International Journal of Medicine and Pharmaceutical Research

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Research Article **Open Access**

Pharmacodynamic Effect of Combination 5-Chlro-Isatin and Gliclazide in STZ Induced Diabetic Rats

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A B S T R A C T

The antidiabetic effect of 5-Choloroisartin in experimental animals examined in this study. This synthetic drug is assumed to have anti-diabetic. The drug (orally) was administered in rat at the doses of 100 mg/kg the drug suppressed increased glucose levels induced by STZ and also exhibited protector effect in STZ-induced diabetes, at 100 mg/kg dose. Since the drug decreases the icreased blood glucose level, it is concluded that it interfere with gabaergic mechanism(s) to exert their antidiabetic effect in addition it reveals the presence of flavonoid and vitamins, phenolic compounds attributed to their anti-diabetic action.

Keywords: Diabetes, STZ, 5-Choloroisartin.

A R T I C L E I N F O

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Article History: Received 28 January 2016, **Accepted** 11 March 2016, **Available Online** 10 April 2016

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Manuscript ID: IJMPR2998

PAPER-QR CODE

Citation: Gampa Vijaya Kumar. Pharmacodynamic Effect of Combination 5-Chlro-Isatin and Gliclazide in STZ Induced Diabetic Rats. *Int. J. Med. Pharm. Res*., 2016, 4(2): 109-113.

1. Introduction

Diabetes mellitus is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago. In 1936, the distinction between type 1 and type 2 DM was clearly made. Type 2 DM was first

described as a component of metabolic syndrome in 1988. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors. People living with type 2 DM are more vulnerable to various forms of both short- and long-term complications, which often lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type 2 DM because of the commonness of this type of DM, its insidious onset and late recognition, especially in resource-poor developing countries. Type 2 DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure. This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells. There is an increase in the breakdown of fat with hyperglycemia. The involvement of impaired alpha-cell function has recently been recognized in the pathophysiology of type 2 DM.

Thiazolidinediones:

Thiazolidinedione is an insulin sensitizer, selective ligands transcription factor peroxisomes proliferator-activated gamma. They are the first drugs to address the basic problem of insulin resistance in type 2 DM patients, whose class now includes mainly pioglitazone after the restricted use of rosiglitazone recommended by Food and Drug Administration (FDA) recently due to increased cardiovascular events reported with rosiglitazone. Pioglitazone use is not associated with hypoglycemia and can be used in cases of renal impairment and thus well tolerated in older adults. On the other hand, due to concerns regarding peripheral edema, fluid retention and fracture risk in women, its use can be limited in older adults with DM. Pioglitazone should be avoided in elderly patients with congestive heart failure and is contraindicated in patients with class III-IV heart failure.

5-chloro-Isatin:

Isatin (indoline-2,3-dione), possessing an indole nucleus with two chemically distinct cyclic carbonyl groups, keto and lactam, has provoked tremendous interest due to its numerous biological and pharmacological activities. The compound exhibiting a wide range of effects including antimicrobial, anti-tubercular, antiviral, anti-inflammatory, anticonvulsant, antihypertensive, anti-viral, anti-fungal, hypoglycemic, cytotoxic, anticancer activity and enzymatic inhibition. In 5-chloro-Isatin, hydrazine moiety is responsible for the hypoglycemic activity.

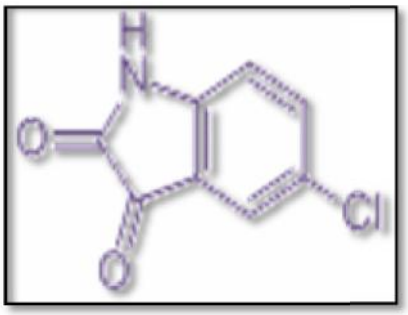

Figure 1: Chemical structure of 5-chloro-Isatin

Molecular formula: $C_4H_8CLNO_2$.

Molecular weight: 181.58.

Solubility: Insoluble in water.

Physical appearance: It is a yellow to red niddle crystalline solid.

2. Materials and Methods

Materials:

Cholesterol Estimation kit, Glucose estimation kit, standard pellet diet, 5-Chloro-Isatin, standard Gliclazide.

