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Comprehensive Assessment of Metabolic and Urinary Alterations in Canine Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HR, NM, AK, PK, RKK and NKP conceptualized the study, designed the methodology, conducted experiments, and analyzed data. Author AK drafted the manuscript for publication. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to assess the metabolic and urinary alterations in canines with diabetes mellitus, focusing on biochemical, hematological, and urinary parameters to better understand the disease and inform treatment strategies.

Study Design: An observational, cross-sectional study.

Place and Duration of Study: The study was conducted at the Department of Veterinary Medicine, PGIVER, Jaipur, India, from August 2024 to January 2025.

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Methodology: The study involved 200 canines exhibiting clinical signs of diabetes mellitus, with 13 diagnosed based on fasting blood glucose levels above 140 mg/dl. A control group of 10 healthy dogs were used as control. Blood and urine samples were collected for analysis of glucose, liver enzymes (ALT, ALP), cholesterol, triglycerides, renal markers (creatinine, BUN), and urine parameters such as glucose, ketones, protein, specific gravity, and pH.

Results: Diabetic dogs showed significantly higher blood glucose (315.84 \pm 30.14 mg/dl) compared to controls (92.60 \pm 2.31 mg/dl, p<0.01). Biochemical analysis revealed elevated ALT (107.23 \pm 4.01 U/L), ALP (177.92 \pm 6.06 U/L), triglycerides (188.61 \pm 6.38 mg/dl), and cholesterol (249.92 \pm 7.71 mg/dl). Renal dysfunction was indicated by increased creatinine (1.69 \pm 0.03 mg/dl) and BUN (30.55 \pm 0.53 mg/dl). The hematological examination indicated the presence of neutrophilia (76.61 \pm 0.36) and lymphopenia (16.23 \pm 0.49). Urinalysis showed increased glucose (834.61 \pm 198.42 mg/dl), ketone bodies (31.15 \pm 8.38 mg/dl), specific gravity (1.025 \pm 0.00), and protein (33.07 \pm 5.78 mg/dl), with a decreased pH (5.53 \pm 0.14, p<0.01).

Conclusion: Diabetic canines exhibit significant metabolic and urinary disturbances, highlighting the need for ongoing monitoring to manage the disease and prevent complications. Further research with larger sample sizes is required for broader validation.

Keywords: Diabetes mellitus; biochemical; hematological; glucose; urinalysis; diagnostic tool.

1. INTRODUCTION

Diabetes mellitus embodies a complex metabolic condition distinguished by elevated blood sugar levels and irregularities in the metabolism of carbohydrates, fats, and proteins, stemming from insufficient insulin production or impaired insulin function, often linked to pancreatic inadequacies (Ahmed & Goldstein, 2006). In diabetic canines, this leads to hyperglycemia as insulin, produced by the β -cells of the pancreas, is unable to facilitate glucose uptake into cells. This condition is not uniform and presents differently across individual dogs, complicating diagnosis and treatment. Often, diabetes is associated with complications such as metabolic acidosis, nephropathy, hepatic lipidosis, and liver failure (Mauna, 1995). With proper management, including tailored care and regular monitoring, diabetes can be controlled, requiring continuous reassessment of hematological, biochemical, and urinary parameters (Greco, 2018).

Diabetic dogs commonly exhibit clinical signs like hyperglycemia, elevated liver enzymes (ALP, ALT), and hypercholesterolemia as observed in other animal models (Alwahsh et al., 2017). In cases of diabetic ketoacidosis (DKA), other complications such as azotemia, electrolyte imbalances, hyperlipidemia, and ketonuria are observed. Hematological issues are frequent in diabetic dogs, with studies showing lower hematocrit and hemoglobin levels in dogs with poor glucose control. In ketoacidosis, relative polycythemia due to dehydration may mask anemia. Elevated ketone and glucose levels cause hyperosmolality, swelling red blood cells,

and false increases in mean corpuscular volume (MCV) (Kelly et al., 1993). Neutrophils, monocytes, and lymphocytes in diabetic dogs often show impaired functions, increasing their susceptibility to infections.

