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M Joshi
M.V.Sc. (Scholar), Department of Veterinary Medicine, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

HK Mehta
Professor, Department of Veterinary Medicine, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

R Chaurasia
Ph.D. (Scholar), Department of Veterinary Medicine, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Corresponding Author
R Chaurasia
Ph.D. (Scholar), Department of Veterinary Medicine, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Comparative efficacy of neutral protamine hagedorn insulin (NPH) and insulin degludec (IDeg) in dogs

M Joshi, HK Mehta and R Chaurasia

Abstract
The present study "Studies on prevalence and comparative efficacy of neutral protamine hagedorn insulin (NPH) and insulin degludec in canine diabetes mellitus in dogs" was conducted at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow (M.P.) and in privet veterinary clinics across Indore. Twelve dogs were selected with the history of Polyuria, Polydipsia, polyphagia, loss of body condition and blood glucose level more than 150mg/dl were diagnosed for diabetes mellitus in dogs and treated in to two groups, six dogs in each group. To evaluate the efficacy of neutral protamine hagedorn insulin and insulin degludec. The efficacy of insulin therapy was judged on the basis of improvement in clinical symptoms and alteration in haemato-biochemical parameters.

Keywords: Neutral protamine hagedorn insulin, insulin degludec, diabetes mellitus, canine, Madhya Pradesh

Introduction
Diabetes mellitus consists of a group of metabolic diseases that are characterized by a chronic excess of blood glucose resulting from defects in insulin secretion, insulin action or both (American Diabetes Association, 2003) [3]. Diabetes mellitus is a chronic disorder Associated with chronic complications including retinopathy, neuropathy, nephropathy and antipathy. It constitutes a major global public health problem in human and also of concern in dogs.
There are two types of diabetes mellitus now known as Insulin Dependent Diabetes Mellitus (IDDM) formerly known as juvenile onset diabetes and Non-Insulin Dependent Diabetes Mellitus (NIDDM), formerly called maturity onset diabetes. IDDM is also referred to as type 1 diabetes which implies certain pathogenic mechanism whilst NIDDM is referred to as type 2 diabetes.
Porcine-source Lente and recombinant human NPH insulin were effective for the treatment of diabetes in dogs and have exactly the same amino acid sequence as canine insulin and induces no anti-insulin antibodies with prolonged use in dogs Monroe *et al*. (2005) [23]. Problems with prolonged duration of insulin effect can occur with both insulin preparations but are not common Fleeman *et al*. (2009) [10].
Problems with short duration of insulin effect despite twice a day administration were more common than problems with prolonged duration of insulin effect, especially with Neutral Protamine Hagedorn (NPH) insulin (Palm *et al*., 2009) [26]. Recombinant human Protamine Zinc (PZI) insulin is commonly used for the treatment of diabetes in cats but published experiences with PZI in diabetic dogs is limited. In a recent study, PZI administered twice a day was effective in improving or maintaining control of glycaemia in the majority of diabetic dogs enrolled in the study, and more than 80% of owners were satisfied with the results of treatment (Della-Maggiore *et al*., 2012) [8]. Human insulin differs by one amino acid from canine insulin, and anti-insulin antibodies have only been detected in one dog treated with recombinant human insulin (Neubauer and Schone, 1978) [24]. Bovine insulin differs by two amino acids from canine insulin. Anti-insulin antibodies have been detected in dogs treated with purified bovine insulin and mixed bovine/porcine insulin (Harb-Hauser *et al*., 1998) [11].
Ode *et al*. (2018) investigated the effect of Insulin Degludec on glycemic control in dogs. Its time action profiles were monitored in healthy dogs using an artificial pancreas apparatus under glycemic conditions. At 9-13.5 hr post- insulin degludec injection, an indistinct peak of glucose level was detected. Moreover, the action of insulin degludec (IDeg) was persistent for >20 hr. both Insulin degludec (IDeg) and neutral protamine hagedorn insulin (NPH) lowered blood glucose concentrations in diabetic dogs, but insulin degludec cause post prandial hyperglycemic and a somewhat lower pre-prandial glucose level than that cause by NPH.