Experimental animals:

Adult albino rats weighing between 150 to 180g (Albino laboratories, miyapur, Hyd.) were used in the study. The animals were acclimatized to standard laboratory conditions of temperature ($27^oC \pm 1^oC$) and maintained on 12:12 h light: dark cycle in animal house of Sree Chaitanya college of pharmacy. They were housed in elevated wire cages and provided with regular rat chow (Standard pellet diet) and distilled water *ad libitum* for 2 weeks. The animal care and experimental protocol were in accordance with CPCSEA/IAEC.

Equipments: UV spectrophotometer, micro centrifuge.

Estimation of glucose in serum samples:

Principle:

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4–aminoantipyrine by catalytic action of peroxidase to form a red colored Quinoneimine dye complex. Intensity of colour formed is directly proportional to the amount of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{gluconate} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidases}} \text{Quinoneiminedye} + H_2O_2$$

Procedure:

Wavelength/filter: 505 nm
Temperature : 37^oC /R.T.
Light path : 1 Cm

Pipette in to clean and dry test tubes labeled as Blank (B), Standard(S) and Test (T).

Table 1

Addition sequence	B (ml)	S (ml)	T (ml)
Glucose reagent (L1)	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose standard	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37 ^oC for 10 min. or at R.T. (25^oC) for 30 min. Measure the absorbance of standard (Abs.S), and Test samples (Abs.T) against the Blank, within 60 min.

Calculation:

$$\text{Total glucose in mg/dl} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 100$$

Estimation of Cholesterol:

Principle: Cholesterol ester breaks down in to Cholesterol and Fatty acids. Cholesterol produced is oxidized in the presence of enzyme Cholesterol oxidase to form Cholesterol-3-one & H_2O_2.Then H_2O_2 reacts with Phenol & 4-Aminoantipyrine to produce Red colored Quinoneimine dye.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholestrol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{4-Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O + \text{4Aminoantipyrine}$$

Procedure:

Wavelength/Filter: 500 nm (Green Filter)
Temperature : 37^{o}C /R.T.
Light path : 1 Cm
Pipette in to clean and dry test tubes labeled as Blank (B), Standard(S), Test (T).

Table 2

Addition sequence	B(ml)	S(ml)	T(ml)
Cholesterol enzyme reagent (L1)	1.0	1.0	1.0
Distilled water	0.01	-	-
Cholesterol standard	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37^{o}C for 5 minutes and Measure the absorbance of standard (Abs.S), and Test samples (Abs.T) against the Blank. The final colour is stable for at least 1 hour.

Calculation:

$$\text{Cholesterol conc. In mg\%} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200(\text{Std.Conc})$$

Experimental models:

Experimental Induction of Diabetes in Rats:

Diabetes was induced in overnight fasted adult albino rats weighing 120-150g by a single intraperitoneal injection of freshly prepared Streptozocin in sodium citrate buffer (60 mg/kg body weight) in a volume 1ml/kg body weight. Hyperglycemia was confirmed by the elevated glucose level in plasma, determined at 48 h after injection.

Study design:

The diabetic rats are divided in to four groups 6 animals in each.

Group I: Control group (standard pellet diet with water)
Group II: Diabetic control (standard pellet diet with water)
Group III: 5-chloro-Isatin (100mg/kg)
Group IV: gliclazide (4mg/kg)
Group V: 5-chloro-Isatin+ gliclazide (100mg/kg+4mg/kg).

Pharmacodynamic study in diabetic rats:

Treatment:

The diabetic rats are divided in to four groups 6 animals in each.

Group I: Control group (standard pellet diet with water)
Group II: Diabetic control (standard pellet diet with water)
Group III: 5-chloro-Isatin (100mg/kg)
Group IV: gliclazide (4mg/kg)
Group V: 5-chloro-Isatin+ gliclazide (100mg/kg+4mg/kg).

Multiple dose study:

The diabetic rats were divided into 5 different treatment groups same as mentioned above and the treatment was carried for 14 days(2 weeks).Different biochemical parameters like glucose, cholesterol concentrations of the overnight fasted rats were determined on 0,7,14th day.

Statistical analysis:

All data are expressed as Mean±Sd. For comparison amongst different groups, One-way analysis of variance (ANOVA) followed by Dunnet test was performed. P value less than 5% (P <0.05) was considered to be statistically significant.

