EFFECT OF NEEM (AZADIRACHTA-INDICA) EXTRACT ON DIABETIC ALBINO RATS- RATTUS NORVEGICUS

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ABSTRACT

The aim of this investigation is to find out possible anti-diabetic mechanism of neem. The experimental model was alloxan treated albino rats and they were treated with aqueous extract of Azadirachta -indica (neem) leaves for 14 days. The effects of this extract on blood glucose concentration, serum α- amylase activity, body weight and pancreatic integrity of normal and diabetic albino rats were studied. The results indicate that treatment with extract caused a significant (p<0.05) reduction in fasting blood glucose, serum α- amylase level in diabetic treated rats as compared to diabetic control rats. Histological examination of pancreas of diabetic control rats showed cellular degeneration which appeared to be reversed in the animals following extract treatment. However there was no any significant difference in the serum α- amylase activity of normal treated rats when compared to the normal control.

Conclusion: The neem extract might have anti diabetic properties which may be associated with enhanced islets cells regeneration.

Key words: Albino rats, diabetes, Neem leaves.

INTRODUCTION

Diabetes, caused by inherited and/or acquired deficiency in production of insulin by the pancreas (type 1) or by ineffectiveness of insulin (type 2), is one of the most burdensome and costly chronic disease in the world [2,11,16]. As a strong oxidant, alloxan is widely used in experimental animals to induce insulin-dependent diabetes (type 1). It works by increasing generation of reactive oxygen species from metabolic reactions in the body, together with massive increase of cytosolic calcium concentration, and it can rapidly cause destruction of pancreatic β-cells [4,5,14]. Neem is a well known herbal anti-diabetic agent [1]. Diabetic patients of rural areas usually do not register themselves in diabetic clinics or hospitals because the medical facilities are mainly concentrated in the urban areas. So, with the ever-increasing incidence of diabetes, it’s management is becoming costly not only for the individual and his/her family but also for the national health care sector. Now-a-days various medicinal plants are becoming very popular for the treatment of different diseases in our country as well as all over the world. For considering all these constraints, in this experiment we wanted to establish indigenous system of medicine (herbal therapy) as anti-diabetic drugs instead of chemical drugs and rat was used as model of animal.

MATERIALS AND METHODS

The experiment was performed in the Department of Animal Science, M.J.P. Rohilkhand University Bareilly
U.P to evaluate the comparative efficacies of Neem on alloxan induced diabetic rats. Eighteen mixed albino rats (Ratus norvegicus) both male and female of body weight between 150 to 200gm were collected from Indian Veterinary Research Institute, Bareilly. All the rats were grouped into three each containing 6. Each group of rats was housed at serene bottomed wire cages arranged in rows and kept in the animal house of this University. Food was given periodically regularl, 100 g/kg. Drinking water was supplied ad libitum. The rats were maintained in this condition for a period of two weeks to acclimatize them prior to experimental uses.

Induction of diabetes
Alloxan induced hyperglycemia has been described as a useful experimental model to study the diabetics. After overnight fasting (deprived of food for 16 hours had been allowed free access to water) diabetes was induced in rats by intraperitoneal injection of Alloxan monohydrate (Sigma, St. Louis, Mo USA) dissolved in normal saline at room temperature and a dose of 150mg/kg body weight was injected via intraperitoneal routes for 14 days. The control rats (Group I) received the same amount of normal saline. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 14 days time for the development of diabetes, the rats with diabetes having hyperglycemia (blood glucose range of above 250-300 mg/dl) were considered as diabetic rats (Group II).

Preparation of Neem leaves extract
Fresh neem leaves were collected from locality. Then cleaned and washed with plain tap water and kept in shade in normal room temperature dried in air by spreading them on large trays for 72 hours. Then neem leaves were grinded in to powder form by grinder. Then 250 mg/kg body weight, of neem leave powder for each rat was dissolved in distilled water and administered orally by nasogastric tube for 10 days (Group III) Blood glucose level was estimated via glucometer and alpha amylase was estimated via methods of Hansawasdi C et al & Dineshkumar B et al [6,7]. Results were expressed as mean values ± standard error of mean (Mean ± SEM) based on five experiments. Significant differences between control and experimental groups were assessed using student’s t-test and the results were considered significant at P-values of less than 0.05 (P<0.05).

