Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 4 |OCT-DEC|2013|107-121

Research Article
Pharmaceutical Sciences

ANTIDIABETIC ACTIVITY OF NEW ISATIN DERIVATIVE – N'- (7- Chloro- 2- oxo -2,3- dihydro- 1H - indol- 3-yl) BENZOHYDRAZIDE IN ALLOXAN-INDUCED DIABETIC RATS

M. Nirmala*, G.E. Suhasini , K.Venkata Lakshmi, Archanagiri, B.Solomon Sunder Raj

[1]Lecturer In Pharmacy, Govt. Polytechnic, Masab Tank, Hyderabad Pin – 500028

[2]HOD in Pharmacy, Govt. Polytechnic, Masab Tank, Hyderabad Pin – 500028

[3]Govt. Polytechnic, Masab Tank, Hyderabad Pin - 500028

[4]Ass Professor, JNTUH, Hyderabad

[5]HOD, Pharmacology, MRIPS, Maisammaguda, Secunderabad

*Corresponding Author Email: pa_amra@yahoo.co.in

ABSTRACT

New Isatin derivative was investigated for its antidiabetic effect in alloxan - induced albino rats. A comparison was made between the derivative and a known anti diabetic drug glibenclamide (10mg / kg b. wt.). The study was conducted for 14 days in 4 different groups -Control, Diabetic control, standard drug and Isatin containing 6 rats in each group. Dose selection was made on the basis of acute oral toxicity study (50-5000 mg/kg b. w.) as per OECD guidelines. The effect of isatin on body weight of the animals, blood glucose, serum lipid profile [cholesterol, triglycerides], serum enzymes [serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminases (SGPT) were measured in the diabetic rats. The isatin derivative at 50mg /kg elicited significant (P < 0.01) reduction of blood glucose level after 3rd day 208.160 ± 12.422 mg/dl comparable to glibenclamide and also showed a significant improvement in oral glucose tolerance test, body weight loss and serum lipid profile.

KEY WORDS

Acute toxicity, Isatin derivative, alloxan, glibenclamide, anti- diabetic activity

INTRODUCTION

Diabetes mellitus is a complex chronic disorder that is a major source of ill health worldwide in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or cells do not respond to the insulin that is produced.[1] This metabolic disorder is characterized primarily by hyperglycemia and disturbances in metabolism of carbohydrate, protein, and fat , 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 macro vascular complications of diabetes that are the major causes of morbidity and death.[2] High blood sugar produces symptoms of polyuria, polydipsia and polyphagia and also damage tiny blood vessels in kidneys, heart, eyes and nervous system.

Diabetes has emerged as a major public health problem in the world According to WHO projections; diabetes will be the 7th leading cause of death in 2030 [3] and 347 million people worldwide have diabetes[4]. In 2004, an estimated 3.4 million people died from consequences of fasting high blood sugar[5]. More than 80% of diabetes deaths occur in low and middle – income countries the prevalence of diabetes is likely to increase by 35%.[6]

According to Diabetes Atlas published by the International Diabetes Federation (IDF), 366 million people have diabetes in 2011; by 2030 this will rise to 552 million. [7]

Reasons for this rise include increase in sedentary lifestyle, consumption of energy- rich diet, obesity, higher life span, etc. The real burden of the disease is due to its associated complications which lead to increased morbidity and mortality.

The management of diabetics is not without side effects and is a challenge to the medical system. Insulin and oral hypoglycemic agents like sulphonyl ureas and biguanides are still the drugs of choice and as these drugs are to be used throughout life and diminution of response after long use and produce side effects. [8]

Because of the drawbacks of the available drugs there is always a need to find novel antidiabetic drugs. In this aspect, the present study was undertaken to evaluate the antidiabetic effect of isatin derivative as the compound is found to be non-genotoxic.[9]

MATERIALS AND METHODS

Materials

Glibenclamide - Hetero chemicals, Alloxan - SD fine chemicals, Cholesterol Kit, Triglycerides Kit , SGPT-SGOT Kit, GOD-POD Kit - Span diagnostics ltd Glucometer – Thyrocare

Maintenance of Animals :(experiments were conducted at MRIPS institution with 1662/PO/a/08/ CPCSEA)

Albino Wistar rats were purchased from mahaveer enterprises. Hyderabad. The animals were acclimatized to the conditions by maintaining them at the experimental conditions for about 7 days prior to dosing. Cage number and individuals Marking on the tail to identify the animals. The animals were housed six per cage of same sex in polypropylene cages with bedding of paddy. Pellet chew feed standard diet under good management conditions and water *ad libitum* was provided to the animals. The temperature 20-25^{o}C and 12 hour each at dark and light cycle was maintained.

Acute Toxicity Studies

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimum number of animals while allowing for acceptable data based scientific conclusion. The method used defined doses (5, 50, 500, 2000 mg/kg b.wt) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of the chemical which causes acute toxicity.

Six Rats weighing between 180-200 gms were used for toxicity. The starting dose level of Isatin derivative at dose of 50 mg/kg b.wt orally as most of the crude extract possesses LD50 value more than 4000 mg/kg b.wt per oral dose was administered to the rats, which were fasted overnight with water *ad libitum*, food was withheld for a further 3-4 hrs. After administration of drugs and observed for another 14 days.