Insulin degludec might be ineffective in concurrently preventing post prandial hypoglycemia and pre-prandial hyperglycemia in a single agent administration

Materials and Methods

The study was carried out in Department of Veterinary Medicine, Veterinary Clinical Complex (V.C.C.), College of Veterinary Science and Animal Husbandry, MHOW (M.P.) and private veterinary clinics across Indore.

Experimental design: The dogs having history and clinical signs of Polydipsia, polyphagia, Polyuria, and loss of body condition and corneal opacity were selected for the study. The blood glucose levels of selected dogs were done for estimation of diabetes mellitus. For efficacy of insulin therapy a total 12 dogs were selected having more than 150 mg/dl glucose. These dogs were divided in to two groups, having six dogs in each groups.

Group 1: The dogs of this group were treated with Neutral Protamine Hagedorn (NPH) insulin subcutaneously @ 0.5 IU/kg body weight just before food BID.

Group 2: The dogs of this group were treated with Insulin Degludec (IDeg) subcutaneously @ 0.5 IU/kg body weight just before food OD. The repetition of Insulin was decided on the basis of post treatment blood glucose level.

Days of observation: For insulin dose the blood collection was done for estimation of fasting blood glucose level that is 0 hr and post insulin/meal at 45 minutes, 2 hrs, 5 hrs, 8 hrs, 12 and 24hrs, at first day the dose was calculated according to post insulin blood glucose level. For efficacy of insulin treatment, diabetes affected dogs were observed at 0, 7 and 14 days of post insulin.

Evaluation of comparative efficacy of insulin: The effect of insulin was evaluate on the basis of improvement in clinical signs, blood glucose values and haemato-biochemical parameters.

Collection of blood: One ml of Blood was collected from cephalic vein, recurrent tarsal vein in sodium fluoride (NaF) and EDTA vial for estimation of fasting Blood Glucose and hematological parameters respectively. For Biochemical examination two ml of Blood was collected in non EDTA vial and allowed the blood to clot in an upright position for at least 60 minutes than centrifuge at 2200-2500 rpm for 15 minutes to separate the serum.

Following observations were recorded based on study

Clinical observations: Different clinical signs were observed as Rectal temperature (°F), Pulse rate (per minute), Respiration rate (per minute), Mucous membrane (color and correlation) other clinical signs as Polyuria, Polydipsia, Polyphagia, Loss of body condition and corneal opacity were also observed.

Observations of Hamate-Biochemical Parameters

Hematological Parameters: hematological parameters were estimated as

1. **Hemoglobin (Hb):** Hemoglobin was determined by using automatic blood cell counter (Diatron group abacus 380) and values were expressed in g/dl.
2. **Differential Leukocyte Count (DLC):** The differential leucocyte count were done by preparing thin blood smear on glass side, after staining with Leishman's stain, were examined under (100X magnification) microscope using oil immersion lens. One hundred cells were counted and values were expressed in percentage
3. **Total Leukocyte Count (TLC):** The counting of white blood cells were done in automatic blood cell counter (Diatron group abacus 380). The values were expressed in thousand/cu.mm.
4. **Packed cell volume (PCV) (%):** Packed cell volume was determined by using automatic blood cell counter (Diatron group abacus 380). The values were expressed in percents (%).
5. **Biochemical parameters**: biochemical parameters were estimated as
6. **Blood glucose (mg/dl):** Blood glucose was estimated by using Erba Manheim Diagnostics Kit through semi-automatic biochemistry analyzer (Tietz *et al.,* 1983) [28].
7. **S.G.P.T. (IU/L):** Serum glutamic pyruvic transaminase (SGPT**)** was estimated by using Erba Manheim Diagnostics Kit (Bradley *et al.,* 1972) [5] and results were expressed in IU/L.
8. **S.G.O.T (IU/L):** Serum glutamic-oxaloacetic transamination (SGOT**)** was estimated using Erba Manheim Diagnostics Kit (Wolf *et al.,* 1972) [30] and results were expressed in IU/L**.**
9. **Alkaline phosphatase (IU/L):** Serum Alkaline Phosphatase were estimated by using Erba Manheim Diagnostics Kit (Tietz *et al.*, 1983) [28] and value were expressed in IU/L.
10. **BUN (mg/dl):** Serum Blood Urea Nitrogen was estimated by DAM method using Erba Manheim Diagnostics Kit as described by (Marsh *et al*., 1965) [22] and the results were expressed in mg/dl.
11. **Serum creatinine (mg/dl):** Serum Creatinine was estimated using Erba Manheim Diagnostics Kit as the results were expressed in mg/dl.

Results & Discussion

On the basis of improvement in clinical signs, blood glucose values, and Hamate-biochemical parameters comparative efficacy of insulin was studied.

On the basis of improvement in clinical signs

1. Rectal temperature (ºF): The mean value of rectal temperature (ºF) on days 0, 7 and 14 in group 1 and group 2 were 101.46±0.34, 101.73±0.35 and 100.98 ± 0.23 and 101.05±0.88, 102.33±1.22and 101.66±0.98 respectively.

The mean rectal temperature values were non-significant between the groups (p=0.251).

There were no significant changes observed in mean rectal temperature between the groups and the rectal temperature was ranged from 100.9ºF to 102.33ºF at different days of intervals.

The findings are in agreement with the findings of (Ettinger and Feldman, 2010) [9] found normal rectum temperature.

In accordance with the findings of Kapoor (2019) [19] who studied canine diabetes mellitus and found mean values of rectal temperature 101.33±0.23 ºF.

2. Pulse rate (per minute): The mean values of pulse rate on 0, 7, and 14 days in group 1 and group 2 were 80.00±2.5, 83.66±2.27 and 87.50±1.82 and 86.16±1.3, 82.00±1.48 and

82.16±1.92respectively.
The mean pulse rate values were non-significant between the groups. It was also non-significant among the days (p=0.811).
There were no significant changes observed in the mean pulse rate (per minute) between the groups and the pulse rate was ranged from 80 to 87.5 per minute at different days of intervals.
Kapoor (2019) [19] found heart rate per minute) as 94.75±1.38. However (Bhat *et al*., 2013) [4] reported higher heart and respiration rates.

3. Respiration rate (per minute): The mean values of respiration rate on 0, 7, and 14 days in group 1 and group 2 were 23.83±0.47, 23.83±0.30 and 24.83±1.07 and 23.50±0.88, 24.33±1.22 and14.66±0.98 respectively.
The mean pulse rate value was non-significant between groups 1 and 2. It was also non-significant among the days (p=0.766)
There were no significant changes observed in mean respiration rate (per minute) between the groups and the respiration rate was ranged from 14.66 to 24.83 per minute at the different day of intervals
Kapoor (2019) [19] observed respiration rate (per minute) as 27.50±1.10 which was in the normal range as found by (Ettinger and Feldman, 2010) [9].

4. Mucous membrane (color and correlation): In 50% (6/12) dogs had hyperemic mucous membrane and 50% (6/12) had normal mucous membrane. A hyperemic mucous membrane was observed due to dehydration. After the treatment, the color of the mucous membrane becomes normal. Kapoor (2019) [19] found on physical examination, conjunctival mucous membranes were found pink and moist. Abdominal palpation revealed no abnormality in healthy dogs and there was no lymph might be due to dehydration.

