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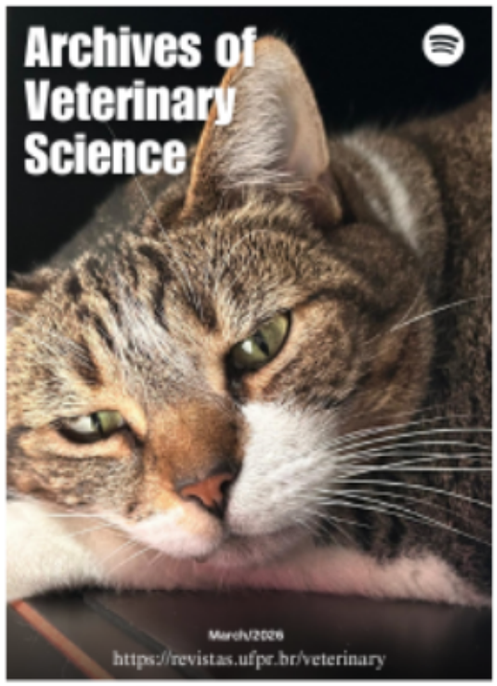

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Preliminary study on ozone hemotherapy for canine ischemic renal failure

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Abstract: In dogs, kidney injury is a sudden deterioration in renal function that can be fatal. Ischemia, inflammation, nephrotoxin exposure, and infectious diseases are the most frequent causes. In several fields of regenerative medicine, ozone therapy has recently attracted considerable attention. Clinical studies have shown that ozone therapy is safe and effective, and that it reduces readmission rates for kidney injury in animal models of renal disorders. This study investigated the efficacy of ozone therapy in experimentally induced ischemic renal failure in dogs. Ten dogs were used in this study. All dogs underwent right nephrectomy and 60 minutes of left renal ischemia followed by reperfusion. The animals were divided into two equal groups. The control group received no treatment, and the O3 group received an ozone–oxygen mixture. All groups were evaluated clinically and biochemically until day 40 after surgery, after which they underwent histopathological assessment. Major ozonated autologous hemotherapy substantially reduced the degree of elevation in the treatment group's serum blood urea nitrogen and creatinine levels relative to the control group. Medical ozone therapy considerably reduced the degree of glomerular filtration rate decline and notable alterations; it resulted in a morphological improvement in renal tubular structure compared with that in the control group, and a well-structured improvement in glomeruli, which were reasonably close to those in the healthy control group. According to the findings, ozone therapy may be an effective way to enhance renal function. However, our results indicate that the aforementioned treatment has potential as a straightforward therapeutic strategy for the management of renal failure that may be caused by ischemia or other factors.
Keywords: Acute Renal Failure, Autohemotherapy, Dogs, Medical ozone, Nephrectomy.

1. Introduction

Renal diseases, which are common in dogs and often associated with a poor prognosis in the late stages, can cause abrupt declines in renal function. About 2%–5% of all dogs suffer from renal failure, one of the most dangerous issues in the canine population that can be gradually brought on by renal diseases (Kumar *et al*., 2023).

Ischemic renal failure occurs when there is a brief disruption and subsequent restoration of blood flow to the kidney. It frequently occurs in clinical settings and could include renal artery blockage, heart surgery, and renal transplantation (Shen *et al*., 2024). It causes nitrogenous wastes to be retained in plasma and a sharp decline in renal function. Dogs frequently contract the illness, which has a high morbidity and fatality rate (Cao *et al*., 2020). Renal failure has a highly complex pathophysiology that includes adenosine triphosphate (ATP) depletion, intracellular Ca+2 and reactive oxygen species buildup, proinflammatory cytokine release, and activation of the apoptotic pathway (Yang *et al*., 2023). Acute renal injury in dogs frequently necessitates extended hospital stays, which can be expensive, and they face the risk of acquiring chronic kidney disease (Chen *et al*., 2023).

Ozone has an antioxidant defense system and medicinal qualities that modulate apoptosis. Although many techniques can be used to administer medical ozone, major ozonated autohemotherapy is the most dependable and sophisticated method (Yang *et al*., 2023; Luo *et al*., 2023). Depending on the intended therapeutic goal, ozone therapy can be administered at doses ranging from 1 µg/mL to 100 µg/mL, with safety and efficacy, through various methods (Serra *et al*., 2023). O3 dose methods include topical and infiltrative therapy (for localized effects, such as musculoskeletal and germicidal), autohemotherapy, and rectal insufflation (for systemic effects), and they vary according to the objectives and site of therapy (Hidalgo-Tallón *et al*. 2022). Ozone therapy reduces oxidative stress and inflammation, which are implicated in organ damage in chronic illnesses (Lino *et al*., 2024).

Moreover, ozone increases the activity of enzymes such as hydrogen peroxide, oxidized glutathione reductase, and superoxide dismutase by efficiently eliminating free radicals, thereby enhancing local tissue metabolism, stimulating fibroblast proliferation, facilitating collagen fiber synthesis, and supporting angiogenesis (Wen and Chen, 2020). Ozone therapy facilitates the secretion of growth factors by macrophages and fibroblasts, promoting angiogenesis and granulation tissue formation, thereby accelerating tissue healing. The therapeutic mechanism of ozone can be attributed to the upregulation of growth factors, including vascular endothelial growth factor, transforming growth factor beta, and platelet-derived growth factor (Sun *et al*., 2024). It increases blood flow and oxygen transport in ischemic tissues (Emre *et al*., 2024). Xanthine oxidase is thought to mediate oxidative damage during renal ischemia. When cells are subjected to ischemia, this enzyme breaks down nucleotides.

Nitric oxide (NO), when supported by ozone therapy, can be a defense mechanism against endothelin-1-induced kidney damage, inflammation, and vasoconstriction (Delgadillo-Valero *et al*., 2023). Ozone never exceeds 5% of the gaseous mixture when therapeutic oxygen is used as the carrier; the highest ozone concentrations in this context are estimated at roughly 100 mg/L (Travagli *et al*., 2023). Ozone therapy works by boosting cellular metabolism, decreasing the production of proinflammatory prostaglandins and allogenic compounds, increasing the release of immunosuppressive cytokines, reducing oxidative stress by inducing the

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