Body weight of the rats before and after treatment were noted and any changes in skin and fur, eyes and mucous membranes and also autonomic, central nervous systems, somatomotor activity and behavior pattern were observed and also signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity was also to be noted (OECD 423).[10]

Method for Antidiabetic activity

Induction of diabetes mellitus in experimental animals

Inbred adult Wistar albino rats, weighing 150-250 g of either sex were fasted for overnight before challenging with single subcutaneous route (sac) of alloxan monohydrate, freshly prepared and injected within 5min of preparation to prevent degradation at a dose of 130mg/kg. After administration of alloxan monohydrate 5% glucose

solution was given for 72hr to prevent hypoglycemic shock. Animals had access to feed and water. The development of hyperglycemic in rats was confirmed by fasting serum glucose estimation 72hr post alloxan monohydrate injectionwhere in the animals were fasted again for 14hr before blood collection. The rats with fasting serum glucose level of above 200mg/dl at 72hr were considered as diabetic and are included in the study[11]

Experimental Design

- Normal Wistar rats of either sex (150-250g) were used in the present study.
- Animals were provided with standard diet and water ad libitum.
- The rats were divided into 4 different groups containing 6 each.
- Group I- Control, administered vehicle (DMSO) at a dose of 50mg/kg.
- Group II- Diabetic control, administered alloxan monohydrate at a dose 130 mg/kg b.wt. intraperitoneally.
- Group III- Administered standard drug at oral dose of 10 mg/kg b.wt.
- Group IV- Administered with Isatin at oral dose of 50mg/kg b.wt.

Preparation of test drug

Test sample was suspended in 10% DMSO and each rat received a daily 1 ml as suspension at a dose of 50mg/kg body weight orally by oral gavage throughout the experimental period.

Determination of the blood glucose levels

Blood was collected from the tip of the tail vein and fasting blood glucose levels were measured using single touch glucometer (Thyro care) based on glucose oxidase method.

Oral glucose tolerance test

The Animals were fasted overnight (16hr), the blood glucose levels of rats were determined and treated with test samples and standard. Test samples and standard were given immediately after the collection of initial blood samples. The blood glucose levels were determined in the following pattern: 15 and 30 min to access the effect of test samples on normoglycaemic animals. The rats were then loaded orally with 2g/kg glucose and the glucose concentrations were determined at 30, 60, 120, 180min, after glucose load [12,13].

Determination of single dose treatment of Isatin on blood glucose level in Alloxan-induced diabetic rats

The test animals were divided into 4 groups. Group I consists of normoglycaemic rats and the remaining 3 groups consisted of six Alloxan-induced diabetic rats, each. In a single dose treatment study, all surviving diabetic animals were fasted overnight. Blood samples were collected from the fasted animals prior to the treatment with dosage schedule and after drug administration at 0, 1, 2, 3 hr time intervals to determine the blood glucose level, by glucometer[14]

Determination of sub-acute treatment of Isatin on changes in body weight in Alloxan-induced diabetic rats

The body weight changes of the control, diabetic control, standard, test groups treated rats were measured throughout the study. The rats were weighed at the beginning of the experiment and then subsequently 1^{st}, 3^{rd}, 7^{th}, 14^{th}day. [15]

Determination of sub-acute treatment of Isatin on blood glucose level in Alloxan-induced diabetic rats

The Animals were divided into 4 groups. Group I consists of normoglycaemic rats and the remaining 3 groups consisted of six Alloxan-induced diabetic rats, each. The treatment schedule was followed for the respective group of animals for 14 days. Blood samples were collected from overnight fasted animals on 1^{st}, 3^{rd}, 7^{th} and 14^{th} day to estimate blood glucose levels using glucometer. On the final day of the study, blood was collected from the retro-orbital plexus. [16]

Estimation of Biochemical parameters

Estimation of total cholesterol*:* CHOD-PAP Method

Principle:

Cholesterol esters are hydrolysed by cholesterol esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, Cholesterol Oxidase (CHOD) oxidises the 3-OH group of free cholesterol to liberate Cholest-4-en-3-one and Hydrogen Peroxide. In presence of Peroxidise (POD), Hydrogen Peroxide couples with 4-Aminoantipyrine (4-AAP) and phenol to produce Red Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample.

$$\text{Cholesterol Esters} \xrightarrow{CE} \text{Cholesterol + Fatty Acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{CHOD} \text{Cholest-4-en-3-one + } H_2O_2$$

$$2\ H_2O_2 + \text{Phenol + 4-AAP} \xrightarrow{POD} \text{Quinoneimine dye + } H_2O$$

Procedure:

Pipette into tubes marked	Blank	Standard	Test
Serum/Plasma	----	----	10 µl
Reagent 2	----	10 µl	-----
Reagent 1	1000 µl	1000 µl	1000 µl

Mix well. Incubate at 37°C for 10 minutes at room temperature (15 to 30 °C) for 30 minutes
Program the analyzer as per assay parameters.

1. Blank the analyzer with reagent blank
2. Measure absorbance of standard followed by test.

a) Calculation:

$$Cholesterol\ Concentration\ \left(\frac{mg}{dL}\right) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times 200$$

Estimation of HDL Cholesterol: PEG-CHOD-PAP method

Principle:

Low Density Lipoprotein (LDL) Cholesterol, Very Low Density Lipoprotein (VLDL) Cholesterol and Chylomicron fractions are precipitated by addition of Polyethylene Glycol 6000 (PEG). After centrifugation, the high density lipoprotein (HDL) fraction remains in the supernatant and is determined with CHOD-PAP method.

Procedure:

Step 1: HDL-Cholesterol separation

Pipette into tubes marked	Test
Serum/Plasma	200 µl
Reagent 3	200 µl

Mix well and keep at room temperature (15 to 30°c) for ten minutes. Centrifuge for 15 minutes at 2000 rpm and separate clear supernatant. Use the supernatant for HDL cholesterol estimation.

Step 2: HDL-Cholesterol estimation

Pipette into tubes marked	Blank	Standard	Test
Supernatant from step 1	----	----	100 µl
Reagent 4	----	100 µl	-----
Reagent 1	1000 µl	1000 µl	1000 µl

Mix well. Incubate at 37°C for 10 minutes at room temperature (15 to 30°C) for 30 minutes.
Program the analyzer as per assay parameters.

