Int J Pharma Res Health Sci. 2020; 8 (2): 3151-54
DOI:10.21276/ijprhs.2020.02.04
Akhil M *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Antidiabetic Activity of Chemically Synthetic Compound on Alloxan Induced Diabetes in Mice

Akhil Moudgil *, Sanjay Kumar, Madhu Bala

Gautam College of Pharmacy Hamirpur, Himachal Pradesh-177001, India.

ARTICLE INFO

Received: 04 Mar 2020
Accepted: 20 Apr 2020

ABSTRACT

Alloxan a nitrosourea derivative is one of the most universally accepted diabetogenic agents. The selective β-cell toxicity of alloxan depends on the degree of DNA alkylation and subsequent activation of poly ADP ribose synthetase in the base excision repair pathway, this stimulated activation of poly ADP ribose synthetase triggers exhaustion of NAD+ in the pancreatic islets that will lead to β-cell death through necrosis. In the present study, the objective was to study the evaluation of the antidiabetic activity of the chemically synthetic compound on alloxan-induced diabetes in mice. Chemically synthetic compounds were given to the mice after the administration of alloxan and glucose levels were estimated using a semi-auto analyzer at a range of 505/670nm. The hyperglycaemic levels due to alloxan administration lead to the development of diabetes. Treatment with chemically synthetic compounds significantly lowers the elevated glucose levels in alloxan-induced diabetic mice. Hence compound number 1321,05152,0717 has potential antidiabetic activity. To explore further exhaustive study is required for the mechanism behind the anti-diabetic activity of their chemical compounds.

Keywords: Alloxan, diabetogenic agent, antidiabetic activity, hyperglycaemic levels.

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

Corresponding author *
Akhil Moudgil
Gautam College of Pharmacy Hamirpur, Himachal Pradesh-177001, India.
E-mail: diwansanjay1981@gmail.com

long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers, and damage to the eyes [1].

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 sulphydryl (-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 alloxanic 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 ^{0}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

Sl.no.	Synthetic Derivatives	R	Molecular Weight	R"
1	TZN4(01456)	C_6H_5	480.56	H
2	TZN4(07165)	C_6H_5OH	496.54	H
3	TZN4(1321)	$CH=CHC_6H_5$	506.5	H
4	TZN4(05152)	$C_6H_50CH_3$	510.59	H
5	TZN4(0717)	$C_6H_4NC_2H_6$	523.21	H
6	TZN4(0514)	NO_2	525.56	H
7	TZN4(07171)	C_6H_5	515	CL

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)** in water

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 (H_2O_2) 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.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4\text{AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 2\,H_2O$$

Table 2: Procedure for preparing the solutions.

Pipette into tubes marked	Blank (B)	Standard (S)	Test (T)
Enzyme Reagent	1000µl	1000µl	1000µl
Standard	--	10µl	--
Test	--	--	10µl

Mix well after each addition and incubate at 37 ^{0}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 3: Effect of the chemically synthetic compound on serum glucose levels in alloxan-induced diabetes in mice

Groups/days	1	2	3	4	5	6	mean
Control	109	100	107	106	100	105	104
Diabetic control	345	336	342	336	343	341	340
1456	316	317	322	323	323	325	320
7165	245	236	243	242	235	235	240
1321	190	195	186	185	192	191	190
5152	125	118	115	123	120	119	120
717	115	110	117	114	116	115	114
514	295	286	285	292	290	291	290
7171	310	300	306	304	302	305	305
Insulin	110	102	101	107	105	106	105

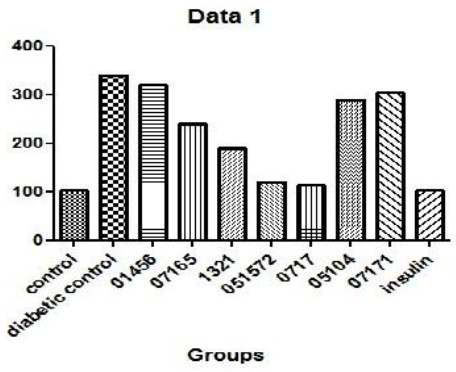

Fig 1: Statistical analyzed in Graph Pad Prism

4. CONCLUSION

The present study demonstrated that ALLOXAN administration resulted in a significant increase in blood glucose levels, decrease in body weight. Chemically synthetic compounds administration for a period of 7 days was found to be effective in maintaining glucose levels.

