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ANTIDIABETIC ACTIVITY OF 1-(4-(DIMETHYLAMINO)BENZYLIDENE)-5-(2-OXO INDOLIN-3-YLIDENE) THIOCARBOHYDRAZONE IN RATS

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Keywords:
Antidiabetic activity; 1-(4-(Dimethylamino)benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone; Isatin derivative; Antihyperglycemic

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ABSTRACT:

Objective: The present study was aimed to evaluate the antidiabetic activity of 1-(4-(Dimethylamino) benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone i.e. a novel Isatin derivative diabetic rats.

Methods: The antidiabetic activity of Isatin derivative was evaluated against alloxan induced diabetes in rats. Diabetes in rats was induced by administration of alloxan monohydrate (120mg/kg, b.w, p.o) as a single dose. The diabetic rats were treated with single dose of test compound at a dose of 50 and 100mg/kg. Blood samples were collected at different intervals and analyzed for fasting glucose levels, total cholesterol (TC), triglycerides (TG) and High density cholesterol (HDL-C) levels using respective kits.

Results: Treatment with test compound with a single dose of 50 and 100mg/kg to diabetic rats showed a significant dose dependant ($p<0.01$) reduction of fasting blood glucose levels along with mild change of lipid profiles (TC, TG, HDL-C) indicating the antidiabetic activity of test compound.

Conclusion: The study results confirmed the antidiabetic activity of 1-(4-(Dimethylamino)benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone in rats.

INTRODUCTION: Diabetes mellitus now affects a higher proportion of persons in many developing countries than it does in western countries where two or three percent of the population is affected.

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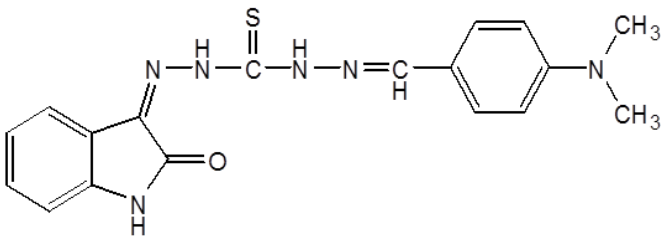
Diabetes mellitus today affects over 50 million people in the world and about one half them are living in the developing world [1.] Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and

macrovascular complications of diabetes that are the major causes of morbidity and death metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes that are the major causes of morbidity and death [2].

Most of the isatin or indole derivatives were reported as anticancer, antimicrobial and antioxidant agents [3, 4]. Based on the thorough literature review and biological importance of indole derivative, we have choosen 1-(4-(Dimethylamino)benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone, i.e a novel synthetic isatin or indole derivative for evaluation for antidiabetic activity against alloxan induced diabetic in rats.

MATERIALS AND METHODS:

Test Compound: The test compound i.e 1-(4-(Dimethylamino)benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone is a as gift sample from Mr. G. Kiran, Asst. Prof of Pharmaceutical Chemistry, Kakatiya Institute of Pharmaceutical Sciences, Hanmkonda, Warangal, India. The following are the structural, physical properties of compound and the suspension of test compound was prepared using 1% sodium carboxy methyl cellulose (Sod. CMC).



1-(4-(DIMETHYLAMINO)BENZYLIDENE)-5-(2-OXO INDOLIN-3-YLIDENE)THIOCARBOHYDRAZONE

TABLE 1: THE PHYSICAL PROPERTIES OF 1-(4-(DIME THYLAMINO) BENZYLIDENE)-5-(2 OXOINDOLIN-3-YSLIDENE) THIOCARBOHYDRAZONE

Property	Mol formula	Mol wt (g)	Melting Point	% yield	Solubility
Value	$C_{18}H_{18}N_6OS$	366	240-242 °C	76	Dimethyl Sulfoxide

Preparation of test suspension: The test isatin derivative was prepared as a suspension by using 0.5% sodium carboxy methyl cellulose (Sod CMC). Fresh suspension was prepared before administration to the animals.