Biochemically, diabetic dogs often show elevated ALT, ALP, and cholesterol levels (Nelson, 2010). ALT levels higher than 600 U/L suggest potential liver conditions unrelated to diabetes. Kidney function, indicated by BUN and creatinine levels, is typically normal but may increase in cases of renal failure or dehydration (Nelson, 2015). diagnosing Urinalysis, crucial for dysfunction, detects abnormalities like glucose, ketones, and protein (Coppens et al., 2010; Delanghe & Speeckaert, 2014). Urine, being easily accessible and non-invasive, provides valuable insight into metabolic diseases like diabetes (Sharma et al., 2020). Healthy dogs typically excrete minimal protein in urine, but proteinuria can indicate prerenal, renal, or postrenal causes (Vaden & Elliott, 2016; Hekmatynia et al., 2016; Harley & Langston, 2012).

The emergence of glucose within the urine becomes evident when the levels of blood glucose surpass the renal threshold or when the reabsorption prowess of the renal tubules is hindered (Melandri et al., 2020). In a similar vein, the presence of ketone bodies in urine can be identified through the application of reagent strips, signifying a metabolic transition to lipolysis, which is a hallmark of diabetic ketosis (Chong & Reineke, 2016). Urine pH, influenced by various factors, provides important information about acid-base balance and renal function

(Reppas & Foster, 2016). These diagnostic markers are essential in managing diabetes in canines, helping veterinarians monitor disease progression, adjust treatments, and prevent complications.

2. MATERIALS AND METHODS

This study, titled "Diagnostic and Clinico-Therapeutic Studies on Diabetes Mellitus in Canines," was conducted at the Department of Veterinary Medicine, Post Graduate Institute of Veterinary Education and Research, Jaipur. The study involved canines presented to the Veterinary Clinical Complexof PGIVER and the Government Veterinary Polyclinic Hospital, Panchbatti, Jaipur, from August 2024 to January 2025. A total of 200 canines, representing various breeds, ages, and sexes, were included. These animals presented with symptoms such as (polydipsia). excessive thirst hunger (polyphagia), obesity, frequent urination (polyuria), rapidly progressing bilateral cataracts, or unexplained weight loss, either individually or in combination.

Blood glucose concentrations were initially assessed with a handy glucometer right at the location. Dogs displaying a spontaneous blood glucose concentration surpassing 140 mg/dl were subjected to a fasting period of 12 hours and were subsequently re-evaluated for their fasting blood glucose levels the following day (Chaudhary, 2021). Canines with fasting blood glucose levels that went beyond 140 mg/dl were included in the investigation and underwent additional assessments involving hematological, biochemical. and urinalvsis examinations. Additionally, a control group of ten healthy dogs was assembled for the sake of comparative order investigation. In to evaluate hematological and biochemical modifications in the diabetic canines, blood samples (7 ml) were procured from either the cephalic or saphenous veins of all subjects under rigorous aseptic protocols. Of the total blood volume, 2 ml was allocated to EDTA vials, while the remaining 5 ml was designated for non-EDTA vials containing a clot activator. The blood contained within the non-EDTA vial was permitted to coagulate and was subsequently centrifuged at 2500 rpm for 30 minutes for the separation of serum. Employing commercially available diagnostic kits (CPC Diagnostics), biochemical metrics including ALP, ALT, triglycerides. glucose. cholesterol, blood urea nitrogen, calcium, creatinine, and phosphorus were quantified

utilizing an automated biochemistry analyzer (Turbochem 100). Urine samples, ranging from 5 to 10 ml, were collected in sterile containers either through spontaneous urination or by catheterization under aseptic conditions. These samples were then immediately transferred to sterile test tubes, centrifuged at 1000 rpm for 20 minutes at 2–8 °C to remove insoluble particles and cellular debris. For routine dipstick urinalysis, the supernatant was placed into small Pyrex tubes and refrigerated until further testing (Teitz, 1990).

provides comprehensive This study а assessment of the metabolic and urinary alterations in diabetic canines, focusing on key hematological, biochemical, and urinary parameters. The findings highlight significant disruptions in glucose metabolism, kidney function, and liver health, emphasizing the need for ongoing monitoring and tailored treatment strategies for managing diabetes mellitus in dogs.

2.1Statistical Analysis

The outcomes were articulated as mean \pm standard error (SE), and comparative analyses between the diabetic cohort and the healthy control cohort were conducted. The statistical significance of the discrepancies between the groups was ascertained using one-way ANOVA, with a significance benchmark established at p<0.01.

