Saudi Journal of Biological Sciences 27 (2020) 3267–3273

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# *In vivo* glucose-6-phosphatase inhibitory, toxicity and antidiabetic potentials of 2-picolylamine thioureas in Swiss albino mice

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ARTICLE INFO

*Article history:*
Received 26 July 2020
Revised 19 September 2020
Accepted 22 September 2020
Available online 29 September 2020

*Keywords:*
Thioureas
Alkaloids
Hyperglycemia
Glucose-6-phosphatse
Hematology
Biochemistry
Histopathology

ABSTRACT

The 2-picolylamine is a simplest analogue of the alkaloid that has secondary and tertiary nitrogen function in its cyclic structure like that of alkaloids that can be derivatized to a number of biologically active compounds. In connection to our previous work, in the present work, three thiourea derivatives (**I** = 1,3-*bis*(2-benzyl-3-phenyl-1-(pyridine-2-yl) propyl) thiourea, **II** = 1,3-bis (pyridin-2-ylmethyl) thiourea, and **III** = 1-(2-benzyl-3-phenyl-1-(pyridine-2-yl) propyl)-3-phenylthiourea) were synthesized using 2-picolylamine template which is a readily available synthetic analogue of naturally occurring alkaloid. The biological effect of the synthesized derivatives were monitored on the activity of glucose-6-phosphatase in Swiss albino mice (21-days). The derivatives were also tested for their potential toxicity in a 28-days sub-chronic toxicity studies by assessing their effects on different parameters like hematological, serum biochemistry and liver histology. The therapeutic effect of the safe derivative (**I**) was examined in streptozotocin-induced diabetic mice as well. The derivatives showed inhibition of the enzyme activity from good to an excellent degree. Compound **I** had the highest inhibition with 21.42 ± 5.113 m g of the released phosphate as compared to that of the positive control group (84.55 ± 3.213 mg). Only **I** turned out to be safe for use in animals without exerting any toxic or lethal effects on any of the assessed parameters in the used animal model. Compound **I** efficiently reversed the effects like hyperglycemia, hyperlipidemia and weight loss in the test animals. Out of these three-tested compounds, **I** was found safe to be use as therapeutic agent in diabetes complications. However, further toxicological studies in other animal models are needed as well.



## 1. Introduction

Glucose-6-phosphatase is a common enzyme in two important metabolic pathways of carbohydrate metabolism, i.e., glycogenolysis and gluconeogenesis. These pathways are responsible for yielding free glucose; as this enzyme catalyzes last step, i.e., hydrolysis of glucose-6-phosphatase to free glucose and inorganic phosphate. Moreover, the activity of glucose-6-phospahtase is higher than normal in patients with type-2 diabetes. Inhibiting/ decreasing the activity of this enzyme can delay the release of free glucose in blood and thus can help in management of hyperglycemia associated with type 2 diabetes (Bhagavan, 2002).

Diabetes mellitus is a chronic condition where the body cannot maintain its blood levels in normal healthy range because of its inability to either produce insulin (Type-1) or to respond to the effects of insulin (Type-2) (Werny et al., 2018). Type-2 is more common and search for the discovering the non-insulin medications of type-2 diabetes is progressing day by day and so are the discoveries in the area. One of such approaches is inhibition of the activity of enzyme/s involved in carbohydrate metabolism (Benalla et al., 2010; Agarwal and Gupta, 2016). The area is yet

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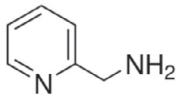
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.09.048
1319-562X/

to be explored and researched further. There is a need to find out the potential inhibitors from different classes of organic compounds that can be effective and safe to inhibit any of the different enzymes involved in carbohydrate metabolism (Naz et al., 2019).

Alkaloids are basic organic nitrogen containing naturally occurring complex compounds having poly-and heterocyclic structure that are primarily derived from amines; as most frequently, tertiary amines are present in their structure. They have remarkable physiological effects and have been extensively used therapeutically as antimicrobials, stimulants, analgesics, and typical anesthetics; but except caffeine all of the alkaloids discovered and used so far are considered to be potentially toxic (Bribi, 2018). Therefore, efforts have been made by researchers to make synthetic analogues of the alkaloids possessing therapeutic potential so as the desired therapeutic results may be obtained with the lesser probability of side effects/toxicity. The 2-picolylamine is a simplest analogue of the alkaloid that has secondary and tertiary nitrogen function in its cyclic structure like that of alkaloids and can be derivatized further to more complex desired derivatives.




Structure of 2-picolylamine

There are several studies in literature reporting many thioureas derivatives that have anti-diabetic properties (Saeed et al., 2017; Larik et al., 2018). The reports suggests the likeliness of the thiourea scaffold to possess antidiabetic potential, keeping in view the antidiabetic nature of the alkaloids and thioureas. The 2-picolylamine has been converted to its thiourea derivatives to take benefit of the combined effects of both of the active pharmacophores there by increasing the probability of synergistic effect.

