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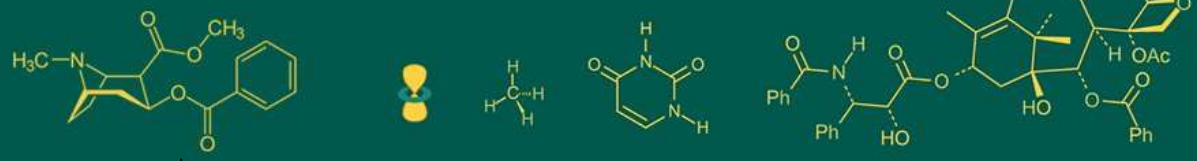

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Clinical manifestations and alterations in urine parameters in canine diabetes mellitus

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Abstract
The current investigation was carried out in the Jaipur, Rajasthan between April 2022 and September 2022 to assess the clinical symptoms and variations in urine parameters in diabetic canines. Two hundred dogs of varying age, sex and breeds were analysed for the presence of the symptoms such as polydipsia, polyuria, weakness or fatigue, polyphagia, obesity, rapid weight loss and rapidly growing bilateral cataracts. In our study, nine positive diabetic canines revealed, the highest cases of diabetes were found in Labrador breed dogs and age group of more than 6 years of age while females are more prone to diabetes than males. Dogs that were thought to have diabetes mellitus had their blood tested for glucose levels using an in-house glucometer. After 12 hours of fasting, dogs with a blood glucose (random) level above 140 mg/dl were retested, and only those with fasting blood glucose above 140 mg/dl were included in the current investigation. The study also included a healthy control group of 10 canines (dogs). Dogs with diabetes mellitus were tested by having their urine analysed with a standard urinalysis dip-stick kit. Nine dogs were found to have diabetes after initial testing revealed the condition. Ketone bodies, specific gravity, protein and glucose were all shown to be significantly elevated in the urine of diabetic canines, while urine pH was significantly decreased (p<0.01). Canines with diabetes did not have blood or nitrite in their urine. The most common clinical indications of diabetes in dogs were polydipsia, polyuria and weight loss, followed by polyphagia, cataract formation and vomiting.

Keywords: Diabetes mellitus, glucose, urinalysis, canines, polydipsia

Introduction
Diabetes mellitus is a disorder characterised by persistent hyperglycaemia caused by a decrease in insulin secretion from pancreatic beta cells or an increase in glucose uptake by tissues that are resistant to insulin. Immune-mediated destruction, vacuolar degeneration, and pancreatitis are the most common causes of rapid and progressive beta-cell loss in dogs (Davison *et al*., 2003) [9] Diabetic mellitus symptoms are caused by high blood glucose levels and the body's inability to utilize glucose for energy. Unchecked hepatic glucose synthesis, impaired glucose entry into tissues, and increased protein and fat catabolism all contribute to hyperglycemia due to insulin insufficiency. As a result of persistent hyperglycaemia, glucosuria occurs when the renal tubular threshold for glucose excretion exceeds 180 to 220 mg/dl. Muscle atrophy and delayed wound recovery are the unfortunate results of elevated proteolysis. Hepatic lipidosis, ketoacidosis due to increased ketone body generation, endothelial damage, and immunological suppression can all occur when rapid lipid catabolism persists (Qadir *et al*., 2015) [23].
Early diabetic symptoms include increased urination, water consumption, hunger and weight loss that does not respond to diet and exercise. An elevated level of glucose in the blood and urine occurs, according to lab results. In advanced canine diabetes instances, the dog may show signs of lethargy, lack of appetite, weakness, vomiting, dehydration and coma; acquire cataracts and hepatomegaly; become more susceptible to infections; and eventually have neurological difficulties if the condition is not treated (Alberti *et al*., 1999; Catchpole *et al*., 2005) [2,4]. Polyuria, polydipsia, polyphagia, and weight loss are the hallmark symptoms of diabetes mellitus. The severity of these symptoms is proportional to the severity of hyperglycemia. Ketoacidosis develops as ketone body production increases to compensate for blood glucose underutilization (Hess *et al*., 2000) [16].

There have been shifts in how diabetes mellitus is diagnosed over time (WHO, 2011) [28]. Since diabetes was formerly identified by its telltale sweet aftertaste in the urine, a lot has changed. Glycosuria was the primary diagnostic criteria for diabetes mellitus in the outset (Banting *et al*., 1922 [3]. Blood and urine glucose levels are used to diagnose diabetes. Normal canine urine does not contain any glucose (Rucinsky *et al*., 2010) [24]. This study is relevant in the canine population because it uses clinical manifestations and changes in urine parameters to identify canine diabetes mellitus early, which helps to save the lives of individual canines.

Materials and Methods

The present study has been carried out at PGIVER, Jaipur. Dogs were enrolled in the study between April and September of 2022 at the Veterinary Clinical Complex of the institute and the Government Veterinary Polyclinic Hospital situated at Panchbatti, Jaipur. Necessary ethical approval was taken from the Institutional Animal Ethics Committee prior to start investigation as per reference of letter No. F. ()/PGIVER/IAEC/2022/21, dated 4/06/2022.

