

## Article

# Antidiabetic, Antihyperlipidemic, and Antioxidant Evaluation of Phytosteroids from *Notholirion thomsonianum* (Royle) Stapf

Mohammad A. Huneif<sup>1</sup>, Shah Fahad<sup>2</sup> , Alqahtani Abdulwahab<sup>1</sup>, Seham M. Alqahtani<sup>1</sup>,  
Mater H. Mahnashi<sup>3,\*</sup> , Asif Nawaz<sup>4</sup> , Fida Hussain<sup>5</sup> and Abdul Sadiq<sup>4,\*</sup> 

- <sup>1</sup> Pediatric Department, Medical College, Najran University, Najran 61441, Saudi Arabia; maalhuneif@nu.edu.sa (M.A.H.); aalsharih@nu.edu.sa (A.A.); drseham2015@gmail.com (S.M.A.)
- <sup>2</sup> Department of Agronomy, Abdul Wali Khan University Mardan, Mardan 23200, KP, Pakistan; shahfahad@awkum.edu.pk
- <sup>3</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Najran University, Najran 61441, Saudi Arabia
- <sup>4</sup> Department of Pharmacy, Faculty of Biological Sciences, University of Malakand, Chakdara 18000, KP, Pakistan; asifnawaz2446@gmail.com
- <sup>5</sup> Department of Pharmacy, University of Swabi, Swabi 23561, KP, Pakistan; fida2k9@yahoo.com
- \* Correspondence: matermaha@gmail.com (M.H.M.); sadiquom@yahoo.com (A.S.); Tel.: +966-508734539 (M.H.M.)

**Abstract:** Diabetes mellitus (DM) is a metabolic complication and can pose a serious challenge to human health. DM is the main cause of many life-threatening diseases. Researchers of natural products have been continuously engaged in treating vital diseases in an economical and efficient way. In this research, we extensively used phytosteroids from *Notholirion thomsonianum* (Royle) Stapf for the treatment of DM. The structures of phytosteroids **NtSt01** and **NtSt02** were confirmed with gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) analyses. Through in vitro studies including  $\alpha$ -glucosidase,  $\alpha$ -amylase, and DPPH assays, compound **NtSt01** was found to be comparatively potent. An elevated dose of compound **NtSt01** was also found to be safe in an experimental study on rats. With a dose of 1.0 mg/kg of **NtSt01**, the effect on blood glucose levels in rats was observed to be  $519 \pm 3.98$ ,  $413 \pm 1.87$ ,  $325 \pm 1.62$ ,  $219 \pm 2.87$ , and  $116 \pm 1.33$  mg/dL on the 1st, 7th, 14th, 21st, and 28th, days, respectively. The in vivo results were compared with those of glibenclamide, which reduced the blood glucose level to  $107 \pm 2.33$  mg/dL on the 28th day. On the 28th day of **NtSt01** administration, the average weights of the rats and vital organs (liver, kidney, pancreas, and heart) remained healthy, with a slight increase. The biochemical parameters of the blood, i.e., serum creatinine, blood urea, serum bilirubin, SGPT (or ALT), and serum alkaline phosphatase, of rats treated with **NtSt01** remained in the normal ranges. Similarly, the serum cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels also remained within the standard ranges. It is obvious from our overall results that the phytosteroids (specifically **NtSt01**) had an efficient therapeutic effect on the blood glucose level, protection of vital organs, and blood biochemistry.

**Keywords:** *Notholirion thomsonianum*; diabetes; in vivo and in vitro studies; phytosteroids; blood biochemistry



**Citation:** Huneif, M.A.; Fahad, S.; Abdulwahab, A.; Alqahtani, S.M.; Mahnashi, M.H.; Nawaz, A.; Hussain, F.; Sadiq, A. Antidiabetic, Antihyperlipidemic, and Antioxidant Evaluation of Phytosteroids from *Notholirion thomsonianum* (Royle) Stapf. *Plants* **2023**, *12*, 3591. <https://doi.org/10.3390/plants12203591>

Academic Editors: Natália Cruz-Martins and Christophe Hano

Received: 15 August 2023

Revised: 3 October 2023

Accepted: 7 October 2023

Published: 17 October 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

As a chronic disease, DM is a metabolic disorder in which insulin production by the pancreas is reduced or the produced insulin may be ineffective, leading to hyperglycemia, which can cause further damage to other body systems like the circulatory and nervous systems. Some of the abnormalities that account for DM include defects in the production of insulin or in its action or secretion and dysfunction in the metabolism of fat, protein, and carbohydrates [1–3]. Polyphagia, polyuria, and polydipsia are some of the symptoms associated with the state of hyperglycemia [4]. There are about 450 million diabetic patients

around the globe, with this figure expected to reach 690 million by the year 2044 [5]. Currently, DM prevalence has been reported as 8.5% of adults globally, and a rapid increase has been observed in countries with a low or middle income [6]. DM is a serious metabolic disease, and chronic hyperglycemia can cause numerous complications and the dysfunction of several organs like the kidneys, nerves, eyes, heart, blood vessels, and liver [2,7,8].

There are different types of DM; the common types are type 1 and type 2 DM. Type 1 DM is insulin-dependent and is due to insulin deficiency, along with an impairment of the  $\beta$  cells of the pancreas [9], whereas type 2 DM is non-insulin-dependent and is due to insulin resistance or decreased insulin secretion [10]: 95% of diabetic patients have type 2 DM [11]. DM development in people with an impaired tolerance of glucose can be prevented or managed with the use of antidiabetic drugs or through changing their lifestyle via exercise, diet control, and/or weight loss [12]. The use of antidiabetic drugs can have number of adverse effects, including hypoglycemia, retention of fluids, osteoporosis, and heart failure, due to which their use is limited [13–15]. Hence, the development of new, effective drugs that have fewer adverse effects is needed to control and manage diabetes. In the development of antidiabetic drugs that are specific for type 2 DM, several biochemical approaches can be used. Among the important biochemical pathways, the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is common. Both of these enzymes break down starch and oligosaccharides into glucose, leading to an increase in the concentration of glucose; hence, their inhibition is important for decreasing glucose absorption in the intestine [16].

For thousands of years, medicinal plants and natural products have been reported for the treatment of many diseases, including diabetes, especially type 2 DM [17–19]. The crude extracts of medicinal plants and their bioactive compounds have been found to be useful in many pharmacological activities [20–22]. Approximately 400 plants have been demonstrated to have antidiabetic activity, but only some of them have been evaluated for their efficacy [23]. A number of natural products of plant origin have been shown to have an antidiabetic activity. The most important reported phytochemicals include alkaloids, carbohydrates, peptidoglycan, amino acids, glycosides, steroids, glycopeptides, galactomannan gum, terpenoids, hypoglycans, guanidine, and inorganic ions [24]. Different plants and microorganisms produce  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors for the regulation of such enzyme activities [25]. Synthetic compounds are also being developed in parallel in order to create  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors [26,27]. Inhibitors of  $\alpha$ -amylase enzymes reduce the conversion of starch into glucose usually after eating a meal, which results in a decrease in the level of glucose in the blood. Therefore,  $\alpha$ -amylase inhibitors are needed to control the glucose levels of diabetic patients.