In this context it would be helpful to know that some of the thiourea derivatives have already been reported that are inhibitors of two important enzymes of carbohydrate metabolism *viz*. alpha-amylase and alpha-glucosidase (Jiang et al., 2007; Taha et al., 2016; Rehman et al., 2017; Taha et al., 2019). This recommends that these derivatives of thioureas should further be assessed for their inhibitory potential for the same or for some other important enzymes involved in carbohydrate metabolism like glucose-6-phosphatase.

While studying a substance for its potential therapeutic effect/s, an important aspect is its toxicity, especially if the substance under investigation is not from natural sources. For this purpose, different parameters like hematology, serum biochemistry and histopathology are examined in the experimental animals after they have been fed with the test substance for a certain period of time. Assessment of the mentioned parameters give a clear idea of its toxicity which are helpful in deciding whether the potential therapeutic compound under study is safe enough to be used as medicine in humans or not (Eaton and Gallagher, 2010; Nugent et al., 2011).

In our previous work, where 3 out of 5 thioureas showed promising antidiabetic potential in term of inhibiting alpha amylase and glucosidase enzymes (Naz et al., 2019). In connection to that work, the current study is concerned with i*n vitro* and *in vivo* inhibition of glucose-6-phosphatase by the previously synthesized compounds, and their toxicological evaluation in Swiss albino mice. Out of the three selected compounds one was found to be safe enough to be use as drug and was thus tested as therapeutic agent for the remedy of diabetes in streptozotocin induced diabetic mice.

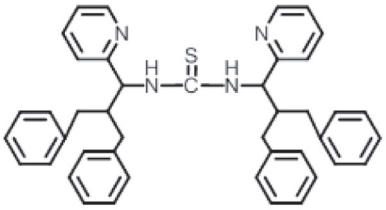
## 2. Material and methods

### 2.1. Chemicals

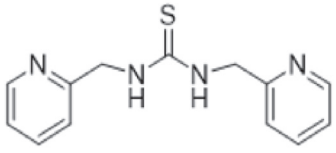
Chemicals, reagents, and solvents like 2-(aminomethyl) pyridine, 2,4-dimethylaniline, sodium hydroxide, phenyl isothiocyanate, benzyl bromide, sodium hydride, ammonium acetate, zinc powder, catalysts like 18-Crown-6, carbon disulphide, n-hexane, chloroform, ethyl acetate, anhydrous acetone, toluene, ethanol, hydroxyl ammonium chloride sodium sulphate, and ammonium hydroxide. The chemicals (anilines, reagents and solvents) used were of analytical grade and purchased from Sigma Aldrich Co, St Louis, MO, USA.

### 2.2. Methods

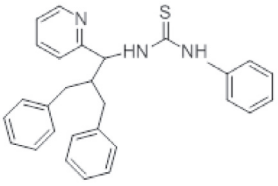
Our research team (Naz et al., 2019) has reported the compound tested here previously. The structures of these compounds are given as follow:




1,3-*bis*(2-benzyl-3-phenyl-1-(pyridine-2-yl)propyl)thiourea (**I**)




1,3-bis(pyridin-2-ylmethyl)thiourea (**II**)




1-(2-benzyl-3-phenyl-1-(pyridine-2-yl) propyl)-3-phenylthiourea (**III**)

### 2.3. Experimental animals and their handling

Swiss albino mice (male) were used in all *in vivo* experiments carried out in the current study. Their weight was in range of 30–35 mg and age from 22 to 24 weeks. They were obtained from National Institute of Health Islamabad and acclimated in animal house University of Malakand where they were kept in plastic

cages provided with good space, aeration and proper bedding of sawdust. The animals were given free access to food and water. A room temperature of 25-29°C and a 12 h light and day cycles were maintained through the experiments.

All the *in vivo* studies and animal treatment were carried out in accordance to instructions of the guidelines "Ethics Committee for Animal Care & Use" Pakistan.

*2.3.1. Groups*

The animals were divided into 4 groups each for the two different *in vivo* studies (glucose-6-phosphatase inhibition and toxicity). One group was kept as control group (having 5 mice) fed only with normal food and water. The 3 other groups were made on the basis of the three derivatives given to each group, which were further sub divided into 3 groups each based on quantity of dose each mice received (subgroup A, B, and C received 0.5, 1.0 and 1.5 mg/kg body weight doses).

