ejpmr, 2018,5(2), 370-374

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

5, 5'-TETRA HYDRO-1H, 3H-FURO [3, 4-c] FURAN-1,4-DIYLBIS (2-CHLORO-1, 3-BENZODIOXOLE, SESAMINE DERIVATIVE, NEUTRITIVE IN LOWERING HYPERGLYCEMIA

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Article Received on 17/12/2017 **Article Revised on 07/01/2018** **Article Accepted on 27/01/2018**

ABSTRACT

Recently, studies have reported that sesame oil lowered blood pressure and improved antioxidant status in hypertensive and diabetic-hypertensive patients. This study was to evaluate the effectiveness of synthetic sesamine derivative (3-o-methyl-d-glucose) in normal and alloxan induced rats. The study included 30 Wistar strain albino rats and randomly divided into 5 equal groups, normal rats (Only fed with normal rat feed), alloxan monohydrate 150mg/kg BW rats, sesamin derivative 10mg/kg BW, alloxan monohydrate 150mg/kg BW rats+ sesamin derivative 10mg/kg BW, Alloxan monohydrate, reference control i.e., Standard drug Glibenclamide 10 mg/kg BW was administered for 28 days for various biochemical analysis. The induction of diabetes has caused significant initial increase in the fasting blood glucose levels of all the groups. The diabetic control group shows significant increase throughout the study period when compared with the normal control group ($p<0.001$). However, the extract treated groups and the standard treated group shows significant decrease in the fasting blood glucose levels when compared with diabetic control ($p<0.001$) which was determined on the 7th and 14th day of experiment. SGOT, SGPT and ALP, show significantly lower levels of SGOT, SGPT and ALP in comparison to the diabetic control group ($p<0.001$). Fat accumulation in liver and inflammation were reduced with sesamine derivative. Sesamine derivative can provide a safe and effective option that may be useful in clinical practice to lower hyperglymia.

KEYWORDS: Diabetes, Alloxan monohydrate, Glibenclamide, ALP.

INTRODUCTION

India has a rich heritage of usage of medicinal plants in the Ayurvedic, Siddha and Unani system. Many Indian plants have been investigated for their beneficial use in different diseases and reports occur in numerous scientific journals.[1] The country has about 15000 medicinal plants that include 7000 plants used in Ayurveda, 700 in Unani, 600 in siddha, 450 in Homeopathy and 30 in modern medicines.[2,3] The plant extracts and its product play an important role in treating many symptoms. Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity.[4,6] They have been also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities.[7,11] World ethnobotanical information about medicinal plants reports almost 800 plants used in the control of diabetes mellitus.[12] During the 18th century, the active principles of a number of antidiabetic plant drugs were isolated; hence it became possible to administer these in standardized dosage forms. Based on a large number of chemical and pharmacological research work, numerous bioactive compounds have been found in medicinal plants for diabetes.[13] Potential mechanism by which compounds can reduce blood glucose include increasing insulin action, decreasing hepatic glucose production, increasing peripheral glucose metabolism independently of insulin and decreasing nutrient ingestion etc. Some compounds such as polyphenols, lectins, fiber and phytic acid are negatively correlated with the glycemic index. Many of the herbal drugs used in diabetes also have antioxidant activity indicating that reduced oxidant state can reduce the extent of glucose level in the body.[14] A number of investigators have shown that cumarins, flavonoids, terpenoids and a host of other secondary plant metabolites, including arginine and glutamic acid, possess hypoglycemic effect in various experimental models.[15] Many of these are being used in ayurvedic system of medicine for the treatment of diabetes. However, most of the species of higher plants have not been screened for chemical or biologically active constituents.[16] It is a big challenge to fully exploit

medicinal biodiversity to look for phytochemicals with insulin mimetic property.

MATERIALS AND METHODS

Experimental animals

Male albino Wistar rats, weighing: 120 ± 20g were used for the present study, procured from Akshaya Biologicals, Hyderabad, India. All the animals were maintained under regulatory laboratory conditions (12L: 12D; Humidity: 76% and temperature: 28 ± 2^0 C) in the Department of Biochemistry, Global Institute of Biotechnology, Hyderabad. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional animal Ethical Committee.