1. Blank the analyzer with reagent blank
2. Measure absorbance of standard followed by test.
3. Calculate results as per given calculation formula.

c) Calculation:

$$\text{HDL} - \textit{Cholesterol Concentration}\ \left(\frac{mg}{dL}\right) = \frac{\textit{Absorbance of Test}}{\textit{Absorbance of Standard}} \times 50 \times 2^{*}$$

*(2 = dilution factor, as Sample is diluted 1:1 in step 1)

LDL-Cholesterol using Friede wald's equation**

$$\text{LDL} - \text{Cholesterol} = \frac{\text{Total Cholesterol} - \text{Triglycerides} - \text{HDL Cholesterol}}{5}$$

VLDL-Cholesterol

$$\text{VLDL} - \text{Cholesterol} = \textit{Total Cholesterol} - \textit{HDL} - \textit{LDL}$$

Estimation of Triglycerides

Method: GPO-PAP Method**.**

Principle:

Triglycerides are hydrolysed by Lipoprotein Lipase (LPL) to produce Glycerol and Free Fatty Acid (FFA). In presence of Glycerol Kinase (GK), Adenosine Triphosphate (ATP) phosphorylates Glycerol to produce Glycerol 3-Phosphate and Adenosine Diphosphate (ADP). Glycerol 3-Phosphate is further oxidised by Glycerol 3-Phosphate Oxidase (GPO) to produce Dihydroxyacetone Phosphate (DAP) and H_2O_2. In presence of Peroxidase (POD), hydrogen peroxide couples with 4-Aminoantipyrine and 4-Chlorophenol to produce red Quinoneiminedye. Absorbance of coloured dye is measured at 505nm is proportional to triglycerides concentration in the sample.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Free Fatty Acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol 3-Phosphate + ADP}$$

$$\text{Glycerol 3-Phosphate + } O_2 \xrightarrow{\text{GPO}} \text{DAP + } H_2O_2$$

$$2\ H_2O_2 + \text{4-Chlorophenol + 4-AAP} \xrightarrow{\text{POD}} \text{Quinoneimine dye +4}H_2O$$

Procedure:

Pipette into tubes marked	Blank	Standard	Test
Serum/Plasma	----	----	10 µl
Reagent 2	----	10 µl	-----
Reagent 1	1000 µl	1000 µl	1000 µl

Mix well. Incubate at 37°C for 10 minutes.

Program the analyzer as per assay parameters.

1 Blank the analyzer with Reagent Blank
2 Measure absorbance of Standard followed by Test.
3 Calculate results as per given calculation formula.

Calculation:

$$\textit{Triglycerides}\ (mg/dL) = \frac{\textit{Absorbance of Test}}{\textit{Absorbance of Standard}} \times 200$$

Estimation of SGPT **Method:**2,4-DNPH method

Principle:

Alanine aminotransferase (ALT) catalyses the transamination of L-alanine and α-Ketoglutarate (α-KG) to form Pyruvate and L-Glutamate. Pyruvate so formed is coupled with 2,4-Dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a

brown coloured complex in alkaline medium and this can be measured colorimetrically.

α- KG + L-Alanine ⟶ Pyruvate + L-Glutamate

Pyruvate + 2,4-DNPH ⟶ Corresponding Hydrazone (Brown colour)

Procedure:

Pipette into tubes marked	Blank (Volume in ml)	Standard (Volume in ml)	Test (Volume in ml)	Control (Volume in ml)
Reagent 1	0.25	0.25	0.25	0.25
Serum	----	----	0.05	----
Standard	----	0.05	----	----
Mix well and incubate at 37°C for 30 minutes				
Reagent 2	0.25	0.25	0.25	0.25
Deionised water	0.05	----	----	----
Serum	----	----	----	0.05
Mix well and allow standing at Room Temperature (15-30°C) for 20 minutes.				
Solution 1	2.5	2.5	2.5	2.5

Mix well and read the O.D against Purified Water in a Colorimeter using Green filter or on Photometer at 505nm, within 15 minutes.

Calculation:

$$\text{ALT (GPT)activity}\left(\text{in}\frac{\text{IU}}{\text{L}}\right) = \frac{Absorbance\ of\ Test - \text{Absorbance of Control}}{Absorbance\ of\ Standard - Absorbance\ of\ Blank} \times Concentration\ of\ Standard$$

Estimation of SGOT **Method:** 2, 4-DNPH method

Principle:

Aspartate aminotransferase (AST) catalyses the transamination of L-Aspartate and α-Ketoglutarate (α-KG) to form Oxaloacetate and L-Glutamate. Oxaloacetate so formed is coupled with 2,4-Dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium and this can be measured colourimetrically.

α- KG + L-Aspartate ⟶ Oxaloacetate + L-Glutamate

Oxaloacetate + 2, 4-DNPH ⟶ Corresponding Hydrazone (Brown colour)

Procedure:

Pipette into tubes marked	Blank (Volume in ml)	Standard (Volume in ml)	Test (Volume in ml)	Control (Volume in ml)
Reagent 1	0.25	0.25	0.25	0.25
Serum	----	----	0.05	----
Standard	----	0.05	----	----
Mix well and incubate at 37°C for 60 minutes				
Reagent 2	0.25	0.25	0.25	0.25

Deionised water	0.05	----	----	----
Serum	----	----	----	0.05
Mix well and allow standing at Room Temperature (15-30^{o}C) for 20 minutes.				
Solution 1	2.5	2.5	2.5	2.5

Mix well and read the O.D against Purified Water in a Colorimeter using Green filter or on Photometer at 505nm, within 15 minutes.

Calculation:

$$AST\ (GOT) activity\ \left(in \frac{IU}{L}\right) = \frac{Absorbance\ \ of\ Test - Absorbance\ \ of\ Control}{Absorbance\ \ of\ Standard\ - Absorbance\ \ of\ Blank} \times Concentration\ of\ standard$$

Statistical Analysis

The results were expressed as mean ± SEM for 6 animals in each group. Data was statistically analyzed by one way ANOVA as primary test followed by Dunnet test using Graph pad Instat 3.0 software. *P* values < 0.05 were considered to be statistically significant. (*p*denotes probability).

