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Evaluation of Anti-Diabetic and Hypolipidemic Activity of Isatin Derivatives in Streptozotocin-Nicotinamide Induced Type II Diabetic Rats

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Abstract: The present study is to evaluate anti-diabetic and hypolipedimic activity of isatin derivatives in Streptozotocin- Nicotinamide induced Type –II diabetic model. The isatin derivatives were given to the animals at the doses of 10,100mg/kg doses to the rats. The hypoglycemia, glucose tolerance were observed with only Id, If, Ih and Ij at both doses show significant (P<0.001) reduction in blood glucose level when compared with control. The antidiabetic activity of isatin derivatives (Id, If, Ih and Ij) showed significant (P <0.001) reduction in blood glucose level at 10mg/kg and 100mg/kg dose levels at 14th day. At 7th day 100mg/kg of these derivatives shows significant (P<0.01) fall in blood glucose level but 10mg/kg also shows significant (P<0.05) fall in blood glucose when compared with diabetic control. Glibenclimide shows significant (P <0.001) antidiabetic activity at 7th and 14th day. The elevated SGOT, SGPT, TG, TC, VLDL-C, LDL-C and decreased HDL-C level was significantly (P<0.001) restored respectively when compared with diabetic control. The glycosylated heamoglobin, insulin levels also restore significantly (P <0.001) by (Id, If, Ih and Ij) when compared with diabetic control.

Key words: Anti-Diabetic · Isatins · Streptozotocin · Nicotinamide · Glibenclimide

INTRODUCTION

Diabetes mellitus is a syndrome characterized by disordered metabolism and inappropriately high blood sugar (hyperglycemia) resulting from either low-level of the hormone insulin or from abnormal resistance to insulin's effects coupled with inadequate insulin secretion [1]. Diabetes mellitus is a disease which has assumed epidemic proportions worldwide with 5.9% of the world's adult population being affected with it. The developed countries, which harbor 80% of world's population with diabetes, bear the brunt of the burden. India, in recent times, has acquired the dubious distinction of being the diabetes capital of the world. According to the International Diabetes Federation (IDF), India is going to have close to 70 million individuals with diabetes by the year 2025 [2]. Currently, India is the diabetes capital of the world. In other words, 1 in every 5 diabetics in the world will live in India [3]. Isatin derivative had wide range of biological activities like antimicrobial [4], anti-tuberculosis [5], anticonvulsant [6], anti-tumor [7], anti-inflammatory, analgesic, antipyretic [8] and diuretic activity [9] respectively. From the above literature, we synthesized novel derivatives of isatin and were screened for their diuretic activity on Wistar albino rats.

MATERIAL AND METHODS

Chemicals: Carboxy methyl cellulose (CMC) was purchased from S.D. Fine, Mumbai, India. Glibenclamide was obtained as a gift sample from Suzikem Drugs private limited, Hyderabad. Streptozotocin was purchased from Sigma Aldrich, Germany. GOD- POD, Total cholesterol and HDL, triglycerides kits and other chemicals were procured from SS pharma, Hanamkonda.

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Synthesis of Isatin Derivatives: Procedure for Synthesis of isatin hydrazones was carried out according to Ragunandhan *et al*., [10].

General Procedure for Synthesis of isatin-3-[N^2-(chloroacetyl)] hydrazones was carried out according to Sarangapani and Reddy [11].

General Procedure for Synthesis of isatin-3-[N^2-(2-aminothiazol-4 yl)] hydrazones was carried out according to John *et al, [*12].

Synthesis of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrzones

A solution of an appropriate isatin-3-[N^2-(2-aminothiazol-4-yl)] hydrazone (0.01 mol) in ethanol (60 ml) added 2-3 drops of glacial acetic acid and various aromatic aldehydes (0.01 mol), the reaction mixture were heated at refluxed for about 8-10 hr. Completion of the reaction was monitored by TLC. Solvent present in the reaction mixture was evaporated under vacuum and the solid was collected and washed with cold petroleum ether, further purified by recrystallization from suitable solvent.