Histology: The pancreas was immediately rinsed and fixed in Bouin’s fluid. Pancreatic scrapes were embedded in paraffin, cut and stained with hematoxylin and eosin (H&E), and graded in a blinded manner. Section cutting was via microtome at 7µ. Slides were observed under light microscope at 10x and 40x.

RESULTS AND DISCUSSION
Effect on body weight in control and experimental rats
The effects of aqueous extracts of neem (Azadirachta indica) leaves, the body weight in normal control, diabetic control and diabetic neem treated rats were shown in Table 1. After 14 days of treatment with Neem leaves, body weights were increased significantly (p < 0.01) in comparison with control. In diabetic control group body weight decreased (4.10%). Results of the present study supports partially the earlier findings of Devi et al. [5], Pari and Saravanan [11].

Effects on blood glucose level in normal and experimental rats
The effect of alloxan by damaging the beta cells of pancreas interfered glucose metabolism resulting increasing blood glucose level. The effect of aqueous extracts of neem leaves on blood glucose in normal control, diabetic and neem treated rats is shown in Table 2. After 14 days of treatment with Alloxan the blood glucose level increase significantly (p < 0.01) which agreed with that of Zhang [19]. After 14 days of treatment with neem, blood glucose were decreased significantly (p < 0.01). The exact mechanism in reducing blood glucose level is not well understood. But the probable cause of reduction of blood glucose might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor in case of neem leaves. In accordance to the present finding some author also reported reduction of blood glucose following administration of insulin and neem such as Pari et al. [12] Khosla et al. [9].

Effects on plasma alpha amylase level in normal and experimental rats
Plasma alpha amylase levels were estimated at the end of treatment period. The results showed that plasma amylase level was significantly (p<0.01) decreased in diabetic rats compared with control. Treatment with neem extract resulted in significant (P <0.01) increase in plasma alpha amylase level when compared with diabetic rats, Ledox et al [10], Vats et al [17].
Effects on Histology of Pancreas in normal and experimental rats

Figure A, Showing pancreatic section in untreated control Group I, normal pancreatic b-cell islets with normal cellularity and normal histology of pancreas. Whereas Alloxan challenge (Group II) caused destruction of the pancreatic tissue structure, characterized by vacuolization of acinar cells and necrosis, as shown in Figure B. Treatment with neem (Group III) significantly reduced the severity of the histological signs of pancreas injury, as shown in Figure C. Table1. Showing Body weight (gm) changes in Albino rats during the experiment.

<table>
<thead>
<tr>
<th>S No.</th>
<th>GROUPS</th>
<th>TREATMENT (14 days)</th>
<th>BODY WEIGHT (Mean ± S D) n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Control</td>
<td>170.80±8.74</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>Alloxan</td>
<td>149.68±9.21</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>Neem leaves extract</td>
<td>158.86± 5.17</td>
</tr>
</tbody>
</table>

Table 2. Showing blood glucose level (m mol/L) in different groups in albino rats.

<table>
<thead>
<tr>
<th>S No.</th>
<th>GROUPS</th>
<th>TREATMENT (14 days)</th>
<th>BLOOD GLUCOSE (n=6) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Control</td>
<td>5.65±0.16</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>Alloxan</td>
<td>33.64±0.91</td>
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<td>3</td>
<td>Group III</td>
<td>Neem leaves extract</td>
<td>14.26±0.21</td>
</tr>
</tbody>
</table>

Table 3. Showing blood α- amylase (1U/L) in different groups in albino rats.

<table>
<thead>
<tr>
<th>S No.</th>
<th>GROUPS</th>
<th>TREATMENT (14 days)</th>
<th>Alpha amylase (Mean±SE) n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Control</td>
<td>1240 ± 20.45</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>Alloxan</td>
<td>1764 ± 18.11</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>Neem leaves extract</td>
<td>1300 ± 30.83</td>
</tr>
</tbody>
</table>
REFERENCES


Figure B: Histological micropicture of pancreas of albino rats in Group II

Figure C: Histological micropicture of pancreas of albino rats in Group III