Experimental animals: Albino Wistar rats (180-200gr) and mice (20-25gr) procured from Mahaveer Enterprises Hyderabad and were used for the study. They were housed in polypropylene cages and were maintained at room temperature of 23± 2^0C and relative humidity 50%. They were exposed to 12:12hr light: dark cycle throughout the period of acclimatization and experimental study. All the study protocols were reviewed and approved by Institutional Animal Ethical Committee (IAEC).

Acute toxicity study: Acute toxicity study of synthetic compound 1-(4-(Dimethylamino) benzylidene)-5-(2-oxoindolin-3-ylidene)thiocarbo hydrazone in mice has carried out according to OECD 423 guidelines [15].

The Isatin derivative was suspended in 0.5% Sodium Carboxy Methyl Cellulose and different doses were administered orally to albino mice. The animals were observed continuously for any change in autonomic or behavioral responses for first few hours and later at 24 hrs intervals for a period of 48 hrs. At the end of this period, the mortality if any, in different dose groups were noted.

Induction of Diabetes in rats: Diabetes mellitus or hyperglycemia was induced in overnight fasted rats by administration of alloxan monohydrate (2, 4, 5, 6,-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) at dose of 120 mg/kg intraperitoneally in normal saline [13]. After one hour of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation for about 48 hours. The animals were kept fasting overnight and fasting blood glucose levels were estimated before and after 72 hrs of alloxan treatment.

Animals showing blood glucose levels >200 mg/dl is considered as diabetic and were used for study.

Study design: The rats were divided into five groups of six each (n = 6) and were treated with single dose of vehicle/test compound to evaluate the antidiabetic activity of Isatin derivative.

Group-I: Served as normal control and received 1 ml/ kg of 0.5% sodium CMC (p.o).

Group-II: Served as disease control and received 1 ml/ kg of 0.5% sodium CMC (p.o).

Group-III: Diabetic rats treated with test compound (50 mg/kg; p.o)

Group-IV: Diabetic rats treated with test compound (100 mg/kg; p.o).

Group-V: Served as standard group and received glibenclamide (2.5mg/kg.,i.p).

Collection and analysis of blood samples: Blood samples were collected from all the groups of animals at 0, 1,3,4,5,7 hr intervals through puncture of retro orbital plexus and were centrifuged at 10,000 revolutions per minute (RPM) for 10 min. Serum was separated and stored at -20^{o}C and used for estimating the fasting blood glucose [5] triglyceride (TG) [6] Total Cholesterol (TC) (Lowe et al., 2009), High Density lipoprotein (HDL-C) [14] levels using respective kits.

Statistical analysis: All the experimental values were expressed as mean ± SD (n=6). One way analysis of variance (ANOVA) and Dunnett's test were used to compare means from the control group and each of the groups exposed to toxicant/disease control and the statistical significance was judged at the $p<0.05$ probability level.

RESULTS:

The acute toxicity studies were performed in mice with various doses of 1-(4-(Dimethylamino) benzylidene)-5-(2-oxoindolin-3-ylidene)thiocarbo hydrazone and it was found that the IC_{50} value is as 1500mg/kg. Based on this, we have chosen 50 and 100mg/kg as a test doses for the evaluation of antidiabetic in rats.

Alloxan monohydrate adminsitartion at a dose of 120mg/kg to rats successfully produced diabetes by elevating blood glucose levels grater than 200mg/dl. The antidiabetic activity of Isatin derivative was evaluated in rats against alloxan induced diabetes model. The effect of Isatin derivative on blood glucose levels were shown in **Table 2.** Administartion of Isatin derivative at a doses of 50 & 100mg/kg to diabetes rats produced a significant reduction in the blood glucose levels in a dose dependant manner ($p<0.01$).

The significant glucose levels were reduced after 1 hr of administartion of Isatin derivative. The maximum reduction of blood glucose levels were observed at 3rd hour i.e 23.3% & 38.5% with doses of 50 & 100mg/kg respectively **(Table 2).** However the antidiabetic activity of Isatin derivative of 100mg/kg was comparable with konown standard drug like glibenclamide.

