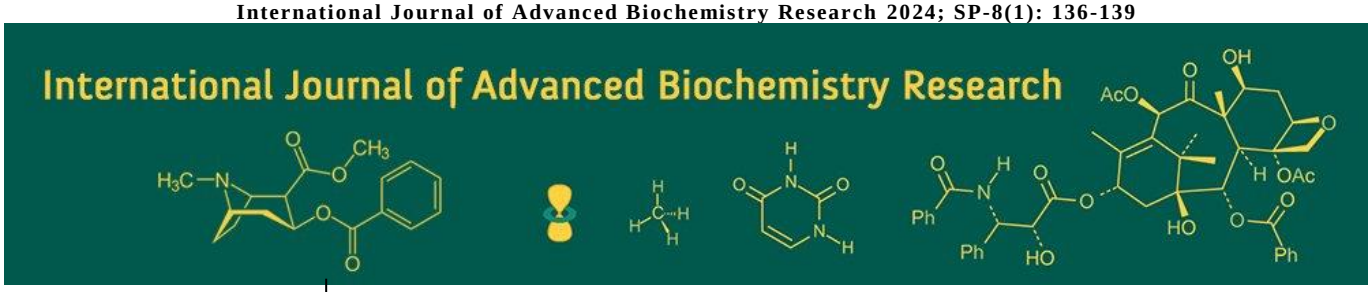

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Haemato-biochemical and urine examination on diabetes mellitus in canine

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Abstract
The present study was conducted to estimate various blood/serum biochemical parameters in diabetic canines in Jaipur region, of Rajasthan during June, 2020 to December, 2020. A total of two hundred canines of different age group, sex and breed with the history of polydipsia, polyphagia, polyuria, obesity, rapidly developing bilateral cataracts, rapid weight loss or in combination thereof were screened.
Blood samples of canines suspected for diabetes mellitus were screened for blood glucose by using on-site glucometer. Those canines showing fasting blood glucose level > 140 mg/dl, were included for the present study. Ten healthy canines (dogs) were also included for healthy control group in the study. On the basis of screening test results, eleven canines were diagnosed as diabetic.
Hematological profile of the diabetic canines revealed neutrophilia and mild lymphocytopenia. Serum glucose, ALT, ALP, triglyceride, total cholesterol, BUN and creatinine were significantly increased in diabetic canines. Urine parameters- ketone bodies, glucose, specific gravity and protein, increased significantly but urine pH was decreased significantly in diabetic canines. The blood was not found in urine of diabetic canines.

Keywords: Diabetes mellitus, biochemical, hematological, polydipsia, polyphagia

Introduction
Diabetes mellitus is a metabolic disorder characterized by high level of glucose in blood and changes in carbohydrate, lipid and protein metabolism which are caused by reduction in insulin secretion and/or in insulin action (Ahmed and Glodstein, 2006) [1]. Glucose is absorbed from the intestines into the bloodstream where it travels to cells throughout the body. Insulin is required for the cells to absorb glucose. Insulin is produced by the pancreas in response to the amount of glucose in the bloodstream. In canine diabetes mellitus, unused glucose builds up in the bloodstream leads to hyperglycemia. Diabetes mellitus in animals shares many similarities to diabetes mellitus in man. It is a multifaceted disease and remains a humbling challenge for the clinician and the researcher. DM is a treatable condition that requires committed effort by veterinarian and client. Each animal needs individualized, frequent reassessment, and treatment may be modified based on response. It is a very complex disease in people and equally so in the diabetes mellitus is a heterogeneous condition in the dog rather than a single disease entity. It is characterised by a relative or absolute deficiency of insulin secreted by the β cells of Islets of langerhans in a pancreas (John, 1999) [11].
Greco (2018) [6] studied common clinico-pathologic features of DM in dogs and cats include fasting hyperglycemia, hypercholesterolemia, increased liver enzymes (ALP and ALT), neutrophilic leucocytosis, proteinuria, increased urine specific gravity and glycosuria. Common clinico-pathologic findings in DKA include all of the foregoing plus azotaemia, hyponatremia, hyperkalaemia, hyperlipaemia, hyperamylasaemia, ketonemia, regenerative or degenerative left shifts, hyperosmolality, ketonuria, bacteriuria, haematuria and pyuria.