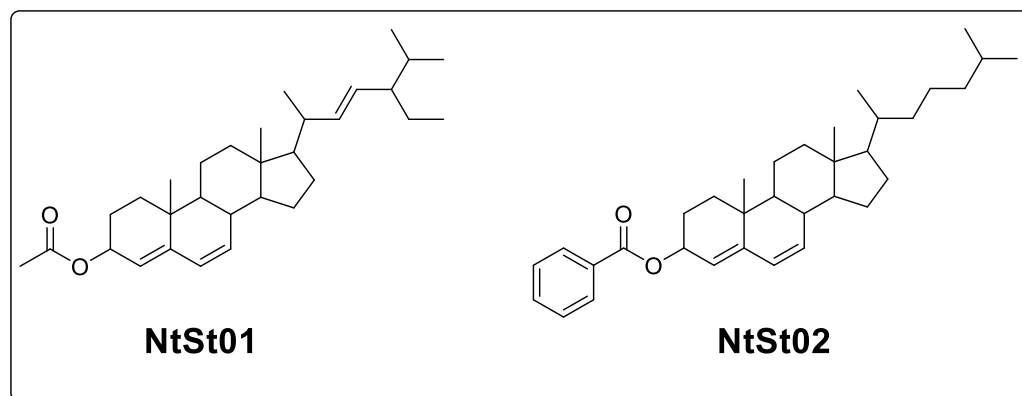
*Notholirion thomsonianum* is a small bulbous plant of the family Liliaceae. This small liliium-like medicinal plant has been studied for various pharmacological effects [28]. The plant can be used to improve the digestive system and in the management of microbial infections [29]. Our group has been exploring the medicinal aspects of this species for a decade. We have previously explored its crude extract for antibacterial, antifungal, and analgesic effects [29,30]. In recent years, we explored the potential of this plant for the management of diabetes mellitus using its various fractions and some bio-guided bioactive compounds following multitarget in vitro and in silico approaches [31]. Based on our previous experience with this plant, its hydroalcoholic extract contains phytosteroids, which have been identified. This current study is extensive compared with our previously published work. In this study, we extensively used these phytosteroids for investigating in vitro and in vivo antidiabetic targets. Furthermore, the beneficial effects of the identified phytosteroids were also explored on the vital organs of the body like the liver, kidney, pancreas, and heart and on blood biochemistry.

## 2. Results

### 2.1. Phytochemistry

In this research, we initially purified and identified two different phytosteroids (**NtSt01** and **NtSt02**, as shown in Figure 1). The isolated amount of **NtSt01** was 830 mg as a white

powder, while 375 mg of **NtSt02** was isolated as a yellowish-brown solid. The structures of these two isolated compounds were initially confirmed with GCMS analysis. The retention time of compound **NtSt01** was 56.964 min, with a base peak value of 55.1 (Table S1 Supporting Information). The fragmentation pattern of compound **NtSt01** is shown in the Figure S1 of the Supporting Information. The spectrum and its fragmentation pattern were compared with the library spectrum and the difference spectrum, as shown in Figures S2 and S3 of the Supporting Information, respectively. The chemical name of the identified compound **NtSt01** is 3- $\beta$ -Acetoxystigmasta-4,6,22-triene with an IUPAC name of (E)-17-(5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate. Similarly, from the same GCMS analysis, the compound **NtSt02** was observed at a retention time of 57.812 min, with a major peak at  $m/z$  135 (Table S2 of Supporting Information). The fragmentation pattern of the compound **NtSt02** is shown in Figure S4 of the Supporting Information. This spectrum and its fragmentation pattern were compared with the library spectrum and difference spectrum, as shown in Figures S5 and S6 of the Supporting Information, respectively. The chemical name of the identified compound **NtSt02** is 4,6-cholestadien-3 $\beta$ -ol, benzoate, and the IUPAC name is 10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl benzoate.



**Figure 1.** Chemical structures of the identified phytosteroids in *Notholirion thomsonianum*.

### 2.2. Alpha Glucosidase Inhibition

The in vitro  $\alpha$ -glucosidase inhibitory results of both phytosteroids of *N. thomsonianum* (**NtSt01** and **NtSt02**) are shown in Table 1. The percent inhibitions were recorded on all concentrations in triplicate. **NtSt01** was three times more potent than **NtSt02**, as observed from their respective  $IC_{50}$  values. **NtSt01** exhibited inhibitions of  $85.00 \pm 1.52$ ,  $81.52 \pm 1.85$ ,  $77.63 \pm 1.56$ ,  $68.78 \pm 1.02$ , and  $61.22 \pm 0.85\%$  at experimental concentrations of 500, 250, 125, 62.50, and 31.25  $\mu\text{g/mL}$ , respectively. The  $IC_{50}$  values of **NtSt01** and **NtSt02** were 7.34 and 22.87  $\mu\text{g/mL}$ , respectively, in comparison to the standard drug, acarbose, with an  $IC_{50}$  value of 2.14  $\mu\text{g/mL}$ .

### 2.3. Alpha Amylase Inhibition

The in vitro  $\alpha$ -amylase activities of **NtSt01** and **NtSt02** were also analyzed in comparison to the standard acarbose, as shown in Table 2. Likewise, **NtSt01** was found with very practical result which was eleven folds more potent than **NtSt02**. The  $IC_{50}$  value of **NtSt01** and **NtSt02** were 4.17 and 46.73  $\mu\text{g/mL}$  respectively in comparison to the standard drug acarbose with the  $IC_{50}$  value of 1.96  $\mu\text{g/mL}$ . The **NtSt01** demonstrated percent inhibitions of  $80.03 \pm 2.11$ ,  $75.52 \pm 0.96$ ,  $71.63 \pm 0.92$ ,  $67.63 \pm 2.51$  and  $62.35 \pm 1.78\%$  at experimental concentrations of 500, 250, 125, 62.50 and 31.25  $\mu\text{g/mL}$  respectively.

**Table 1.** Alpha-glucosidase inhibitions of the phytosteroids.

Comp/Standard	Conc (µg/mL)	Percent Inhibition (Mean ± SEM)	IC <sub>50</sub> (µg/mL)
NtSt01	500	85.00 ± 1.52 ***	7.34
	250	81.52 ± 1.85 ***	
	125	77.63 ± 1.56 ***	
	62.50	68.78 ± 1.02 ***	
	31.25	61.22 ± 0.85 ***	
NtSt02	500	77.56 ± 3.22 ***	22.87
	250	70.63 ± 2.45 ***	
	125	64.52 ± 3.15 ***	
	62.50	59.98 ± 1.88 ***	
	31.25	52.63 ± 1.52 ***	
Standard Drug	500	92.65 ± 0.55	2.14
	250	89.53 ± 1.45	
	125	83.89 ± 2.65	
	62.50	78.63 ± 1.98	
	31.25	70.52 ± 2.63	

All the values are expressed as mean ± SEM compared with the standard. Two-way ANOVA followed by Dunnett's test was applied. \*\*\*, significantly different ( $p < 0.001$ ) compared with standard drug.

**Table 2.** Alpha amylase inhibitions of the phytosteroids.

Comp/Standard	Conc (µg/mL)	Percent Inhibition (Mean ± SEM)	IC <sub>50</sub> (µg/mL)
NtSt01	500	80.03 ± 2.11 **	4.17
	250	75.52 ± 0.96 **	
	125	71.63 ± 0.92 **	
	62.50	67.63 ± 2.51 **	
	31.25	62.35 ± 1.78 **	
NtSt02	500	74.99 ± 1.53 ***	46.73
	250	64.32 ± 1.85 ***	
	125	60.04 ± 0.86 ***	
	62.50	53.10 ± 2.05 ***	
	31.25	46.84 ± 0.67 ***	
Standard Drug	500	91.01 ± 1.36	1.96
	250	87.79 ± 1.27	
	125	82.33 ± 1.00	
	62.50	75.63 ± 0.86	
	31.25	71.07 ± 1.82	

All the values are expressed as mean ± SEM compared with the standard. Two-way ANOVA followed by Dunnett's test was applied. \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  compared with standard drug.

#### 2.4. Antioxidant Assay

Antioxidant activity is a very important supplementary activity in antidiabetic studies. Antioxidants combat excessive free radicals within the body. This concept applies to the reduction of free radicals in the pancreas, which partially protects the pancreas from damage and helps with diabetic control. Keeping this concept in mind, we also determined the antioxidant activity of the phytosteroids. Though both of our phytosteroids demonstrated low antioxidant activity profiles, they were both notable as supplementary targets. The observed IC<sub>50</sub> values for NtSt01 and NtSt02 were 142.76 and 223.43 µg/mL, respectively, as shown in Table 3.

**Table 3.** Antioxidant potential of the phytosteroids.

Comp/Standard	Conc (µg/mL)	Percent Inhibition (Mean ± SEM)	IC <sub>50</sub> (µg/mL)
<b>NtSt01</b>	500	61.05 ± 2.05 ***	142.76
	250	55.79 ± 0.92 ***	
	125	47.08 ± 2.41 ***	
	62.50	42.53 ± 0.69 ***	
	31.25	37.25 ± 2.66 ***	
<b>NtSt02</b>	500	59.12 ± 1.96 ***	223.43
	250	52.01 ± 1.07 ***	
	125	42.99 ± 2.70 ***	
	62.50	33.08 ± 1.17 ***	
	31.25	28.52 ± 2.63 ***	
<b>Standard Drug</b>	500	91.52 ± 2.52	0.74
	250	85.04 ± 0.63	
	125	81.54 ± 1.28	
	62.50	77.87 ± 1.49	
	31.25	75.37 ± 1.69	

All the values are expressed as mean ± SEM compared with the standard. Two-way ANOVA followed by Dunnett's test was applied. \*\*\* =  $p < 0.001$  compared with the standard drug.

### 2.5. In Vivo Results

Based on the enzymatic assays, we observed that **NtSt01** is a potential inhibitor of  $\alpha$ -glucosidase and  $\alpha$ -amylase. With these results, we further analyzed the compound **NtSt01** in in vivo studies using experimental rats following ethical guidelines. The dose of **NtSt01** was started at 200 and was increased up to 2000 mg/kg body weight. The rats remained healthy; no morbidity, mortality or irritation was observed for the tested doses during the acute toxicity studies.

Phytosteroid **NtSt01** (1.0 mg/kg) was administered to alloxan-induced fasting diabetic rats, and the blood glucose levels were observed until 28 days, as shown in Table 4. The blood glucose level in the normal control group remained within the normal range during the observational time. In contrast, the blood glucose level of the diabetic control group remained elevated throughout the experiment. At 1.0 mg/kg of **NtSt01**, the effect on the blood glucose levels in rats were  $519 \pm 3.98$ ,  $413 \pm 1.87$ ,  $325 \pm 1.62$ ,  $219 \pm 2.87$  and  $116 \pm 1.33$  mg/dL on days 1, 7, 14, 21, and 28, respectively. In comparison, in the glibenclamide control group (0.5 mg/kg), the blood glucose level dropped from  $517 \pm 0.77$  (day 1) to  $103 \pm 1.70$  mg/dL (day 28).

**Table 4.** Observational changes in blood glucose level in alloxan-induced fasting diabetic rats in mg/dL.

Groups	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	102 ± 2.36	106 ± 0.98	105 ± 1.77	110 ± 0.91	109 ± 1.07
Diabetic control	521 ± 1.28	517 ± 2.06	526 ± 1.00	524 ± 2.66	529 ± 2.80
Glibenclamide (0.5 mg/kg)	517 ± 0.77	398 ± 2.08	302 ± 1.38	207 ± 1.91	103 ± 1.70
NtSt01 (1.0 mg/kg)	519 ± 3.98	413 ± 1.87	325 ± 1.62	219 ± 2.87	116 ± 1.33

During the observational time period, the effect on the body weight (in grams) of the rats was also closely observed (as shown in Table 5). In the normal control group, the body weight of the rats remained the same throughout the four weeks. In the diabetic control group, we observed a body weight loss of 18 g of rats at four weeks. With the administration of phytosteroid **NtSt01** at a concentration of 1.0 mg/kg, the rats became healthy, and there was a gain in body weight. The body weight changed from  $238 \pm 0.33$  g

(day 1) to  $246 \pm 0.11$  g (day 28) in our sample. In comparison, the weight of the rats in the glibenclamide control group also increased slightly.

**Table 5.** The effect of NtSt01 on body weight in grams of fasting rats.

Groups	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	$226 \pm 0.98$	$227 \pm 1.27$	$228 \pm 0.63$	$229 \pm 1.67$	$233 \pm 0.48$
Diabetic control	$232 \pm 2.69$	$229 \pm 1.98$	$225 \pm 1.39$	$217 \pm 2.69$	$214 \pm 3.09$
Glibenclamide (0.5 mg/kg)	$235 \pm 1.36$	$236 \pm 1.37$	$239 \pm 0.67$	$244 \pm 2.38$	$249 \pm 3.18$
NtSt01 (1.0 mg/kg)	$238 \pm 0.33$	$240 \pm 0.49$	$242 \pm 1.04$	$245 \pm 1.11$	$246 \pm 0.11$

Diabetes mellitus is a perilous disease, and it affect all the vital organs of the body. After the in vivo experiments, the rats from the different groups were euthanized following the ethical procedure. The vital organs were isolated, and the weights were recorded, as shown in Table 6. The weights of the liver, kidney, pancreas, and heart were  $9.83 \pm 0.92$ ,  $1.01 \pm 0.15$ ,  $0.92 \pm 0.12$ , and  $1.04 \pm 0.13$  g, respectively, in the NtSt01-administered group. In diabetic control group, we observed a drastic effect on the average weights of the vital organs.

**Table 6.** The effect of NtSt01 on vital organ weights in grams of fasting rats.

Groups	Liver	Kidney	Pancreas	Heart
Normal control	$9.16 \pm 0.45$	$0.97 \pm 0.13$	$0.88 \pm 0.02$	$1.02 \pm 0.11$
Diabetic control	$6.38 \pm 0.10$	$1.26 \pm 0.37$	$0.71 \pm 0.13$	$0.83 \pm 0.08$
Glibenclamide (0.5 mg/kg)	$9.21 \pm 0.66$	$0.99 \pm 0.16$	$0.84 \pm 0.21$	$0.90 \pm 0.02$
NtSt01 (1.0 mg/kg)	$9.83 \pm 0.92$	$1.01 \pm 0.15$	$0.92 \pm 0.12$	$1.04 \pm 0.13$

The results of the renal function and liver function tests are summarized in Table 7. The serum creatinine, blood urea, serum bilirubin, ALT, and serum alkaline phosphatase of the normal, diabetic, and standard (glibenclamide) groups were compared with the results of phytosteroid NtSt01. In the normal group, all the parameters were within the normal range. In the diabetic group, elevated blood urea, ALT, and serum alkaline phosphatase levels were observed. In a pattern like that of the standard group, the phytosteroid NtSt01 group exhibited normal serum creatinine, blood urea, and serum bilirubin values. However, the ALT and serum alkaline phosphatase values were near the upper normal limit. These overall results showed that compound NtSt01 was effective in protecting the vital organs from damage over the four weeks of the experiment.

**Table 7.** Biochemical profile of blood in alloxan-induced diabetic rats.

Test	N.Control	D.Control	Standard	NtSt01 (1.0 mg/kg)	Unit	Reference Range
S.Creatinine	$0.42 \pm 0.02$	$0.96 \pm 0.10$	$0.43 \pm 0.01$	$0.6 \pm 0.02$	mg/dL	0.4–0.8
Blood Urea	$19.1 \pm 0.17$	$187 \pm 3.65$	$20.2 \pm 0.33$	$19.1 \pm 2.36$	mg/dL	15–22
S.bilirubin	$0.71 \pm 0.24$	$0.96 \pm 0.01$	$0.81 \pm 0.03$	$0.72 \pm 0.77$	mg/dL	Up to 1.0
SGPT(ALT)	$29.4 \pm 1.07$	$268 \pm 1.37$	$37 \pm 1.11$	$32 \pm 0.25$	U/L	17–30
S.ALK.Phosphatase	$114.7 \pm 0.39$	$159 \pm 1.07$	$125 \pm 1.22$	$134 \pm 2.33$	U/L	30–130

The lipid profile is a major concern in the evaluation of serum triglycerides, total cholesterol, high-density cholesterol (HDL), and low-density cholesterol (LDL). The results