TABLE 2: EFFECT OF ISATIN DERIVATIVE ON SERUM GLUCOSE LEVELS (MG/DL) IN DIABETIC RATS

Group/interval	0hr	1hr	3hr	5hr	7hr
Normal control	77.8±6.9	78.7±8.4 (1.15)	81.9±8.7 (5.26)	81.4±7.3 (4.62)	83.4±6.3 (7.19)
Diabetic control	240.7±18.5	238.0±14.6 (1.12)	237.2±20.2 (0.74)	238.2±17.3 (1.03)	234.3±21.2 (2.65)
Isatin derivative (50mg/kg)	210.8±10.8	191.6±17.2***** (9.10)	161.6±22.9****** (23.3)	139.5±21.6****** (33.8)	117.7±16.1****** (44.1)
Isatin derivative (100mg/kg)	232.2±20.5	195.6±16.6****** (15.7)	142.6±22.1****** (38.5)	115.3±17.5****** (50.3)	101.8±13.7****** (56.1)
Glibenclamide (2.5mg/kg)	228.3±19.6	192.6±16.1****** (15.6)	139.7±14.9****** (38.8)	109.9±12.6****** (51.8)	92.0±17.3****** (59.7)

All the values of Mean±SD; n=6; *p<0.01, **p<0.001 vs diabetic control, ( ) indicates % reduction of Serum Glucose levels.

Effect on Lipid Profiles: The effect of Isatin derivative administration on lipid profiles was evaluated in diabetes rats and the results were showed in **Tables 3-5.**

TABLE 3: EFFECT OF ISATIN DERIVATIVE ON TOTAL CHOLESTEROL LEVELS (MG/DL) IN DIABETIC RATS

Group/interval	0hr	1hr	3hr	5hr	7hr
Normal control	97.6±6.9	98.1±9.2 (0.51)	95.3±10.7 (2.35)	99.1±8.3 (1.53)	95.2±9.1 (2.45)
Diabetic control	143.8±12.6	145.4±11.8 (1.11)	146.7±9.7 (2.01)	147.2±12.3 (2.36)	146.9±11.3 (2.15)
Isatin derivative (50mg/kg)	137.5±8.7	131.2±9.8* (4.58)	126.7±10.6* (7.85)	114.1±9.6** (17.0)	111.7±8.5** (18.7)
Isatin derivative (100mg/kg)	134.6±10.8	128.4±12.1** (4.60)	116.8±8.7** (13.2)	109.5±9.4** (18.6)	108.1±10.2** (19.6)
Glibenclamide (2.5mg/kg)	134.9±7.9	127.1±9.3** (5.78)	113.5±7.4** (15.8)	110.6±7.9** (18.0)	108.2±6.4** (19.7)

All the values of Mean±SD; n=6; *p<0.05, **p<0.01 vs diabetic control, ( ) indicates % reduction of TC.

Similar to the glibenclamide, administration of Isatin derivative at doses of 50&100mg/kg to diabetic rats resulting the significant (p<0.05) reduction of the serum cholesterol levels after 3hr of its administration. The reduction of total cholesterol in rats was observed in dose dependant manner (p<0.01) **(Table 3).** The maximum reduction of total cholesterol was found at 5thhr i.e., 17.01% and 18.64% at doses of 50 and 100mg/kg.

TABLE 4: EFFECT OF ISATIN DERIVATIVE ON HDL-C LEVELS (MG/DL) IN DIABETIC RATS

Group/Interval	0hr	1hr	3hr	5hr	7hr
Normal control	44.7±3.1	45.6±2.4 (2.01)	42.7±6.7 (4.47)	47.1±5.0 (5.36)	46.3±5.0 (3.57)
Diabetic control	25.8±2.9	26.7±6.5 (3.48)	27.0±8.7 (4.65)	26.9±8.0 (4.26)	28.2±2.6 (9.30)
Isatin derivative (50mg/kg)	24.6±7.6	25.0±8.4 (1.62)	23.9±3.7* (2.84)	26.4±9.2** (7.31)	22.5±10.1** (8.53)
Isatin derivative (100mg/kg)	25.01±6.9	24.3±4.3 (3.18)	27.6±9.1* (9.96)	28.3±7.5** (12.7)	28.8±8.0** (14.7)
Glibenclamide (2.5mg/kg)	24.7±3.9	29.4±5.0 (19.0)	29.9±10.4** (21.5)	30.2±9.8** (22.2)	31.3±11.3** (26.7)

All the values of Mean±SD; n=6; *p<0.05, **p<0.01 vs diabetic control, ( ) indicates % increase of HDL-C.