Animals: Healthy Wistar albino rats of either sex weighing 150–200 g were used for the studies which were procured from Mahaveer Enterprises, Hyderabad. Housed individually in polypropylene cages, maintained under standard conditions (12h light and 12h dark cycle, 25±2°C, 35–60% relative humidity), the animals were fed with standard rat pellet diet and water ad libitum. The experiments planned after the approval of Institutional Animal Ethical Committee (IAEC), Vaagdevi College of Pharmacy, Warangal and A.P.

Experimental Procedures

***In vivo* Screening of Isatin Derivatives for Hypoglycemic Activity in Normal Rats:** Selected animals were divided into different groups (n=6) were fasted overnight. The selected animals were administered with 0.1% sodium CMC for control and isatin derivatives (**Ia-Ij**) for test groups at a dose of 10 mg/kg and 100 mg/kg. Glibenclamide (10 mg/kg) was used as a standard drug. The blood was withdrawn by retro orbital plexus at 0hr, 2hrs, 4hrs and 6hrs from test and control groups. The plasma was separated and blood glucose level was estimated by using Glucose Oxidase- Peroxidase (GOD-POD) method [13].

Oral Glucose Tolerance Test: The oral glucose tolerance test was performed in overnight fasted normal rats. Rats divided into different groups (n=6) were administered 0.1% sodium CMC for control and isatin derivatives (**Ia-Ij**) for test groups at a dose of 10 mg/kg and 100 mg/kg b.w. Glibenclamide (10 mg/kg) was used as a standard drug. Glucose (3g/kg b.w) was fed 30 min after treatment. The blood was withdrawn by retro orbital plexus at 0, 30, 60 and 120 min from test and control groups [14]. The plasma was separated and blood glucose level was estimated by using Glucose Oxidase- Peroxidase (GOD-POD) method [15].

***In vivo* Screening of the Isatin Derivatives for Antidiabetic Activity Using Streptozotocin-nicotinamide Induced Model**

Induction of Diabetes: NIDDM was induced [16] in overnight fasted adult Wistar strain albino male rats weighing 150–200 g by a single intraperitoneal injection of 60 mg/kg streptozotocin is dissolved in in citrate buffer (pH 4.5), 15 min after the *i.p* administration, 120 mg/kg of nicotinamide was given *i.p.* Nicotinamide was dissolved in normal saline [17]. This model has been used earlier to induce NIDDM. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hrs after administration. Animals with blood glucose concentration more than 126mg/dl were used for study [18].

Assessment of Serum Glucose in Hyperglycemic Rats: After induction of diabetic the selected animals are divided into different groups (n=6). The first group was normal control received 0.1% sodium CMC, second group was diabetic control, third group was received standard (glibenclimide 10 mg/kg) and remaining groups were treated with test compounds (**Ia-Ij**, 10 and 100 mg/kg, *p.o*). The drugs were given every day for 14 days. Blood was withdrawn by retro orbital plexus at 1, 7 and 14 day after dosing and the plasma was estimated for glucose levels were determined by using GOD-POD method using commercial kit (Span diagnostics, India). Glucose levels were expressed as mg/dl [18].

Bio Chemical Analysis: The blood samples were centrifuged at 4000rpm for 15 min and serum was separated and stored at -20°C until analysis was done. Triglyceride, Total cholestrol and High density lipoprotein was estimated by using commercially available kits by GPO/PAP method [19-21]. Low density lipoprotein and Very Low density lipoprotein cholesterol were calculated by using Friedevald's equation [22] as described below.

VLDL: TG/5.
LDL: TC–(HDL+VLDL).

Glycosylated haemoglobin and serum insulin was estimated by ACS: 180 automated chemiluminescence system. On 14th day serum glycosylated hemoglobin and insulin estimations were carried out at Vijaya diagnostic center, Hanamkonda, A.P. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) was measured spectroscopically by utilizing the method of Reitman and Frankle [23]. The body weight was calculated on day 1 and 14th day of the experiment.

Statistical Analysis: Results were expressed as Mean±SD, statistical significance was calculated by applying one way ANOVA. $P<0.05$ was considered as significant (Newman-Keuls multiple comparison test).