3. RESULTS AND DISCUSSION

3.1 Hematological Alterations

The empirical data delineated in Table 1 and Figs. 1 and 2 demonstrated an absence of statistically significant disparities between the cohort of diabetic canines and the healthy control group concerning hemoglobin concentration, total leukocyte count, packed cell volume, erythrocyte monocyte levels, total eosinophil counts, basophil counts, and mean corpuscular volume. Nevertheless, a statistically significant diminution (p<0.01) in lymphocyte count was noted within the diabetic cohort in comparison to the control dogs. This observed decline may be ascribed to alterations in the membrane protein composition of lymphocytes, as evidenced by SDS-PAGE, which indicated a marked reduction in the deformability of lymphocytes in diabetic dogs (Kaymaz et al., 2007). Such modifications may also suggest a condition of immunodeficiency, thereby enhancing the vulnerability of diabetic dogs to prevalent infections (Mori et al., 2008). These outcomes are consistent with the research conducted by Valilou and Loftia (2011), Xu et al. (2013), Patel et al. (2019), and Chaudhary (2021).

Moreover, the neutrophil count exhibited a significant elevation in the diabetic cohort when juxtaposed with the healthy control subjects, corroborating the findings delineated by Xu et al. (2013), Kapoor (2019), and Chaudhary (2021). This observation implies that the heightened presence of neutrophils may be associated with perturbations in glucose and glutamine

metabolism, given that neutrophils necessitate these substrates to sustain their elevated metabolic activity. An increased total leukocvte count (TLC) alongside elevated neutrophil levels serve as indicative biomarkers hyperglycemic episodes in canines afflicted with diabetes (Xu et al., 2013). Furthermore, the enhanced expression of tumor necrosis factor and interleukin 6 (IL-6) in reaction to pathogenassociated molecular patterns (PAMPs) is anticipated to contribute significantly to the neutrophilia observed in individuals with diabetes mellitus. These cytokines act as chemotactic mediators, thus promoting leukocytosis and neutrophilia in the context of diabetic pathology (Declue et al., 2012).

Table 1. Hematological parameters in healthy and diabetic canines (Mean ± SE)

S.No.	Parameter	Healthy Control Group (n=10)	Diabetic Group (n=13)
1.	Hemoglobin (gm/dl)	13.31 ± 0.21	12.83 ± 0.13
2.	Total Erythrocyte Count (x 10^6/µl)	6.51 ± 0.09	6.35 ± 0.06
3.	Packed Cell Volume (%)	41.50 ± 0.40	41.61 ± 0.70
4.	Mean Corpuscular Volume (fl)	63.79 ± 0.50	65.52 ± 1.10
5.	Platelet (x 10^3/µl)	380.10 ± 15.33	391.38 ± 12.14
6.	Total Leukocyte Count (x 10^3/µl)	8.31 ± 0.15	8.46 ± 0.25
7.	Lymphocytes (%)**	18.30 ± 0.49	16.23 ± 0.49
8.	Neutrophils (%)**	73.80 ± 0.57	76.61 ± 0.36
9.	Monocytes (%)	4.70 ± 0.42	4.15 ± 0.22
10.	Eosinophils (%)	3.00 ± 0.33	2.84 ± 0.22
11.	Basophils (%)	0.20 ± 0.13	0.23 ± 0.12

^{**}Statistically significant differences (p<0.01) between the diabetic and healthy control groups were observed in lymphocytes and neutrophils

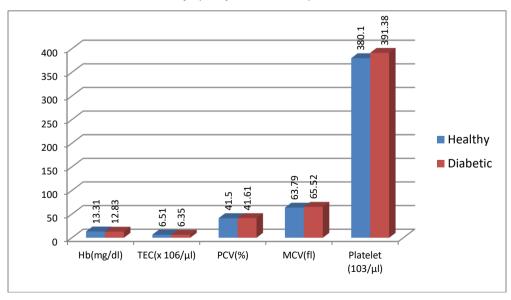


Fig. 1. Mean values of Hb, TEC, PCV, MCV and platelet in healthy control group and diabetic canines (pre-treatment)

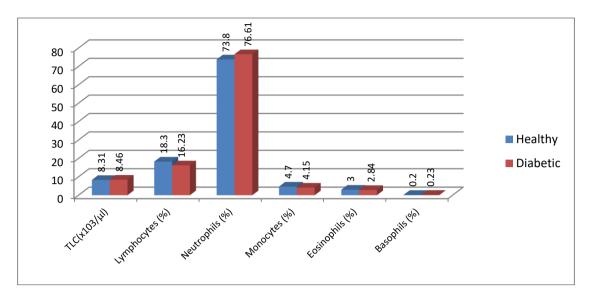


Fig. 2. Mean values of TLC and DLC in healthy control group and diabetic canines (pretreatment)

