Effect of insulin zinc suspension on experimentally induced diabetic rabbits

S. H. Mir, R. Yousuf, *M. M. Darzi and *M. S. Mir
Postgraduate Department of Zoology, University of Kashmir, Srinagar-190 006.
*Division of Veterinary Pathology, FVSC and AH Shuhama (Alustang), SKUAST (K) Srinagar-190 006.
*Corresponding Author:

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ABSTRACT
An investigation was carried out to study the effect of insulin zinc suspension on the level of blood glucose and haematological parameters in alloxan induced diabetic rabbits. Ten healthy male rabbits were made diabetic by intraperitoneal administration of alloxan (@ 80mg/kg, b.w.) and were randomly divided into two groups of five each. Group A was kept as control and received normal saline whereas Group B received subcutaneous injections of insulin (2u/kg b.w.). Blood glucose levels were significantly (P<0.01) reduced in Group B in comparison to Group A of rabbits. Total erythrocyte count (TEC) and total leukocyte count (TLC) were not changed significantly. From the present study, it may be inferred that the drug insulin zinc suspension possess blood glucose lowering effect and the present experiment proves to be a good laboratory model for study of other aspects of diabetes mellitus under control condition.

Key words: Insulin, Induced Diabetes, Rabbits, Haematology.

INTRODUCTION:
Diabetes mellitus is one of the most common endocrine disorders leading to abnormal carbohydrate, protein and lipid metabolism. In this metabolic disorder there is defective or deficient insulin secretion resulting in persistent hyperglycemia (Mohan, 2000). Due to inadequate presence of insulin there are disorders of all kinds of metabolism (Frank, 1962; Nelson, 1985). It has been suggested that red and white blood cell (RBC and WBC) counts are lower in type diabetes than in non-diabetic individuals (Yenigun, 1997 Mohan, 2000 and Ganong, 2006).

Diabetes mellitus has been shown to be associated with abnormalities in the zinc metabolism. Scott and Fisher (1938) first recognized the relationship between zinc and insulin. Urinary excretion of zinc has been shown in diabetic patients than non-diabetic ones (Pidduck et al., 1970; Kumar and Rao, 1974).

Keeping in view the clinical significance of the disease, the present investigation was carried out to determine whether the insulin zinc suspension (Insulin Lente) is effective in restoring the biochemical and haematological values to normal.

Materials and Methods:
Animals and experimental design: Ten New Zealand male rabbits of 10 months old were obtained from FVSC and AH, Shuhama Srinagar. Before the start of experiment rabbits were acclimatized to standard laboratory conditions for ten days and the fasting blood sugar level and some haematological parameters were estimated. All the rabbits were kept fasting overweight and made diabetic by four doses of alloxan (Loba Chemie) administered intraperitoneally @ 80mg/kg between at weekly intervals. Seven days after injections blood sample were collected from marginal ear veins of the animals and diabetes mellitus was confirmed from each animal by estimation of blood sugar levels (F) using Glucometer Gx (Bayer Diagnostic Ltd. India). Half of the animals were kept as control which received normal saline whereas the remaining half received daily subcutaneous 2U/kg injections of insulin zinc suspension (Insulin Lente) for a period of 20 days. Assessment of treatment was based on the improvement of blood sugar level (F).

Haematological Analysis: When the blood sugar level reached normal after administration of insulin zinc suspension for a period of 20 days, the rabbits were fasted for 12 hours and blood samples were taken for the determination of RBC and WBC counts. The RBC and WBC counting methods were based on the dilution of obtained blood with dilution fluids (Hayem and Turk) in RBC and WBC counting pipettes (Mitruka and Rawnsley, 1977). Individual cells were then counted in the counting chamber (haemocytometer). Giemsa's staining method was based for the differential count of WBC.

Statistical Analysis: The data were expressed as mean ± standard deviation (SD) and analyzed using students 't' test (Prasad, 2000). 'P' value was obtained
from the distribution of 't' probability chart.

**Results:**

The efficiency of insulin zinc suspension on the alloxan induced diabetic rabbits is shown in Table I.