Administration of single dose of Isatin derivative at a doses of 50&100mg/kg to diabetic rats significantly (p<0.05) increased the serum HDL-C levels after 3hr of its administration. The maximum increase in HDL-C levels was observed at 5thhr were 7.31% and 12.7 % with the doses of 50&100mg/kg **(Table 4).**

Administration of Isatin derivative at doses of 50&100mg/kg to diabetic rats resulted in a significant (p<0.05) reduction in the serum triglycerides levels after 3hr of its administration in a dose dependant manner. The maximum reduction of serum triglycerides in rats was observed at 5thhr were 15.40% and 17.44% with 50 and 100mg/kg (p<0.01) **(Table 5).**

TABLE 5: EFFECT OF MEHB ON TRIGLYCERIDE LEVELS (MG/DL) IN DIABETIC RATS

Group/interval	0hr	1hr	3hr	5hr	7hr
Normal control	80.9±5.8	78.4±6.9 (3.09)	81.3±3.8 (0.49)	77.6±9.1 (4.07)	79.2±8.3 (2.10)
Diabetic control	130.9±10.8	136.8±4.1 (0.07)	142.4±9.6 (2.55)	148.5±5.4 (3.36)	147.6±10.6 (4.16)
Isatin derivative (50mg/kg)	129.2±11.2	126.3±7.6* (2.24)	118.7±4.6** (8.12)	109.3±8.2** (15.4)	108.1±10.5** (16.3)
Isatin derivative (100mg/kg)	130.1±9.3	124.2±8.3** (4.53)	112.6±10.1** (13.4)	107.4±12.4** (17.4)	101.8±5.3** (21.7)
Glibenclamide (2.5mg/kg)	128.4±11.7	123.1±9.8** (4.12)	103.6±2.1** (19.0)	97.4±3.5*** (24.1)	91.6±9.3*** (28.6)

All the values of Mean±SD; n=6; *P<0.05,**p<0.01, ***p<0.001 vs diabetic control, ( ) indicates % reduction of TG levels.

DISCUSSION: In the present study, we have evaluated the antidiabetic activity of 1-(4-(Dimethylamino) benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone a novel isatin derivative against alloxan induced diabetes in rats.

Diabetes mellitus is a disease in which homeostasis of carbohydrates, protein and lipid metabolism is improperly regulated by the insulin resulting in elevation of fasting and post prandial blood glucose levels [12.] Diabetes mellitus is an independent predictor of high risk for Coronary Heart Disease (CHD). About 70-80% of deaths in diabetic patients are due to vascular disease. Glucose control is essential, but this provides only minimal benefit with respect to CHD prevention. An ideal treatmant for diabetes would be a drug that are not only controls the glycemic levels but also prevents the development of arteriosclerosis and other complications of diabetes [7].

The present study results suggest that the Isatin derivative exhibited significant antihyperglycemic activity in alloxan induced diabetic rats. Fasting blood glucose level in diabetic rats is an important basal parameter for monitoring diabetes [8] and it has shown that the Isatin derivative causes the antihyperglycemic effect by reducing ($p<0.001$) the fasting blood glucose level in a dose dependant manner (Table 2).

Diabetes mellitus is always be associated with hyperlipidemia [9]. A reduction in serum lipids, particularly of the LDL and VLDL fraction and triglycerides, should be considered as being beneficial for the long-term prognosis of coronary artery disease patients [10]. Lowering of blood glucose and plasma lipid levels through dietary modification and drug therapy seems to be associated with a decrease in the risk of vascular disease.

Treatment with Isatin derivative produced significant beneficial effects in the lipid profile in diabetic rats by reducing triglycerides, total cholesterol, and increasing HDL-C levels (Tables 3-5). The higher lipid levels seen in diabetic rats was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones [11].

CONCLUSIONS: Based on the above all results, it was confirmed the antidiabetic activity of novel isatin derivative i.e 1-(4-(Dimethylamino) benzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazone in rats. Further studies are required to elucidate the mechanism of action of the test Isatin derivative for the hepatoprotective activity.

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