs, young dogs are also susceptible to CKD due to the occurrence of congenital renal disease (Polzin, 2011). Increased urbanization, environmental pollution, unscientific feeding and abuse of common therapeutic agents were associated with a higher incidence of CKD in dogs (Katoch *et al*., 2017).

Oxidative stress is an important component of CKD. As the renal cells are metabolically most active, kidneys maintain persistently high levels of mitochondrial oxidative phosphorylation. This provides a suitable environment for the generation of reactive oxygen species (ROS) which can contribute to renal interstitial fibrosis, glomerulosclerosis and systemic and renal inflammation thereby promoting the progression of CKD. Kogika *et al*. (2014) reported that circulating uremic toxins and chronic inflammatory processes in CKD patients played an important role in ROS production and the development of oxidative stress. Renal adaptive responses to the loss of functional nephrons in CKD patients resulted in increased cellular oxidative phosphorylation and thereby oxidative stress which further led to the progression of CKD (Brown, 2008). Thus, the present study was carried out to assess oxidative stress in dogs with CKD as well as to evaluate response to N- acetylcysteine therapy in the management of oxidative stress.

Materials and methods

Selection of animals

Dogs presented to the Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai with clinical signs suggestive of CKD were screened for the study. Twelve dogs diagnosed with stage III CKD as per the guidelines of the International Renal Interest Society (IRIS) were selected and divided into two groups of six animals each. Animals of group I were subjected to standard therapy for fifteen days, whereas animals of group II were subjected to N-acetyl cysteine therapy along with standard therapy. Six apparently healthy adult dogs brought for vaccination or health check-up were selected and the parameters under study were estimated for comparison.

Oxidative stress parameters

Blood samples (4mL) were collected in clot accelerator tubes for assessing malondialdehyde (MDA) level and total antioxidant status (TAS). Serum was separated by centrifuging at 1459 x g for 15 min. Whole blood samples were also collected into a vacutainer containing heparin (75 USP) for glutathione peroxidase (GSH-Px) estimation. Oxidative stress parameters were analysed on days 0 and 15 of treatment.

Serum lipid peroxides level was determined by the method of Yagi (1984) by estimating MDA level. Thiobarbituric acid reactive substance test was used to assess MDA concentration (McMichael, 2007).

Total antioxidant status was measured by using ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996).

Glutathione peroxidase activity in plasma was measured using the procedure by Paglia and Valentine (1967) with modifications as elaborated by Pleban *et al*. (1982).

The absorbance was measured using Perkin Elmer Lambda 25 UV-VIS spectrometer.

Therapeutic management

All the dogs in groups I and II with stage III CKD were managed with parenteral administration of fluids, pantoprazole @ 1 mg/Kg body weight, intravenously (IV) once daily (OD), ondansetron @ 0.5 mg/Kg body weight, IV twice daily (BID) and intramuscular administration of B vitamins. Acid-base and electrolyte imbalances were corrected according to the results of blood gas and electrolyte analysis. Oral therapy was instituted with benazepril @ 0.25 mg/Kg body weight, OD, sevelamer hydrochloride @ 40 mg/Kg body weight, BID, sodium bicarbonate @ 10 mg/Kg body weight, thrice daily (TID), omega-3 polyunsaturated fatty acids (syrup Vitabest derm @ 10 ml OD) and commercial preparation of prebiotics and probiotics (Renodyl capsule @ 1 capsule BID). Amoxicillin-sulbactam @ 12.5 mg/Kg body weight, IV, BID was administered in all three dogs with pyometra and one with urinary tract

infection (UTI). Three dogs (two dogs in group I and one in group II) with anaemia (VPRC ?20 per cent) were treated with darbepoetin alfa @ 1µg/Kg body weight subcutaneously (SC), weekly once for 3 weeks along with oral haematinics (syrup Hemobest @ 5ml BID). Sucralfate @ 1g/dog, TID, PO was given to dogs with melena. Dietary modifications like the restriction of phosphorus and protein intake and initiation of commercially available renal diet were advised for all stage III CKD-affected dogs. In addition to these, animals of group II were given N-acetylcysteine @ 70 mg/Kg, IV, OD for 10 days.