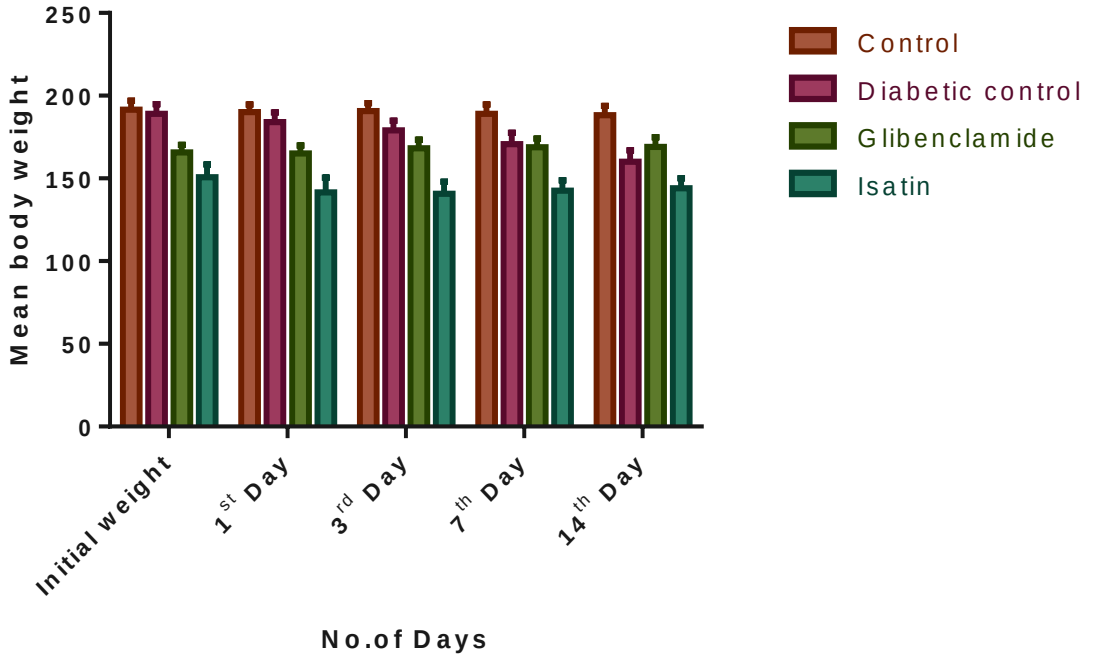
RESULTS

Table 1 Effect of sub-acute treatment of Isatin derivative on body weight changes in Alloxan induced diabetic rats. *Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

Group	Treatment	Dose (Kg^{1}Body Weight)	Body Weights (gms)				
			Intial Weight	1st Day	3rd Day	7th Day	14thDay
I	Control(DMSO)	50mg	191.666± 5.270	190± 4.655	190.833± 4.729	189.166± 6.760	188.333± 5.578
II	Diabetic control	130mg	189.166± 5.833^{ns}	184.166± 5.689^{ns}	179.166± 5.689^{ns}	170.833± 6.760^{ns}	160± 6.952**
III	Standard (GBC+Alloxan)	10mg	165.833± 4.549*	165± 4.830^{ns}	168.33± 5.110^{ns}	168.833± 5.270^{ns}	169.166± 5.689^{ns}
IV	Test(Isatin+Alloxan)	50mg	150.833± 7.683**	141.666± 8.819**	140.833± 7.120*	142.5± 6.423*	144.166± 5.974^{ns}

During the experiment period 14 days, the body weight significantly (p<0.01) decreased in Alloxan-treated group when compared to control group. The Isatin at a dose of 50mg/kg b.w/p.o showed a significantly (p<0.05) decrease in the body weight on 3rd, 7th day and did not show any significant improvement on 14thdayof treatment when compared with diabetic control. The standard drug glibenclamide did not show any significant improvement throughout the study when compared with diabetic control.

Effect of sub-acute treatment of Isatin derivative on body weight changes in Alloxan induced diabetic rats



*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

Table 2: Effect of Isatin derivative on normoglycaemic and glucose fed-hyperglycemic rats [NG-OGTT]

Group	Treatment	Dose (Kg^{1}Body Weight)	Blood glucose levels(mg/dl) 0 min	15 min	30 min (glucose)	60 min	120 min	180 min
I	Control (DMSO)	50mg	82.833± 0.833	80.333± 1.342	81.67± 1.22	119.166± 1.276	106.166± 2.561	82.166± 0.5426
II	Standard (GBC)	10mg	81.5± 1.147^{ns}	67.666± 1.838**	63± 1.506**	90± 2.633**	71± 2.236**	58.333± 1.978**
III	Test(Isatin)	10mg	86.5± 1.310^{ns}	81.666±1.202^{ns}	76.166± 1.222^{ns}	114.166± 3.919^{ns}	98± 9.241*	72.666± 0.954**
IV	Test(Isatin)	50mg	84.666± 0.988^{ns}	78.166±0.833^{ns}	72± 4.023*	109.166± 1.447**	75.333± 1.483^{ns}	55.833± 1.299**

*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Experimental groups values are compared with control group.*

The Isatin at a dose of 10mg/kg did not show any significant hypoglycemic effect in fasted normal rats after 30 min of administration and at a high dose of 50mg/kg shows reduced blood glucose in normal rats significantly (p<0.01) after 30 min of drug administration. In the same group of rats which are loaded with glucose (2gm/kg b.w/p.o) after 30 min of drug administration a low dose of 10mg/kg shows reduced blood glucose levels with less significance(p<0.05) but at a high dose of 50mg/kg

shows reduced blood glucose levels significantly($p<0.01$). The standard drug glibenclamide (10mg/kg) treatment showed significant reduction in blood glucose levels in both normal and glucose-induced hyperglycemic rats ($p<0.01$).