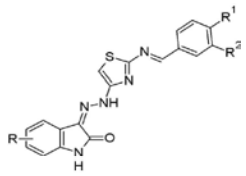
RESULTS

Synthesis of isatin-3-[N^2(2-benzalaminothiazol-4-yl)]hydrazones: The isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones have been synthesized by the following sequence of chemical reactions. The respective isatins were reacts with 99% hydrazine hydrate offered the isatin hydrazones. The isatin-3-[N^2-(chloroacetyl)] hydrazones were prepared by a reaction of respective isatin hydrazones with chloroacetyl chloride. Condensation of chloroacetyl derivatives of isatin hydrazones with thiourea in absolute ethanol given isatin-3-[N^2-(2-aminothiazol-4-yl)]hydrazone. Finally the title compounds prepared by respective isatin aminothiazolyl hydrazone were condensed with different aromatic aldehydes. However, intermediates and final compounds have been further purified by recrystallization from appropriate solvents(s) and characterized by their physical and spectral data. The representative compound in the series **Ia** [isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones] was characterized by their spectral data. ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) at 10.94 (s, 1H, lactam), 8.67 (s, 1H, NNH), 7.91 – 8.07 (m, 9H, Ar-H), 7.47 (s, 1H, N=CH), 6.97 (s, 1H, thiazole-H). Mass spectrum, m/z: 347.7 (7%), 272.7 (100%) and 244.7 (25%). The details of isatin derivatives were given in **Table 1**.

Hypoglycemic Activity: After the oral administration of isatin derivatives **(Ia-Ij)** with different doses (10mg/kg and 100mg/kg) to normal rats. The hypoglycemia was observed with only **Id, If, Ih** and **Ij** at both doses 2hr after the oral administration show significant ($P<0.001$) reduction in blood glucose level when compared with control. This effect is persisted up to 6hrs. Oral administration of vehicle did not change significant level of basal blood glucose. After 2 hrs, glibenclamide significantly ($P<0.001$) decreased the blood glucose. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in **Table 2** as mean ±SD.

Oral Glucose Tolerance Test (OGTT): In oral glucose tolerance test with isatin derivatives (**Id, If, Ih and Ij**) an increase in blood glucose levels at 30 min followed by decrease in blood glucose levels from 60 minutes onwards was observed at various doses. Whereas at 10mg/kg and 100mg/kg doses of isatin derivatives (**Id, If, Ih and Ij**) causes significant ($P<0.001$) decrease in glucose levels. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in **Table 3** as mean ±SD.

Table 1: Phys ical data of Is atin-3-[N^2-(2-be nzalaminothiazol-4-yl)]hydrazones



Compound	R	R^1	R^2	MF	MW
Ia	H	H	H	$C_{18}H_{13}N_5OS$	347
Ib	H	Cl	H	$C_{18}H_{12}ClN_5OS$	381
Ic	H	$N(CH_3)_2$	H	$C_{20}H_{18}N_6OS$	390
Id	H	OH	OCH_3	$^{C}19^{H}15^{N}5^{O}3^{S}$	393
Ie	5-CH_3	Cl	H	$C_{19}H_{14}ClN_5OS$	395
If	5-CH_3	OH	OCH_3	$^{C}20^{H}17^{N}5^{O}3^{S}$	407
Ig	5-CH_3	H	H	$C_{19}H_{15}N_5OS$	361
Ih	5-Cl	OH	OCH_3	$C_{19}H_{14}ClN_5O_3S$	427
Ii	5-Cl	Cl	H	$C_{18}H_{11}Cl_2N_5OS$	416
Ij	5-NO_2	OH	OCH_3	$^{C}19^{H}14^{N}6^{O}5^{S}$	438