Statistical analysis

The statistical analysis of data obtained was done using the SPSS software, version 24.0. Comparison of parameters between groups I, II and control were done by using one-way ANOVA followed by the Duncan Multiple Range test (DMRT). A comparison of all parameters between group I and II were done using independent t-test. Comparison of days 0 and 15 values of variables within each group was done using paired t-test.

Results and discussion

The mean values of oxidative stress parameters, *viz.* lipid peroxidation level, TAS and GSH-Px level in group I, group II and healthy animals are represented in Tables 1 and 2.

Lipid peroxidation level

Compared to healthy dogs (2.12 ± 0.07 nM/mL), a significant increase in mean lipid peroxidation level before treatment was noticed in animals of group I (5.42 ± 0.66 nM/mL) and group II (5.46 ± 0.67 nM/mL) ($p<0.01$). This was in accordance with the findings of Kogika *et al*. (2014), who documented a significantly higher concentration of malondialdehyde (MDA), the breakdown product of lipid peroxidation in all dogs diagnosed with CKD compared to healthy controls. Similar findings were recorded by Gultekin and Voyvoda (2017), who observed a significant increase in plasma MDA levels in dogs with non-regenerative anaemia associated with CKD. This might be due to oxidative stress in CKD patients. Lipids were common substrates attacked by ROS and MDA was the most extensively studied marker of oxidative stress (Meagher and FitzGerald, 2000). In contrast to the present study finding, Silva *et al*. (2013) reported a significant reduction in MDA concentration in dogs with CKD compared with healthy controls. The authors also reported that the thiobarbituric acid reactive substance test used to assess MDA concentration was insufficiently sensitive.

After treatment, a significant reduction in mean lipid peroxidation level was observed in animals of group II (3.04 ± 0.16 nM/mL) whereas a significant increase was noted in animals of group I (6.85 ± 0.76 nM/mL). This might be due to the antioxidant action of NAC which alleviated oxidative stress and slowed down the progression of CKD in animals of group II. McMichael (2007) stated that NAC helped to reduce oxidative stress by replenishing endogenous glutathione reserves and by scavenging hydroxyl radicals and hypochlorous acid.

Total antioxidant status

A significant increase in the mean total antioxidant level was found in animals of group I (434.94 ± 25.28 µM/L) and group II (449.05 ± 21.59 µM/L) compared to healthy dogs (292.31 ± 11.14 µM/L) before treatment. This was in accordance with the findings of Gultekin and Voyvoda (2017), who documented that plasma total antioxidant status values were found to be higher in dogs with non-regenerative anaemia associated with CKD. This might be due to the development of elaborate antioxidant defence mechanisms to limit the damage caused by ROS (McMichael, 2007).

In contrast to the present study finding, Zel *et al*. (2014) observed that there was no significant difference in total antioxidant status in CKD patients of any stage. Similarly, Halfen *et al*. (2020) mentioned that no significant change could be detected in total antioxidant capacity in dogs with CKD compared to healthy controls, suggesting that the systemic antioxidant system was not exhausted in CKD patients.

A significant increase in the mean

value of total antioxidant status was found in animals of group I (694.85 ± 48.40 µM/L) after treatment compared to before treatment, whereas a significant decrease was noticed in group II (286.87 ± 6.92 µM/L). A significant decrease in mean value was noted in animals of group II after treatment compared to group I. This might be due to the antioxidant action of NAC which alleviated oxidative stress and slowed down the progression of CKD in animals of group II. Small *et al*. (2012) reported that the limiting precursor to glutathione biosynthesis, L-cysteine and NAC helped to replenish intracellular glutathione stores in humans.