3.2 Biochemical Alterations

The biochemical parameters pertaining to both the healthy control and diabetic cohorts are succinctly encapsulated in Table 2 and visually represented in Figs. 3 and 4. The data unequivocally indicate that serum glucose concentrations were markedly elevated (p<0.01) in the diabetic canines (315.84 \pm 30.14 mg/dl) in comparison to their healthy counterparts (92.60 ± 2.31 mg/dl), thereby corroborating a condition of hyperglycemia. This augmentation is emblematic of a hyperglycemic crisis, frequently observed in insulin-deficient diabetes attributed autoimmune-mediated destruction of β-cells. In these diabetic canines, stimulation of the pancreatic β-cells within the islets of Langerhans via glucose or glucagon did not elicit a concomitant increase in insulin or C-peptide levels, thereby implying that the β-cells exhibited an unresponsive state, resulting in persistently elevated blood glucose levels (Catchpole et al., 2008). These findings are congruent with the research conducted by Sundararajan al. (2022), Kwong et al. (2023), and Calibo (2024).

In the context of hepatic function, the noted elevation in hepatic enzymes, particularly ALT and ALP, was found to be significantly greater (p<0.01) within the diabetic group in contrast to the control cohort. This observation implies hepatic involvement in the etiopathogenesis of diabetes mellitus, potentially as a consequence

of hepatic lipidosis, a condition frequently observed in diabetic canines (Hess et al., 2000). Moreover, diabetic canines may encounter additional hepatic complications, including hepatic necrosis or enlargement (Hiblu et al., 2015). The accelerated catabolism of proteins and lipids in the diabetic state may contribute to these hepatic alterations (Qadri et al., 2015; Kumar et al., 2014).

In association with the increase in hepatic enzyme concentrations, the cohort diagnosed with diabetes additionally demonstrated elevated levels of triglycerides and overall cholesterol when compared to the control group. This in biqil profile elevation parameters presumably attributable to insulin deficiency, which diminishes the activity of lipoprotein lipase, the enzyme responsible for the catabolism of triglyceride-rich lipoproteins. This dysfunction results in the accumulation of lipids within the circulatory system (Durocher et al., 2008). These results align with the conclusions presented by Jena et al. (2019).

Additionally, renal dysfunction in diabetic canines, commonly manifested as azotemia (Huang, 2012), was evident in this investigation, as evidenced by significantly elevated serum creatinine and BUN levels in the diabetic subjects. These findings align with those documented by Kapoor (2019) and Patel et al. (2019), further substantiating renal impairment in these animals.

Table 2. Mean ± SE of biochemical parameters in healthy and diabetic groups

S.No.	Parameter	Healthy Control Group (n=10)	Diabetic Group (n=13)
1.	Glucose **	92.60 ± 2.31	315.84 ± 30.14
2.	ALT **	42.40 ± 1.79	107.23 ± 4.01
3.	ALP **	63.80 ± 5.46	177.92 ± 6.06
4.	Triglycerides **	47.60 ± 3.09	188.61 ± 6.38
5.	Cholesterol **	139.90 ± 4.58	249.92 ± 7.71
6.	BUN **	21.90 ± 1.55	30.55 ± 0.53
7.	Creatinine **	1.14 ± 0.09	1.69 ± 0.03
8.	Calcium **	9.27 ± 0.11	12.03 ± 0.11
9.	Phosphorus	5.46 ± 0.30	5.96 ± 0.25

^{**}The differences in mean values between the diabetic and healthy control groups were highly significant (p<0.01)

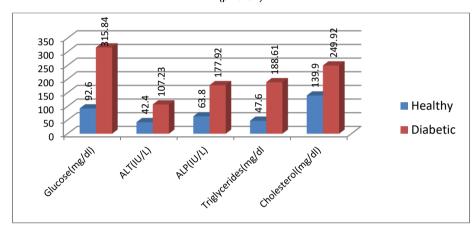


Fig. 3. Mean values of glucose, ALT, ALP, triglycerides and cholesterol in healthy control group and diabetic canines (pre-treatment)