1. Healthy Wistar strain albino rats were selected and randomly divided into five groups with six animals in each group serving as
Group 'A' = Normal rats (Only fed with normal rat feed)
Group 'B'= Alloxan monohydrate (AM) (150 mg/kg BW) rats
Group 'C' = Sesamin derivative-04 10 mg/kgBW
Group 'D' = AM group (150 mg/ kgBW) + Sesamin derivative-0410 mg/kgBW
Group 'E' = Reference control i.e. Standard drug (Glibenclamide, 10 mg/kgBW).

Acute toxicity

Male rats (*Rattus novergicus*) of Wistar strain weighing 159.00 ± 7.20g were obtained from the animal house of the Global Institute of Biotechnology, Hyderabad. They were kept in aluminum cages placed in well ventilated house conditions (temperature 23 ± 1^oC; photoperiod: 12 h light and 12 h dark cycle; humidity: 40-45%). The animals were allowed free access to rat pellets and distilled water. The acute toxicity of the synthesized compound-04 was evaluated in rats (6 rats per group) by preparing five different doses (100, 500, 1000, 2000 and 4000 mg/kg) and administered orally using gavages. Animals were kept without food for 18 h prior to dosing and were monitored continuously on a daily basis for 3 days after dosing for any sign of toxicity. Animals showing any symptom of toxicity were immediately sacrificed. LD50 value of the synthesized compound was calculated arithmetically using the method described by Hamilton et al. 1977.[17]

Dosage (mg/kg) = Volume of synthesized compound (ml) × Concentration of synthesized compound (mg/ml)/Weight of animal (kg).

Preparation of serum

The procedure described by Yakubu et al. (2005) was adopted for the preparation of serum. An aliquot (2 ml) of the blood was collected from the tail vein of the animals into sample bottles containing EDTA for the haematological analysis while another 5 ml was allowed to clot at room temperature for 10 min. This was centrifuged at 1282 g x 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300 Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay of biochemical parameters.[18]

Haematological analyses

The full blood count (FBC) including red blood cells (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell distribution width (RCDW), haematocrit, white blood cell (WBC), basophils, neutrophils, eosinophils platelet and monocytes were determined using a semi-automated hematology analyzer (Horiba ABX 80 Diagnostics, ABX Pentra Montpellier, France).

Biochemical parameters

Estimation of Glucose and other clinical parameters

Sample was collected (preferably in glass tubes) and left for 1 hr at 37°C to allow it to clot. Using a glass pasteur carefully the clot was loosened from the sides of the tube. The serum was centrifuged at 3000 rpm for 10 min at 4°C. The serum was removed from the clot by gently pipetting off into a clean tube using a glass pasteur or a micropipette. The serum was labeled with the animal number and the estimations were made. The serum glucose level; the enzymes SGOT, SGPT and ALP level was determined enzymatically on Robonik semi-autoanalyser.

Screening of dose dependence

In order to check the dose dependence different doses of the synthesized compound(s) like 10, 30, 50, mg/kg BW, were given to the different groups of rats following the same methodology as above. Different doses of the synthesized compound-04 were given to the different groups in order to determine the dose dependence of the synthesized compounds and the results are shown in Table 1. It shows that the reduction in fasting blood glucose level by the ethyl acetate and the aqueous synthesized compounds is significant ($p<0.001$) at all the concentrations that were taken i.e., from 200mg/kg body weight up to 1000mg/kg body weight, however the percentage variation that represents the percentage increase/decrease in the blood glucose levels from Day0 to Day14 shows a systematic increase in the activity of the synthesized compounds in a dose dependent manner.

Oral Glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted (18hr) normal rats as per Bonner, 1988. Healthy rats were randomly selected and distributed into five groups (n=6). One of those groups was administered distilled water and the rest four groups and glibenclamide (30mg/kg bw) groups. Glucose (2g/kgBW) was fed 1 hr after the administration of the active compound and glibenclamide. Blood was withdrawn from the tail vein at 0, 60, 90, 120 and 150 min of glucose administration and glucose levels were

estimated using Accucheck Go blood glucose monitoring kit. The blood glucose level of the rats was measured after overnight fasting. Group 'A' was fed with simple drinking water which served as normal control and rest of the groups were fed with the respective active compound, mentioned above, i.p., following standard methodology. All the groups were given respective treatments daily for 14 days. Blood was collected again on the 7th day and 14th day of dosing, through the retro orbital sinus of the rats. The serum from the blood was separated and labeled with the animal number. The estimation of glucose level was measured enzymatically on an auto analyser.[19]

