doi.org/10.36721/PJPS.2026.39.5.REG.15213.1

Synthesis, characterization, blood biochemical parameters evaluation and antidiabetic potential of thiobarbiturate derivatives

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Abstract: **Background**: Diabetes mellitus (DM) is a long-term metabolic disorder that poses a significant challenge to healthcare systems worldwide and is considered one of the top five leading causes of death globally. **Objectives**: The current study was designed to evaluate the two newly synthesized thiobarbituric acid analogues for anti-hyperglycemic potential. **Method**: In experimental animal diabetes was induced using alloxan. The compounds designated as SL1 and SL2 were fed to animals to ameliorate the symptoms of induced diabetes. Level of blood glucose and weight of animals along with other biochemical parameters were monitored daily for four weeks. Antioxidant enzymes catalase, superoxide dismutase and malondialdehyde (MDA) levels were also evaluated. **Results**: Safety profile of compounds SL1 and SL2 was confirmed up to 250 mg/kg. Groups treated with SL1 had a substantial reduction in blood glucose levels (127.25±4.81mg/dL and 115.61±4.65 mg/dL) at respective doses, whereas for SL2 the recorded values were 148.98±4.36 mg/dL and 129.81±4.59 mg/dL (p<0.001). The diabetic group of animals treated with SL1 and SL2 significantly reduced HbA1c (p <0.001), total cholesterol (p<0.001), low-density lipoprotein (LDL), triglycerides (p<0.001), alkaline phosphatase (ALP), bilirubin and creatinine. Furthermore, the activity of superoxide dismutase (SOD) and catalase (CAT) was effectively enhanced in the treated groups (p<0.001). **Conclusion**: Both the compounds were significantly effective in normalizing the symptoms of induced diabetes and changes brought about in other blood parameters in experimental animals.

Keywords: Antioxidant enzymes; Blood glucose level; Diabetes; Mice; Serum biomarkers; Thiobarbituric acid analogues

Submitted on 15-09-2025 – Revised on 25-10-2025 – Accepted on 13-11-2025

INTRODUCTION

A huge and growing threat to the general population, healthcare systems and economy is posed by diabetes mellitus outbreak across the world because of long-term cascade of diseases that is set off by diabetes manifestation (Goodman and Gilman, 1975). DM is a complicated condition characterized by increased hepatic glucose synthesis, diminished insulin production and decreased insulin action ultimately contributing to an increase in blood glucose levels and it is reported that around 5% of human fatalities can be attributed to diabetes. Diabetes affects the blood vessels, causing them to constrict and so reduce blood flow producing a wide range of complications in the body. It affects the vital organs including the brain, eyes, heart, kidneys and nerves, resulting in paralysis, blindness, heart attack, loss of sensation in the feet (diabetes-related nephropathy) and chronic renal failure (Saeedian Moghadam *et al*., 2021). In particular, the Eastern Mediterranean showed the greatest starting prevalence of diabetes mellitus in 1980, subsequently followed by the fastest speed of the epidemic noticed by a 0.23% yearly increase that led to a greater incidence (2.3-fold) in 2014 (Wang *et al*., 2025). Contrarily, although having the second-highest incidence in the world in 1980, the diabetes pandemic trends were better managed in Europe than in any other area in the world (Duarte *et al*., 2018). The International Diabetes Federation predicts that by 2045, 693 million people will have diabetes (Ali *et al*., 2020).

Pancreatic amylase (digestive enzyme) operates as a catalyst in reactions involving the hydrolysis of the 1,4 glycosidic bonds of starch, glycogen, amylose, amylopectin and various maltodextrins (Corkovic *et al*., 2022). The other major enzyme is glucosidase that catalyzes the last phase of carbohydrate digestion mostly starch, by acting on 1,4 alpha bonds and creating glucose as the end result (Dirir *et al*., 2022). Larger molecules like starch, cannot cross the blood brain barrier (BBB). The brain solely relies on its nourishment molecule, glucose, therefore it must be able to pass through the BBB. To counter this issue, amylase breaks the complex starch molecule into simple sugar in order to pass the barrier. If

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there is a surplus conversion to sugars from starch, the sugar level in blood will rise and insulin will manage to metabolize the surplus sugar molecules. However, in some cases, high blood sugar levels rise, resulting in hyperglycemia due to amylase enzyme hyperactivity and insulin resistance or shortage. The suppression of amylase enzyme activity is being considered for the treatment of hyperglycemia. However, excessive inhibition of pancreatic alpha-amylase might result in bacterial fermentation of undigested carbohydrates in the colon, causing gas and diarrhea (Agarwal and Gupta, 2016). Derivatives of barbituric acid have been reported to have antihypertensive (Park *et al*., 2008), anti-cancer (Penthala *et al*., 2010), anti-convulsant (Goodman and Gilman, 1955), anti-inflammatory (Radwan *et al*., 2007), anti-psychotic (Madadi *et al*., 2013) and anti-diabetic agents (Barakat *et al*., 2015). On the other hand, thiobarbituric acid analogues have been reported to have anti-inflammatory (Radwan *et al*., 2007), anticonvulsant (Cliffs *et al*., 1977) and anti-hypnotic (Cliffs *et al*., 1977), α-glycosidase inhibition effects (Barakat *et al*., 2017). Synthetic barbiturates as well as thiobarbiturates have been documented for their *in-vitro* alpha amylase inhibitory action and antioxidant effects (Kulsoom *et al*., 2022). Based on the therapeutic significance of barbituric acid derivatives, the search of new compounds containing thiobarbituric acid moieties is a desirable goal, especially given the importance of thiobarbituric acid (TBA) derivatives in medicinal chemistry.

MATERIALS AND METHODS

Chemicals

Chemicals used in this study were of synthetic grade and purchased from local supplier. TLC plate (Kiesel gel WG, Merck), FTIR spectra were determined by Bruker Tensor 37 FTIR. ^{1}H-NMR and 13C-NMR spectra were determined by JEOL spectrophotometers. Chemical shifts (δ) are expressed in parts per million. SOD ELISA kit (Sigma-Aldrich, Germany: CS0009), thiobarbituric acid (TBA; Sigma-Aldrich, Germany, CAS Number: 504-17-6), H_2O_2 solution (Sigma-Aldrich, Germany, CAS 7722-84-1) and Tween-80 (Merck, Germany) were purchased for analysis.

Synthesis

The selected novel thiobarbiturate derivatives (SL1 and SL2) were synthesized by reacting equimolar amount of *N,N*-diethylthiobarbituric acid with substituted benzaldehydes (2,3,4-trihydroxybenzaldehyde and 3-nitrobenzaldehyde) under reflux for about 30 minutes at room temperature (Khan *et al*., 2019) (Fig. 1).

1,3-Diethyl-2-thioxo-5-(2,3,4-trihydroxybenzylidene) dihydropyrimidine-4,6(1H,5H)-dione (SL1)

^{1}H-NMR: (300 MHz, DMSO-d_6): 1.18 (t, 6H, J = 6.9 Hz, -$(CH_3)_2$), 4.42 (q, 4H, J = 6.9 Hz, $(NCH_2)_2$), 6.44 (d, 1H, $J_{5,6}$ = 9.0 Hz, H-5), 8.35 (d, 1H, $J_{6,5}$ = 9.0 Hz, H-6), 8.34 (br.s, 1H, -OH), 8.92 (s, 1H, *vin*. H), 10.07 (br.s, 1H, -OH), 10.87 (br.s, 1H, -OH). The structure of synthetic molecule SL1 was determined through H-NMR spectroscopy. The physical properties (m.p, solubility and R_f), C-NMR, MS: m/z and CHN analyses were found to be satisfactory and in agreement with the calculated values (Khan *et al*., 2019).

1,3-Diethyl-5-(3-nitrobenzylidene)-2-thioxodihydro-pyrimidine-4,6(1H, 5H)-dione (SL2)

^{1}H-NMR (300 MHz, DMSO-d_6): 1.19 (t, 6H, J = 6.6 Hz, -$(CH_3)_2$), 4.45 (q, 4H, J = 6.6 Hz,-$(NCH_2)_2$), 6.36 (s, 1H, *vin*. H), 7.52-7.44 (m, 2H, H-4/5), 7.75 (s, 1H, H-2), 7.97 (d, 1H, $J_{6,5}$ = 7.5 Hz, H-6). The structure of synthetic molecule SL2 was determined through H-NMR spectroscopy. The physical properties (m.p, solubility and R_f), C-NMR, MS: m/z and CHN analyses were found to be satisfactory and in agreement with the reported values (Khan *et al*., 2019).

Animals

Mice (Balb/C) 22–28 gm were procured from the VRI (Veterinary Research Institute, Peshawar) and kept under suitable conditions in the animal house by providing a standard diet and water, with a temperature range of 25 ± 2°C and humidity of 55–65%, and a 12 h light/dark cycle.

Assessment of acute toxicity study

Acute toxicity was carried out as per Lorke method with slight modification in two phases. In Phase-1 animals received different oral low doses (10, 25 and 50 mg/kg, n=4) and the animals were observed for 24 to 72 h. In 2nd phase, three other groups. each group consisted of four mice (n=4) were given higher doses of (75, 125 and 250 mg/kg b.w, respectively). The animals were observed for 24 to 72 h, followed by 14 days for signs of fatigue, drooling, convulsions, tremors, drowsiness and change in body weight (Lorke, 1983).

Induction of type II diabetes

For induction of diabetes with slight modification, alloxan (150 mg/kg) was administered intraperitoneally (i.p.) to the overnight fasted animals (72 in number). Post 72 hours of alloxan administration blood glucose level was determined using SD glucometer (Accu-Chek Active blood glucose meter, Korea). The animals with blood glucose levels higher than 250 mg/dL were selected for study (Ndile *et al*., 2025).

Experimental protocol for antidiabetic activity

After the induction, animals were divided into 7 groups (n=8) test groups receiving 3 mg and 6 mg/kg b.w of SL1 and SL2 were administered, diabetic and control groups were administered with Tween-80 and the standard group received standard drug. The samples (3 mg and 6 mg/kg b.w) in Tween-80 (2%) and standard were administered orally using feeding tube. The data was recorded from number of animals in group (n=8) that were survived

during the entire study. The animals were excluded from study that died during induction and 28 days treatment (Fig. 2).

Estimation of weight and blood glucose level

On the first day of each of the four weeks of treatment, weight and blood glucose of animals were measured (Kumar *et al*., 2016).

Evaluation of serum profile

After 28th day of the study animals were humanely sacrificed using isoflurane and blood samples from all groups were taken by cardiac puncture to assess biochemical parameters including serum profile (Doğan and Çelik, 2016). For MDA estimation, 1 mL 10% TCA, 0.5 mL distilled water and 0.5 mL volume of sample were mixed well and centrifuged (3000 rpm) followed by addition of 0.375% of thiobarbituric acid to get absorbance using a spectrophotometer. Superoxide dismutase level was estimated by mixing the sample with 20 µL of enzyme solution, 200 µL of working solution and 20 µL buffer, which was mixed together followed by incubation at 37°C for 20 min. The absorbance was noted at 450 nm using a spectrophotometer. The level of catalase was determined by mixing 1 mL of the sample, 5mL of phosphate buffer (pH 7.0 having pH 7.0 and addition of 4 mLof H_2O_2 at room temperature. Changes in absorbance were recorded using a spectrophotometer at 570 nm (Wahid *et al*., 2022).

Statistical analysis

Each data point (n = 8) was represented as mean ± SEM. Significance of variance was evaluated by using GraphPad Prism. One-way ANOVA was performed following which Dunnett's post hoc multiple tests were carried out.

RESULTS

Acute toxicity studies

Following the administration of thiobarbiturate derivatives (SL1 and SL2), none of the animals showed evidence of discomfort, death, or behavioral abnormalities. The weight of animals in each group was normal and animals did not show any promising changes in gross morphology of vital organs. Therefore, up to 250 mg/kg body weight (b.w), these compounds are safe. The effective doses 3 and 6 mg/kg were selected upon acute toxicity test.

Determination of blood glucose level

After treatment on days 14th and 21st, groups treated with SL1 had significantly lower blood glucose levels. In contrast to the diabetic group (511.75±4.65 mg/dL, n= 8, p<0.001), the level was calculated as 344.17±4.91 mg/dL and 301.52±4.60 mg/dL on the fourteenth day respectively at 3 mg and 6 mg/kg (b.w). SL1 lowered glucose level to 249.15±4.77 mg/dL and 207.28±4.79 mg/dL (p<0.01, p<0.001) on the 21st day at 3 mg and 6 mg/kg doses, showing further significant decreases in contrast to the diabetic control (517.80±4.87 mg/dL, p<0.001, n = 8). After 4 weeks (28 days) of therapy, the glucose level in the treated groups was significantly lower than in the diabetic animal group; at 3mg and 6mg/kg of SL1, the levels were measured at 127.25±4.81 mg/dL and 115.61±4.65 mg/dL (p<0.001, n=8), respectively (Fig. 3).

Similarly, SL2 administered groups at 3mg and 6 mg/kg significantly decreased the blood glucose after the second and third week of administration. In association to the diabetes control group (517.80±4.87 mg/dL, n=8, p<0.001), the glucose levels for SL2 were measured as follows: 379.84±4.98 mg/dL and 346.05±4.96 mg/dL on the 14th day and 275.03±4.80 mg/dL and 231.56±4.73 mg/dL on the 21st day (p<0.001, n = 8) for 3mg and 6mg/kg, respectively. Significant decreases in glucose levels were detected at the end of the 28day treatment, with levels of 148.98±4.36 mg/dL and 129.81±4.59 for the group of animals treated with SL2 at 3 mg and 6 mg/kg.

Effect on body weight

Over the course of 28-day trial period, effect of medication on body weight of mice was determined. Considerable reduction in body weight in diabetic group was noticed when compared to normal animals. The treated groups' body weight loss was reversed (Table 1).

Effect of thiobarbiturate derivatives (SL1 and SL2) on HbA1c level

The HbA1c levels of the diabetic control group's animals significantly increased (p<0.001, n=8). In contrast to the diabetic control group of animals, the sample-treated groups (SL1 and SL2) demonstrated a statistically significant decrease in HbA1c levels (Table 2).

Estimation of serum profile

In this investigation, compared to normal animals, Alloxan administration increased the plasma level of triglycerides, LDL and total cholesterol (Table 3). Nevertheless, the lipid profile values were dramatically altered after treatment with the sample.

Effect on liver function tests

The assessment of abnormal liver and renal function resulting from diabetes was conducted through the measurement of serum levels of ALP, bilirubin, urea and creatinine. The result of serum profile levels after the test samples were administered suggests that the damage to the liver tissue has been reversed and the plasma membrane has stabilized (Table 4).

Investigation of stress marker levels and antioxidant enzymes

MDA is a byproduct of lipid peroxidation and a marker of tissue degradation; it was shown that the diabetic group had higher levels of this compound. On the other hand,

Table 1: Effect of SL1 and SL2 on body weight of animals.

Treatment groups		Body weight (mg) 1st day	Body weight (mg) 14th day	Body weight (mg) 28th day
Control (normal)		23.45±3.87	24.31±3.70	26.06±4.96
SL1	3 mg/kg	24.60±5.05	25.09±4.83**	24.73±4.80**
	6 mg/kg	25.19±4.96	24.77±4.58**	25.16±4.76***
SL2	3 mg/kg	23.84±4.89	23.50±4.81**	22.71±4.67*
	6 mg/kg	26.11±5.40	25.28±4.94***	24.38±4.90**
Diabetic control		24.67±4.93	22.78±4.70!!!	21.80±4.66!!!
Glibenclamide	0.5 mg/kg	24.06±4.87	24.81±4.81***	25.03±4.68***

Data has been reported as mean with SEM, data was evaluated statistically and was considered significant when !!! $p<0.001$ by comparing to normal animals and * represent $p<0.05$, ** represent $p<0.01$ and *** represent $p<0.001$ upon comparing to diabetic group.

Table 2: Effect of SL1 and SL2 on HbA1c level.

Treatment groups		HbA1c (%)
Normal control		5.04
SL1	3 mg/kg	5.77***
	6 mg/kg	5.56***
SL2	3 mg/kg	6.41**
	6 mg/kg	6.17**
Diabetic control		10.70!!!
Glibenclamide	0.5 mg/kg	5.44***

Data has been reported as mean with SEM, data was evaluated statistically and was considered significant when !!! $p<0.001$ by comparing to normal animals and * represent $p<0.05$, ** represent $p<0.01$ and *** represent $p<0.001$ upon comparing to diabetic group.

Table 3: Effects of SL1 and SL2 on antihyperlipidemic profile.

Treatment groups		Total cholesterol (mg/dL)	LDL (mg/dL)	TG (mg/dL)
Normal control		71.75±3.98	29.15±1.87	86.50±3.19
Diabetic control		250.82±4.91!!!	122.59±2.62!!!	235.87±4.16!!!
Glibenclamide (0.5)	mg/kg	78.61±3.51***	30.27±2.35***	82.71±2.79***
SL1	3 mg/kg	73.89±2.97***	54.73±2.34**	118.90±2.11**
	6 mg/kg	62.70±2.81***	47.62±2.01***	101.29±1.93***
SL2	3 mg/kg	105.31±3.81**	63.70±2.66**	129.44±2.67**
	6 mg/kg	85.06±2.92**	48.62±2.61***	116.26±2.41***

Data has been reported as mean with SEM, data was evaluated statistically and was considered significant when !!! $p<0.001$ by comparing to normal animals and * represent $p<0.05$, ** represent $p<0.01$ and *** represent $p<0.001$ upon comparing to diabetic group.

Table 4: Effect of SL1 and SL2 on liver function test

Treatment groups		ALP (IU)	Urea (mg/dL)	Bilirubin (mg/dL)	Serum creatinine (mgldL)
Normal control		121.44±2.91	21.50±1.97	0.91±0.09	0.61±0.21
Diabetic control		240.67±3.98!!!	329.70±4.11!!!	0.62±0.07!!!	3.19±0.49!!!
Glibenclamide	0.5 mg /kg	124.60±3.49***	20.18±1.88***	0.93±0.06***	0.68±0.24***
SL1	3 mg/kg	151.87±4.12**	60.11±2.51*	0.77±0.05*	0.75±0.33*
	6 mg/kg	144.12±4.31***	37.59±2.03***	0.84±0.11***	0.73±0.27**
SL2	3 mg/kg	166.70±4.39*	59.18±2.90**	0.79±0.19*	0.81±0.53*
	6 mg/kg	149.81±3.98***	45.21±2.67**	0.87±0.06***	0.77±0.34*

Data has been reported as mean with SEM, data was evaluated statistically and was considered significant when !!! $p<0.001$ by comparing to

the MDA level significantly decreased in the diabetic groups treated with the sample, suggesting a decrease in tissue damage and lipid peroxidation. Additionally, SL1 and SL2 treated groups had significantly higher levels of the antioxidant enzyme superoxide dismutase (SOD), reaching levels that were comparable to the diabetic group of animals ($p<0.001$, n=8). When other factors were taken into account, comparable outcomes were seen in the activity of catalase (CAT), an antioxidant enzyme, indicating that the samples may have a protective effect due to their antioxidant qualities (Table 5).

Table 5: Effect of SL1 and SL2 on stress markers level and antioxidant enzymes.

Treatment groups		TBARS (nmol MDA/mg protein)	CAT (mU/mg protein)	SOD (mU/mg protein)
Control (normal)		1.71±0.44	31.87±1.80	11.09±1.41
Diabetic control		4.01±0.57 $^{!!!}$	7.96±0.96 $^{!!!}$	2.21±0.11 $^{!!!}$
Glibenclamide	0.5 mg/kg	1.89±0.40***	27.89±2.11***	9.03±0.83***
SL1	3 mg/kg	2.11±0.34**	26.50±2.02***	7.58±0.81***
	6 mg/kg	2.07±0.40**	27.11±1.97***	8.30±0.96***
SL2	3 mg/kg	2.39±0.49**	24.92±2.07**	7. 34±0.67**
	6 mg/kg	2.08±0.44**	27.41±2.10**	7.86±0.80***

Data has been reported as mean with SEM, data was evaluated statistically and was considered significant when $^{!!!}$ p<0.001 by comparing to normal animals and * represent p<0.05, ** represent p<0.01 and *** represent p<0.001 upon comparing to diabetic group.

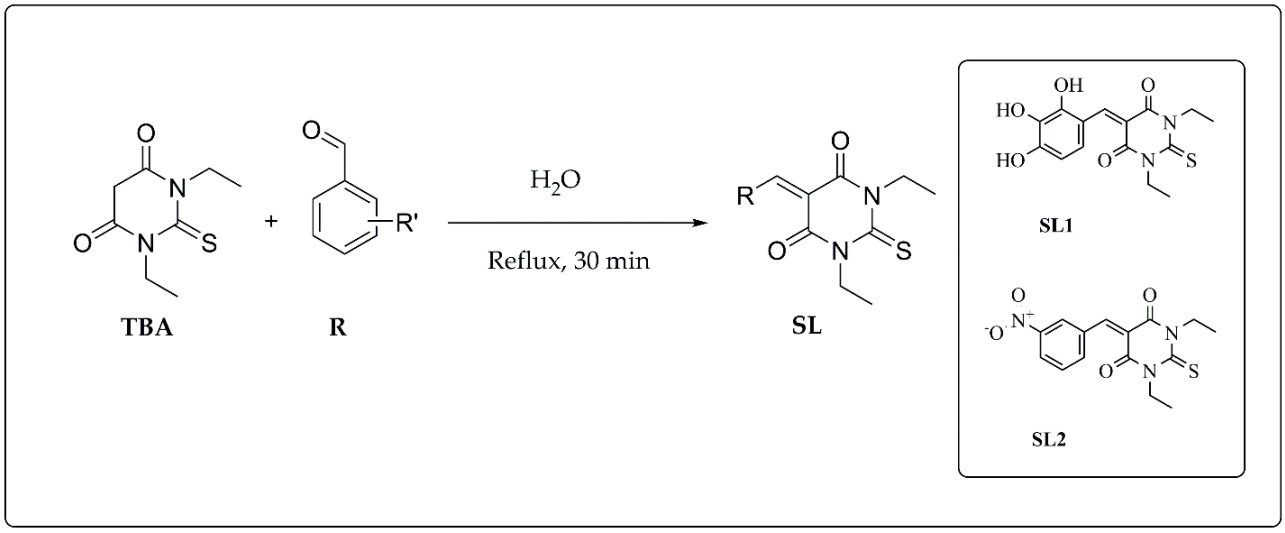

Fig. 1: Scheme for thiobarbiturate derivatives (SL1 and SL2)

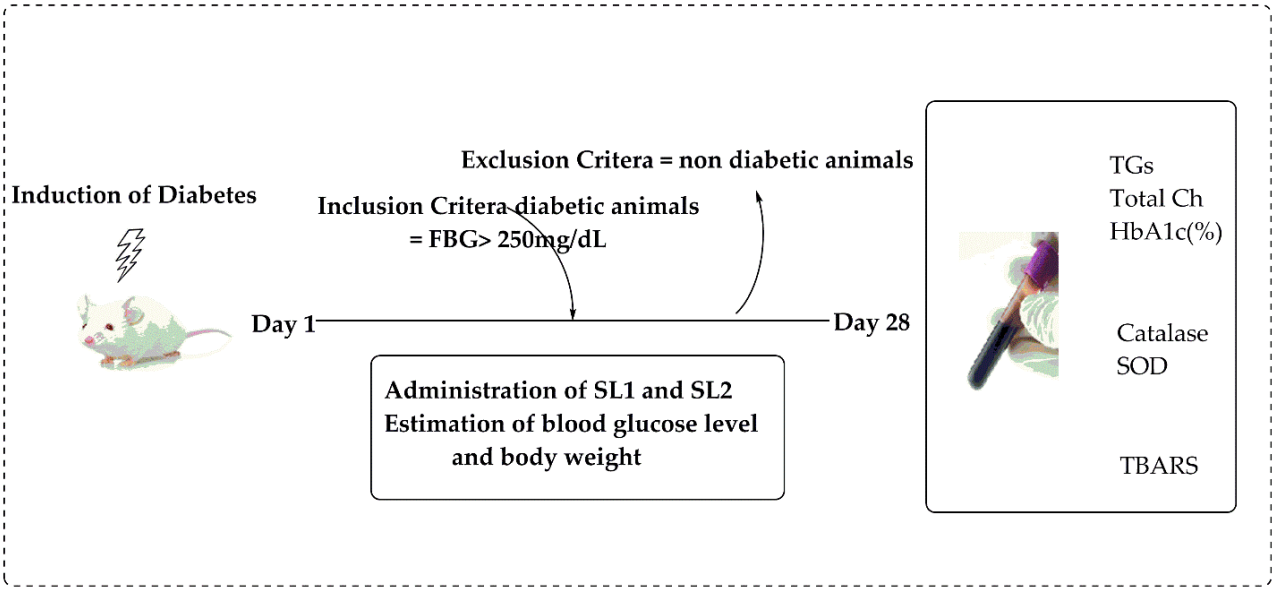

Fig. 2: Treatment schedule and assessment flow chart of study.

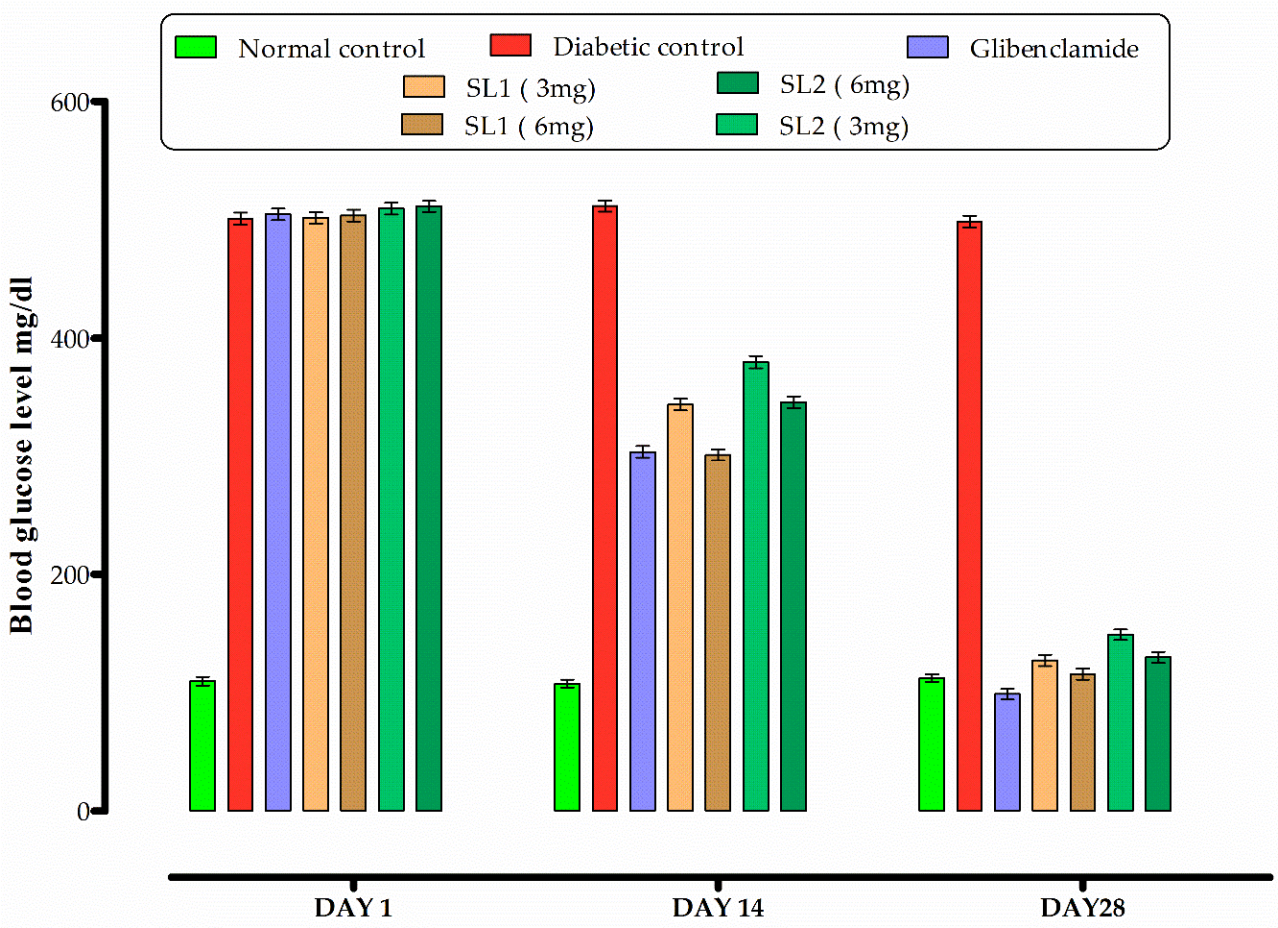

Fig. 3: Effect of SL1 and SL2 on blood glucose level.
Data has been reported as mean with SEM, data was evaluated statistically and was considered significant when $^{!!!}$ $p<0.001$ by comparing to normal animals and * represent $p<0.05$, ** represent $p<0.01$ and *** represent $p<0.001$ upon comparing to diabetic group.

DISCUSSION

Globally, about 415 million individuals suffer from diabetes and by 2040, the figure is expected to increase to 642 million (Roglic *et al*., 2016). A typical hallmark of diabetes mellitus (DM) is abnormalities in the metabolism of carbohydrates, fats and proteins. These abnormalities are produced by insulin's ineffectiveness on target tissues, namely skeletal muscle, body fat and the liver (Deshmukh *et al*., 2015).

Following digestion, the resultant glucose is absorbed into the bloodstream, raising the amount of postprandial hyperglycemia (Shibib *et al*., 2024). The streptozotocin and alloxan are the most widely utilized diabetogenic agents to evaluate the antidiabetic potentials of test sample in diabetic model (Macdonald Ighodaro *et al*., 2017). The results of this investigation indicate that the pretreatment of alloxan-induced diabetic mice with SL1 and SL2 has a modulatory effect and decreases the fasting blood glucose level in groups, suggesting the antidiabetic potential in comparison to the positive control group.

Dyslipidemia is associated with diabetes and leads to vascular complications as well as neurodegenerative processes. In diabetic animal model, the levels of cholesterol, LDL and triglycerides were elevated and level of HDL declined. These results are according to the previously reported studies that diabetes intensifies oxidative stress which enhances neurodegeneration (Chatterjee *et al*., 2020). Mitigating the lipid profile may have a dual effect on diabetes as well as on cognitive outcomes (Zeng *et al*., 2025). The elevated level of liver enzymes such as ALT and AST and dysregulated glucose in diabetic animals are the signs of this hepatic damage (Daneshvar-Ghahfarokhi *et al*., 2025).

Several findings have established a strong relation of oxidative stress between diabetes and neurodegeneration supporting the findings in the current study. The level of catalase and SOD is decreased while MDA level has increased in oxidative stress leading to neuronal damage. Studies reported previously have confirmed that oxidative stress is a factor in memory impairments and neuronal loss (Nandi *et al*., 2019). The compounds SL1 and SL2

have shown a positive modulatory impact on the stress biomarkers in diabetes model.

Medicinally, the SL1 possessed promising effects in comparison to SL2 based on the structural chemical nature. In SL1 compound the attachment of three hydroxyl groups to benzene ring has increased the overall electronic density of molecule by donating electrons making it more promising. On the other hand, in SL2 compound the strong electron withdrawing nitro group is pulling the electron density towards itself thus decreasing the overall electron density of the molecule. Considering this study, it may be concluded that the compounds synthesized in this study have the ability to reverse the changes caused by diabetes, the compounds are safe up to 250 mg/kg and even at small doses such as 3 and 6 mg/kg can produce significant results.

CONCLUSION

This study aimed to synthesize two novel analogues of thiobarbituric acid designated as SL1 and SL2 and explore their potential against hyperglycemia by using alloxan induced diabetic model as well as enzymatic activities were also carried out. Acute toxicity findings in this study suggest that these compounds are safe up to 250mg/kg. Results find that both compounds possess significant anti-hyperglycemic activity and lower blood glucose levels substantially compared to control group. SL1 and SL2 improved biochemical parameters linked to diabetes like glycated hemoglobin (HbA1c), lipid profile (HDL, LDL, triglycerides and total cholesterol) and serum alkaline phosphatase (ALP). Enzymatic anti-oxidant activities like superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were also evaluated. The study finds that SL1 and SL2 increased SOD and CAT activities while decreasing levels of MDA which testify to the promising anti-oxidant effects of SL1 and SL2. The findings of this research open a new window to explore and identify powerful inflammatory markers by immunohistochemistry, enzyme-Linked immunosorbent assay and Western blot of vital organs associated with diabetes.

Acknowledgements
The authors wish to thank Princess Nourah bint Abdulrahman University Researchers Supporting Project (number PNURSP2026R33), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, for the financial support.

Authors' contributions
SM, MK and SM: Performed the experiments; AK and MK: Conceptualized the study and devised the experimental design; SM, MZ, RU and AA: Wrote the initial draft of the paper. All authors have read the paper and consent to publish the data in this journal.

Funding
The project has received no funding.

Data availability statement
The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval
The study was designed and carried out according to "University of Malakand Animals Byelaws 2008 (Scientific Procedures Issue-I)" and was approved by the Department of Pharmacy Ethical Committee vide notification number Pharm/EC-Tb-SL12/02-10/23.

Conflict of interest
All the authors of this article have no conflict of interest.

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