Effect of Isatin derivative on normoglycaemic and glucose fed-hyperglycemic rats [NG-OGTT]

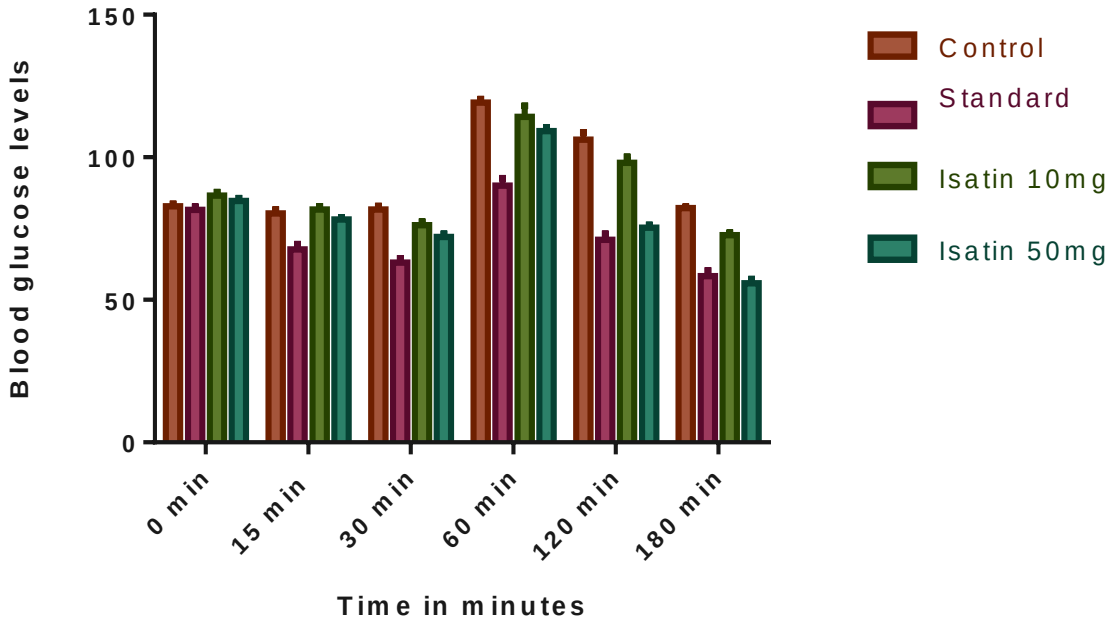


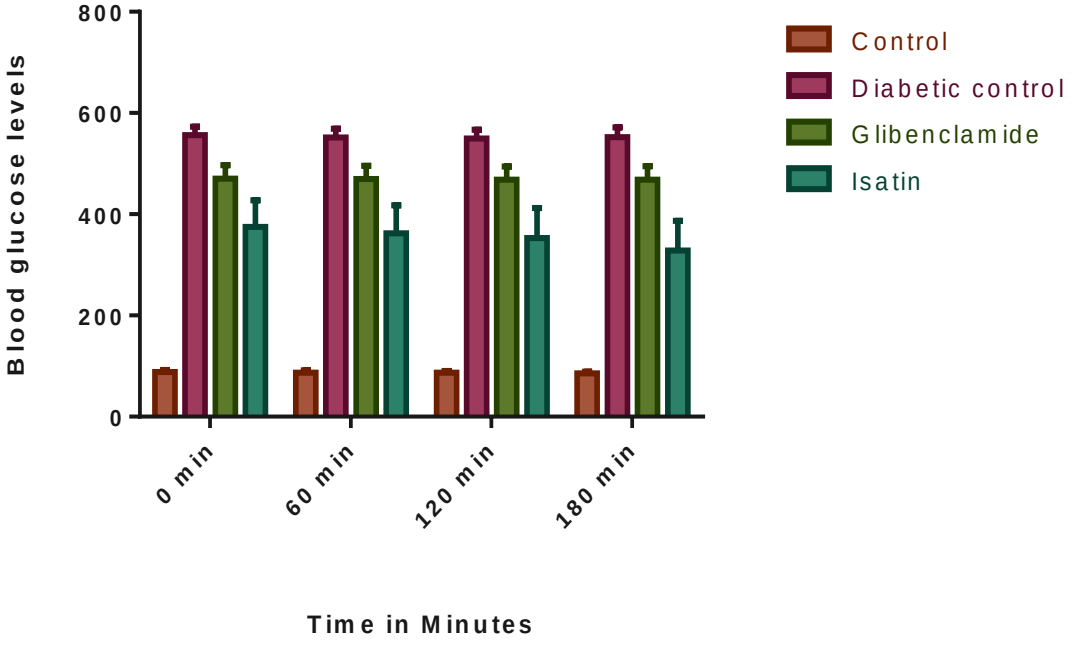
Table 3 Effect of single dose treatment of Isatin derivative on blood glucose in Alloxan induced diabetic rats.

Group	Treatment	Dose (Kg^{-1} Body Weight)	Blood Glucose Levels(mg/dl)			
			0 min	60 min	120 min	180 min
I	Control(DMSO)	50mg	88.666± 3.528	87.166± 4.230	86.833± 3.655	85.666± 3.565
II	Diabetic control	130mg	556.333± 16.556**	551.833± 17.467**	549.666± 17.437**	552.5± 19.259**
III	Standard (GBC+Alloxan)	10mg	470.166± 26.523^{ns}	462.666± 26.006^{ns}	424.333± 25.980*	396.5± 26.830**
IV	Test(Isatin+Alloxan)	50mg	375± 52.477**	362.5± 55.373**	352.833± 59.607**	328± 58.948**

*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group*

The effect of Isatin was evaluated at a single dose administration of 50mg/kg orally at the 0, 60, 120 and 180 min. This reduced the blood glucose level significantly when compared with diabetic control. The Isatin at 50mg/kg showed significant ($p<0.01$) reduction in the blood glucose level in Alloxan induced diabetic rats. The standard drug glibenclamide 10mg/kg showed a less significant ($p<0.05$ and $p<0.01$) reduction in the blood glucose levels in Alloxan induced diabetic rats, at 120 and 180 min, respectively.