Materials and Methods

The present research work entitled "Clinical Studies on Diabetes Mellitus in Canine" has been carried out at the Department of Veterinary Medicine, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur. The animals taken for study, were canines brought to the Veterinary Clinical Complex (VCC) of PGIVER, Jaipur and Government Veterinary Polyclinic Hospital, Panchbatti, Jaipur during June, 2020 to December, 2020.

Screening of animals

A total of two hundred canines of different age, sex and breed with the history of polydipsia, polyphagia, polyuria, obesity, rapidly developing bilateral cataracts, rapid weight loss or in combination thereof were screened. Blood samples of canines suspected for diabetes mellitus were screened for blood glucose by using on-site glucometer. The canines showing random blood glucose level above 140 mg/dl, were tested for fasting blood glucose next day after 12 hours of fast (Deepa, 2014 and Jatav, 2015) [4, 10] and those canines showing fasting blood glucose level above 140 mg/dl, were included for the present study.

Sampling Procedure

Collection of blood and serum

In order to study haemato-biochemical alterations in diabetic canines, blood samples were collected from all canines following all the aseptic precautions. Using 22 or 24 gauge sterilized needle and 10 milliliter (ml) disposable syringe, total 7 ml of blood was collected from the cephalic or saphenous vein.

Out of this, 2 ml blood was collected in sterilized test tubes containing disodium salt of ethylene diamine tetra acetic acid (EDTA) @1 mg/ ml of blood) as an anticoagulant while remaining 5 ml blood was collected in another sterilized test tube without any anticoagulant for the separation of serum and thereafter blood slants were made and incubated for one hour at 30 ºC. Then the tubes were refrigerated for some time (allowing retraction of clot) and centrifuged at 2500 rhythm per minute (rpm) for 30 minutes in order to separate serum. On separation, serum was immediately transferred into sterilized screw capped vial; a drop of Merthiolate solution (1:10,000) was added and stored in deep freeze at -40 ºC until used for biochemical estimations. The blood samples were also collected from 10 healthy canine (Healthy control group) as described above and subjected for the estimation of haematological and biochemical values.

Collection of urine

About 5-10 ml of urine was collected in a clean, dry container either during spontaneous urination by following the necessary precautions or by catheterization. Urine samples were collected aseptically in test tubes directly and/or centrifuged for 20 minutes to remove insoluble impurity and cell debris at 1000 rpm at 2-8 ºC. Clear supernatant of urine was collected in small pyrex tubes for routine urinalysis using dip-stick and was refrigerated immediately until analysis.

Clinical Diagnosis

Clinical diagnosis was carried out through history, clinical manifestations, clinical examinations, biochemical estimations and laboratory tests for blood glucose. Blood samples were collected from all affected canines and assayed for different laboratory tests. Urine samples were also collected for routine urinalysis using dip-stick. Urine and blood samples were collected again 7 days post treatment to evaluate the response of therapy.

Statistical Analysis

Data were analyzed for one way ANOVA (Snedecor and Cochran, 2004) [18]. Means showing significant differences were compared by Duncan's new multiple range test (Duncan, 1955) [5] statistical significance was accepted at $p \leq 0.05$.

Results and Discussion

Haematology Changes

Table 1: Mean ± SE value of hematological parameters in apparently healthy and diabetic canines (pre and post treatment).

S. No.	Parameters	Healthy control group (n=10)	Pre-treatment (n=11)	Post treatment (n=11)
1	Haemoglobin (gm/dl)	13.98±0.23	13.46±0.24	13.7±0.24
2	Total Erythrocyte Count (x 10^6/µl)	5.96±0.20	5.87±0.20	5.73±0.19
3	Packed Cell Volume (%)	43.2±0.91	44±0.61	43.73±1.01
4	Total Leukocyte Count (x10^3/µl)	8.15±0.34	8.4±0.30	8.13±0.30
5	Lymphocytes (%) (*)	18.6^b±0.58	16.91^a±0.56	18.82^b±0.44
6	Monocytes (%)	5.00±0.33	4.19±0.26	4.37±0.33
7	Neutrophils (%) (**)	73.9^a±0.67	76.37^b±0.36	74.19^a±0.53
8	Eosinophils (%)	2.3±0.3	2.37±0.31	2.55±0.31
9	Basophils (%)	0.2±0.13	0.19±0.12	0.1±0.09

* The variations in mean values were significant ($p<0.05$) when compared with the mean value of healthy control group.

