APPENDICES
Biochemical and Therapeutic Studies on the Alloxan - Induced Diabetes Mellitus in Rabbits

Abdul Baqui, Sajad Hussain Mir, M. M. Darzi* and Masood Saleem Mir*
Post Graduate Department of Zoology, University of Kashmir, Srinagar - 190 006, Kashmir
*Division of Veterinary Pathology, SKUAST (K), Shuhama (Alusteng) Srinagar - 190 006, Kashmir

Abstract

The establishment of diabetes mellitus in rabbits by alloxan administration (80 mg/kg, b.w.i.p.) exhibited polyuria, polydipsia, loss of appetite, decreased physical activity, loss of body weight and biochemical alterations like changes in blood sugar, blood urea and serum creatinine (p < 0.001) compared to control. A significant improvement in behavioural patterns and biochemical levels (p < 0.001) of alloxan-induced diabetic rabbits were reported following the administration of antidiabetic drugs like glibenclamide (@ 5mg/day); biguanide (Metformin @ 500mg/day) and a homoeopathic drug Cephelendra indica Q (@ 10 drops/day). This shows the favourable response of drugs against alloxan-induced alterations in biochemical and behavioural patterns.

Key words: Diabetes mellitus; alloxan; biochemical; Rabbits.

Material and Methods

Twenty rabbits of either sex weighing on an average 1.99 ± 0.09 kg were selected for the study. Blood sugar, blood urea and serum creatinine estimated at day 0 before inducing diabetes mellitus. Clinical observations like urinations, food intake, physical activities were recorded.

The rabbits were divided into five groups of four each. Group I was kept as control and received normal saline intraperitoneally for a period of four weeks. Other groups of rabbits received alloxan @ 80mg/kg of body weight intraperitoneally for a period of four weeks following 12 hours fasting.

These rabbits were observed daily for body conditions, food intake, urinations and other clinical signs throughout the period of study. Biochemical parameters and body weight were observed at weekly intervals. The blood sugar level of rabbits was estimated using Glucometer Gx (Bayer Diagnostic India Ltd.), blood urea by “Dam Method” and serum creatinine by “Alkaline Picrate Method” using commercially available kits.

Treatment with antidiabetic drugs was started after 6 weeks when the diabetes was well established based on blood sugar levels. Group II received normal saline orally and served as control. Group III received glibenclamide (@ 5mg/day for a period of 15 days. Group IV received biguanide (Metformin) @ 500 mg/day for a period of 15 days and group V received homoeopathic drug Cephelendra Indica Q (41% w/w alcoholic extract of the wild variety of Cephelendra indica Naud.) (Wellman’s Homoeopathic laboratory Ltd Wazirpur India) @ 10 drops/day for 15 days. All these drugs were fed with the help of intragastric intubations.

Student’s 't' test was used for statistical analysis of the data (Prasad, 2002).
Results and Discussion

Diabetes mellitus in rabbits of group II, III, IV and V was induced on day 7th of alloxan administration which might be due to rapid inhibition of insulin secretary mechanism (Grodsky et al., 1982) of alloxan and its tendency of selective cytotoxicity and concentrations in pancreatic islet tissue relative of some other tissue (Malaisse, 1982). The subsequent effects of hyperglycemia in alloxanized diabetic rabbits exhibited polyuria, polydipsia, general weakness, lethargy and decreased physical activity. These findings are in agreement with earlier observations recorded by other workers in sheep (McCandlers et al., 1984), goats (Prasad et al., 1985), dogs (Nelson et al., 1990; Rao et al., 1998) and rats (Balasubramanian, 1991). The signs of polyuria and polydipsia might be compensatory mechanism for excretion of high levels of glucose in the blood (Doxy et al., 1985).

Mean body weight of group II, III, IV, and V rabbits from 1.99 ± 0.09 kg continued to decrease to 1.67 ± 0.16 kg upto 6th week in contrast to saline treated rabbits which showed an increased tendency of body weight upto 2.40 ± 1.01 kg (Figure 1). The decrease in body weight of alloxan-induced diabetic rabbits might be due to insulin insufficiency leading to decreased accumulations of body reserve and an increased mobilization of endogenous energy store particularly fat (Edward, 1977).

Figure 1. Changes in Body Weight throughout the period of study.

Mean blood sugar level (F) from 89.25 ± 1.29 mg/dl of Group II, III, IV and V rabbits showed a significant increase upto six weeks reaching 225 mg/dl in comparison to saline-treated group of rabbits which remained almost constant at 70.25 ± 5.69 mg/dl of blood sugar level. Mean blood sugar of alloxanized diabetic rabbits was on peak during the 6th week (Table 1). Blood urea and serum creatinine in alloxan-treated groups of rabbits increased from 26.5 ± 1.47 mg/dl and 0.56 ± 0.03 mg/dl to 49.86 ± 1.40
mg/dl and 2.11 ± 0.02 mg/dl respectively in comparison to control rabbits which remained almost constant at 0.55 ± 0.02 mg/dl (Table 1). The increase in biochemical parameters was suggestive of renal damage which was in congruence to earlier findings (Dubey et al., 1994).

Table 1. Changes in Blood Glucose (F), Blood Urea and Serum Creatinine in Alloxan-induced Diabetic Rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial Value</th>
<th>1st Week</th>
<th>2nd Week</th>
<th>3rd Week</th>
<th>4th Week</th>
<th>5th Week</th>
<th>6th Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (F) (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>70.6±1.11</td>
<td>89.25±1.29</td>
<td>69.25±4.64</td>
<td>112.25±4.45</td>
<td>69.25±5.49</td>
<td>132.25±1.55</td>
<td>70.6±1.13</td>
</tr>
<tr>
<td>T</td>
<td>24.2±1.43</td>
<td>26.5±1.47</td>
<td>29.8±1.43</td>
<td>24.5±1.27</td>
<td>30.1±1.05</td>
<td>39.4±1.18</td>
<td>154±8.75</td>
</tr>
<tr>
<td></td>
<td>0.54±0.02</td>
<td>0.56±0.03</td>
<td>0.92±0.03</td>
<td>0.56±0.04</td>
<td>1.66±0.09</td>
<td>1.87±0.06</td>
<td>0.55±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Urea (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.11±0.02</td>
<td>2.11±0.02</td>
<td>2.11±0.02</td>
<td>2.11±0.02</td>
<td>2.11±0.02</td>
<td>2.11±0.02</td>
<td>2.11±0.02</td>
</tr>
<tr>
<td>T</td>
<td>213.33±3.74</td>
<td>210.33±4.32</td>
<td>170.33±10.5</td>
<td>119.33±11.76</td>
<td>130.33±4.25</td>
<td>130.33±4.72</td>
<td>130.33±4.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.54±0.02</td>
<td>0.56±0.03</td>
<td>0.92±0.03</td>
<td>0.56±0.04</td>
<td>1.66±0.09</td>
<td>1.87±0.06</td>
<td>0.55±0.01</td>
</tr>
<tr>
<td>T</td>
<td>213.33±3.74</td>
<td>210.33±4.32</td>
<td>170.33±10.5</td>
<td>119.33±11.76</td>
<td>130.33±4.25</td>
<td>130.33±4.72</td>
<td>130.33±4.72</td>
</tr>
</tbody>
</table>

C = Control (Saline-treated normal rabbits);
T = Treated (alloxan-induced diabetic rabbits). Values are mean ± SE; *P < 0.001 compared to control.

Table 2. Effect of Different Antidiabetic Drugs on the Blood Sugar Level (mg/dl) of Diabetic Rabbits

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage (Oral route)</th>
<th>Blood Sugar (F) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Value</td>
<td>After 5th Day</td>
</tr>
<tr>
<td>Saline</td>
<td>@ 5ml/day</td>
<td>197 ± 3.74</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>@ 5mg/day</td>
<td>202 ± 4.32</td>
</tr>
<tr>
<td>Biguanide (Metformin)</td>
<td>@ 500 mg/day</td>
<td>223.33 ± 10.5</td>
</tr>
<tr>
<td>Cephalandra indica Q</td>
<td>@ 10 Drops/day</td>
<td>210 ± 3.74</td>
</tr>
</tbody>
</table>

* P<0.001 (in comparison to saline-treated alloxanized rabbits).
A significant decrease in blood sugar level (F) of Group III rabbits after oral application of glibenclamide from 202 ± 4.32 mg/dl to 77.67 ± mg/dl was recorded (Table 2). Group IV and V of rabbits also showed a decrease from 223.33 ±10.5 mg/dl and 210 ± 3.74 mg/dl to 84 ± 2.16 mg/dl and 91.33 ± 0.98 mg/dl respectively (Table 2). These changes were significant in comparison to Group II control rabbits which showed an increase in blood sugar from 197 ± 3.74 mg/dl to 205 ± 3.30 mg/dl (Table II). The improvement in blood sugar level following treatment with drugs might be attributable to the stimulation of endogenous insulin release from spared alloxan unaffected pancreatic β cells. (Herman, 1979; Rastogi et al, 1988; Rao et al., 1998).

Summary

Intraperitoneal administration of alloxan (@ 80mg/kg b.w.) in rabbits induced diabetes mellitus characterized clinically by polyuria, Polydipsia, decreased physical activity, Sluggishness and weight loss. The biochemical parameters indicated alterations in blood sugar, blood urea and serum creatinine. The symptoms recorded in biochemical and behavioural changes retained to normal after administration of drugs. The study suggests that induced diabetes in rabbits shows a relapse following the administration of the drugs.

References


Pathoanatomy of Experimental Diabetes in Rabbits (*Oryctolagus cuniculus*)

Sajad Hussain Mir, Abdul Baqui, M.M. Darzi, Masood Saleem Mir

Post Graduate Department of Zoology, University of Kashmir, Srinagar-190006, Kashmir
Division of Veterinary Pathology, SKUAST (K), Shuhama, (Alusteng) Srinagar-190006

Introduction

Diabetes mellitus is the most prevalent endocrine disorder, and in animals it has been experimentally found to be responsible for structural alterations of almost every tissue and organ. Animal models have been frequently used to investigate the various biomedical aspects of the disease. Current study was aimed at elucidating the pathomorphological alterations in pancreas and kidneys following induction of diabetes in rabbits by alloxan.

Material and Methods

Eight rabbits, of either sex weighing on an average 1.75 ± 0.15 kg, were selected for the present study. Prior to the experimentation, the rabbits were acclimatized to the standard laboratory conditions for ten days and the fasting blood sugar level was measured using Glucometer Gx (Bayer Diagnostic India Ltd.). The rabbits were divided into two groups of four each. Diabetes mellitus was induced in group I of rabbits by intraperitoneal injections of alloxan @ 80mg/kg of body weight at weekly intervals for a period of four weeks following 12 hours fasting prior to each injection where as Group II was kept as control and received normal saline only.

When diabetes mellitus got established, observed by behavioural alterations and high blood sugar level, the rabbits were sacrificed. The pancreas and kidneys of both the groups of rabbits were fixed in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris’ Haematoxylin and Eosin (Luna, 1968).

Results and Discussion

The establishment of diabetes mellitus was confirmed by persistent hyperglycemia in Group I rabbits compared to Group II rabbits which remained normal throughout the study. The blood sugar level of diabetic rabbits increased consistently up to sixth week reaching 238 ± 16 mg/dl and in the seventh week the value remained almost constant. The development of increased blood sugar level might be due to the rapid
inhibition of insulin secretion by β cells of the islets following damage by alloxan (Grodsky et al., 1982). Further, the diabetic rabbits showed a change in behaviour such as dullness, lethargy, tendency to lie down, polyuria and polydipsia. These changes were in consonance with the findings of earlier workers (Nelson et al., 1990; Balasubramanian, 1991). The excessive thirst and urination might be due to the compensatory mechanism for excreting the high levels of glucose in the blood (Doxy et al., 1985).

Pancreatic sections stained with Haematoxylin and Eosin showed that alloxan caused severe necrotic changes of pancreatic islets. Vacuolation, degeneration and pancreatic congestion were observed (Fig. 1 & 2). Group II rabbits showed normal histomorphology of the pancreas. The degenerative changes in pancreatic islets are in agreement with the observations of earlier workers (Dunn et al., 1943; Leukenes, 1948 and Bansal et al., 1994). The pancreatic islet damage might be due to alloxan and its metabolites which specifically concentrate in pancreatic islet tissue (Malaisse, 1982) leading to its irreversible toxic damage (Dunn et al., 1943). The kidney sections of group I rabbits showed renal damage characterized by necrosis and lower nephron nephrosis (Fig. 3 and 4). Degeneration in sub-capsular region was also evident in some kidney sections (Fig. 5). The histopathological observations of kidneys support the earlier findings (Doxy et al., 1985; Nakayama et al., 1986 and Bansal et al., 1994) The possible explanation for histopathological changes of kidneys in diabetic rabbits might be due to the increased renal threshold for hyperglycemia. The present study showed that the pathomorphology in pancreas and kidney are the feature of alloxan induced diabetes in rabbits.

Fig. 1: Pancreatic section showing congestion (H&E × 100)

Fig. 2: Section of pancreatic islet showing degeneration & necrosis (H&E × 400)
Fig 3. Kidney section showing degeneration in subcapsular region (H&E × 400)

Fig 4. Kidney section showing tubular degeneration (H&E × 400)

Fig 5. Kidney section showing nephrosis (H&E × 400)
Summary:

This study was conducted to determine the histological studies of pancreas and kidneys of rabbits following intraperitoneal administration of alloxan (at 80mg/kg b.w.) at weekly intervals. Establishment of diabetes mellitus was based on hyperglycemia. It was concluded that the pancreas and kidneys of diabetic rabbits showed structural alterations when compared with normal rabbits.

Acknowledgement:

The authors are thankful to the Head, P.G. Department of Zoology, University of Kashmir for the facilities provided to carry out the above study.

References:


Effect of Insulin Zinc Suspension on the Experimentally Induced Diabetic Rabbits

Department of Zoology, University of Kashmir, Srinagar
*Division of Veterinary Pathology, SKUAST (K) Shuhama (Alustang), Srinagar

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 intraperional 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 where as 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 disorder leading to abnormal carbohydrate, protein and lipid metabolism. In this metabolic disorder there is defective or deficient insulin secretar>- response 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 diabetics than in non-diabetic individuals (Yenigun, 1997).

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 diabetes than non-diabetes (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 All, 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 intraperitonally @ 80mg/kg b.w. 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 10 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 (Mitraka and Rawnsley, 1977). Individuals cells were then counted in the counting chamber (haemocytometer). Giemsa’s staining method was used 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 efficacy of insulin zinc suspension on the alloxan induced diabetic rabbits is shown in Table 1.

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 (x10⁹/μl)</td>
<td>5.8 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>WBC (x10³/μl)</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.
** P<0.05 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, behavioural changes such as sluggishness, a tendency to lie down and general weakness were observed in diabetic rabbits.
The injections of 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 neutrophills 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 Baqui, 2005). 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 defence mechanism against bacterial infections (Ganong, 1991) 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 defence mechanism of the body against the infections in diabetic rabbits.

Insulin treatment 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:

Baqui, A.; Mir Sajad Hussain; Darzi M. M and Masood Saleem Mir


DRUG-INDUCED DIABETES MELLITUS IN RABBITS

SAJAD HUSSAIN MIR AND ABDUL BAQUI
P.G. Department of Zoology.
University of Kashmir. Srinagar - 190006.

Abstract: Diabetes mellitus is the most prevalent endocrine disease with a numerical incidence of three hundred million diabetics all over the world. According to the report of the International Diabetes Institute and the World Health Organization that diabetes mellitus "appears to be epidemic in many regions of the world" and the figure will become double or even triple by the year 2010. The disease has been reported to affect almost all organs of the body causing nephropathy, retinopathy, neuropathy, microangiopathy and a number of other diseases. The disease has been extensively studied in respect of the clinical and chemotherapeutic aspect. However, sufficient information is not available regarding the histopathological/biochemical aspects of the disease. In view of the clinical significance of the disease, diabetes mellitus has been induced experimentally in rabbits by intraperitoneal injection of diabetogenic agent, alloxan, @ 80mg/kg.b.w. Establishment of the disease was confirmed by the appearance of hyperglycemia which was checked by periodic estimation of blood sugar level of alloxan-induced rabbits, along with certain KFT parameters. Pancreas was removed under aseptic conditions from alloxan-induced rabbits for histopathological study, which revealed vacuolation, degeneration and necrosis of pancreatic beta cells. Besides, deleterious effects of hyperglycemia in the form of polyuria, polydipsia, weight loss and sluggishness were noted in alloxan-induced rabbits.

Key words: Induced diabetes mellitus; Alloxan; Rabbits; Histopathology.
ABSTRACT

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and / or insulin action. The characteristic symptoms that have been recognized clinically include excessive thirst, polyuria, pruritus, otherwise unexplained weight loss, or one or more of the many complications associated with or attributable to the disease. The disease has been studied in respect of the chemotherapeutic aspects in experimental animals. However, there is scanty information available regarding the histopathological aspect of the disease in rabbits.

In view of greater clinical significance of this illness, diabetes mellitus has been induced experimentally in rabbits by intraperitoneal injections of alloxan @ 80 mg/kg b.w. at weekly intervals following 12 hours fasting. When the diabetes mellitus was well established which was confirmed periodically by elevated levels of blood sugar, blood urea and serum creatinine, histopathological analysis was done. The pancreatic sections of alloxan-induced diabetic rabbits showed pancreatic congestion and in the islets of Langerhan's degeneration, vacuolation and necrosis of β-cells were found. The kidney sections showed renal damage. Nephrosis and degeneration were grossly observed. The tubular epithelium showed hyperplasia and vacuolar degeneration. Polygonal cells were found to be proliferated which occluded the lumen of tubules. Some cells towards lumen were found to have lost their nuclei. In some glomeruli there was hypercellularity. Subcapsular regions showed degeneration. Lower nephron nephrosis was also observed. Moreover, behavioural changes were observed in alloxanized diabetic rabbits.

Key words: Pathology; Drug Induced Diabetes Mellitus; Rabbits; Alloxan
In present communication the detailed histochemical localization of cholinesterase at the tissue level of the amphistome *Cotylophoron cotylophorum* has been described. *Cotylophoron cotylophorum* worms were recovered from the rumen of cattle from the local abattoirs. Frozen sections were cut and histochemical localization of cholinesterase was done by using Myristoylcholine method (Pearse, 1972). Cholinesterase activity was demonstrated in various tissues like musculature of oral sucker, acetabulum, tegument, digestive system, reproductive system and parenchymal cells. The possible functional activity of this enzyme in different tissues has been discussed. Cholinesterase are closely associated with nervous system, but not exclusively so. Presence of cholinesterase in the musculature of suckers is suggestive of their nervous control for the attachment with the host tissue. Cholinesterase also acts as decelerator for peristaltic activity and membrane transport. Cholinesterase in reproductive system may help in discharge of sperms and eggs, and further, procure sperms in sperm ductules. Presence of cholinesterase in the integument suggests its role in absorption and transport of metabolites in the worm.

**Key words:** Histochemistry, cholinesterase, *Cotylophoron cotylophorum.*
Seasonal Incidence of *Paramphistomum cervi* in Sheep in Kashmir Valley

Tariq Ahmad, Abdul Baqui, Parvaiz Ahmad and Sajad Hussain Mir
P. G. Department of Zoology, University of Kashmir, Srinagar

A total of 512 sheep were examined during the period from January 2004 to December 2004 to establish seasonal trends of *P. cervi* infection in sheep and its relationship to the climatic factors. The sheep were chosen from different areas of valley, but the parasite *P. cervi* was found throughout the year with prevalence rate of 42%. Prevalence increased in the rainy and post-monsoon seasons and decreased slightly in the winter and summer. Adult parasites were found predominantly in the oesophageal end, reticular end and papillers of the rumen. Immature parasites were predominant in dorsal, ventral sacs of the rumen and duodenum.

**Key words:** paramphistomum cervi, prevalence, rumen, sheep.