Effect of single dose treatment of Isatin derivative on blood glucose in Alloxan induced diabetic rats



*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

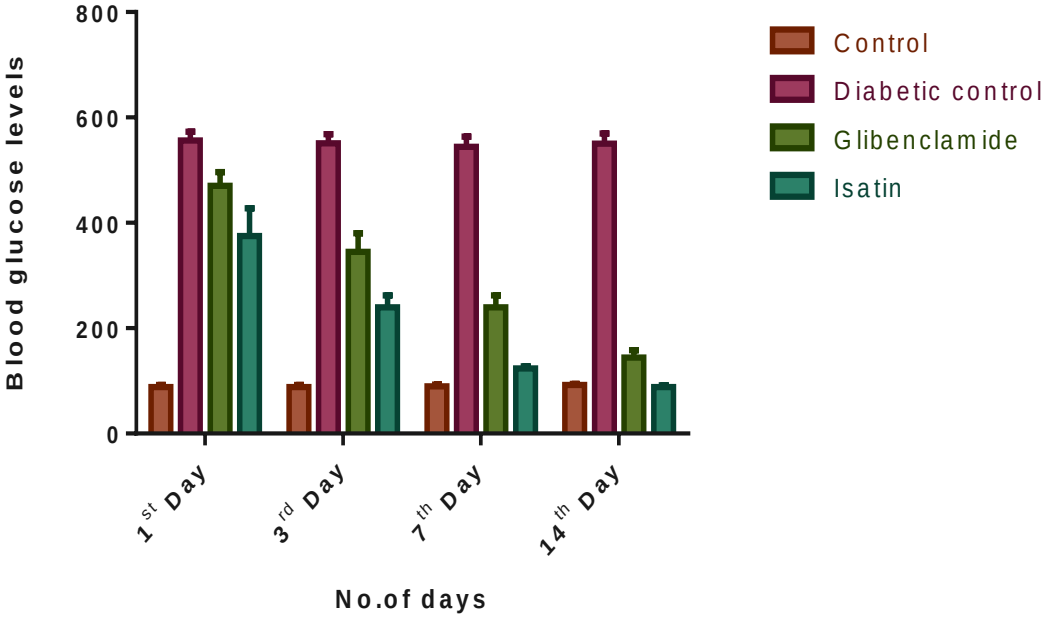
Table 4: Effect of sub-acute treatment of Isatin derivative on blood glucose in Alloxan induced diabetic rats.

Group	Treatment	Dose (Kg^{-1}Body Weight)	Blood Glucose Levels(mg/dl)			
			1st Day	3rdDay	7thDay	14thDay
I	Control(DMSO)	50mg	88.666± 3.528	88.5± 3.695	89.666± 3.051	92.333± 2.290
II	Diabetic control	130mg	556.333± 16.556*	551± 17.104**	544± 19.541**	550.166± 19.331**
III	Standard(GBC+Alloxan)	10mg	438.416± 26.322*	345.166± 35.137**	239.5± 22.937**	144.166± 14.115*
IV	Test(Isatin+Alloxan)	50mg	375± 52.477**	208.160± 12.422**	123.666± 3.792**	88.166± 3.311**

*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns - non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

During the experiment period 14 days, the blood glucose levels increases significantly (p<0.01) in Alloxan treated group when compared with control group. In the sub-acute study, the Alloxan induced diabetic rats were treated with Isatin 50mg/kg for the duration of 14 days. Treatment with Isatin 50mg/kg significantly (p<0.01) decreased the blood glucose levels after 3rd day. Treatment with Glibenclamide 10mg/kg significantly (p<0.01) decreases the blood glucose levels after 3rd day respectively.

Effect of sub-acute treatment of Isatin derivative on blood glucose in Alloxan induced diabetic rats.



*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns - non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

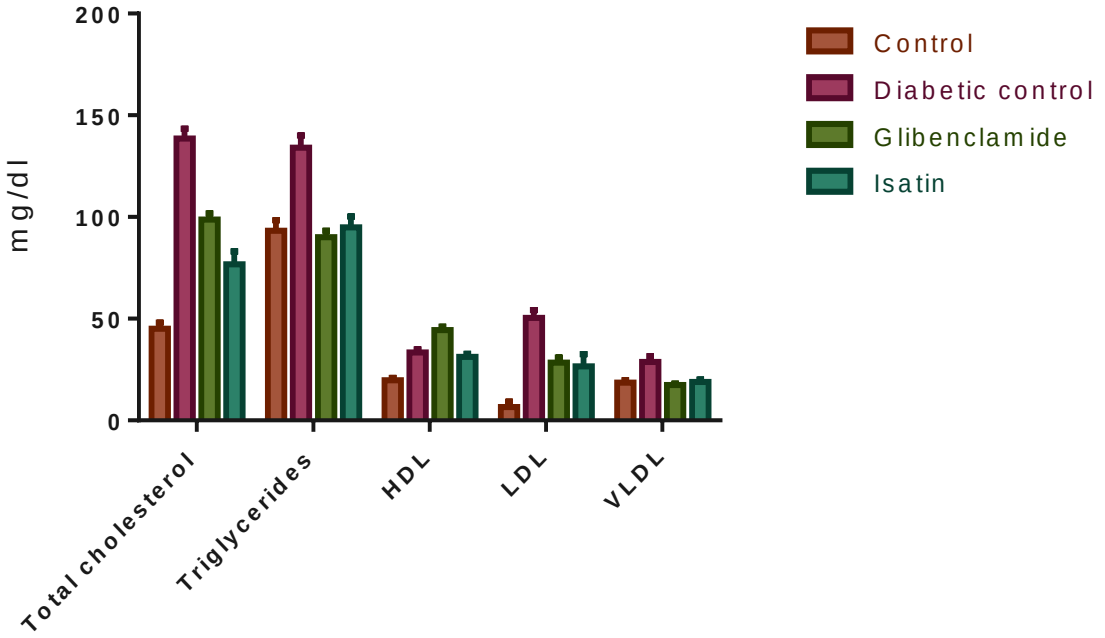
Table 5: Effect of Isatin derivatives on serum lipid profiles in Alloxan induced diabetic rats.