5. Other clinical signs: The signs of Polydipsia, Polyuria, and polyphagia were disappeared in all dogs at 14 days of observation. The body condition of all dogs was partially improved. While corneal opacity was not disappeared in all the dogs.
Hess *et al*. (2001) [15] reported that 82% of dogs Polyuria and Polydipsia, 57% lethargy, 45% inappetence or anorexia, 40% vomiting, 39% weight loss, 22% polyphagia, and 13% diarrhea. Systemic signs like vomiting and anorexia could develop due to ketosis and metabolic acidosis.
Clinical findings recorded in the present study were similar to those reported by (Heritage, 2009 and Rucinsky *et al*., 2010 and Kumar *et al*. 2014) [27, 21].
The onset of diabetic cataracts involves several factors like osmotic changes in the lens, glycosylation of structural proteins, and a decreased concentration of antioxidants.
Kapoor, (2019) [19] studied and found that 58.33% (7/12) of dogs were constipated and 50% (6/12) dogs were dehydrated. 25% (3/12) of dogs were anorectic. Only 2 out of 12 (16.66%) dogs showed vomiting and diarrhea was present in only one dog (8.33%). She also found that 41.66% (5/12) of dogs had developed ocular changes like cataract and uveitis and abnormal gait was observed in 1 dog out of 12.
Choudhary *et al.* (2021) [6] studied clinical findings and recorded in diabetes mellitus affected canines were Polydipsia (81.81%), polyphagia (63.63%), Polyuria (72.72%), weight loss (72.72%), vomiting (18.18%) and cataract formation (27.27%).

Table 1: Symptom wise improvement in dogs

		Group 1		Group 2	
S. No.	**Clinical signs**	**No. of dogs**	**Improvement (%)**	**No. of dogs**	**Improvement (%)**
1.	Hyperemic mucous membrane	3	3 (100%)	3	1(16.66%)
2.	Polyuria	6	6(100%)	6	2(33.33%)
3.	Polydipsia	6	6(100%)	6	3(50%)
4.	Polyphagia	5	5(100%)	6	2(33.33%)
5.	Loss of body condition	4	Partial improvement	4	No improvement
6.	Corneal opacity	2	No improvement	1	No improvement

Haemato-Biochemical Parameters
Hematological Parameters
1. Hemoglobin
The results of different treatments and their interactions on the levels of hemoglobin were studied. The mean values of hemoglobin (g/dl) on days 0, 7, and 14 in group 1 and group 2 were 13.45±1.15, 13.65±0.91 and 14.03±0.94 and 14.69±0.62, 14.61±0.74 and 15.00±0.73 respectively.
The mean hemoglobin values were non-significant between group 1 and group 2. It was also non-significant among between the days (p=0.666). The hemoglobin values were ranged between 13.45 to 15.00 g/dl in between the groups. While Hess *et al*. (2001) [15] found that 24% of dogs were anemic.
Kapoor (2019) [19] studied the hematological profile of dogs suffering from diabetes mellitus. The respective mean values of hemoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC), and total leukocyte count (TLC) were recorded to be 14.5±1.21 g/dl, 43.68±3.48%, 6.59±0.48 × 1012/L, and 14.00±2.10 ×109/L respectively. There was no significant difference between the mean values of Hb, PCV, and TEC in diabetic dogs as compared to healthy dogs.

2. Differential Leucocyte Count (DLC)
2.1 Neutrophils (%)
The results of different treatments and their interactions on the levels of Neutrophils were studied. The mean values of neutrophils counts (%) on days 0, 7, and 14 in group 1 and group 2 were 80.50±3.67, 79.5±3.28 and 80.83±3.35 and 77.00±1.17, 73.00±2.17 and 75.50±1.19 respectively.
Neutrophils counts were significantly low between the two groups. (p<0.05) and non-significant within the two groups.
Herrera *et al*. (2007) [12] studied canine diabetes mellitus and evaluated 40 dogs. They found neutrophil with the shift to left and also observed changes in other leukocytes count.
Similar findings were observed by Valilou and Lofti (2011) where neutrophils (82.3%) were higher and lymphocyte (14.59%) and monocyte (1.81%) were lower in diabetic dogs as compared to the control group (72.38%, 22.79%, and 2.05%). The probable cause of neutrophilia in diabetic patients can be up regulation of tumor necrosis factor and Interleukin 6 in response to PAMP motifs. These cytokines