*2.3.1.1. In vivo assessment of glucose-6-phosphatase inhibitory potential.* On 22nd day, the animals were sacrificed and their livers samples were collected for assessing glucose-6-phosphatase activity. Livers were fast frozen and their homogenates were prepared in iced water. Enzyme extract was prepared by placing the homogenate in shaker at 0 °C for an hour with constant addition of ice to the homogenates; the supernatant obtained (after filtration) were used as source of glucose-6-phosphatase. Then 1 mL of the filtered liver homogenate (enzyme source), 1.5 mL tris (hydroxyl methyl) amino methane buffer (pH 6.7) and 2.5 mL of 0.01 M glucose-6-phosphate (substrate) taken in a flask and incubated for an hour at 33 °C. The reaction was stopped by addition of 1 mL of 10% trichloroacetic acid. Inorganic phosphate released (one of the products) during the reaction was measured by method of Fiske and Subbarow (1925) to monitor the reaction progress.

*2.3.1.2. Toxicity studies.* For toxicological screening, mice were sacrificed on 28th day, their blood, and livers were collected for assessment of following different parameters.

*2.3.1.2.1. Hematological parameters.* Blood (0.2 mL) of each animal was drawn in a tube containing 10% EDTA as anticoagulant. Concentration of RBC parameters, WBC and platelets was determined using Abbot CELL-DYN 3200 (automated hematology analyzer).

*2.3.1.2.2. Serum biochemistry parameters.* Blood (0.2 mL) samples were collected in tubes without any addition of EDTA and serum of each sample was obtained after centrifugation. Serum biochemical parameters like blood sugar, total cholesterol, triglyceride, ALT and HDL were assessed using HUMAN kit (HUMAN diagnostic Germany).

*2.3.1.2.3. Histopathological examination of liver.* Liver samples were taken, fixed in 10% formalin and dehydrated using alcohol. Thin sections (about 8–10 μm) were cut from specimens, stained and studied under light microscope (model No. M 7000 D, SWIFT, Japan).

*2.4. Anti-diabetic studies in STZ induced diabetic mice*

One compound that was found 'safe'/least toxic was used in this study to find out that whether or not it can be used treat diabetes in STZ-induced diabetic mice.

*2.4.0.1. Animal groups*

The animals were divided into 5 groups (each having 6 mice) as follows;

Normal control (NL): normal with no induced diabetes.

Diabetic control (DL): STZ-induced diabetic, no treatment with glibenclamide or exposure to any test compound.

Glibenclamide medicated (GM); treated with commercially available medicine glibenclamide for treatment of diabetes.

SA and SB: mice were given the safe compound at dose of 0.8 and 1.6 mg/kg body weight.

*2.4.0.2. Induction of diabetes mellitus*

Streptozotocin (STZ) was dissolved in 0.01 M citrate buffer pH 4.5, shortly (not more than 5 min) before use and was injected intraperitoneally at 160 mg/Kg body weight. The doses were calculated according to the body weights. Development of diabetes was monitored by measuring blood glucose level; blood sugar gradually increased from 151.6 ± 4.4 measured at day 1 of STZ treatment

**Table 1**
Effect of the 2-picolylamine based thiourea derivatives on the activity of glucose-6-phosphatase.

| *Compounds* | *Sub-Groups* | *Total Activity** |
|---|---|---|
| **I** | **A** | 26.43 ± 2.412*** |
| | **B** | 24.89 ± 1.649*** |
| | **C** | 21.42 ± 5.113*** |
| **II** | **A** | 56.54 ± 3.735*** |
| | **B** | 47.2 ± 3.904*** |
| | **C** | 38.33 ± 2.641*** |
| **III** | **A** | 51.11 ± 4.8536*** |
| | **B** | 47.56 ± 3.589** |
| | **C** | 40.02 ± 2.001*** |
| *Control* | **84.55 ± 3.213** | |

*activity has been expressed as mg of inorganic phosphate released from the potassium salt of glucose-6-phosphate per hour (at 33 °C., pH 6.7). whereas, A, B and C represent dose rate of 0.5, 1.0 and 1.5 mg/kg body weight respectively.