Table 2: Effect of isatin derivatives on blood glucose level in normal rats

		Blood glucose levels(mg/dl)			
Group	Dose (mg/kg)	0hr	2hrs	4 hrs	6 hrs
Control	0.1% Sodium CMC	80.41±4.87	78.62±5.93	75.86±3.77	77.24±1.68
Glibenclamide		75.72±3.37	65.51±3.32	59.31±3.30***	55.75±3.31***
Ia	10	79.39±4.29	76.45±4.16	74.92±3.16	74.99±2.16
	100	77.25±3.96	75.16±4.08	73.05±4.01	74.35±2.52
Ib	10	79.15±4.21	75.36±3.98	74.52±4.14	74.91±2.68
	100	76.28±3.92	74.14±3.45	74.29±4.08	73.90±2.36
Ic	10	78.26±4.07	75.80±3.26	74.65±4.09	75.75±2.59
	100	76.15±3.29	75.06±3.92	74.14±4.62	74.28±2.90
Id	10	78.56±3.57	68.47±2.52	63.16±2.35***	66.82±3.46***
	100	76.43±2.26	65.32±2.29	59.75±2.75***	61.96±3.21***
Ie	10	79.65±3.01	75.67±3.85	74.28±3.52	73.14±2.43
	100	78.46±3.26	73.45±3.25	72.16±3.45	70.29±2.82***
If	10	77.38±3.42	68.42±2.35	63.08±2.92***	65.92±3.76
	100	76.21±2.29	64.62±2.18	58.96±2.58***	61.38±3.95***
Ig	10	78.52±2.95	75.38±3.45	73.89±3.26	74.25±2.63
	100	77.35±2.26	74.29±3.21	72.75±3.62	73.36±2.41
Ih	10	77.29±3.37	68.25±2.16	62.89±2.52***	65.02±3.45***
	100	76.18±2.08	64.98±2.11	58.75±2.16***	62.75±3.08***
Ii	10	78.36±2.02	75.23±3.21	73.16±3.82	74.08±2.32
	100	76.16±2.45	75.08±3.03	72.08±3.65	73.19±2.21
Ij	10	78.62±3.92	67.84±2.56	61.75±2.65***	64.91±3.25***
	100	76.45±2.16	63.29±2.52	58.02±2.36***	61.82±2.92***

Values are Mean ± SD, n=6 in each group.*P<0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test)

Table 3: Effect of isatin derivatives on Oral Glucose tolerance in normal fasted rats (OGTT)

		Blood glucose (mg/dl) (Mean ± SD)				
Group	Dose (mg/kg)	0min	30min	60min	90min	120min
Normal	0.1% Sodium CMC	85.20±2.60	130.45±3.56	145.45±4.30	120.56±3.85	102.84±2.60
Glibenclamide	10	87.45±3.05	120.50±4.85**	100.66±3.98***	92.08±5.65***	80.53±4.32***
Id	10	88.45±3.65	126.50±2.50	130.82±4.02***	110.20±2.54***	94.45±3.58***
	100	86.56±4.04	124.82±4.68	110.40±5.60***	100.89±3.89***	90.67±4.98***
If	10	84.78±5.25	127.29±3.78	135.56±3.67***	108.46±4.92***	92.50±2.90***
	100	86.45±3.89	123.80±5.05	114.56±4.80***	98.52±3.55***	88.66±5.81***
Ih	10	84.34±5.86	128.56±3.56	132.88±4.28***	105.68±3.40***	90.80±4.90***
	100	85.40±4.20	123.56±4.85	105.45±3.64***	94.55±3.62***	86.34±3.88***
Ij	10	86.44±2.99	124.02±3.36	130.45±4.55***	101.55±3.88***	91.65±3.56***
	100	87.34±3.56	123.12±4.78	102.56±3.70***	93.56±3.67***	84.50±3.60***

Values are Mean ± SD, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test).

Effect on Blood Glucose Levels in Streptozotocin-nicotinamide Induced Diabetic Rats: The antidiabetic activity of isatin derivatives (**Id, If, Ih** and **Ij**) showed significant (P <0.001) reduction in blood glucose level at 10mg/kg and 100mg/kg dose levels at 14th day. At 7th day 100mg/kg of these derivatives shows significant (P <0.01) fall in blood glucose level but 10mg/kg also shows significant (P<0.05) fall in blood glucose when compared with diabetic control. Glibenclimide shows significant (P<0.001) antidiabetic activity at 7th and 14th day. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in **Table 4** as mean ±SD.