** The variations in mean values were highly significant ($p<0.01$) when compared with the mean value of healthy control group.

Result showed that there was value of haemoglobin, total erythrocyte count, packed cell volume, total leukocyte count, monocytes, eosinophils, and basophils no significant changes in diabetic group as compared to healthy control group and no significant change between pre and post treatment groups, when compared to healthy control group. There was no statistical difference in values of between healthy control group and diabetes affected group (post treatment).

The lymphocyte count in canines affected with diabetes mellitus on 1st day (pre-treatment) was decrease significantly ($p<0.05$) in comparison to healthy control and post treatment groups. There was no statistical difference in value of lymphocyte count between control group and post treatment of affected group.

Alterations in peripheral lymphocyte membrane protein content were investigated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). When

compared to controls were found that lymphocyte deformability was significantly decreased in diabetic dogs (Kaymaz *et al*., 2007) [13].

Result showed that there was highly significant increase (p<0.01) in the value of neutrophil count in diabetic group (pre-treatment) as compared to healthy control and post treatment groups. There was no statistical difference in value of neutrophil count between control group and post treatment of affected group.

Suggested that impaired glucose and glutamine metabolism in diabetes and high demands of neutrophils for these substances may cause neutrophils to rise in diabetic dogs. Higher total leukocyte and neutrophil count had a significant ability to reflect the presence of hyperglycemic emergencies (Xu *et al*., 2013) [20].

Biochemical Changes

Table 2: Mean ± SE value of biochemical parameters in apparently healthy and diabetic canines (pre and post treatment)

S. No.	Parameters	Healthy control group (n=10)	Pre-treatment (n=11)	Post treatment (n=11)
1	Serum Glucose (mg/dl) (**)	92.8^{a}±3.25	247.64^{b}±26.58	117.73^{a}±2.49
2	Serum Alanine Aminotransferase (IU/L) (**)	42.9^{a}±1.71	113^{b}±4.74	45.73^{a}±1.60
3	Serum Alkaline Phosphatase (IU/L) (**)	65.1^{a}±4.55	273.37^{b}±6.95	74.37^{a}±3.11
4	Serum Triglyceride (mg/dl) (**)	48.8^{a}±3.25	179.19^{c}±5.39	120^{b}±5.53
5	Serum Total Cholesterol (mg/dl) (**)	177.9^{a}±9.02	252.28^{b}±9.44	174.67^{a}±4.97
6	Blood Urea Nitrogen (mg/dl) (**)	20.4^{a}±1.71	31.46^{b}±1.61	19.75^{a}±0.51
7	Serum Creatinine (mg/dl) (**)	1.15^{a}±0.1	1.63^{b}±0.05	0.95^{a}±0.07

** The variations in mean value were highly significant (p<0.01) when compared with the mean value of healthy control group.

The serum glucose, serum alanine aminotransferase, serum alkaline phosphatase, serum triglyceride, serum total cholesterol, blood urea nitrogen (BUN) and serum creatinine was increased highly significant (p<0.01) in diabetic group (pre-treatment) in comparison to healthy control group and post treatment of diabetic group. There was no statistical difference in values between healthy control group and post treatment of diabetic group.

Catchpole *et al*. (2008) [3] studied insulin deficient diabetes in dogs caused by auto-immune-mediated destruction of β cells. In insulin deficient diabetic dogs, there was no increase in insulin or C-peptide by stimulation of β cells of pancreas located in islets of langerhans with glucose or glucagon which is suggestive of the unresponsiveness of β cells.

In diabetes mellitus (DM) there is accelerated protein and fat metabolism that is responsible for hepatic changes (Kumar *et al*. 2014 and Quadri *et al*., 2015) [14, 17]. Altered lipoprotein metabolism results in the hypertriglyceridaemia that occurs in DM (Iwasaki *et al*., 2007 and Huang, 2012) [9, 8].