Alkaline phosphatases (ALP) determination

The ALP reagents were used to measure ALP activity by a kinetic rate method using a 2-amino-2- methyl-1-propanol (AMP) buffer. 5 µl of serum sample was mixed with 228 µl of AMP (pH 10.3) and 22 µl of p-nitrophenyl phosphate, allowed to react together for 2 min at room temperature. The enzyme (ALP) catalyzes the hydrolysis of p-nitrophenyl phosphate (colourless) to p-nitrophenol (yellow). The calculation was performed by the system to produce the final result.[20]

Estimation of Cholesterol content

The reagents were used to measure cholesterol in the sample by time-end point method as described by Henry (1991). Serum sample (5 µl) was mixed with 290 µl of reagent A (211 IU/L cholesterol esterase, 216 IU/L of cholesterol oxidase, 6667 IU/L peroxidase) with 10 µl reagent B (0.28 mmol/L 4- aminoantipyrine, 8.06 mmol/L phenol). They are allowed to mix together at room temperature for 2 min. The absorbance was measured at 510 nm to determine cholesterol level in the sample. In the reaction, cholesterol esterase hydrolyzed cholesterol esters in the sample to free cholesterol and fatty acid. Cholesterol oxidase acted on the free cholesterol to cholestene-3-one and hydrogen peroxidase. The enzyme peroxidase catalyzes the reaction of hydrogen peroxide and 4-aminoantipyrine to quinoneimine which is then measured.[21]

RESULTS AND DISCUSSION

Acute toxicity testing

Acute toxicity studies revealed that the synthesized compounds were safe up to 2000 mg/kg of body weight and approximate LD_{50} is more than 2000 mg/kg. No lethality or any toxic reactions or moribund state was observed up to the end of the study period.

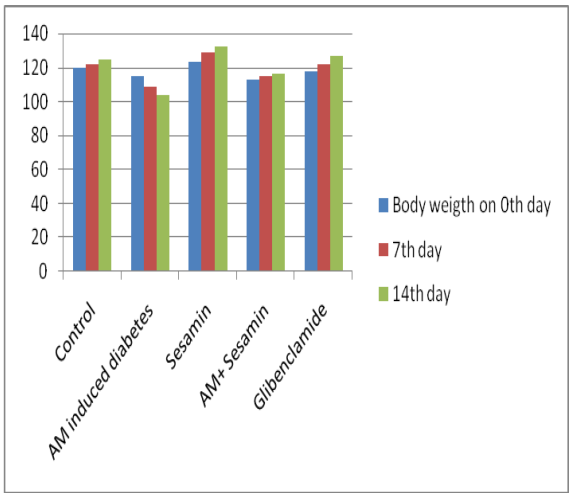

Fig. 1: Effect of extracts of Sesamin and its derivatives on body weight of Alloxan monohydrate induced diabetic rats.

Effect of different sesamin and it's deivatives on fasting blood glucose levels

As shown in Table, the induction of diabetes has caused significant initial increase in the fasting blood glucose levels of all the groups. The diabetic control group shows significant increase throughout the study period when compared with the normal control group ($p<0.001$). However, the extract treated groups and the standard treated group shows significant decrease in the fasting blood glucose levels when compared with diabetic control ($p<0.001$) which was determined on the 7th and 14th day of experiment. The effect is more pronounced in standard (10mg/kg) group, followed by ethyl acetate (500mg/kg) group, aqueous (500mg/kg) group, ethyl acetate (250mg/kg) group, aqueous (250mg/kg) group, methanol (500mg/kg) group and methanol (250mg/kg) group with the percentage variations as shown in Table 6. On the basis of these observations only ethyl acetate and aqueous extracts were selected for further analysis of antidiabetic activity.

Table 1: Effect of Sesamin on fasting blood glucose levels of alloxan monohydrate induced diabetic rats.

Groups	Blood glucose level (mg/dl)			
	0th day	7th day	14th day	% Variation
Control	84.01±4.64	82.21±5.58	80.29±6.53	4.43
AM-induceddiabetic rats (150mg/kgbw)	334.77±13.51*	356.20±16.53*	374.00±12.48*	-11.72
Sesamin (10mg/kgbw)	90.80±11.14	99.30±15.15*	135.40±11.00*	47.78
AM-induced Diabetes + Sesamin	330.10±10.2	250.80±9.67*	184.90±8.99*	38.77
Glibenclamide (10mg/kgbw)	90.90±14.22	258.9±16.06*	104.40±16.88*	51.73

Data represented as mean ± S.D values of 6 animals each. *p<0.001, **p<0.05 (Dunnett t-test); diabetic control was compared with the normal, extract and standard treated groups were compared with the diabetic control.

Effect of different extracts of Sesamin and its derivatives on SGOT, SGPT and ALP levels

SGOT, SGPT and ALP, as shown in Table 4, both the extracts show significantly lower levels of SGOT, SGPT and ALP in comparison to the diabetic control group (p<0.001). Here the maximum reduction was observed for standard followed by Sesamin.

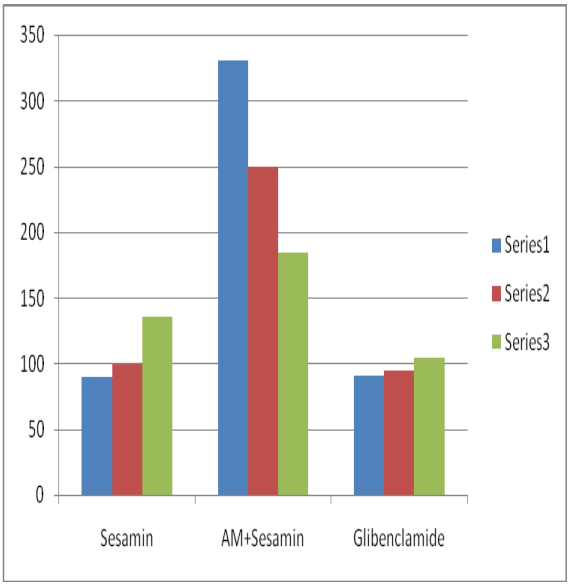

Fig. 2: Effect of sesamin on SGOT, SGPT and ALP levels of alloxan monohydrate induced diabetic rats.

An increase in the SGPT, SGOT and ALP activities was recorded in diabetic rats in comparison with non diabetic rats, indicating an altered liver function in diabetic condition. Sesamin and its derivatives extracts significantly controlled SGOT, SGPT and ALP values in the alloxan induced diabetic rats. In diabetic animals a change in the serum enzymes is directly related to changes in the metabolism in which these enzymes are involved.[23] The increased levels of transaminases which are active in the absence of insulin because of increased availability of aminoacids in diabetes are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes. In the present study, the sesamin and its derivatives significantly decreased SGOT and SGPT enzyme activities. Hence, the improvements noticed in the levels of these enzymes are as a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of SGOT and SGPT levels after treatment also indicates a revival of insulin secretion. Elevation of ALP has been reported in diabetic rats and rabbits. This increase in ALP was significantly reversed by the Sesamin and derivatives.

Moreover, hyperglycemia in diabetic rats was associated with a high serum concentration of total cholesterol and triglycerides as present in the normal diabetic conditions. However, sesamin and derivatives at a dose level of 500 mg/kg and 250mg/kg reversed the diabetes-induced hyperlipidemia compared to the diabetic control group. In extract treated rats, there was a reduction in the levels of cholesterol and triglycerides, showing the hypolipidemic effect of this plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis. In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia.[24,26] The significant reduction of serum lipid levels in diabetic rats after treatment with extracts of Sesamin derivatives may be directly attributed to improvements in insulin levels.

CONCLUSION

In this study the effectiveness of synthetic sesamine derivative-04 in normal and alloxan induced rats was studied. The study included 30 Wistar strain albino rats and randomly divided into 5 equal groups, normal rats (Only fed with normal rat feed), alloxan monohydrate 150mg/kg BW rats, sesamin derivative 10mg/kg BW, alloxan monohydrate 150mg/kg BW rats+ sesamin derivative 10mg/kg BW, Alloxan monohydrate, reference control i.e., Standard drug Glibenclamide 10 mg/kg BW was administered for 28 days for various biochemical analysis. The induction of diabetes has caused significant initial increase in the fasting blood glucose levels of all the groups. The diabetic control group shows significant increase throughout the study period when compared with the normal control group (p<0.001). However, the extract treated groups and the standard treated group shows significant decrease in the fasting blood glucose levels when compared with diabetic control (p<0.001) which was determined on the 7th and 14th day of experiment. SGOT, SGPT and ALP, show significantly lower levels of SGOT, SGPT and ALP in comparison to the diabetic control group (p<0.001). Fat accumulation in liver and inflammation were reduced with sesamine derivative-04. Sesamine derivative -04 can provide a safe and effective option that may be useful in clinical practice to lower hyperglymia.

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