Hypolipidemic Effect of Isatin Derivatives: Streptozotocin treated animals shows significant (P<0.001) elevation of TG, TC, VLDL-C, LDL-C and reduction of HDL-C levels as compared to the normal control rats. Isatin derivatives (**Id, If, Ih and Ij** 10 and 100mg/kg) and glibenclimide (10mg/kg) showed significant (P<0.001) reduction in elevated TG, TC, VLDL-C, LDL-C and HDL-C level was

Table 4: Effect of isatin-derivatives on Streptozotocin- Nicotinamide induced Diabetic rats (Long term effect up to 14 days daily dose)

		Blood glucose (mg/dl) (Mean ± SD)		
Group	Dose (mg/kg)	0^{th} day	7^{th} day	14^{th} day
Normal	0.1% Sodium CMC	111.34±3.62	111.42±4.65	111.33±3.80
Diabetic control	---	300.17±2.45	320.37±3.67	318.86±5.40
Glibenclamide	10	293.45±3.86	220.56±4.23***	195.43±4.56***
Id	10	298.50±4.18	313.42±4.74*	250.61±6.65***
	100	295.84±5.24	250.67±2.05***	219.09±2.98***
If	10	297.34±4.02	314.40±3.46*	240.34±2.66***
	100	293.68±2.61	256.60±4.46***	215.06±2.17***
Ih	10	298.62±3.56	315.36±2.02*	247.90±3.41***
	100	295.80±5.00	245.50±5.56***	224.57±4.77***
Ij	10	297.46±4.68	313.53±2.41*	243.57±4.26***
	100	295.86±3.80	235.80±4.74***	219.54±2.47***

Values are Mean ± SD, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test).

Table 5(a): Effect of isatin derivatives on lipid parameters (Serum Cholesterol, Serum triglyceride)

		Serum Cholesterol(mg/dl)		Serum triglyceride	
Groups	Dose(mg/kg)	0^{st}day	14^{th}	0day	14^{th}
Normal control	0.1% Na CMC	53.81±3.82	55.14±4.01	33.47±2.90	35.42±4.30
Diabetic control	---	180.62±11.47	210.89±8.20	125.00±9.23	139.33±5.35
Glibenclimide	10	168.24±11.23	98.69±5.65***	115.00±8.45	58.91±2.45***
Id	10	176.56±6.84	123.42±4.64***	120.60±3.57	66.82±5.24***
	100	171.24±9.88	109.45±5.41***	115.24±5.49	63.41±2.84***
If	10	174.95±7.62	125.43±3.34***	123.86±3.56	66.45±5.22***
	100	170.55±7.80	110.34±4.30***	118.42±5.09	61.79±2.34***
Ih	10	177.82±6.94	127.67±3.94***	120.66±3.02	68.02±3.34***
	100	172.64±7.33	104.34±4.20***	116.28±5.34	61.09±3.65***
IJ	10	179.40±5.11	123.12±5.56***	119.56±5.21	69.91±4.45***
	100	173.24±5.01	101.09±3.23***	115.52±4.72	62.43±3.29***

Values are Mean ± SD, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test).

Table 5(b): Effect of isatin derivatives on lipid parameters (HDL, VLDL &LDL)

		HDL(mg/kg)		VLDL(mg/kg)		LDL(mg/kg)	
Groups	Dose(mg/kg)	0day	14^{th}day	0day	14^{th}day	0day	14^{th}day
Normal control	0.1% Na CMC	40.29±2.84	42.45±3.83	6.69±2.50	7.08±3.20	6.83±3.08	5.88±3.05
Diabetic control		32.48±8.42	18.41±2.45	25.00±2.54	27.86±3.48	123.14±4.58	164.61±4.98
Glibenclimide	10	24.48±5.82	39.82±3.94***	23.00±2.30	11.78±2.10***	120.76±5.90	47.09±5.50***
Id	10	24.94±5.49	29.69±3.72***	24.12±2.35	13.36±3.72***	127.50±5.20	80.37±6.86***
	100	25.71±5.75	34.00±3.85***	23.04±2.47	12.68±2.34***	122.81±3.02	62.77±4.70***
If	10	24.45±5.32	30.32±3.23***	24.77±2.80	13.29±2.02***	125.73±6.84	81.82±5.90***
	100	25.45±5.65	35.56±3.32***	23.68±3.95	12.35±3.85***	121.42±5.38	62.43±4.67***
Ih	10	23.12±4.87	29.18±3.03***	24.13±2.01	13.60±2.02***	130.57±3.65	84.89±3.20***
	100	24.42±5.98	37.75±3.92***	23.25±3.72	12.21±3.80***	124.97±4.45	54.38±4.40***
IJ	10	24.32±3.02	31.76±5.20***	23.91±3.59	13.98±3.04***	131.89±5.90	77.38±5.40***
	100	25.99±4.02	39.19±3.18***	23.10±2.12	12.48±2.22***	124.15±6.40	49.42±4.38***

Values are Mean ± SD, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test).

restored respectively when compared to diabetic control. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in Table **5a&5b** as mean ± SD.

Effect of Hepatic Marker Enzymes in Serum: The SGPT and SGOT levels in streptozotocin induced diabetic rats were elevated. The elevated levels of SGPT SGOT, should be restored with isatin derivatives (**Id, If, Ih and Ij** 10 mg/kg and 100mg/kg) significantly (P <0.001) at the end of

Table 6: Effect of isatin derivatives on liver enzymes (SGPT, SGOT), Body weight

				BODY WEIGHT	
Groups	Dose (mg/kg)	SGPT (IU/L)	SGOT (IU/L)	Initial	Final
Normal control	0.1% Sodium CMC	43.02±4.20	36.31±3.53	174.32±4.47	198.56±8.54
Diabetic control	---	102.15±5.23	84.40±5.50	178.84±5.50	158.60±5.64
Glibenclimide	10	50.25±3.20***	48.52±6.26***	175.70±5.52	220.30±6.52***
Id	10	84.80±5.64***	68.22±4.16***	169.45±4.40	192.24±5.48***
	100	62.28±5.20***	57.08±5.90***	169.70±5.13	204.58±4.47***
If	10	80.50±4.04***	65.02±4.50***	170.40±5.42	190.04±4.40***
	100	60.18±5.00***	54.23±4.42***	172.70±5.13	200.38±6.08***
Ih	10	86.50±4.60***	64.34±4.32***	169.15±4.61	185.24±4.24***
	100	65.20±4.55***	52.03±4.20***	169.70±5.13	196.58±5.81***
IJ	10	78.54±5.60***	60.04±5.22***	170.05±5.60	194.34±4.48***
	100	58.20±5.55***	50.23±5.63***	174.30±5.53	203.28±5.17***

Values are Mean ± SD, n=6 in each group* P <0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test).

Table 7: Effect of isatin derivatives on serum insulin and glycosylated hemoglobin

Groups	Dose(mg/kg)	Insulin(μIU/ml)	Glycosylated hemoglobin (%)
Normal control	0.1% Na CMC	14.50±1.02	5.13±1.55
Diabetic control	---	6.24±2.02	11.56±1.24
Glibenclimide	10	12.40±0.98***	5.02±1.45***
Id	10	9.42±1.08*	9.05±1.95*
	100	10.65±2.42**	7.65±1.34**
If	10	9.58±1.95*	8.94±1.85*
	100	11.04±2.02***	7.60±1.56**
Ih	10	9.25±1.56*	8.00±2.54**
	100	11.56±1.98***	6.55±1.98***
IJ	10	9.50±2.24*	7.55±1.44**
	100	11.98±2.16***	6.10±1.65***

Values are Mean ± SD, n=6 in each group* P <0.05, ** P <0.01, *** P <0.001 when compared with control group (Newman-Keuls multiple comparison test).

the study. Glibenclimide (10mg/kg) also restore the liver enzymes significant (P<0.001) when compared with diabetic control. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and isatin derivatives further strengthen the antidiabtoegenic effect of these derivatives. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in **Table 6** as mean ±SD.

Effect on Serum Insulin and Glycosylated Haemoglobin: The glycosylated haemoglobin levels were increased in diabetic rats when compared with normal rats. The low dose (10 mg/kg) of **Id, If** significantly (P<0.05) decreased the glycosylated hemoglobin levels. Whereas high dose (100 mg/kg) of these two compounds and low dose (10 mg/kg) **Ih, Ij** was significantly (P <0.01) decreased the glycosylated hemoglobin levels when compared with diabetic control group. High dose (100 mg/kg) of **Ih, Ij** compounds significantly (P<0.001) reduced levels which were similar to glibenclimide (P<0.001).The insulin levels were reduced in diabetic groups compared with control animals. The low dose (10mg/kg) of **Id, If Ih** and **Ij** were significantly (P<0.05) increased the insulin levels. The High dose (100mg/kg) of **Id, If Ih** and **Ij** significantly (P <0.001) increase the insulin levels when compared with diabetic control. Glibenclimide (10mg/kg) also significantly (P <0.001) increased the insulin level. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in **Table 7** as mean ±SD.

Effect of Isatins on Body Weight: In diabetic control rats there was decrease in body weight, the groups treated with Isatin derivatives (**Id, If, Ih** and **Ij**;10 & 100 mg/kg) showed a significant (P<0.001) increase in body weight when compared to diabetic control group. The glibenclamide treated group also increased the body weights significantly (P<0.001) when compared with diabetic control. Statistical analysis was done by using One Way ANOVA followed by Newmann Keul's multiple comparison test by using graph pad prism 5.0 version and the data was represented in **Table 6** as mean ±SD.

DISCUSSION

In oral glucose tolerance test, at 90 and 120 min, a significant decrease in the blood glucose levels were observed in treated rats when compared with control rats. In the present study, diabetes mellitus was induced in rats through a STZ injection that causes the destruction of β-cells of islets of Langerhans, as proposed by many authors [24, 25]. This effect was represented in the current study through the elevation of blood glucose and a decrease of insulin levels in diabetic control rats. The elevated plasma glucose levels in diabetic rats were lowered through the administration of Isatin derivatives which showed an elevated plasma insulin level compared to diabetic control rats. The action of Isatin derivatives seems to be similar to that of glibenclamide. The dyslipidemia is associated with uncontrolled diabetes mellitus. The plasma levels of TC, LDL-C and TG increases, while the HDL levels decline, contributing to secondary complications of diabetes [26, 27]. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in an increased production of LDL-C particle [28]. In the present study, diabetic rats exhibited a significant elevation of TC, TG, LDL-C, while HDL-C was decreased. Isatin derivatives administration resulted in lowering the plasma levels of TC, TG and LDL-C with elevation of HDL-C level. It is known that the administration of insulin to diabetic subjects not only elevates lipoprotein lipase activity, but also lowers the plasma TG concentrations [29]. Glycosylated hemoglobin has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin [30]. The rate of glycation is proportional to the concentration of blood glucose. In the present study, the diabetic rats showed higher levels of HbA_1C compared to those in normal rats. Treatment with isatins and glibenclamide showed a significant decrease in HbA_1C levels in diabetic rats that could be due to an improvement in glycemic status. The isatin derivatives increased serum insulin level significantly, indicating that they might have insulin secretaogues activity, which in turn controls the hyperglycemia state of type-2 diabetes. Diabetic rats treated with the isatin derivatives showed restoring of body weight compared to the diabetic control, which may be due to its effect in controlling muscle wasting. The isatin derivatives lowered serum SGPT, SGOT levels which shows the protective effect and normal functioning of liver in reversing the organ damage due to diabetes which is clearly observed by high levels of SGOT and SGPT in diabetic control.

CONCLUSION

From the above results, it can be concluded that isatin derivatives have potential anti-diabetic and hypolipidemic activity.

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