**Table I:** Effect of Insulin zinc suspension on the blood sugar level of diabetic rabbits.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial Value</th>
<th>Diabetic</th>
<th>Diabetic treated with insulin zinc suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sugar (F) (mg/dl)</td>
<td>76 ? 6.78</td>
<td>240 ? 5.62*</td>
<td>91 ? 5.71*</td>
</tr>
<tr>
<td>RBC (x10^6/?l)</td>
<td>5.8 ? 0.2</td>
<td>4.6 ? 0.3</td>
<td>5.1 ? 0.1</td>
</tr>
<tr>
<td>WBC (x10^3/?l)</td>
<td>5.5 ? 0.6</td>
<td>4 ? 0.1</td>
<td>4.5 ? 0.3</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>31.2 ? 3.4</td>
<td>24 ? 3.2**</td>
<td>30 ? 3.6**</td>
</tr>
<tr>
<td>Basophils</td>
<td>3.0 ? 0.5</td>
<td>2.5 ? 0.5</td>
<td>2.5 ? 0.4</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>3 ? 0.1</td>
<td>2.7 ? 0.2</td>
<td>4 ? 0.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>62.1 ? 7.3</td>
<td>57 ? 5.9</td>
<td>56 ? 6.3</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.5 ? 2.4</td>
<td>3.1 ? 2.3</td>
<td>3 ? 2.2</td>
</tr>
</tbody>
</table>

* P<0.001 in comparison to saline treated diabetic rabbits.

**From the present study the intraperitoneal administration of alloxan @ 80mg/kg.b.w. exhibited diabetes mellitus in the experimental animals. The establishment of diabetes mellitus was confirmed by increase in blood sugar level (F). Moreover, behavioral changes such as sluggishness, a tendency to lie down and general weakness were observed in diabetic rabbits.**

The injections insulin zinc suspensions for a period of 20 days reduced the blood sugar level (F) from an initial value of 240 ± 5.62mg/dl to 91 ? 5.71mg/dl. However, control rabbits did not show any significant decreases in blood sugar level. Instead, a fluctuation of blood sugar level was observed. It was also found that the RBC and WBC counts especially the neutrophils decreased in diabetic rabbits and increased to some extent in treated group of rabbits. However, these haematological parameters were still lower than normal values taken initially from rabbits prior to the start of the experiment.

**Discussion:**

Establishment of diabetes mellitus in the rabbits by intraperitoneal administration of alloxan observed in the present study is in consistent with earlier observations (Rastogi et al., 1988; Baqui et al., 2005; Mir and Darzi, 2009). It might be attributable to specific irreversible toxic effects of alloxan on beta cells of pancreas (Dunn et al., 1943; Lukenes, 1948) inhibiting insulin secretory mechanism (Grodsky et al., 1982). The subsequent effects of diabetes mellitus exhibited behavioral changes such as lethargy, general weakness, a tendency to lie down and decreased physical activity.

These findings have been reported earlier in rabbits (Baqui et al., 2005).

The improvement in blood sugar level (F) of diabetic rabbits was observed by subcutaneous injections of insulin zinc suspension. The exogenous insulin is required for Type I diabetic individuals. Alloxan diabetes inhibits insulin secretory mechanisms (Grodsky et al., 1982) and serves as a model of insulin dependent diabetes mellitus (Szkudelski, 2001). Further the relationship between insulin and zinc has been recognized and it has been found that normal pancreas contained significant quantities of zinc in comparison to diabetic pancreas which contained very little (Scott and Fischer, 1931). In this experiment, we did not determine the zinc levels in diabetic rabbits. However, in animal experiments Kadota (1950) reported that organic compounds capable of reducing the zinc content of pancreas were diabetogenic.

The improvement in some haematological parameters such as RBC and WBC counts is inconsistent with earlier observation of experiments (Meral et al., 2004). However, the values were still lower than normal values taken initially from rabbits prior to the start of experiment. Neutrophils which are considered as body's first line of defense mechanism against bacterial infections (Ganong, 2006) have been found to be disturbed in diabetes (Yenigun, 1997). In the present experiment it was demonstrated that insulin zinc suspension treatment lowered neutrophil percentage of WBC near normal level. It thus, indicated that insulin zinc suspension increases the defense mechanism of
the body against the infections in diabetic rabbits. Insulin treatment however, remains fraught with difficulties because of imprecise manner in which subcutaneous insulin is delivered. It is further complicated by how, when, and in what quantities food and other dietary substances are ingested, and the effects of activity and day to day life stresses.

In conclusion, the findings of the present study suggests that daily injection of insulin zinc suspension might decrease the diabetes induced glycemic and haematological alterations.

REFERENCES: