$$T = \langle \chi_f(k_f, r_2) \varphi_f(r_1) \frac{1}{r_{12}} \psi^*(r_1, r_2) \rangle$$
Observations following administration of alloxan in the common carp Cyprinus carpio (L.) fed on yellow soybeans Glycine max

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On the administration of a total dose of 1g/kg of alloxan to Cyprinus carpio(L.) fish in five divided doses, the pancreatic islets showed no histologic alteration from those of the normal control animals. The anticipated beta cells destruction and consequent hyperglycemia did not ensue. However, in the individuals fed soybean alone there was a significant rise in the serum blood sugar with a concomitantly very significant fall in the total serum cholesterol. A toxic effect on the liver and kidney as evidenced by localized lesions in these organs was apparent. It is suggested that the degenerative changes noticed in the liver are caused by the direct action of alloxan on the organ. The treatment had significant decrease in the total blood cholesterol level in common carp fed on pellets and soybeans (50:50).

Key Words: soybean; alloxan; hyperglycemia; diabetes; cytotoxic; alpha & beta cells

INTRODUCTION

Administration of alloxan produced hyperglycemia in many species of snakes with slight lesions in the beta cells (Saviano & De Francisis, 1948; Saviano, 1955). On the other hand, alloxan administration failed to produce diabetes in the snake, Zenodon merremii (Houssay & Pnehos, 1960).

The discovery by Dunn et al. (1943), that alloxan produces selective necrosis of the beta cells in the pancreatic islets of rabbit led many workers to extend similar observations in other species (Baiky et al., 1944. In lizards, Veranus monitor (Rangnekar & Sabnis, 1976) and Uromastix hardwickii (Rangneker & Suryawanshi, 1969) alloxan resistance was noted. Turtles became diabetic after alloxan administration, typical lesions of beta cells were seen (Garcia Romos, 1944: Lopes, 1995; Cardeza, 1957).

Despite administration of total dose 2g/kg of alloxan, the pancreatic islets showed no histologic alteration from those of the normal control animals (Padgaonkar & Rangnekar, 1973), in an experiment with Nitrix piscator.

The purpose of studying the action of alloxan on the alpha & beta cells respectively, in this fish was to induce selective destruction of the cell elements and follow derangement, if any, in blood sugar homeostasis, consequent upon the loss of secretion of the respective hormone. In order to study the extra pancreatic effects, if any, of this cytotoxic agent, histological observations have also been made on the liver and kidney.

MATERIALS AND METHODS

Experimental fish were obtained from Sagana Fish Culture Farm of the Ministry of Tourism and Wildlife, Government of Kenya. Upon arrival in the laboratories, they were allowed to acclimatize in glass aquarium (74 x 95 x 45 cm) with aeration for two weeks at a temperature range of 18–24°C. Fifteen growing healthy fishes of either sex each weighing 6.5 ± 0.3g (SE) were used. During the course of acclimatization six fish died hence the experiment was done with the remaining 9 fishes. They were kept in separate tanks. After 24 hours of fasting, the fish were divided into 3 groups of 3 fish each and fed ad lib. Group I was a control where the fish were given a standard fish pellet diet (Unga Feeds) alone. Groups II and III were the experimental animals. In group II the fish were given food in a ratio 50:50, fish pellet and soybean coarse powder at 3% of body weight daily. The fish in group III were given coarse soybean powder
alone. The fish were weighed separately on an electric balance at weekly intervals.

Alloxan (200mg/kg) from British Drugs House (BDH), dissolved in water was given to the six experimental fishes every day for 5 days. Bleeding was done from zero hours to five weeks, at weekly intervals without any anaesthesia. Control group fish were maintained and injected with equivalent quantities of distilled water. All injections were given intramuscularly.

Blood glucose estimations were made using Nelson-Somogyi's method (Hawk et al., 1954). The cholesterol concentration in the blood plasma was estimated by the method Bloor, Peken and Ellen as modified by Tikekar (1984). For histological observation the pancreas and part of the liver from the control and experimental fish were fixed in Bouin's fluid and 10% Formal Saline. The paraffin section of the different tissue cut at 5μm were processed by the routine way. The sections of the pancreas were stained by chromalum Haemotoxylin-floxin method (Gomori, 1941). The sections of the liver were stained with Haemotoxylin/Eosin.

RESULTS

The amounts recorded for total serum cholesterol and serum blood sugar levels in common carps over a study period of five weeks were as indicated in Table 1.

Table 1. Mean total serum cholesterol and serum blood sugar (mg/dl) (X±SE)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>WEEK</th>
<th>CHOLESTEROL</th>
<th>SUGAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PELLETS ALONE</td>
<td>0</td>
<td>5.73±0.153a</td>
<td>35.84±0.058a</td>
</tr>
<tr>
<td>(Control)</td>
<td>1</td>
<td>6.03±0.006ab</td>
<td>35.34±0.171be</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.87±0.014acg</td>
<td>35.789±0.143aef</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.50±0.164adgh</td>
<td>35.26±0.214cefgh</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.77±0.022aeh</td>
<td>35.77±0.220aefgh</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.36±0.142afh</td>
<td>35.43±0.050degh</td>
</tr>
<tr>
<td>PELLETS AND</td>
<td>0</td>
<td>6.05±0.002a</td>
<td>41.96±0.179a</td>
</tr>
<tr>
<td>SOYBEAN IN 50:50</td>
<td>1</td>
<td>5.36±0.0018b</td>
<td>42.63±0.006bd</td>
</tr>
<tr>
<td>GROUP II (Experimental)</td>
<td>2</td>
<td>4.97±0.132cg</td>
<td>42.72±0.147cdg</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.20±0.045dgh</td>
<td>42.14±0.086ae hi</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.87±0.131egi</td>
<td>41.78±0.249a f gh</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.51±0.153f</td>
<td>42.21±0.335ad gh i</td>
</tr>
<tr>
<td>SOYBEAN ALONE</td>
<td>0</td>
<td>6.05±0.004a</td>
<td>42.42±0.016a</td>
</tr>
<tr>
<td>GROUP III (Experimental)</td>
<td>1</td>
<td>5.33±0.119b</td>
<td>43.54±0.089b</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.36±0.082c</td>
<td>44.15±0.053c</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.99±0.059d</td>
<td>45.36±0.020d</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.47±0.022e</td>
<td>43.78±0.433b ce</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.97±0.048f</td>
<td>46.11±0.25f</td>
</tr>
</tbody>
</table>

Means followed by different letters within each of the six cases above for cholesterol and sugar are significantly different at P<0.05 using Tukey test (Zar, 1996).
In the common carps fed on pellets and soybeans the total serum cholesterol level decreased significantly by the fifth week (b=0.265; t₄= 4.96; p<0.01) with the serum blood sugar level showing a non significant rise (b=0.55; t₄= 0.0580; p>0.05).

However, in those individuals fed on soybean alone there was a significant rise in the serum blood sugar level (b=0.528; t₄= 2.89; p<0.05) with a concomitant very significant fall in the total serum cholesterol level (b=0.613; t₄= 12.5; p<0.001). There was a non-significant fall in both the total serum cholesterol level (b=0.0365; t₄= 0.55; p>0.05) in the common carps fed on pellets only.

**Histological Observations**

(a) **Pancreas**
Treatment of Alloxan administration did not cause any damage to the beta cells of the pancreatic islets (Fig. 1).

(b) **Liver**
Significant depletion of glycogen granules was noticed in the cytoplasm of the liver cells of the treated animals. The remaining granules were extremely coarse in nature. Frequently, remains of pycnotic nuclei separated from cells were noticed. The liver cells at places showed large cytoplasmic vacuoles. On the whole sinusoids were collapsed or distorted (Fig. 2–3).

(c) **Kidney**
The renal tubules showed variations in the degrees of injury in the treated animals. In some of them the epithelial cells showed atrophic degeneration while in others distinct signs of necrosis were noticed. In the latter case, the cells became swollen, gradually lost their protoplasmic contents and thus became honey-combed. However, they retained their outlines and nuclei but the tubular pattern of the nephron was lost. Sometimes cellular debris was noticed in the lumen of the tubule. The nuclei showed signs of pycnosis (Fig. 4–5).

**DISCUSSION**

In the fish *Cyprinus carpio* (L.) a total dose as high as 200mg/kg/day for 5 days and sacrifice at the end of the fifth week did not cause any damage to the beta cells of the pancreatic islet tissue. The average values of blood sugar and plasma cholesterol remained within their respective normal ranges and the anticipated hyperglycemia did not ensue: except that there was a significant rise in serum blood sugar level in those individuals fed soybean alone (Experimental, group II). This observation made on the beta cells suggests that the fish under study is resistant to the action of alloxan. Similar observation was made by Padgaonkar and Rangnekar (1973) on (*Natrix piscator*). In the snake *Xenodon merremii*, Houssay and Penhos (1960) observed that alloxanisation produced a transitory increase of glycemia followed by a marked and lasting hypoglycemia, but there was no diabetes. Resistance to alloxan treatment as seen in *Cyprinus carpio* was also recorded in snake (*Natrix piscator*) (Padgaonkar and Rangnekar, 1973), in the lizards *V. monitor* (Rangnekar and Subnis, 1967) and *U. hardwickii* (Rangnekar and Suryawanshi, 1969) after administration of 1.8g/kg of alloxan given in six divided doses. Hyperglycemia and hypercholesterolemia in fish fed on soybeans alone can be due to soybean diet (Bagalkote et al., 1994,1995).

In contrast to the alloxan resistance noted in the two species of lizards mentioned earlier, it was observed that in the lizard of the genus *Eumeces* (Miller and Wuster 1956, 1958) a total amount of 1.5gms/kg of alloxan given in five divided doses (and sacrifice on the fourteenth day) made the animals hyperglycemia and produced beta cell destruction in the islet tissue. Likewise, in turtles, alloxanisation induced hyperglycemia, glycogenic infiltration and necrosis of some cells (Garcia Ramos, 1944; Lopes, 1955; Cardeza 1959). It is evident that the effects of alloxanisation are variable not only in the representatives of different classes of animals but also in the members of the same class.

Alloxan administration also produced pathological changes in the kidney. Dunn Sheehan and Moletchi (1943) also noticed that alloxan when injected in rabbits intravenously or intraperitoneally in doses of 100mg/kg had a specific necrosing action on both pancreatic islet cells and epithelium of the renal
convoluted tubule. In rat (Lazarow et al., 1946) the severity of injury varied from atrophic degeneration to necrosis of the tubules. Jarrett (1946) observed gross kidney damage in sheep. Similarly, De Pietro and Cardeza (1947) also observed degenerative and necrotic changes without apparent lesion of the glomeruli after alloxan administration in dog.

Histological examination of the liver of the alloxanised animals revealed parenchymatous damage to some extent. A significant loss of cytoplasmic granularity was noticed within the cells. The remaining granules were extremely coarse in nature. On the whole sinusoides were collapsed or distorted. Rarely, remains of pyknotic nuclei separated from cells were noticed. The liver cells at places showed large cytoplasmic vacuoles. In agreement with the observations made by Padgaonkar and Rangnekar (1973) in snake and Lazorrow and Berman (1947) in toad fish, it may be said that the toxic effect of alloxan on the liver following intraperitoneal injections may be related to the hepatic portal circulation in as much as large part of the injected alloxan goes in the liver. Injury to the liver following alloxinisations was also observed in rats (Lazarow et al., 1946) in sheep, (Jerrett, 1946) in rabbit (Herbut, Watson and Perkins, 1946) and in dog (De Piero and Cardeza, 1947).

CONCLUSIONS

Following administration of a total dose of 1gm/kg of alloxan in five divided doses to fish the pancreatic islets showed no histologic alteration from those of the normal control animals. The anticipated beta cells destruction and consequent hyperglycemia did not ensue. However, in the individuals fed soybean alone there was a significant rise in the serum blood sugar with a concomitantly very significant fall in the total serum cholesterol. A toxic effect on the liver and kidney as evidenced by changes noticed in the liver are caused by the direct action of alloxan on the organ. The treatment significantly reduced the total blood cholesterol level in the common carps on pellets and soybeans (50:50).

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REFERENCES


PLATE 1

Fig. 1. Photomicrograph of pancreatic islet of an alloxan treated animal.
A — alpha cell
B — beta cell
(Bouin – fixed and chrom – alum – haemotoxylin – phloxin stained sectioned at 5µ)

Fig. 2. Photomicrograph of T.S. of liver of a normal fish.

Fig. 3. Photomicrograph of T.S. of liver of an alloxan treated fish showing large cytoplasmic vacuoles and collapsed and distorted sinusoids.

Fig. 4. Photomicrograph of T.S. of Kidney of a normal fish.

Fig. 5. Photomicrograph of T.S. of the kidney of an alloxan treated fish showing necrosis of the kidney tubules (at 5µ).