Group	Treatment	Dose (Kg^{-1}Body Weight)	Lipid Profiles(mg/dl)				
			Total Cholesterol	Triglycerides	HDL	LDL	VLDL
I	Control(DMSO)	50mg	45.015± 2.997	93.235± 5.221	19.76± 1.224	6.608± 2.577	18.647± 1.044
II	Diabetic control	130mg	138.635± 4.764**	134.136± 5.989**	33.498± 1.442**	50.438± 3.880**	28.7483± 2.863**
III	Standard (GBC+Alloxan)	10mg	98.765± 3.023*	90.063± 3.120**	44.418± 1.714*	28.356± 2.533**	17.446± 0.668**
IV	Test(Isatin+Alloxan)	50mg	76.794± 6.332**	94.932± 5.372*	31.2616± 1.532^{ns}	26.547± 6.044**	18.985± 1.074*

*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

The diabetic control animals showed a significant ($p<0.01$) increase in total cholesterol, triglycerides, HDL, LDL, VLDL when compared with control group. The serum total cholesterol, triglycerides, HDL, LDL, VLDL levels in Isatin 50mg/kg and Glibenclamide 10mg/kg shows a significant($p<0.05$ and $p<0.01$) decrease respectively when compared with Alloxan-induced diabetic rats.

Effect of Isatin derivatives on serum lipid profiles in Alloxan induced diabetic rats.



*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

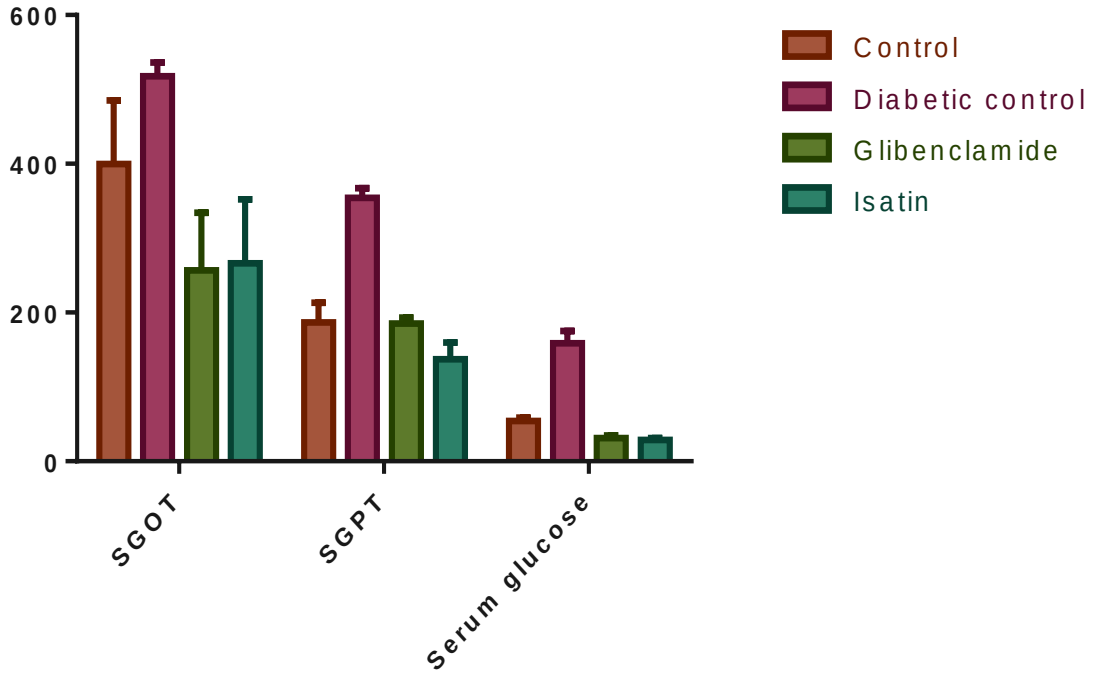
Table 6: Effect of Isatin derivative on SGOT,SGPT and serum glucose in Alloxan induced diabetic rats.

Group	Treatment	Dose (Kg^{-1} Body Weight)	SGOT(IU/L)	SGPT(IU/L)	Serum glucose (mg/dl)
I	Control(DMSO)	50mg	339.6±85.637	186.523±26.860	54.465±4.352
II	Diabetic Control	130mg	517.683±18.694^{ns}	354.066±13.420**	158.696±16.464**
III	Standard (GBC+Alloxan)	10mg	256.95±77.476*	185.2±7.834**	31.1833±3.746**
IV	Test(Isatin+Alloxan)	50mg	266.28±85.734^{ns}	137.085±22.854**	28.815±2.237**

*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental group values are compared with diabetic control group.*

The diabetic control animals did not show significant levels in SGOT when compared with control group. The test group levels of SGOT did not show significant levels when compared with diabetic control group. Glibenclamide levels show significant decrease ($p<0.05$) when compared with diabetic control. The SGPT, Serum glucose levels of diabetic control shows significantly ($p<0.01$) increased when compared with control group. The SGPT, Serum glucose levels of Isatin 50mg/kg and Glibenclamide 10mg/kg shows significant decrease ($p<0.01$) when compared with diabetic control group.

