1. INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious
Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Prevention and treatment involve a healthy diet, physical exercise, not using tobacco, and being a normal body weight. Blood pressure control and proper foot care are also important for people with the disease. Type 1 diabetes must be managed with insulin injections. Type 2 diabetes may be treated with medications with or without insulin. Insulin and some oral medications can cause low blood sugar [2, 3]. Weight loss surgery in those with obesity is an effective measure in those with type 2 DM. Gestational diabetes usually resolves after the birth of the baby.

- Diabetes is a long-term condition that causes high blood sugar levels.
- In 2013 it was estimated that over 382 million people throughout the world had diabetes (Williams textbook of endocrinology).
- Type 1 Diabetes - the body does not produce insulin. Approximately 10% of all diabetes cases are type 1.
- Type 2 Diabetes - the body does not produce enough insulin for proper function. Approximately 90% of all cases of diabetes worldwide are of this type.
- Gestational Diabetes - This type affects females during pregnancy.
- The most common diabetes symptoms include frequent urination, intense thirst and hunger, weight gain, unusual weight loss, fatigue, cuts and bruises that do not heal, male sexual dysfunction, numbness and tingling in hands and feet [4].
- If you have Type 1 and follow a healthy eating plan, do adequate exercise, and take insulin, you can lead a normal life.
- Type 2 patients need to eat healthily, be physically active and test their blood glucose. They may also need to take oral medication, and/or insulin to control blood glucose levels [5].
- As the risk of cardiovascular disease is much higher for a diabetic, it is crucial that blood pressure and cholesterol levels are monitored regularly.
- As smoking might have a serious effect on cardiovascular health, diabetics should stop smoking.
- Hypoglycemia - Low blood glucose - can have a bad effect on the patient. Hyperglycemia - when blood glucose is too high - it can also have a bad effect on the patient [6, 7].

Alloxan

One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan. It is a well-known diabetogenic agent that is used to induce Type I diabetes in experimental animals. Alloxan is a urea derivative that causes selective necrosis of the β-cells of pancreatic islets. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice, and dogs with different grades of disease severity by varying the dose of alloxan used [8, 9]. As it has been widely accepted that alloxan selectively destroys the insulin-producing beta cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. The toxic action of alloxan on pancreatic beta cells involves oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals, and disturbances in intracellular calcium homeostasis. The underlying mechanism involves the selective uptake of the compound due to its structural similarity to glucose as well as the highly efficient uptake mechanism of the pancreatic beta-cells. The aim of the present review is to explicate the mechanisms involved in alloxan-induced induction of experimental diabetes mellitus. Moreover, the biological effects produced by alloxan have also been discussed in various review articles [10-12].

Mode of action

Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta-cell [13, 14], and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta-cell [15, 16].

Beta-cell selectivity of alloxan

Alloxan is a very unstable chemical compound with a molecular shape resembling glucose. Both alloxan and glucose are hydrophilic and do not penetrate the lipid bilayer of the plasma membrane [17]. The alloxan molecule is structurally so similar to glucose that the GLUT2 glucose transporter in the beta-cell plasma membrane accepts this glucomimetic and transports it into the cytosol. Alloxan does not inhibit the function of the transporter, and can therefore selectively enter beta cells in an unrestricted manner. It is therefore not toxic to insulin-producing cells that do not express this transporter. The half-life of alloxan is short; in aqueous solution, it spontaneously decomposes into non-diabetogenic alloxamic acid within minutes. Because of this, it must be taken up and accumulated quickly in the beta cell and is therefore ineffective when blood flow to the pancreas is interrupted for the first few minutes after alloxan injection. N-substituted alloxan derivatives with a long carbon side chain, such as butylalloxan, differ chemically from alloxan in that they are lipophilic. Butylalloxan acts in a similar manner to alloxan and preferentially damages beta cells. But since derivatives such as butylalloxan are lipophilic they can also penetrate plasma membranes that do not express the GLUT2 transporter. Nephrotoxicity is a dominating feature of the toxicity of lipophilic derivatives after systemic
administration. This nephrotoxicity is so severe that it causes fatal renal failure in the animals before diabetes can develop. The susceptibility of the kidney to the toxic action of these lipophilic alloxan derivatives is the result of their preferential accumulation in the tubular cells of the kidney, which, like the beta cells, express the GLUT2 glucose transporter [18].

2. MATERIAL AND METHODS

Alloxan (Purchased from NP chemicals Pvt.Ltd BOMBAY). The Pyrimidine derivatives of Thiazolidinedion synthesized compounds TZN4(a), TZN4(b), TZN4(c), TZN4(d), TZN4(e), TZN4(f), TZN4(g), TZN4(h), TZN4(i), TZN4(j), TZN4(k) obtain from Comprime Lab (Hyderabad). Mice weighing 25g-30g were used and were procured from animal house facility which was inbred earlier. They were housed in a group of seven-under environmentally controlled room with 23-25 °c, 35 to 60% humidity, 12-h light/dark cycle and given standard pellet diet, Provimi animal nutrition Limited (India), provided ad libitum. After seven days of acclimatization period, they were randomly selected for different experimental groups. All the experimental procedures were carried out in accordance with the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee (I/IAEC/LCP041/2014/SM-40♂).

Table 1: Pyrimidine derivatives of Thiazolidinedion

<table>
<thead>
<tr>
<th>Sl.no.</th>
<th>Synthetic Derivatives</th>
<th>R</th>
<th>Molecular Weight</th>
<th>R&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TZN4(01456)</td>
<td>C6H5</td>
<td>480.56</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>TZN4(07165)</td>
<td>C6H5OH</td>
<td>496.34</td>
<td>H</td>
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<tr>
<td>3</td>
<td>TZN4(1321)</td>
<td>CH=CHC6H5</td>
<td>506.5</td>
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<tr>
<td>4</td>
<td>TZN4(05152)</td>
<td>C6H50CH3</td>
<td>510.59</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>TZN4(0717)</td>
<td>C6H5NC2H5</td>
<td>523.21</td>
<td>H</td>
</tr>
<tr>
<td>6</td>
<td>TZN4(0514)</td>
<td>NO2</td>
<td>525.36</td>
<td>H</td>
</tr>
<tr>
<td>7</td>
<td>TZN4(07171)</td>
<td>C6H5</td>
<td>515</td>
<td>CL</td>
</tr>
</tbody>
</table>

Experimental study design:

Induction of diabetes: Overnight-fasted swiss albino mice are injected with alloxan (75mg/kg) in saline buffer intraperitoneally. After 72 hours blood glucose levels were determined and mice having blood glucose levels <145mg/dl were excluded from experiment and rest were divided into ten groups [19].

Group –I(control): Normal mice injected with saline.

Group-II (diabetic control): animals were given alloxan(75mg/kg) in saline.

Group-III (01456): Diabetic animals treated with chemically synthetic compound (01456)(50mg/kg) (P.O) for 7 days

Group-IV (07165): Diabetic animals treated with chemically synthetic compound (07165)(50mg/kg) (P.O) for 7 days

Group-V (1321): Diabetic animals treated with chemically synthetic compound (1321) (50mg/kg) (P.O) for 7 days.

Group–VI (05152): Diabetic animals treated with chemically synthetic compound (05152)(50mg/kg) (P.O) for 7 days.

Group-VII (0717): Diabetic animals treated with chemically synthetic compound (0717)(50mg/kg) (P.O) for 7 days.

Group-VIII (05104): Diabetic animals treated with chemically synthetic compound (05104) (50mg/kg) (P, O) for 7 days.

Group- XI (07171): Diabetic animals treated with chemically synthetic compound (07171) (50mg/kg) (P.O) for 7 days.

Standard group (Insulin): Animals were given insulin as a standard

During the experimental period, body weight and food intake were measured daily. Glucose levels were measured at the end of the study period.

Estimation of fasting blood glucose

Glucose estimation was done by using a standard kit obtained from ERBA Diagnostics, USA.

3. RESULTS AND DISCUSSION

Principle:

Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide (H2O2) in the presence of glucose oxidase. The enzyme peroxidase catalyzes the oxidative coupling of 4 amino antipyrine with phenol to yield a colored quinoneimine complex, with absorbance proportional to the concentration of glucose in the sample.

Glucose oxidase

\[
\text{Glucose + O}_2 + \text{H}_2\text{O} \rightarrow \text{Gluconic acid} + \text{H}_2\text{O}_2
\]

Peroxidase

\[
\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{AAP} \rightarrow \text{Quinoneimine Dye} + 2\text{H}_2\text{O}
\]

Table 2: Procedure for preparing the solutions.

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Test (T)</th>
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<tbody>
<tr>
<td>Enzyme Reagent</td>
<td>1000μl</td>
<td>1000μl</td>
<td>1000μl</td>
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<tr>
<td>Standard</td>
<td></td>
<td>10μl</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td>10μl</td>
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</table>

Mix well after each addition and incubate at 37 °C for 5 minutes. Read absorbance of standard and test against reagent blank at 505/670 nm.

Calculation

Glucose = (Absorbance of test/Absorbance of Std.) X Conc of Std. (100mg/d)

Effect of chemically synthetic compounds on serum glucose levels:

Alloxan administration leads to a significant increase in glucose levels when compared to normal control mice. Treatment with chemically synthetic compounds for seven
days significantly reduced the glucose level when compared to diabetic mice. Out of seven synthetic compounds, compound number 1321,05152, and 0712 showed significantly decreased in glucose level as compared to standard treatment with insulin [20, 13].

<table>
<thead>
<tr>
<th>Groups/days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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5. REFERENCES


Conflicts of Interest: None

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