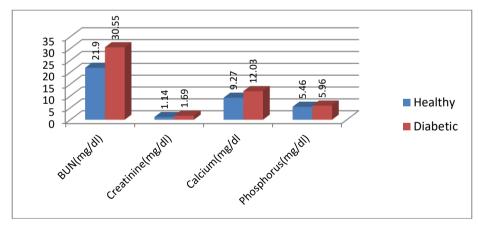


Fig. 4. Mean values of BUN, creatinine, calcium and phosphorus in healthy control group and diabetic canines (pre-treatment)

Table 3. Mean ± SE of biochemical parameters in healthy and diabetic canines

S.No.	Parameter	Healthy Group (n=10)	Diabetic Group (n=13)
1.	Glucose (mg/dl) **	0 ± 0	834.61 ± 198.42
2.	Ketone Bodies (mg/dl) **	0 ± 0	31.15 ± 8.38
3.	Specific Gravity **	1.0085 ± 0.00	1.025 ± 0.00
4.	pH **	6.40 ± 0.10	5.53 ± 0.14
5.	Protein (mg/dl) **	0 ± 0	33.07 ± 5.78

^{**}The differences in mean values between the diabetic and healthy groups were highly significant (p<0.01)

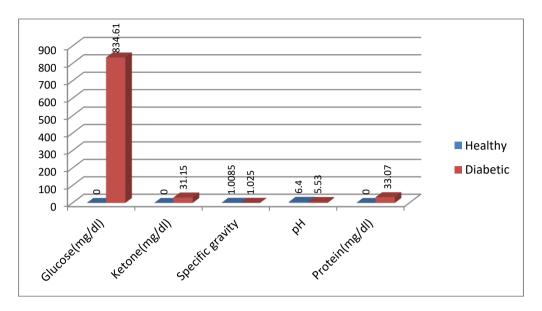


Fig. 5. Mean value of different urine parameters in healthy control group and diabetic canines (pre-treatment)

3.3 Urinary Changes

the current investigation, remarkable elevations in the levels of ketone bodies, specific gravity of urine, protein content, and glucose measurements were observed in the diabetic cohort when compared to the healthy control cohort. The ongoing hyperglycemia observed in diabetic dogs triggers glucosuria, an abnormal condition that arises when the renal threshold for glucose elimination surpasses 180 mg/dl. Prolonged elevated glucose levels also promote muscle wasting and hinder wound healing, with excessive proteolysis being a significant factor in complications. Accelerated these bigil metabolism in diabetic dogs can result in endothelial damage and immune suppression, potentially causing hepatic lipidosis and, in some cases, ketoacidosis due to the increased production of ketone bodies. As a result of hyperglycemia, diabetic dogs commonly experience glycosuria and osmotic diuresis, as the renal tubules are overwhelmed and cannot effectively reabsorb glucose. Qadri et al. (2015) provided insight into the mechanisms behind glycosuria in canine diabetes. Dogs with poor glycemic control typically exhibit elevated urine specific gravity, which is directly related to the elevated blood glucose levels (Akarsu et al., 2006). The chronic nature of poorly controlled diabetes can contribute to diabetic nephropathy, with glucose-induced microvascular damage playing a central role (Nelson & Couto, 2014). Furthermore, chronic diabetes can lead to proteinuria and mild pyuria, which are commonly

seen in kidney function assessments of affected dogs. The alteration of acid-base balance in diabetic dogs also results in a reduced ability to excrete acids, contributing to the development of uric acid urolithiasis (Maalouf et al., 2010). These findings are consistent with the results reported by Kapoor (2019).

4. CONCLUSION

The findings of this study demonstrate that significantly diabetes mellitus alters biochemical and urinary profiles in affected canines, with hematological abnormalities being less frequently observed. The examination of hematological specimens indicated the presence of neutrophilia and lymphopenia, concomitant with the detection of elevated concentrations of ALT, triglycerides, ALP, total cholesterol, BUN, creatinine, and calcium during the biochemical evaluation. Alterations in urinary composition were characterized by a substantial increase in ketone bodies, specific gravity, glucose, and protein, whereas the urine pH exhibited a significant decline.

5. STUDY LIMITATIONS

- The cohort is modest, comprising merely 13 diabetic canines and 10 robust ones, which constrains statistical strength and heightens the chances of erroneous positive and negative results.
- The research unfolded within a distinct geographical landscape, limiting the

- applicability of the findings to diverse groups beyond this area.
- A larger, more geographically diverse sample would likely produce more robust and reliable results, improving the overall validity and applicability of the conclusions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative Al technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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