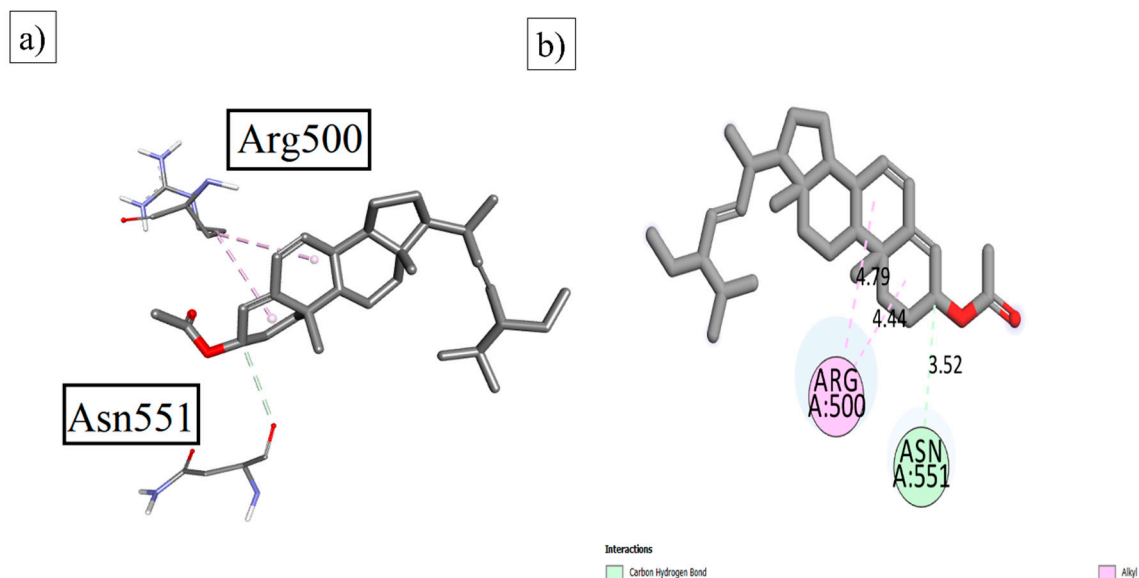
of lipid profile of all the experimental groups are summarized in Table 8. Except for the serum cholesterol, all other values were within the normal ranges.

**Table 8.** Antilipidemic effects of NtSt01 on alloxan-induced diabetic rats.

Groups	S.Cholesterol	S.Triglycerides	HDL	LDL
Normal control	52.06 ± 1.04	93.54 ± 1.37	43.50 ± 2.35	23.88 ± 0.82
Diabetic control	280.63 ± 2.33	456 ± 3.21	40.76 ± 1.49	165.5 ± 3.65
Glibenclamide (0.5 mg/kg)	59.98 ± 0.99	112 ± 0.99	32.55 ± 2.35	34.16 ± 2.66
NtSt01 (1.0 mg/kg)	77.45 ± 1.72	125 ± 3.65	38.10 ± 1.64	48.85 ± 1.08
References Range	10–54	26–145	Up to 50	10–54

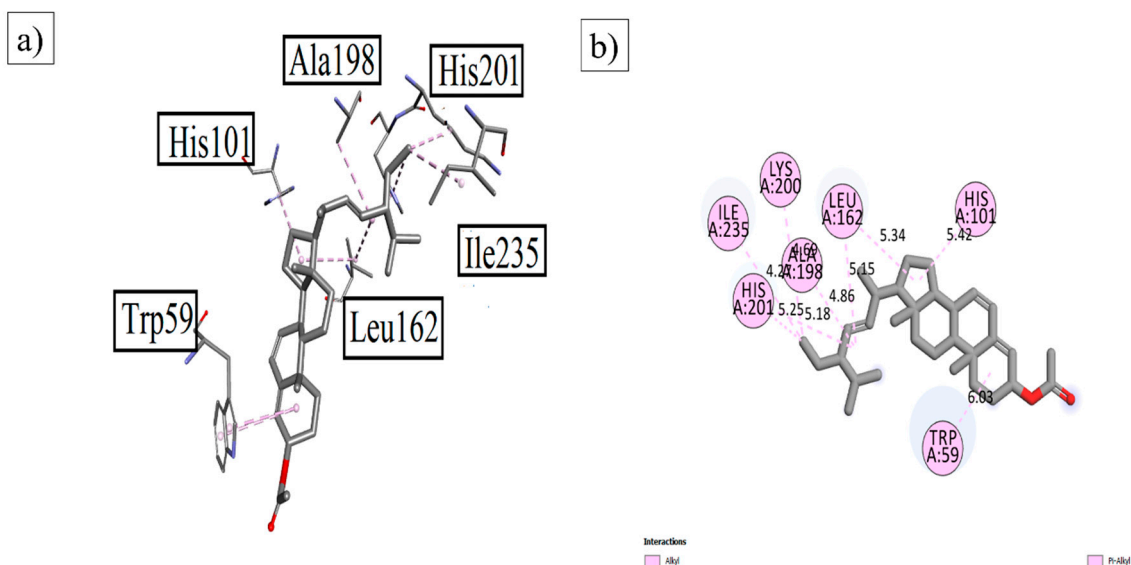
### 2.6. Molecular Docking Studies

For the determination of the pharmacological parameters of the ligand with targeted protein moieties, using the fit model theory with ligand–enzyme interactions, docking studies were performed. These docking results were examined to understand the different interaction parameters. **NtSt01** was docked with the targeted macromolecules to analyze the binding affinity. It was found to have excellent binding affinity with an energy of  $-8.84$  Kcal/mol against  $\alpha$ -glucosidase 7K9Q and  $-8.9$  Kcal/mol against  $\alpha$ -amylase 4W93. The results of the interaction with  $\alpha$ -glucosidase are depicted in Figure 2. The prominent interactions are Arg 500 and Asn 551, respectively.



**Figure 2.** The (a) 3D and (b) 2D visualizations of NtSt01 with  $\alpha$ -glucosidase.

**NtSt01** also displayed excellent results, with great binding affinities against  $\alpha$ -amylase. The results are depicted in Figure 3. The prominent interactions were found to be with Trp 59, His 101, Leu 162, His 201, Ala 198, and Ile 235, respectively.



**Figure 3.** The (a) 3D and (b) 2D visualization of *NtSt01* with  $\alpha$ -amylase.

### 3. Materials and Methods

#### 3.1. Phytochemistry

We collected rhizomes of *Notholirion thomsonianum*, which were also used in our previous research [29–31]. In this study, we subjected the hydroalcoholic extract of *N. thomsonianum* to isolation. Initially, we used a large-diameter gravity column to partially purify the sample. The solvent system used in this chromatography included n-hexane and ethyl acetate. Initially, the column was started with pure n-hexane for a 100 mL elution. Then, the polarity of solvent was gradually increased by adding 5% ethyl acetate each time, and the fractions were collected. The fractions were preliminary checked via thin-layer chromatography (TLC) analysis. One of the fractions containing two major and a few minor spots was concentrated and subjected to a small column for further purification. The elution fractions were monitored closely and collected in separated vials. The vials with major coeluted spots were combined and dried via a rotary evaporator. The two major spots were subjected to GCMS analysis for the identification of the components as per the previously described procedure [32].

#### 3.2. Alpha-Glucosidase Inhibition

The alpha-glucosidase inhibitory effects of our compounds were determined via a reported protocol [31]. Sample dilutions (50  $\mu$ L) and 100  $\mu$ L of  $\alpha$ -glucosidase solution (0.5 U/mL) were mixed. Then, 600  $\mu$ L of phosphate buffer (0.1 M, pH 6.9) was added, and the mixture was incubated at 37  $^{\circ}$ C for 15 min. After this, 5 mM substrate (p-nitrophenyl  $\alpha$ -D-glucopyranoside) solution (20  $\mu$ L) in 0.1 M phosphate buffer (pH 6.9) was added and again incubated under the same conditions. Sodium carbonate was added to stop the reaction; at 405 nm, the absorbance was noted using a spectrophotometer. A mixture without  $\alpha$ -glucosidase served as the blank, and a mixture without the test compound served as the control. The percent enzyme inhibition was calculated as:

$$\% \text{ Alpha glucosidase inhibition} = \frac{\text{Control abs.} - \text{Sample abs.}}{\text{Control abs.}} \times 100 \quad (1)$$

#### 3.3. Alpha-Amylase Inhibition

The inhibitory activity of our compounds against alpha-amylase enzymes was determined as per the previously reported protocols [31]. Test samples of 100  $\mu$ L were mixed with an enzyme solution (200  $\mu$ L) and 100  $\mu$ L of phosphate buffer (pH 6.9, 2 mM). The mixture was then incubated at 25  $^{\circ}$ C for 20 min, followed by the addition of 100  $\mu$ L of



starch solution (1%). The same procedure was followed for positive controls, where phosphate buffer was added instead of enzyme solution (200  $\mu$ L). After 5 min of incubation, 3,5-dinitrosalicylic acid reagent (500  $\mu$ L) was added to the test samples and control group. The mixtures were incubated again for 10 min; at 580 nm, the absorbance was recorded using a spectrophotometer. The alpha-amylase percent inhibition was calculated as:

$$\% \text{ Amylase inhibition} = \left[ 1 - \left( \frac{\text{Test sample abs.}}{\text{Control abs.}} \right) \right] \times 100 \quad (2)$$

### 3.4. Antioxidant Assay

To evaluate the antioxidant potential of our compounds, the DPPH free radical scavenging assay developed by Brand William was used [33]. The DPPH solution was made by dissolving 20 mg of DPPH in 100 mL of methanol, and its absorbance at 517 nm was adjusted to 0.75. Then, 2 mL of DPPH solution was added to 2 mL of sample dilutions ranging from 31.25 to 500  $\mu$ g/mL, and the mixture was incubated at room temperature in the dark for 15 min. The absorbance was noted at 517 nm, and the following formula was used to determine the % inhibition of DPPH free radicals.

$$\% \text{ Inhibition} = \frac{\text{Control abs.} - \text{Sample abs.}}{\text{Control abs.}} \times 100 \quad (3)$$

### 3.5. Molecular Docking Studies

The docking studies were performed using Auto Dock Vina 1.2.2. PyRx. The three-dimensional structure of ligand **NtSt01** was drawn in ChemDraw 20.0 software and saved as a Mol.file. The structure was modified by adding polar hydrogen using Discovery Studio Visualizer and saved in PDB format. The three-dimensional structures of both targeted proteins,  $\alpha$ -glucosidase and  $\alpha$ -amylase, were acquired from the RCSB protein data bank (<http://www.rcsb.org> accessed on 22 September 2023) as PDB id 4W93 and 7K9Q, respectively, and saved in PDB format. Before starting the computational studies on the ligand, the docking process protocols were validated through a redocking process. These ligands and targeted protein structures were permitted for energy minimization through the Charm force field factor, which detached the unwanted crystallographic observations. The ligand and targeted protein structures were opened in Autodock Vina and converted to ligands and macromolecules as Pdbqt molecules. The grid box was adjusted as center X: 10.112, Y: 68.356, and Z: 32.853, with dimensions (Angstrom) X: 75.2435, Y: 105.3254, and Z: 103.4758. The results were visualized through Discovery Studio Visualizer software 2017 R2 [22].

### 3.6. In Vivo Experiments

#### 3.6.1. Experimental Animals and Ethical Approval

The experimental rats were purchased from the Breeding House of National Institute Health, Islamabad, Pakistan. The average weights of these rats ranged from 220 to 250 g. The animals were handled as per the guidelines of the University of Malakand animals By-Laws 2008 (Scientific Procedure Issue I) under the approval of the ethical committee via letter No. DREC-140/B. The food, water, and light/dark cycles provided to animals were observed by the ethical committee [34]. The animals were euthanized as per the AVMA guidelines version 2020. The animals were subjected to slow exposure of halothane vapors to induce anesthesia. There was a gradual increase in the dosage of halothane vapors, which eventually euthanized the animals [35].

#### 3.6.2. Acute Toxicity

In experimental animals, acute toxicity studies were performed to determine the safe dose of our test compounds for in vivo studies. Test compounds were administered at increasing doses of up to 2000 mg/kg, and rats were observed for any aberrant behavior or lethality [34].

### 3.6.3. Induction of Diabetes

Alloxan monohydrate (Sigma Aldrich; Steinheim, Switzerland) was used to induce diabetes at a dose of 160 mg/kg in the experimental animals [36]. The experimental animals were kept in fasting mode for 8–12 h but allowed water only before being subjected to the bioassay. The level of blood glucose was checked after 48 h of administration of alloxan using a glucometer, and only the animals that were diabetic were considered for the study, having a blood glucose level of more than 200 mg/dL [34].

### 3.6.4. Experimental Design

The antidiabetic activity of our test compounds was studied in alloxan-induced diabetic animals. All the experimental animals were placed into four different groups, with 6 animals in each group.

**Group 1:** Only normal saline i.p was given to this nondiabetic group throughout the experimental period.

**Group 2:** Alloxan was administered i.p to this group for diabetes induction and rats were under observation without any treatment throughout the experiment.

**Group 3:** This group was the alloxan-induced diabetic group, which was treated with 0.5 mg/kg of the standard drug, glibenclamide.

**Group 4:** This group was the alloxan-induced diabetic group, which was treated with 1.0 mg/kg of NtSt01 intraperitoneally.

### 3.6.5. Lipid Profile

Standard methods were used for the analysis of total cholesterol, serum triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Briefly, 10 mL of serum sample was added to 1000 mL solution of triglyceride. The mixture was incubated for 10 min at 37 °C and the absorbance was recorded at 546 nm. To monitor blood cholesterol, diagnostic kits were utilized following the specifications of the manufacturer. A total of 10 µL of serum sample was added to 1000 µL of cholesterol solution, followed by incubation for 10 min at 37 °C; the absorbance against blank was noted at 546 nm. For HDL concentration determination, 200 µL of serum sample and 500 µL of HDL solution were combined, and the mixture was allowed to stand at room temperature for 5 min. Again, the mixture was mixed and subjected to further centrifugation for 5 min, followed by supernatant collection. Then, 50 µL of supernatant of HDL was added to 500 µL of solution of cholesterol; the mixture was incubated at 37 °C for 5 min. Then, at 546 nm, the sample absorbance was measured. Similarly, LDL was determined in all groups following the manufacturer's specifications [37].

$$\text{LDL} = \text{Total cholesterol} + \text{HDL} - \text{Triglycerides}/5 \quad (4)$$

### 3.6.6. Renal Functions Tests

Renal functions tests were performed to determine blood urea and serum creatinine [38]. To determine blood urea, enzyme reagent 1 (1000 µL) and 10 µL of serum were mixed together and incubated for 5 min at 25 °C, followed by the addition of 1000 µL of reagent 2 to the mixture. After 5 min, the absorbance was recorded at 578 nm. The serum creatinine was measured very carefully, as it is highly sensitive reaction to temperature. Then, 500 µL of reagent and 50 µL of serum were mixed, followed by incubation for 1 min at 37 °C, and the absorbance was noted at 500 nm.

### 3.6.7. Liver Functions Tests

Serum glutamate pyruvate transaminase (SGPT/ALT), alkaline phosphatase (ALP), and bilirubin tests were performed following standard protocols using micro lab 300 and tecno plus biochemistry analyzers [39]. To perform the SGPT/ALT test, 50 µL of serum sample was added to 500 µL of reagent (400 µL of reagent 1 and 100 µL of reagent 2). The mixture was incubated at 37 °C for 30 s, and the absorbance was noted at 340 nm. To

determine ALP, a kit was used following the manufacturer's specifications: 10  $\mu\text{L}$  of serum sample was added to 500  $\mu\text{L}$  of reagent (400  $\mu\text{L}$  of reagent 1 and 100  $\mu\text{L}$  of reagent 2). The mixture was then incubated at 37  $^{\circ}\text{C}$  for 30 s, and the absorbance was noted at 405 nm. Similarly, the concentration of bilirubin was evaluated using standard protocols. Four types of reagents, R1, R2, R3, and R4, were used in this test. R2 (25  $\mu\text{L}$ ) was added to R1 (100  $\mu\text{L}$ ). After this, 100  $\mu\text{L}$  of serum sample and 500  $\mu\text{L}$  of R3 were added and allowed to stand at 25  $^{\circ}\text{C}$  for 5 min. Then, 500  $\mu\text{L}$  of R4 was added; at 25  $^{\circ}\text{C}$  for 5 min, the mixture was incubated. The absorbance of the sample was recorded at 546 nm.

#### 3.6.8. Statistical Analysis

Two-way ANOVA followed by Dunnett's post-test were applied for the comparison of the positive control with the test groups using GraphPad prism 8.0.1 software.  $p$  values less than or equal to 0.05 were considered statistically significant. The findings of the statistical analysis are shown as mean  $\pm$  SEM.

### 4. Discussion

For thousands of years, number of medicinal plants and natural products have been reported for the treatment of many diseases including diabetes [40–42]. Metformin, obtained from *Galega officinalis*, has been the first-line drug used for 60 years for the treatment of type 2 diabetes [43]. Plant-based drugs have minimal or no side effects compared with synthetic drugs; therefore, researchers have mainly focused on natural products for developing new drugs. In DM, the main treatment strategy involves controlling high glucose levels in the blood. Apart from this,  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes inhibitions are important strategies for controlling hyperglycemia, as these enzymes convert starch into glucose, resulting in increased levels of blood glucose [44]. For *Notholirion thomsonianum*, the in vitro antidiabetic properties of the in vitro targets of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and tyrosine phosphatase 1B, and the antioxidant potential have been studied using free radical assays of ABTS, DPPH, and  $\text{H}_2\text{O}_2$  [1]. In this study, we determined the antidiabetic potential of *Notholirion thomsonianum* through in vitro ( $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition) and in vivo studies.

The in vitro antidiabetic properties of *Notholirion thomsonianum* were determined against  $\alpha$ -amylase and  $\alpha$ -glucosidase.  $\alpha$ -Amylase converts starch into disaccharides and oligosaccharides, whereas  $\alpha$ -glucosidase hydrolyzes disaccharides into glucose [45].  $\alpha$ -Amylase and  $\alpha$ -glucosidase enzymes, if inhibited, decrease the glucose level via the breakdown of starch in GIT, which is retarded via the inhibition of these enzymes, which ameliorates hyperglycemia in diabetic patients. In the  $\alpha$ -glucosidase inhibition assay, the compounds **NtSt01** and **NtSt02** exhibited inhibitions of  $85.00 \pm 1.52$  and  $77.56 \pm 3.22\%$  at the highest concentration (500  $\mu\text{g}/\text{mL}$ ), with  $\text{IC}_{50}$  values of 7.34 and 22.87  $\mu\text{g}/\text{mL}$ , respectively. Compounds **NtSt01** and **NtSt02** exhibited  $80.03 \pm 2.11$  and  $74.99 \pm 1.53\%$  inhibition at 500  $\mu\text{g}/\text{mL}$  against  $\alpha$ -amylase with  $\text{IC}_{50}$  values of 4.17 and 46.73  $\mu\text{g}/\text{mL}$ , respectively. However, at the same tested concentration (500  $\mu\text{g}/\text{mL}$ ), the standard drug acarbose demonstrated  $92.65 \pm 0.55\%$  inhibition against  $\alpha$ -glucosidase and  $91.01 \pm 1.36\%$  inhibition against  $\alpha$ -amylase with  $\text{IC}_{50}$  values of 2.14 and 1.96  $\mu\text{g}/\text{mL}$ , respectively.

In the blood glucose test, diabetes induction with alloxan was confirmed, and the blood glucose levels in the diabetic group were observed as  $521 \pm 1.28$ ,  $517 \pm 2.06$ ,  $526 \pm 1.00$ ,  $524 \pm 2.66$ , and  $529 \pm 2.80$  mg/dL on days 1, 7, 14, 21 and 28, respectively. Treatment of diabetic animals with **NtSt01** at a dose of 1.0 mg/kg produced a decline in blood glucose levels, i.e.,  $519 \pm 3.98$ ,  $413 \pm 1.87$ ,  $325 \pm 1.62$ ,  $219 \pm 2.87$ , and  $116 \pm 1.33$  mg/dL on the 1st, 7th, 21st, and 28th days of the treatment, respectively.

No statistical differences in the weights of the liver, kidney, pancreas, and heart were observed among all groups after completion of the experiments. The kidney function profile, lipid profile, and biochemical profile of the test animals in all groups were also determined. In the biochemical tests, blood urea, serum bilirubin, serum creatinine, SGPT(ALT), and serum alkaline phosphatase levels were high in the diabetic disease group

and decreased after treatment with 1.0 mg/kg **NtSt01**, as shown in Table 7. In the lipid tests, considering serum cholesterol, serum triglycerides, LDL, and HDL, a significant decline was demonstrated in our sample (**NtSt01** 1.0 mg/kg) in comparison with the diabetic animals, as shown in Table 8. Both the in vitro and in vivo antidiabetic results for **NtSt01** revealed the potential of this compound for the management of DM and as a multitarget antidiabetic agent.

Molecular docking is an important approach to determine the binding energies and interactions of a drug molecule for its target. We docked our isolated compounds against  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes. The molecular docking studies revealed encouraging binding interactions with the target proteins for our compounds.

Though the whole of our work is based on the development of an antidiabetic drug from medicinal plant source, significant efforts have also been made by synthetic organic chemists for the discovery of new antidiabetic agents [46]. Among the synthetic approaches, the modification of immunosugar has been identified to develop potential inhibitors of alpha-glucosidase [47–49].

## 5. Conclusions

Herein, we explored the antidiabetic potential of phytosteroids from *Notholirion thomsonianum*. In an attempt to combat diabetes and its consequences, we used phytosteroid **NtSt01** as a natural drug. In the in vitro  $\alpha$ -glucosidase,  $\alpha$ -amylase, and DPPH assays, compound **NtSt01** was found to be potent enough to be tested in experimental animals. The compound was initially found safe in experimental rats and was then found effective in combating induced diabetes over the four weeks of the experiment. After the experiment, we also observed that the weights of the liver, kidney, pancreases, and heart and their functions were within the allowed limits. Our overall results show that phytosteroids (specifically **NtSt01**) have an efficient therapeutic effect on the blood glucose level and other protective effects on organs like the liver, kidney, pancreases, and heart, so may serve as a multitarget antidiabetic drug.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants12203591/s1>, Table S1: GCMS results of compound **NtSt01**. Figure S1: MS spectrum and fragmentation pattern of **NtSt01**. Figure S2: Library spectrum and fragmentation pattern of **NtSt01**. Figure S3: Difference spectrum of **NtSt01**. Figure S4:  $^1\text{H}$  NMR spectrum of **NtSt01**. Table S2: GCMS results of compound **NtSt02**. Figure S5: MS spectrum and fragmentation pattern of **NtSt02**. Figure S6: Library spectrum and fragmentation pattern of **NtSt02**. Figure S7: Difference spectrum of **NtSt02**. Figure S8:  $^1\text{H}$  NMR spectrum of **NtSt02**.

**Author Contributions:** M.A.H., S.F., A.A., S.M.A., A.N. and F.H.: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, and writing—review and editing; M.H.M. and A.S.: Conceptualization, methodology, validation, investigation, resources, writing—original draft preparation, supervision, writing—review and editing, visualization, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research obtained funding under the National Research Priorities and Najran Area Research funding program grant code number NU/NRP/MRC/12/40.

**Institutional Review Board Statement:** The animals were handled as per the guidelines of the University of Malakand animals By-Laws 2008 (Scientific Procedure Issue I) under the approval of the ethical committee via letter No. DREC-140/B.

**Data Availability Statement:** The data are presented in the manuscript and Supporting Information.

**Acknowledgments:** The authors acknowledge support from the Deanship of Scientific Research, Najran University, Kingdom of Saudi Arabia, for funding this work under the National Research Priorities and Najran Area Research funding program grant code number NU/NRP/MRC/12/40). We are also grateful to the Department of Pharmacy, University of Malakand, Pakistan, for the laboratory facilities.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Booth, G.; Lipscombe, L.; Butalia, S.; Dasgupta, K.; Eurich, D.; Goldenberg, R.; Khan, N.; MacCallum, L.; Shah, B.; Simpson, S.; et al. Pharmacologic Management of Type 2 Diabetes: 2016 Interim Update. *Can. J. Diabetes* **2016**, *40*, 484–486. [[CrossRef](#)] [[PubMed](#)]
2. Huneif, M.A.; Alqahtani, S.M.; Abdulwahab, A.; Almedhesh, S.A.; Mahnashi, M.H.; Riaz, M.; Ur-Rahman, N.; Jan, M.S.; Ullah, F.; Aasim, M.; et al.  $\alpha$ -glucosidase,  $\alpha$ -amylase and antioxidant evaluations of isolated bioactives from wild strawberry. *Molecules* **2022**, *27*, 3444. [[CrossRef](#)] [[PubMed](#)]
3. Lin, Y.; Sun, Z. Current views on type 2 diabetes. *J. Endocrinol.* **2010**, *204*, 1–11. [[CrossRef](#)]
4. Ahmed, F.; Urooj, A. Antihyperglycemic activity of *Ficus glomerata* stem bark in streptozotocin-induced diabetic rats. *Global J. Pharmacol.* **2008**, *2*, 41–45.
5. Cho, N.H.; Shaw, J.E.; Karuranga, S.; Huang, Y.; da Rocha Fernandes, J.D.; Ohlrogge, A.W.; Malanda, B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* **2018**, *138*, 271–281. [[CrossRef](#)] [[PubMed](#)]
6. Mathers, C.D.; Loncar, D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* **2006**, *3*, e442. [[CrossRef](#)] [[PubMed](#)]
7. Labazi, H.; Trask, A.J. Coronary microvascular disease as an early culprit in the pathophysiology of diabetes and metabolic syndrome. *Pharmacol. Res.* **2017**, *123*, 114–121. [[CrossRef](#)]
8. Konig, M.; Lamos, E.M.; Stein, S.A.; Davis, S.N. An insight into the recent diabetes trials: What is the best approach to prevent macrovascular and microvascular complications? *Curr. Diabetes Rev.* **2013**, *9*, 371–381. [[CrossRef](#)]
9. Chillarón, J.J.; Le-Roux, J.A.; Benaiges, D.; Pedro-Botet, J. Type 1 diabetes, metabolic syndrome and cardiovascular risk. *Metabolism* **2014**, *63*, 181–187. [[CrossRef](#)]
10. DeFronzo, R.A. Pathogenesis of type 2 diabetes mellitus. *Med. Clin.* **2004**, *88*, 787–835. [[CrossRef](#)]
11. Thomas, C.C.; Philipson, L.H. Update on Diabetes Classification. *Med. Clin. N. Am.* **2015**, *99*, 1–16. [[CrossRef](#)] [[PubMed](#)]
12. Franz, M.J.; Boucher, J.L.; Rutten-Ramos, S.; VanWormer, J.J. Lifestyle Weight-Loss Intervention Outcomes in Overweight and Obese Adults with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *J. Acad. Nutr. Diet.* **2015**, *115*, 1447–1463. [[CrossRef](#)] [[PubMed](#)]
13. Ghosh, D.; Parida, P. Drug Discovery and Development of Type 2 Diabetes Mellitus: Modern-Integrative Medicinal Approach. *Curr. Cancer Drug Targets* **2016**, *13*, 60–67. [[CrossRef](#)] [[PubMed](#)]
14. Phung, O.J.; Scholle, J.M.; Talwar, M.; Coleman, C.I. Effect of noninsulin antidiabetic drugs added to metformin therapy on glycemic control, weight gain, and hypoglycemia in type 2 diabetes. *JAMA* **2010**, *303*, 1410–1418. [[CrossRef](#)]
15. Van Staa, T.; Abenham, L.; Monette, J. Rates of hypoglycemia in users of sulfonylureas. *J. Clin. Epidemiol.* **1997**, *50*, 735–741. [[CrossRef](#)]
16. Gin, H.; Rigalleau, V. Post-prandial hyperglycemia. post-prandial hyperglycemia and diabetes. *Diabetes Metab.* **2000**, *26*, 265–272.
17. He, L.; Wang, H.; Gu, C.; He, X.; Zhao, L.; Tong, X. Administration of traditional Chinese blood circulation activating drugs for microvascular complications in patients with type 2 diabetes mellitus. *J. Diabetes Res.* **2016**, *2016*, 1081657. [[CrossRef](#)]
18. Pang, B.; Zhou, Q.; Li, J.L.; Zhao, L.H.; Tong, X.L. Treatment of refractory diabetic gastroparesis: Western medicine and traditional Chinese medicine therapies. *World J. Gastroenterol.* **2014**, *20*, 6504. [[CrossRef](#)]
19. Xie, W.; Du, L. Diabetes is an inflammatory disease: Evidence from traditional Chinese medicines. *Diabetes Obes. Metab.* **2011**, *13*, 289–301. [[CrossRef](#)]
20. Shah, S.M.M.; Sadiq, A.; Ullah, F. Antioxidant, total phenolic contents and antinociceptive potential of *Teucrium stocksianum* methanolic extract in different animal models. *BMC Complement. Altern. Med.* **2014**, *14*, 181. [[CrossRef](#)]
21. Mahnashi, M.H.; Alyami, B.A.; Alqahtani, Y.S.; Jan, M.S.; Rashid, U.; Sadiq, A.; Alqarni, A.O. Phytochemical profiling of bioactive compounds, anti-inflammatory and analgesic potentials of *Habenaria digitata* Lindl.: Molecular docking based synergistic effect of the identified compounds. *J. Ethnopharmacol.* **2021**, *273*, 113976. [[CrossRef](#)] [[PubMed](#)]
22. Huneif, M.A.; Alshehri, D.B.; Alshaibari, K.S.; Dammaj, M.Z.; Mahnashi, M.H.; Majid, S.U.; Javed, M.A.; Ahmad, S.; Rashid, U.; Sadiq, A. Design, synthesis and bioevaluation of new vanillin hybrid as multitarget inhibitor of  $\alpha$ -glucosidase,  $\alpha$ -amylase, PTP-1B and DPP4 for the treatment of type-II diabetes. *Biomed. Pharmacother.* **2022**, *150*, 113038. [[CrossRef](#)] [[PubMed](#)]
23. Bailey, C.J.; Day, C. Traditional plant medicines as treatments for diabetes. *Diabetes Care* **1989**, *12*, 553–564. [[CrossRef](#)] [[PubMed](#)]
24. Prabhakar, P.K.; Doble, M. A target based therapeutic approach towards diabetes mellitus using medicinal plants. *Curr. Diabetes Rev.* **2008**, *4*, 291–308. [[CrossRef](#)] [[PubMed](#)]
25. Choudhury, A.; Maeda, K.; Murayama, R.; DiMagno, E. Character of a wheat amylase inhibitor preparation and effects on fasting human pancreaticobiliary secretions and hormones. *Gastroenterology* **1996**, *111*, 1313–1320. [[CrossRef](#)]
26. Sadiq, A.; Mahnashi, M.H.; Rashid, U.; Jan, M.S.; Alshahrani, M.A.; Huneif, M.A. 3-(((1S,3S)-3-((R)-Hydroxy (4-(trifluoromethyl)phenyl) methyl)-4-oxocyclohexyl) methyl) pentane-2,4-dione: Design and Synthesis of New Stereopure Multi-Target Antidiabetic Agent. *Molecules* **2022**, *27*, 3265. [[CrossRef](#)]
27. Mahnashi, M.H.; Alam, W.; Huneif, M.A.; Abdulwahab, A.; Alzahrani, M.J.; Alshaibari, K.S.; Rashid, U.; Sadiq, A.; Jan, M.S. Exploration of Succinimide Derivative as a Multi-Target, Anti-Diabetic Agent: In Vitro and In Vivo Approaches. *Molecules* **2023**, *28*, 1589. [[CrossRef](#)]