Screening of animals

Two hundred dogs of varying age, sex and breeds were analysed for the presence of the symptoms polydipsia, obesity, polyuria, polyphagia, weakness or fatigue, rapid weight loss and rapidly growing bilateral cataracts. Dogs that were thought to have diabetes mellitus had their blood tested for glucose levels using an in-house glucometer. Dogs with a random blood glucose reading over 140 mg/dl had their fasting blood sugar checked the next day after fasting for 12 hours, and only those dogs with a fasting blood glucose reading over 140 mg/dl were included in the current study (Deepa *et al*., 2014; Jatav, 2015 and Chaudhary, 2021) [11, 17, 6]. After that Diabetic canines were treated with insulin @0.25 IU/kg body weight subcutaneously after meal twice a day resulted significant decrease in blood glucose levels over the period of time. Supportive therapy, balanced diets rich in high fiber, complex carbohydrates, high quality protein content and a low restricted fat along with regular, scheduled feeding always at same amount twice daily and regular exercise should also advised on daily basis.

Sampling Procedure

Collection of urine

A sterile container was used to collect urine samples ranging from about 5-10 ml during spontaneous urination or catheterization under strict safety conditions. Direct collection of urine samples in sterile test tubes was followed by centrifugation at 1000 rpm for 20 minutes at 2-8^0 C to remove insoluble impurities and cell debris. Urine was collected for routine dip-stick urinalysis in tiny pyrex tubes, and the supernatant was refrigerated until analysis (Teitz, 1990) [27].

Clinical Diagnosis

Laboratory tests for blood glucose and other biochemical estimates and clinical examinations were used to arrive at a clinical diagnosis. All the sick dogs had blood drawn to test for a variety of conditions in the lab. Additionally, dip-stick urinalysis was performed on collected urine samples. Seven days after finishing medication, doctors took another round of urine and blood samples to see how well the body was responding.

Statistical Analysis

ANOVA was used to analyse the data (Snedecor and Cochran, 2004) [26]. The Duncan's new multiple range test (Duncan, 1955) [12] was used for comparing means that showed significant differences; statistical significance was accepted at a P-value of 0.05.

Results and Discussion

A significant increase in ketone bodies, specific gravity, protein and glucose in the urine of the diabetic group was observed when comparing the pre-treatment diabetic group with the healthy control group and post-treatment diabetic group. The values of urine glucose in the healthy control group and the diabetes affected group (after therapy) did not differ statistically.

As a result of persistent hyperglycaemia, glucosuria occurs when the renal tubular threshold for glucose excretion exceeds 180 mg/dl. Muscle atrophy and delayed wound recovery are the unfortunate results of elevated proteolysis. Endothelial damage and immunological suppression may follow prolonged accelerated lipid catabolism, which causes hepatic lipidosis and, potentially, ketoacidosis due to increased ketone body synthesis. Diabetic dogs usually experience glycosuria and osmotic dieresis due to hyperglycaemia, which exceeds the proximal tubules' capacity to resorb glucose. Qadri *et al*., (2015) [23] elucidated the underlying pathophysiology responsible for glycosuria in canine diabetes.

Patients with poor glycaemic control typically exhibit an increased urine specific gravity due to high blood glucose levels (Akarsu *et al*., 2006) [1]. The development of diabetic nephropathy can be attributed to the central role glucose plays in microvascular damage (Nelson and Couto, 2014) [21]. Chronic, poorly controlled diabetes in dogs can cause proteinuria and moderate pyuria in the kidneys (Chandler *et al*., 1984) [5].

Compared to the healthy control group and the treated diabetic group, the diabetic group (before treatment) had a higher urine pH. The pH readings in the healthy control group and the diabetic group (after therapy) were not significantly different. In diabetes, net acid excretion (NAE) and ammonia buffer utilization are decreased, resulting in uric acid urolithiasis (Maalouf *et al*., 2010) [20]. After treatment, the results of the diabetic group showed that blood was not present in the urine (before treatment) when compared to the healthy control group. This result is consistent with that found by Kapoor (2019) [18] and Chaudhary (2021) [6]. No nitrite was detected in the urine of either the diabetic group (before treatment) or the healthy control group. These results are consistent with those found by Kapoor (2019) [18].

Most of the cases of diabetes were found in Labrador breed dogs, due to genetic predispositions, familial associations, pedigree analysis, and genomic studies aimed at identifying susceptibility and protective major histocompatibility complex haplotypes (Guptill *et al*., 2003 and Fall *et al*., 2007) [15, 14]. Diabetes mellitus in dogs has been linked to MHC class II genes (dog leucocyte antigen; DLA) on canine chromosome 12, with similar haplotypes and genotypes seen in the most susceptible breeds (Catchpole *et al*., 2005) [4]. MHC class II is in charge of delivering antigen to T

lymphocytes. Preference for DM-prone breeds is one factor of increased DM incidence in the canine population (Klinkenberg *et al*., 2006) [19].