are chemotactic in nature and cause leukocytosis and neutrophilia in diabetic patients (DE Clue *et al*., 2012) [7].
Kapoor (2019) [19] studied that neutrophils were none significantly increased in diabetic dogs. These observations also were in agreement with the findings of (Xu *et al*., 2013) [31] which showed higher TLC and neutrophils in diabetic dogs.
The reason behind the Neutrophilia was the chemotactic nature of cytokine that causes Leukocytosis and Neutrophilia in diabetic dogs.

2.2 Lymphocyte (%)
The results of different treatments and their interactions on the levels of lymphocyte (%) were studied and their mean values on days 0, 7, and 14 in groups 1 and 2 were 15.00±2.46, 16.16±0.16 and 14.83±2.24 and 20.66±0.714, 21.83±1.53 and 21.00±0.51 respectively.
Lymphocyte counts ware non-significant between the groups. It was also non-significant among the days (p>0.05).
Herrera *et al*. (2007) [12] studied canine diabetes mellitus and evaluated 40 dogs. They found lymphocytosis and also observed changes in other leukocytes count.
In contrast to the study, the mean value of lymphocytes was significantly reduced in diabetic dogs as compared to healthy dogs. Similar findings were observed by Valilou and Lofti (2011) where neutrophils (82.3%) were higher and lymphocyte (14.59%) and monocyte (1.81%) counts were lower in diabetic dogs as compared to the control group.
Kapoor (2019) [19] observed that Differential leucocyte count revealed 77.04±3.37% neutrophils, 19.58±3.16% lymphocytes, and 3.38±0.38% monocytes. The TLC and neutrophils were non-significantly increased in diabetic dogs. The mean value of lymphocyte and monocyte were non-significantly reduced in diabetic dogs as compared to the healthy group.

2.3 Monocyte (%)
The results of different treatments and their interactions on the levels of Monocyte (%) were studied and their mean values are on days 0, 7, and 14 in group 1 and group 2 were 3.16±1.16, 2.00±0.894 and 2.83±1.19 and 1.33±0.49, 2.33±0.66 and 1.93±0.60 respectively.
Monocytes counts were non-significant between group 1 and group 2. It was also non-significant among the days (p>0.05).
In contrast to the study, findings were observed by (Valilou and Lofti, 2011) were neutrophils (82.3%) were higher and lymphocyte (14.59%) and monocyte (1.81%) were lower in diabetic dogs as compared to the control group (72.38%, 22.79%, and 2.05%).
In accordance with the study, (Abakpa *et al.*, 2017) [1] found that the white blood cells (WBC) insignificantly increased in diabetic groups post-induction and post-treatment.

2.4 Eosinophil (%)
The results of different treatments and their interactions on the levels of eosinophil (%) were studied and their mean values are presented on days 0, 7, and 14 in group 1 and group 2 were 2.00±0.85, 2.33±0.66 and 2.00±0.85 and 1.33±0.61, 2.83±1.01 and 1.66±0.76 respectively.
The eosinophil count was non-significant between groups 1 and 2. It was also non-significant among the days (p<0.05).
Herrera *et al*. (2007) [12] recorded Leukogram alterations in dogs with diabetes mellitus were eosinophilia and eosinopenia.