Hiblu *et al*. (2015) [7] were of the opinion that diabetes mellitus is a common condition in dogs with many concurrent complications such as cataract, urinary tract infection, metabolic acidosis, nephropathy, hepaticlipidosis and liver failure. Alterations in lipid (fat) metabolism because of diabetes may also contribute to increases in these liver enzymes. This altered fat metabolism may be noted by increases in serum cholesterol concentrations (Sridhar *et al*., 2005) [19]. Evidence for renal failure in diabetic dogs reveals azotemia, increased serum creatinine and BUN (Huang, 2012) [8].

Urine examination

Table 3: Mean ± SE value of urine parameters in apparently healthy and diabetic canines (pre and after treatment)

S. No.	Parameters	Healthy control group (n=10)	Pre-treatment (n=11)	Post treatment (n=11)
1	Ketone Bodies (mg/dl) (**)	0^{a}±0.0	64.09^{b}±16.59	2.27^{a}±0.78
2	Glucose (mg/dl) (**)	0^{a}±0.0	1318.18^{c}±213.44	18.18^{a}±12.19
3	pH (**)	6.4^{b}±0.1	5.45^{a}±0.15	6.09^{b}±0.06
4	Specific gravity (**)	1.005^{a}±0.001	1.02^{b}±0.001	1.00^{a}±0.001
5	Protein (mg/dl) (**)	0.0^{a}±0.0	95.0^{b}±32.28	3.0^{a}±2.0
6	Blood (RBC/µl)	0.0±0.0	0.0±0.0	0.0±0.0

** The variations in mean value were highly significant (p<0.01) when compared with the mean value of healthy control group.

The ketone bodies, Glucose, Specific gravity and Protein in urine was significantly higher (p<0.01) in diabetic group (pre-treatment) in comparison to healthy control group and post treatment of diabetic groups. There was no statistical difference in values of urine ketone bodies between healthy control group and post treatment of diabetic group.

Persistent hyperglycemia results in glucosuria when the renal tubular threshold for glucose excretion more than 180 mg/dl. Increased proteolysis leads to muscle wasting and poor wound healing. As the accelerated lipid catabolism persists, hepatic lipidosis develops and ketoacidosis could result secondary to enhanced ketone body production, endothelial damage and immune suppression ultimately occurs (Qadir *et al*., 2015) [17].

Glycosuria was predominantly seen in diabetic dogs due to hyperglycemia overwhelming the capacity of the proximal tubules to resorb glucose leads to glycosuria and osmotic dieresis. The pathology causing glycosuria in diabetic dogs was described (Qadir *et al*., 2015) [17].

The patients with poor glycemic control elevated blood glucose levels should result in an increased urine specific gravity (Akarsu *et al*., 2006) [2]. Proteinuria in the present study may be due to the central role of glucose in the development of microvascular damage resulting in diabetic nephropathy as opined by (Nelson and Couto, 2014) [16].

In present investigation, urine pH decreased significantly (p<0.01) in diabetic group (pre-treatment) in comparison to healthy control group and post treatment of diabetic group.

There was no statistical difference in values of urine pH between healthy control group and post treatment of diabetic group.

The lower pH in urine is due to a combination of greater net acid excretion (NAE) and lower use of ammonia buffers in patients with diabetes, which predisposes them to uric acid urolithiasis (Maalouf *et al*., 2010) [15].

The results revealed that blood was not found in urine of diabetic group (pre-treatment) same as that compared to healthy control group and post treatment of diabetic group. The present observation corroborate with the finding of Kapoor (2019) [12].

Conclusion

Hematological profile of the diabetic canines revealed neutrophilia and mild lymphocytopenia.

Serum glucose, ALT, ALP, triglyceride, total cholesterol, BUN and creatinine were significantly increased in diabetic canines. The urine parameters i.e. ketone bodies, glucose, specific gravity and protein were increased significantly but urine pH was decreased significantly in diabetic canines. The blood was not found in urine of diabetic canines.

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