The canines of age group more than six years of age are more susceptible to canine diabetes, the likely cause was insulin resistance, which was observed in adult canine diabetes mellitus cases and was attributed to exogenous corticosteroids, progestagen treatment, or endocrinopathies such as hyperadrenocorticism and hypothyroidism (Hess *et al*., 2000) [16]. Aging has been identified as a major component in canine diabetes (Davison *et al*., 2005) [10].

Females canines are more exposed to diabetes than males. The cause for the increased female predisposition to diabetes mellitus has been postulated to be a hormonal influence, namely during the protracted diestrus phase of the oestrus cycle of intact female dogs (Klinkenberg *et al*., 2006) [19]. Insulin resistance strong enough to result in diabetes can develop in whole females during the progesterone dominant phase of diestrus, when growth hormone synthesis by the mammary glands also contributes to poor glucose tolerance and diabetes (Selman *et al*., 1994) [25].

Clinical manifestations

Among the clinical findings observed in dogs with diabetes mellitus, there were polydipsia (77.77%), weight loss (66.66%), polyuria (66.66%), polyphagia (55.55%), cataract development (33.33%) and vomiting (22.22%). Due to an entire lack of insulin production by the beta cells, hyperglycemia develops when there is an unusually high quantity of glucose in the blood. This results in decreased tissue utilisation of glucose, amino acids, and fatty acids and accelerated hepatic glycogenolysis and gluconeogenesis. When blood glucose levels in dogs rise above 180 mg/dl to 220 mg/dl, the kidneys ability to reabsorb glucose from the glomerular ultrafiltrate is overwhelmed, resulting in glycosuria. This condition causes an osmatic dieresis, characterized by compensatory polyuria and polydipsia. The body's attempt to counteract the false perception of hunger causes weight loss due to decreased peripheral tissue utilization of ingested glucose. The satiety center temporarily suppresses the feeding center after eating, while the feeding center remains active and is responsible for eliciting eating habits. An individual's level of hunger is clearly linked to how much glucose enters the cells of the satiety center. Insulin mediates glucose's uptake by cells in the hunger control hub. When a diabetic doesn't have enough insulin, glucose can't get into the cells that regulate satiety, so the brain doesn't know how to turn off the eating center. Thus, a polyphagic state has developed (Ettinger and Feldman, 2010) [13].

The ability of the renal tubular cells to reabsorb glucose from the glomerular filtrate may be compromised by an increase in blood sugar. When a dog's blood glucose level is over 180 mg/dl, this is a common occurrence. According to Nelson and Reusch (2014) [22], polyuria and polydipsia result from the osmotic diuresis that glucose intoxication causes. Osmotic alterations in the lens, glycosylation of structural proteins, and a reduction in antioxidant content are all involved in the development of diabetic cataract (Comazzi *et al*., 2008) [7]. Cataracts frequently occur suddenly in diabetic dogs (Curtis, 1988) [8].

Table 1: An analysis of the mean and standard error of urine parameters in apparently healthy and diabetic dogs (Before and after treatment)

S. No.	Parameters	Healthy control group (n=10)	Pre-treatment (n=9)	Post-treatment (n=9)
1	Ketone Bodies (mmol/l) (**)	0.00^{a}±0.00	9.95^{b}±1.90	0.33^{a}±0.16
2	Glucose (mmol/l) (**)	0.00^{a}±0.00	66.67^{b}±11.66	0.17^{a}±0.08
3	pH (**)	6.30^{b}±0.10	5.56^{a}±0.17	6.11^{b}±0.07
4	Specific gravity (**)	1.00051^{a}±0.00	1.024^{b}±0.00	1.007^{a}±0.00
5	Protein (g/l) (**)	0.00^{a}±0.00	10.11^{b}±3.13	0.10^{a}±0.05
6	Blood (RBC/µl)	0.00±0.00	0.00±0.00	0.00±0.00
7	Nitrite	0.00±0.00	0.00±0.00	0.00±0.00

** Compared to the mean value of the healthy control group, there was a highly significant difference in mean value (p<0.01)

Table 2: Canines with diabetes mellitus exhibiting major clinical manifestations

S. No.	Clinical manifestation	Numbers of positive cases showed (9)	Percentage (%)
1	Polydipsia	7	77.77%
2	Polyphagia	5	55.55%
3	Polyuria	6	66.66%
4	Weight loss	6	66.66%
5	Vomiting	2	22.22%
6	Cataract formation	3	33.33%

Conclusion

Ketone bodies, specific gravity, protein and glucose were all shown to be greatly elevated in the urine of diabetic canines, whereas urine pH was found to be dramatically decreased. Canines with diabetes did not have blood or nitrite in their urine. Polydipsia, polyuria, weight loss, polyphagia, cataract formation, and vomiting were the most often reported clinical signs of diabetes in canines.

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Conflict of interest

There are no conflicts of interest to declare by any of the authors.

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