2.5 Total Leukocyte Count (TLC) (thousand/cu.mm.)
The results of different treatments and their interactions on the levels of total leukocyte count (thousand/cu.mm.) were studied and their mean values on days 0, 7, and 14 in group 1 and group 2 were 213033.33±1000.20, 13514.30±907.70 and 13488.33±1024.82 and 10640.00±1471.12, 10998.38±1397.57 and 10872.67±1539.714 respectively.
Total leucocyte counts (TLC) were significant between groups 1 and 2 on 0 days (p=0.05). It was non-significant among the days (p=0.952).
In accordance with the study by Abakpa *et al.* (2017) [1] found that the white blood cells (WBC) are significantly increased in diabetic groups post-induction and post-treatment.
Kapoor (2019) [19] studied that differential leucocyte count revealed 77.04±3.37% neutrophils, 19.58±3.16% lymphocytes, and 3.38±0.38% monocytes. The TLC and neutrophils were non-significantly increased in diabetic dogs. The mean value of lymphocyte and monocyte were non-significantly reduced in diabetic dogs as compared to the healthy group. These observations were in agreement with the findings of (Xu *et al*., 2013) [31] which showed higher TLC and neutrophils in diabetic dogs.

2.6 Packed cell volume (PCV) (%)
The results of different treatments and their interactions on the levels of packed cell volume (%) were studied and their mean values on days 0, 7, and 14 in groups 1 and 2 were 43.83±1.46, 44.46.±1.55 and 44.44±1.80 and 47.85±2.05, 48.06±2.51 and 49.01±2.53 respectively.
Packed cell volume (PCV) was non-significant between the groups (p=0.071). It was also non-significant among the days (p=0.83).
Kothari *et al*. (2012) reported anemia and PCV in diabetic dogs and described it due to dehydration in diabetic dogs.
In consonance with the study of Jena *et al*. (2019) [18] found PCV level was not significantly changed in any group of diabetic dogs.

Biochemical parameters
Blood glucose (mg/dl)
1. Blood glucose- Value with insulin NPH was given BID and insulin degludec given SID.

Blood glucose level: To compare the efficacy of NPH given BID and insulin degludec as SID. The mean blood glucose values were recorded and changes were observed.
In dogs of group 1, the mean blood glucose level was recorded as 0 hours, 45 minutes, 2, 5, 8, 12 and 24 hours the blood glucose levels were 478.05, 465.32, 280.55, 180.60, 164.41, 247.38, and 325.97 mg/dl as presented in Table 2.
The mean blood glucose level was started to increase after 8 hours of post insulin and was found 325.97mg/dl at 24 hours of post insulin.
The mean blood glucose level was reduced to 207 mg/dl at 12 hours after the 2nd dose of insulin NPH. Hence it is observed that the two-dose of insulin NPH maintained the blood glucose level between 247.38mg/dl to 207.03mg/dl.
The mean blood glucose level in dogs of group 2nd at 0 hours, 45 minutes, 2, 5, 8, 12, and 24 hours. The blood glucose levels 411.50, 373.03, 332.56, 308.99, 383.50, 375.11 and 352.96mg/dl respectively as presented in Table2.
The 2nd dose of insulin degludec was administered at 12hours post insulin the means blood glucose value was 349.16mg/dl after 24 hours of 2nddose. On the basis of blood glucose level

in dogs of group 2nd the levels were maintained at a high level as the minimum mean blood glucose level was observed 308.99 mg/dl at 5hours post insulin. As a result, the insulin degludec has failed to maintain a normal blood glucose level.

Table 2: Comparative efficacy of NPH and insulin degludec

	Time interval	Comparative efficacy of NPH and insulin degludec	
		Group1	Group 2
1st dose of insulin	0 hr.	478.05	411.50
	45 minute	465.32	373.03
	2 hrs.	280.55	332.56
	5 hrs.	180.60	308.99
	8 hrs.	164.41	383.5
	12 hrs.	247.38	375.11
	24 hrs.	325.97	352.96
2nd dose of insulin	12hrs. after 2nd dose	207.03	349.16

Response of insulin NPH in clinical signs of dogs of group I was considered the most effective for canine diabetes mellitus as compared with insulin degludec in dogs of group II. Improvement in corneal opacity was not observed in any group.

The results of different treatments and their interactions on the levels of blood glucose (mg/dl) were studied and their mean values are on days 0, 7, and 14 in group 1 and group 2 were 268.08±29.37, 312.21±31.54 and 274.09±34.42 and 260.73±40.77, 383.38±22.94 and 394.86±19.06 respectively.