In conclusion, the results of the present study indicated that chemically synthetic compounds 1321,05152,0717 have a good therapeutic effect on maintaining the glucose levels in the diabetic condition.

5. REFERENCES

1. Adler A I, Boyko E J, Ahroni J H, Stensel V, Forsberg R C, Smith D G. Risk factors for diabetic peripheral sensory neuropathy. Results of the Seattle prospective diabetic foot study. Diabetes Care 1997; 20, 1162−1167.
2. Ahmed N. Advanced glycation endproducts-role in pathology of diabetic complications. Diabetes Res. Clin. Pract 2005; 67: 3−21
3. Allian C C, Poon L S, Chan C S G, Richmand W, Fu P. Determination of total cholesterol by enzymatic method. Clin Chem 1974; 20: 470–475.
4. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414: 813−820.
5. Burnstock G, Mirsky R, Belai A. Reversal of nerve damage in streptozotoc in diabetic rats by acute application of insulin in vitro. Clin Sci 1988; 75: 629–635.
6. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzyme. Clin Chem 1973; 19: 476-482.
7. Cameron N, Eaton S E, Cotter M A, Tesfaye S. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. Diabetologia 2001; 44: 1973–1988.
8. Bousta D, Boukhira S, Aafi A, Ghanmi M, El Mansouri L. Ethnopharmacological Study of anti-diabetic medicinal plants used in the Middle-Atlas region of Morocco (Sefrou region). Int J Pharma Res Health Sci 2014;2(1):75-9
9. Campana W M, Myers R R. FASEB J 2001; 15: 1804–1806.
10. Chong Z Z, Kang J Q, Maiese K. Angiogenesis and plasticity: role of erythropoietin in vascular systems. J. Hematother. Stem Cell Res 2002; 11,863–71.
11. Cohen A M. Erythropoietin and G-CSF, in A.H.C.Kung, R.A. Baughman and J.W. Larrick (Eds.) Therapeutic Proteins: Pharmacokinetics and Pharmacodynamics, W.H. Freeman and Company, New York1992; 165-186.
12. Cohen K L, Harris S. Efficacy and safety of nonsteroidal anti-inflammatory drugs in the therapy of diabetic neuropathy. Arch Intern Med 1987; 147: 1442−1444.
13. Diabetes Control and Complication Trial Research Group. The effect of intensive diabetes therapy on the development and progression of neuropathy. Ann Intern Med 1995;122:561-8.
14. Delaney C A, Dunger A, Matteo M, Cunningham J M, Green M H, Green I C. Comparison of inhibition of glucose-stimulated insulin secretion in rat islets of Langerhans by streptozotocin and methyl and ethyl nitrosoureas and methanesulphonates. Lack of correlation with nitric oxide-releasing or O6-alkylating ability. Biochem Pharmacol 1995; 50: 2015-2020.
15. Roy H. Formulation of sustained release matrix tablets of metformin hydrochloride by polyacrylate polymer. Int J Pharma Res Health Sci 2015; 3(6):900-6.
16. Du X L, Edelstein D, Rossetti L, Fantus I G, Goldberg H, Ziyadeh F. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1

expression by increasing Sp1 glycosylation. Proc Natl Acad Sci 2000; 97: 12222−6.
17. Digicaylioglu M, Garden G, Timberlake S, Fletcher L, Lipton S A. Acute neuroprotective synergy of erythropoietin and insulin-like growth factor I. Proc Natl Acad Sci 2004; 101, 9855–9860.
18. Dyck P J, Kratz K M, Karnes J L, Litchy W J, Klein R, Pach J M. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology 1993; 4: 817−824.
19. Kannan M, Kumar TS, Rao MV. Antidiabetic and antioxidant properties of Waltheria indica L., an ethnomedicinal plant. Int J Pharma Res Health Sci 2016;4(5):1376-84.
20. Feldman E L, Stevens M J, Greene D A. Pathogenesis of diabetic neuropathy. Clin Neurosci 1997; 4: 365−370.

Conflict of Interest: None
Source of Funding: Nil