3. Results and Discussion

Table 3: Blood glucose levels in control, diabetic, 5-chloro-Isatin treated groups

Groups	0thday	7th day	14th day
Control	90±2.840	92.5±1.857	90.0±2.84
Diabetic control	92.5±2.76	240.0±5.00	238.7± 6.805***
Test treated	93.83±2.845	137.5±5.881	122.0± 4.041***
Standard treated	83.67±0.494	125.3±4.014	114.3± 3.547**
Test+Standard treated	90.33±1.626	117.8±4.868	108.3± 3.432*

All treatment groups were compared with diabetic control group. The significance between two groups was determined by one way ANOVA.

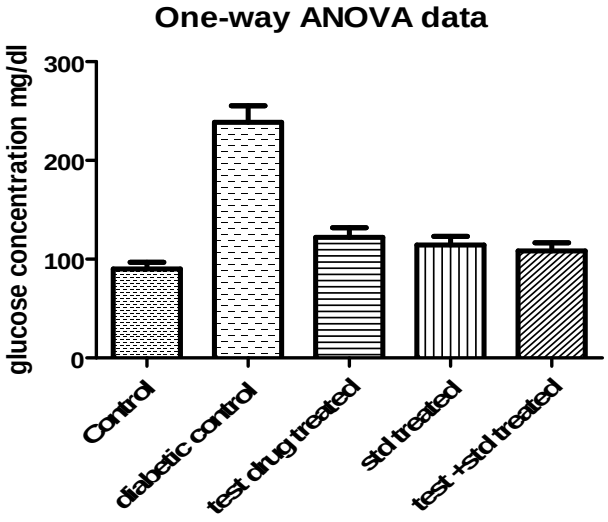

Figure 9: Serum glucose levels in control, diabetic, 5-Chloroisatin treated groups

5-Chloro-Isatinsignificantly decreases glucose levels in treated groups when compared to diabetic control group. The data was expressed as mean± SD,(n=6) and evaluated by ANOVA followed by Dunnett test *p<0.05(v_s Control)

Table 4: Serum cholesterol levels in control, diabetic, 5-chloro-Isatin treated groups

S.N	Contrl	Diabetic control	Test treated	Std treated	Test+std treated
1	100	220	167	150	142
2	102	217	165	140	135
3	98	215	175	138	128
4	99	230	172	135	130
5	104	225	164	132	120
6	105	219	155	139	132

All treatment groups were compared with diabetic control group. The significance between two groups was determined by one way ANOVA.

Discussion:

The present work was aimed to study the anti diabetic activity of 5-chloro-Isatin in STZ-induced diabetic rats. For this we took 5 different groups in each group containing six animals. They induced diabetes with streptozocin. Streptozotocin (STZ) is an antibiotic obtained from Streptomyces achromogenes.

STZ enters the pancreatic cells via a glucose transporter-GLUT2 and causes alkylation of Deoxyribonucleic acid (DNA) leading to pancreatic damage. Its toxicity depends upon the potent alkylation properties combined with the synergistic action of nitric oxide and reactive oxygen species that continue to DNA fragmentation. As a result of STZ action,

Streptozotocin pancreatic cells are destroyed by necrosis. STZ is not only damaging to the pancreatic cells but also to hepatocytes, nephrons and cardiomyocytes. In the present study, hyperglycemia was observed in rats after 3 days of STZ-induction. Treatment with gliclazide (4mg/kg) and 5-chloro-Isatin (100mg/kg) in STZ-induced diabetic rats, glucose and cholesterol levels were measured for every 7 days till 2 weeks.

At 0^{th} day of the study, we observed that the except normal control group all groups having the elevated glucose levels.

At 7^{th} day of the study, the blood glucose levels were reduced in the group which was given with a combination of 5-chloro-Isatin + Gliclazide. The animals showed significant response to gliclazide and 5-chloro-Isatin.

At 14^{th} day of the study, we observed that the blood glucose levels were reduced in the group which was given a combination of 5-chloro-Isatin + gliclazide. The animals showed more response to gliclazide and 5-chloro-Isatin alone and also showed better response when compared to the 7^{th} day.

4. Conclusion

Results proved that the pharmacodynamics interactions are found between 5-chloro-Isatin and Gliclazide. These findings investigated that the combination of 5-chloro-Isatin and Gliclazide showed the beneficial glucose and cholesterol reduction indicating additive effect than 5-chloro-Isatin and Gliclazide alone. However, the present study warrants further studies to find out the relevance of the interaction in human beings.

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