Effect of Isatin derivative on SGOT, SGPT and serum glucose in Alloxan induced diabetic rats.



*Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One way ANOVA followed by Dunnet's test). Diabetic control values are compared with control group. Experimental groups values are compared with diabetic control group.*

DISCUSSION

Management of diabetes is still a challenge to the medicinal systems. Though, various types of oral anti-hyperglycaemic agents are available in addition to insulin for the treatment of diabetes mellitus but these agents are having more side effects. [17]

In the present study the hypoglycaemic activity of isatin derivative was evaluated in alloxan induced diabetic rats. Alloxan is widely used to induce the experimental diabetes in animals. It acts on the β cells of the pancreas. The cytotoxic action of this is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells. [18]

The continuous treatment of the isatin derivative for a period of 15 days produced a significant decrease in blood glucose level in diabetic rats which is comparable to that of standard drug Glibenclamide which is used in treatment of type II diabetes mellitus. The standard drug Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism

by which the isatin derivative decreases the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from betacells of islets of langerhans or by increase in peripheral glucose uptake.

The glibenclamide fed alloxan induced diabetes wistar rats shown the changes in the levels of blood glucose. The isatin 50mg/kg has shown the maximum reduction of blood glucose levels when compared at initial day with the blood glucose levels of the 14th day. The isatin has shown the significant effect $p<0.01$ on the 14th day. From the study, it is suggested that the possible mechanism by which the Isatin decreases the blood glucose levels may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from β-cells of islets of Langerhans or by increase in peripheral glucose uptake.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart diseases. The marked hyperlipidemia that characterizes the diabetic states may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. Lowering of serum lipid concentration through dietary or drug therapy seems to be associated with a decrease in the risk of vascular diseases. The result of this study reveals that the dose of 50 mg/kg not only lowered TC, TG and LDL, but also enhanced the cardioprotective lipid HDL severely diabetic group after 14 days of treatment with the most effective dose further confirms our findings.

CONCLUSION

The derivative exhibited significant hypoglycemic activity and also lowered lipid profile in alloxan induced diabetic rats. It has the potential to develop as an antidiabetic drug.

REFERENCE

1. Shoback, edited by David G. Gardner, Dolores (2011). Greenspan's basic & clinical endocrinology (9th ed.).New York: McGraw-Hill Medical. pp. Chapter 17. ISBN 0-07-162243-8.
2. Apparao C, Kameswararao B, Kesavulu MM. Evaluation of antidiabetic effect of Momordica cymbalaria fruit in alloxan-diabetic rats. Fitoterapia. 2003;74:7–13. [PubMed]
3. Global status report on noncommunicable diseases 2010. Geneva, World Health Organization, 2011.
4. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet, 2011, 378(9785):31–40.
5. Global health risks. Mortality and burden of disease attributable to selected major risks. Geneva, World Health Organization, 2009.
6. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med, 2006, 3(11):e442.
7. International diabetic Atlas 5 edition
8. Moller DE: New drug targets for type 2 diabetes and metabolic syndrome. Nature 2001, 414:821.
9. M.Nirmala,G.E.Suhasini,Archana Giri and Rashmi Shiva kumar Genotoxicity study of newly synthesized Isatin derivative - N'- (7- Chloro- 2- oxo - 2,3- dihydro- 1H - indol- 3-yl) BENZOHYDRAZIDE Biosciences Biotechnology Research Asia, December 2012 vol.9(2), 843-847
10. OECD GUIDELINE FOR TESTING OF CHEMICALS Acute Oral Toxicity – Acute Toxic Class Method
11. Rai PK, Sing SK, KesariAN andWatal G, glycemic evaluation of Psidiumguajava in rats, Indian J Medical Research, 12:224-227 (2007).
12. Mustafa A, Orhan DD and Orhan N. In-vivo antidiabetic and antioxidant potential of Helichrysumplicatum ssp. Plicatumcapitulums in streptozocin-induced diabetic rats, Journal of Ethnopharmacology, 109: 54-59 (2007)
13. Antidiabetic effect of hydroalcoholic extract of B Research gate by NP Babre 1158 International Journal of Pharmaceutical Sciences and Nanotechnology ; Volume 3 , Issue 3 ,October-December 2010.
14. Orhan DD, Mustafa A, Sendogdu A, Ergun F and Yesilada F. Evaluation of hypoglycemic effect and antioxidant activity of three vicum album subspecies in streptozocin-induced diabetic rats. Journal of Ethanopharmacology, 98:95-102 (2005).
15. Nagappa AN, ThakurdesaiPA,Rao NV and Singh J. Antidiabetic activity of Terminiliacatappa Linn fruits. Journal of Ethanopharmacology, 88:45-50 (2003)
16. Malti r, Jana D, Ghosh D, Antidiabetic effect of aqueous extract of Tamarindusindica in streptozocin induced diabetic rats, Journal of Ethanopharmacology, 92:85-91 (2004).

17. Kamtchouing P, Kahpui SM, DjomeniDzeufiet PD, Tedong L, Asongalem EA, Dimo T. Anti-diabetic activity of methanol/methylene chloride extracts of Terminiliasuperba and canariumschweinfurthii on stretozocin-induced diabetic rats. J Ethnopharmacol. 2006; 104:306-09.
18. Szkudelski T. The mechanism of alloxan and streptozocin action in β-cells of the rat pancreas. Physiol res. 2001;50:536-46.

***Corresponding Author:**
M. NIRMALA,
Lecturer In Pharmacy,
Govt. Polytechnic, Masab Tank,
Hyderabad Pin - 500028
Email-pa_amra@yahoo.co.in