On comparison of the value of blood glucose were significantly differ on day 14 (p=0.00). It indicates the efficacy of insulin NPH is comparatively more in group one.

In dogs, the activity of NPH lasts between 4 and 8 hours. Although NPH insulin is preferred by certain doctors, the duration of activity in some dogs is too short for twice-daily administration.

Oda *et al.* (2018) [25] studied that there was no significant difference in mean glucose concentration, time spent in hyperglycemia (blood glucose > 400 mg/dl), time spent in hypoglycemia (blood glucose < 60 mg/dl), and maximum and minimum glucose concentrations between NPH and Insulin degludec in the four diabetic dogs. Insulin degludec maintained a mean glucose concentration of 180 ± 65 mg/dl, whereas NPH maintained 225 ± 53 mg/dl in all diabetic dogs. The maximum and minimum glucose concentrations were 356 ± 98 and 132 ± 70 mg/dl, respectively, after NPH treatment, and 362 ± 98 and 59 ± 20 mg/dl, respectively, after Insulin Degludec treatment.

The percentage of time spent in glucose levels greater than 400 mg/dl for NPH and Insulin Degludec were 3.8 ± 7.7 and 11.5 ± 18.3%, respectively. Moreover, dogs treated with NPH and Insulin Degludec spent 11.5 ± 23.1% of their time with glucose concentrations ns less than 60 mg/dl, respectively.

According to a study by (Oda *et al*., 2018) [25] was observed the time action profile and effect on glycemic control of insulin degludec in diabetic dogs. It was observed that the action of insulin degludec was persistent for >20 hours. Both NPH and insulin degludec lowered blood glucose concentration in diabetic dogs, but IDeg caused postprandial hyperglycemia and a somewhat lower pre-prandial glucose level than that caused by NPH.

2. Serum Glutamic Pyruvic Transaminase (SGPT) (ALT) (IU/L)

The results of different treatments and their interactions on the levels of serum glutamic pyruvic transaminase (SGPT) (IU/L) were studied and their mean values of serum glutamic pyruvic transaminase (SGPT) (IU/L) on days 0, 7, and 14 in groups 1 and 2 were 32.90±7.94, 32.00±5.67 and 33.65±5.10 and 79.81±17.49, 69.95±5.41and 68.62±5.03 respectively.

On comparison of the values of SGOT were found highly significant between the groups (p<0.01).There were no significant changes observed between the days of observation in both the groups.

Diabetic dogs may also be associated with other diseases like hepatic necrosis and hepatic enlargement (Hiblu *et al*., 2015) [16].

The findings are in agreement with the findings presented by Kapoor (2019) [19] as the mean activities of ALT, AST, and ALP were 41.1±5.11 IU/L, 48.8±4.19 IU/L, and 86.7±4.5 IU/L respectively.

Alkaline phosphatase, alanine transaminase, and Aspartate aminotransferase are markedly increased in diabetic dogs, as compared to healthy dogs. These suggest hepatic involvement in DM. Diabetic dogs often show increased alkaline phosphatase and alanine aminotransferase (Jena *et al.*, 2019) [18].

3. Serum Glutamic Oxaloacetic Transaminase (SGOT) (AST) (IU/L)

The results of different treatments and their interactions on the levels of serum glutamic oxaloacetic transaminase (SGOT**)** (IU/L) were studied and their mean values of serum glutamic oxaloacetic transaminase (SGOT**)** (IU/L) on days 0, 7, and 14 in groups 1 and 2 were 37.63±4.48, 44.72±5.02 and 47.16±6.13 and 54.98±2.57, 55.91±3.57and 61.73±2.10 respectively.

On day 0, the differences between the groups were extremely significant (p=0.00).

When the observation was analysed by days, there were no significant differences among the day's 0, 7, and 14.

The observation as in agreement with the study of Behrend *et al.* (2018) [3] as Diabetic dogs often show increased alkaline phosphatase and alanine amino transferase.

4. Alkaline phosphatase (IU/L)

The results of different treatments and their interactions on the levels of serum alkaline phosphatase (IU/L**)** were studied and their mean values of serum alkaline phosphatase (IU/L**)** on days 0, 7, and 14 in both the groups were 37.81±2.67, 37.42±2.76 and 38.97±2.40 and 37.38±0.78, 38.16±1.53 and 41.60±2.54 respectively.

When the groups were compared, there was non-significant difference (p>0.05). As the levels of alkaline phosphatase in neither group were reduced.

In contrast to our study Kapoor (2019) [19] observed the mean activities of alkaline phosphatase was recorded as 86.7±4.5

IU/L.
Jena *et al.* (2019) [18] studied canine diabetes mellitus and found an alkaline phosphatase level of 514.88±21.36 IU.

5. Blood urea nitrogen (BUN) (mg/dl)
The results of different treatments and their interactions on the levels of serum blood urea nitrogen (mg/dl) were studied and their mean values of blood urea nitrogen (mg/dl) on days 0, 7, and 14 in groups 1 and 2 were 16.93±1.42, 17.99±1.14 and 17.50±1.25 and 21.53±1.44, 21.70±0.93 and 21.79±1.27 respectively.
The BUN level was highly significant ($p<0.01$) between the groups. The level of BUN was reduced after treatment and group 1 was better than group 2.
On comparison as per days, non-significant difference was found among the days. According to the study, diabetic dogs' serum creatinine and BUN levels differ significantly from healthy dogs. Evidence for renal failure in diabetic dogs reveals azotemia, increased serum creatinine, and BUN (Huang, 2012 and Jena *et al.*, 2019) [18].
Kapoor (2019) [19] studied diabetic dogs and found the level of blood urea nitrogen was 25.3±2.64 mg/dl.

6. Serum creatinine (mg/dl)
The results of different treatments and their interactions on the levels of serum creatinine (mg/dl) were studied and their mean values of serum creatinine (mg/dl) on days 0, 7, and 14 in both groups were 0.87±0.12, 0.90±0.18 and 0.84±0.04 and 0.88±0.05, 1.01±0.12 and 1.02±0.08 respectively.
On comparison of between the groups, there was no significant difference observed between the groups ($p>0.05$).
In agreement with the study of Jena *et al.* (2019) [18] found Serum creatinine and BUN are significantly different in diabetic dogs, as compared to healthy. Evidence for renal failure in diabetic dogs reveals azotemia, increased serum creatinine, and BUN. She found Creatinine level was 1.80±0.03 mg/dl.
Changes in Haematological parameters were observed as Haemoglobin was non-significant between groups ($p=0.271$). Neutrophils were significantly low between the groups ($p<0.05$).It means that the value of neutrophils were not significantly changed. Total leucocyte count (TLC) was significant between the groups ($p=0.05$). Packed cell volume (PCV) was non-significant between the groups.
Changes in biochemical parameters were observed as blood glucose was highly significant between the groups ($p<0.01$). SGPT was highly significant between the groups ($p<0.01$). SGOT was highly significantly differ on day 0 ($p=0.00$). Alkaline phosphatase was no significant ($p>0.05$) between the groups. BUN level was highly significant ($p<0.01$) between the groups, creatinine was no significant difference between the groups ($p>0.05$).

Conclusion
The improvement in clinical signs *viz* Polyuria, Polydipsia, polyphagia loss of body condition was observed more in group 1st. Insulin NPH was considered the most effective for canine diabetes mellitus. While no changes were observed in clinical symptoms in group 2nd treated with insulin degludec. Improvement in corneal opacity was not observed in any group. Haematological parameters like, TLC and neutrophils and biochemical parameters such as SGPT, SGOT, BUN and blood glucose were found associated with canine diabetes mellitus.

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