28. Ajaib, M.; Ali, S.; Khan, Z. Antioxidant and antimicrobial activities of an ethnobotanically important plant *Notholirion thomsonianum* from district Kotli, Azad Jammu & Kashmir. *J. Anim. Plant Sci.* **2014**, *24*, 774–780.
29. Sadiq, A.; Ahmad, S.; Ali, R.; Ahmad, F.; Ahmad, S.; Zeb, A.; Ayaz, M.; Ullah, F.; Siddique, A.N. Antibacterial and antifungal potentials of the solvents extracts from *Eryngium caeruleum*, *Notholirion thomsonianum* and *Allium consanguineum*. *BMC Complement. Altern. Med.* **2016**, *16*, 478. [[CrossRef](#)]
30. Mahmood, F.; Ali, R.; Jan, M.S.; Chishti, K.A.; Ahmad, S.; Zeb, A.; Ayaz, M.; Ullah, F.; Aasim, M.; Khan, N.Z.; et al. Chemical characterization and analgesic potential of *Notholirion thomsonianum* extract. *Lat. Am. J. Pharm.* **2019**, *38*, 807–812.
31. Mahnashi, M.H.; Alqahtani, Y.S.; Alqarni, A.O.; Alyami, B.A.; Jan, M.S.; Ayaz, M.; Ullah, F.; Rashid, U.; Sadiq, A. Crude extract and isolated bioactive compounds from *Notholirion thomsonianum* (Royale) Stapf as multitargets antidiabetic agents: In-vitro and molecular docking approaches. *BMC Complement. Med. Ther.* **2021**, *21*, 270. [[CrossRef](#)] [[PubMed](#)]
32. Sadiq, A.; Zeb, A.; Ullah, F.; Ahmad, S.; Ayaz, M.; Rashid, U.; Muhammad, N. Chemical Characterization, Analgesic, Antioxidant, and Anticholinesterase potentials of essential oils from *Isodon rugosus* Wall. ex. Benth. *Front. Pharmacol.* **2018**, *9*, 623. [[CrossRef](#)] [[PubMed](#)]
33. Shah, S.M.M.; Ahmad, Z.; Yaseen, M.; Shah, R.; Khan, S.; Khan, B. Phytochemicals, in vitro antioxidant, total phenolic contents and phytotoxic activity of *Cornus macrophylla* Wall bark collected from the North-West of Pakistan. *Pak. J. Pharm. Sci.* **2015**, *28*.
34. Huneif, M.A.; Mahnashi, M.H.; Jan, M.S.; Shah, M.; Almedhesh, S.A.; Alqahtani, S.M.; Alzahrani, M.J.; Ayaz, M.; Ullah, F.; Rashid, U.; et al. New Succinimide–Thiazolidinedione Hybrids as Multitarget Antidiabetic Agents: Design, Synthesis, Bioevaluation, and Molecular Modelling Studies. *Molecules* **2023**, *28*, 1207. [[CrossRef](#)] [[PubMed](#)]
35. Underwood, W.; Anthony, R. *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*; American Veterinary Medical Association: Schaumburg, IL, USA, 2020.
36. Sadiq, A.; Rashid, U.; Ahmad, S.; Zahoor, M.; AlAjmi, M.F.; Ullah, R.; Noman, O.M.; Ullah, F.; Ayaz, M.; Khan, I.; et al. Treating hyperglycemia from *Eryngium caeruleum* M. Bieb: In-vitro  $\alpha$ -glucosidase, antioxidant, in-vivo antidiabetic and molecular docking-based approaches. *Front. Chem.* **2020**, *8*. [[CrossRef](#)]
37. Ponnulakshmi, R.; Shyamaladevi, B.; Vijayalakshmi, P.; Selvaraj, J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicol. Mech. Methods* **2019**, *29*, 276–290. [[CrossRef](#)]
38. Hasan, M.; Mohieldein, A. In vivo evaluation of anti diabetic, hypolipidemic, antioxidative activities of Saudi date seed extract on streptozotocin induced diabetic rats. *J. Clin. Diagn. Res.* **2016**, *10*, FF06. [[CrossRef](#)]
39. Galli, A.; Crabb, D.W.; Ceni, E.; Salzano, R.; Mello, T.; Svegliati-Baroni, G.; Ridolfi, F.; Trozzi, L.; Surrenti, C.; Casini, A. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. *Gastroenterology* **2002**, *122*, 1924–1940. [[CrossRef](#)]
40. Aslam, H.; Khan, A.-U.; Naureen, H.; Ali, F.; Ullah, F.; Sadiq, A. Potential application of *Conyza canadensis* (L) Cronquist in the management of diabetes: In vitro and in vivo evaluation. *Trop. J. Pharm. Res.* **2018**, *17*, 1287–1293. [[CrossRef](#)]
41. Farooq, U.; Naz, S.; Shams, A.; Raza, Y.; Ahmed, A.; Rashid, U.; Sadiq, A. Isolation of dihydrobenzofuran derivatives from ethnomedicinal species *Polygonum barbatum* as anticancer compounds. *Biol. Res.* **2019**, *52*, 1. [[CrossRef](#)]
42. Mahnashi, M.H.; Alyami, B.A.; Alqahtani, Y.S.; Alqarni, A.O.; Jan, M.S.; Hussain, F.; Zafar, R.; Rashid, U.; Abbas, M.; Tariq, M.; et al. Antioxidant molecules isolated from edible prostrate knotweed: Rational derivatization to produce more potent molecules. *Oxidative Med. Cell. Longev.* **2022**, *2022*, 3127480. [[CrossRef](#)] [[PubMed](#)]
43. Bailey, C.; Day, C. Metformin: Its botanical background. *Pract. Diabetes Int.* **2004**, *21*, 115–117. [[CrossRef](#)]
44. Buttermore, E.; Campanella, V.; Priefer, R. The increasing trend of Type 2 diabetes in youth: An overview. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2021**, *15*, 102253. [[CrossRef](#)] [[PubMed](#)]
45. Mahnashi, M.H.; Alqahtani, Y.S.; Alqarni, A.O.; Alyami, B.A.; Alqahtani, O.S.; Jan, M.S.; Hussain, F.; Islam, Z.U.; Ullah, F.; Ayaz, M.; et al. Phytochemistry, anti-diabetic and antioxidant potentials of *Allium consanguineum* Kunth. *BMC Complement. Med. Ther.* **2022**, *22*, 154. [[CrossRef](#)] [[PubMed](#)]
46. Hussain, F.; Khan, Z.; Jan, M.S.; Ahmad, S.; Ahmad, A.; Rashid, U.; Ullah, F.; Ayaz, M.; Sadiq, A. Synthesis, in-vitro  $\alpha$ -glucosidase inhibition, antioxidant, in-vivo antidiabetic and molecular docking studies of pyrrolidine-2,5-dione and thiazolidine-2,4-dione derivatives. *Bioorganic Chem.* **2019**, *91*, 103128. [[CrossRef](#)] [[PubMed](#)]
47. Tseng, P.S.; Ande, C.; Moremen, K.W.; Crich, D. Influence of side chain conformation on the activity of glycosidase inhibitors. *Angew. Chem.* **2023**, *135*, e202217809. [[CrossRef](#)]
48. Compain, P.; Martin, O.R. (Eds.) *Iminosugars: From Synthesis to Therapeutic Applications*; John Wiley & Sons: New York, NY, USA, 2007.
49. Rajasekaran, P.; Ande, C.; Vankar, Y.D. Synthesis of (5,6 & 6,6)-oxa-oxa annulated sugars as glycosidase inhibitors from 2-formyl galactal using iodocyclization as a key step. *Arkivoc* **2022**, *2022*, 5–23. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.