Glutathione peroxidase level

A significant decrease in mean glutathione peroxidase level was found in animals of group I (1947.5 ± 254.5 IU/L) and group II (1946.33 ± 179.66 IU/L) compared to healthy animals (5630 ± 207.14 IU/L) on the day of presentation. This was in agreement with the findings of Silva *et al*. (2013), who described that plasma-derived GSH Px activity was found to be decreased in dogs with CKD when compared to the control group. Similarly, Gultekin and Voyvoda (2017) reported a significant decrease in GSH Px activity in dogs with non-regenerative anaemia associated with

Table 1. Comparison of oxidative stress parameters among group I, II and healthy animals

Variable	Healthy animals	Group I	Group II	t-value	P-value
Lipid peroxidation level (nM/mL)	2.12 ± 0.07[b]	5.42 ± 0.66[a]	5.46 ± 0.67[a]	12.419**	0.001
Total antioxidant status (µmol/L)	292.31 ± 11.14[b]	434.94 ± 25.28[a]	449.05 ± 21.59[a]	18.348**	<0.001
Glutathione peroxidation level (IU/L)	5630 ± 207.14[a]	1947.5 ± 254.5[b]	1946.33 ± 179.66[b]	96.927**	<0.001

*** Significant at 0.01 level (p<0.01)*

Means having different superscript differ significantly within a row

Table 2. Comparison of oxidative stress parameters between groups and between days 0 and 15

Variable	Group	Day 0	Day 15	t-value (P-value)
Lipid peroxidation level (nM/mL)	Group I	5.42 ± 0.66	6.85 ± 0.76	3.453* (0.018)
	Group II	5.46 ± 0.67	3.04 ± 0.16	4.677** (0.005)
	t-value (P-value)	0.044 (0.966)	4.883** (0.004)	
Total antioxidant status (µmol/L)	Group I	434.94 ± 25.28	694.85 ± 48.40	6.182** (0.002)
	Group II	449.05 ± 21.59	286.87 ± 6.92	8.536** (<0.001)
	t-value (P-value)	0.425 (0.680)	8.345** (<0.001)	
Glutathione peroxidase level (IU/L)	Group I	1947.5 ± 254.50	1412.17 ± 145.64	3.667* (0.014)
	Group II	1946.33 ± 179.66	3868.67 ± 352.67	5.923** (0.002)
	t-value (P-value)	0.004 (0.997)	6.438** (<0.001)	

*** Significant at 0.01 level (p<0.01); * Significant at 0.05 level (p<0.05)*

CKD. Costa-hong *et al*. (2009) opined that the chronic inflammatory process in CKD patients resulted in decreased synthesis of GSH Px and further led to ROS production, thereby contributing to oxidative stress. Contradictory to this, Zel *et al*. (2014) reported, no significant variation in plasma GSH Px activity in stage I to III CKD patients.

After treatment, a significant increase in mean value was observed in animals of group II (3868.67 ± 352.67 IU/L), whereas a significant decrease could be noticed in group I (1412.17 ± 145.64 IU/L). A significant increase in mean glutathione peroxidase level was recorded after treatment in animals of group II compared to animals of group I. This might be due to the antioxidant action of NAC which alleviated oxidative stress and slowed down progression of CKD in animals of group II. Kaur *et al*. (2022) documented that NAC, a robust synthetic antioxidant, would restore endogenous glutathione reserves and scavenge superoxide, hydrogen peroxides and hydroxyl radicals.

Conclusion

After treatment, significant decline in serum MDA and TAS and significant increase in GSH-Px activity were observed in animals of group II. Administration of NAC @ 70 mg/Kg body weight I/V, OD for 10 days was found to be effective in management of oxidative stress in CKD dogs.

Acknowledgements

The authors would like to thank the authorities of Kerala Veterinary and Animal Sciences University for the facilities provided.

Conflict of interest

The authors declare that they have no conflict of interest.

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