**Table 2**
Effect of thioureas on hematological parameters in Swiss albino mice.

| *Compound* | *Sub-groups* | *PCV (%)* | *RBCs ($10^6$ μL)* | *Hb g/dL* | *WBCs ($10^3$ μL)* | *Differential count ($10^3$ μL)* | | *Platelets ($10^3$ μL)* |
|---|---|---|---|---|---|---|---|---|
| | | | | | | **L** | **M** | |
| **I** | **A** | 42.08 ± 4.66 | 7.43 ± 1.86 | 14.4 ± 2.16 | 6.6 ± 3.41 | 5.24 ± 1.78 | 2.00 ± 0.00 | 137 ± 3.2*** |
| | **B** | 43.37 ± 1.89 | 7.36 ± 1.58 | 14.79 ± 4.56 | 7.8 ± 2.17 | 5.4 ± 1.16 | 2.00 ± 0.00 | 143 ± 2.43*** |
| | **C** | 42.45 ± 3.74 | 7.21 ± 1.48 | 14.25 ± 1.28 | 7.9 ± 1.24 | 5.15 ± 1.83 | 2.00 ± 0.00 | 146 ± 4.73*** |
| **II** | **A** | 38.33 ± 2.81*** | 6.13 ± 1.22 | 10.40 ± 1.58** | 11.40 ± 1.87** | 6.27 ± 1.33 | 2.00 ± 0.00 | 77 ± 2.31*** |
| | **B** | 35.77 ± 1.88*** | 5.55 ± 1.97 | 9.78 ± 2.79** | 11.86 ± 2.72*** | 7.21 ± 3.12 | 2.00 ± 0.00 | 65 ± 1.92*** |
| | **C** | 31.58 ± 3.41*** | 4.87 ± 2.58 | 9.34 ± 4.27*** | 12.23 ± 1.19*** | 7.6 ± 2.222 | 2.00 ± 0.00 | 69 ± 2.73*** |
| **III** | **A** | 41.55 ± 2.38** | 6.11 ± 1.88 | 12.2 ± 2.47* | 8.79 ± 1.94 | 5.4 ± 2.32 | 2.00 ± 0.00 | 64 ± 2.52*** |
| | **B** | 40.61 ± 1.43*** | 5.71 ± 2.87 | 11.86 ± 3.47* | 9.2 ± 1.38 | 5.8 ± 1.24 | 2.00 ± 0.00 | 61 ± 2.62*** |
| | **C** | 39.54 ± 4.25*** | 5.16 ± 1.83 | 11.12 ± 4.13** | 9.6 ± 2.19 | 5.6 ± 2.42 | 2.00 ± 0.00 | 58 ± 3.57*** |
| Control | | 44.62 ± 1.58 | 7.76 ± 3.79 | 14.86 ± 2.75 | 7.16 ± 2.83 | 5.24 ± 1.78 | 2.00 ± 0.00 | 141 ± 4.69 |

Data is expressed as mean ± SD of n = 6. **I-III** represent derivatives, A, 0.5 mg/kg body weight; B, 1 mg/kg body weight; C, 1.5 mg/kg body weight. Significant difference was measured using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. Whereas, *p < 0.05, and***p < 0.001vs. control group. PCV: Packed cell volume, RBC: red blood cell, Hb: hemogloin, WBC: white blood cells, L: Lymphocytes, M: Monocytes, PLT: Platelets.

**Table 3**
Effect of thioureas on biochemical parameters in Swiss albino mice.

| Compound | *Sub-Groups* | *Glucose (mg/dL)* | *Triglyceride (mg/dL)* | *Cholesterol (mg/dL)* | *HDL (mg/dL)* | *ALT (μ/L)* |
|---|---|---|---|---|---|---|
| **I** | **A** | 71 ± 2.62*** | 104 ± 2.26*** | 77 ± 1.29*** | 52.64 ± 4.2### | 48 ± 4.22 |
| | **B** | 67 ± 1.36*** | 90.64 ± 2.08*** | 74 ± 2.1*** | 52.77 ± 3.21### | 48 ± 1.69 |
| | **C** | 64.86 ± 4.15*** | 89.81 ± 3.07*** | 71 ± 1.71*** | 54.64 ± 1.78 | 53 ± 1.12 |
| **II** | **A** | 88.13 ± 3.65 | 119.3 ± 2.61### | 105 ± 2.44### | 36.10 ± 3.15*** | 71 ± 2.87### |
| | **B** | 91.61 ± 2.94## | 121 ± 3.15### | 105 ± 4.09### | 34.80 ± 1.17*** | 75 ± 2.12### |
| | **C** | 90.53 ± 1.57## | 120 ± 2.19### | 111 ± 3.14### | 33 ± 1.25*** | 82 ± 3.32### |
| **III** | **A** | 86.34 ± 2.16 | 114 ± 2.71 | 96 ± 3.31 | 38.64 ± 2.25*** | 60 ± 5.08### |
| | **B** | 85.72 ± 5.2 | 116 ± 2.11 | 96 ± 2.91 | 37.77 ± 1.34*** | 57 ± 1.34### |
| | **C** | 85.15 ± 3.71 | 118 ± 1.67### | 105.2 ± 2.84 | 36.74 ± 1.67*** | 57 ± 2.64### |
| Control | | 87.2 ± 2.49 | 110.4 ± 2.68 | 82.6 ± 3.78 | 45.4 ± 1.63 | 49.2 ± 2.87 |

Data is expressed as mean ± SD of n = 6. **I-III** represent derivatives, whereas, A, B and C represent dose rate of 0.5, 1.0 and 1.5 mg/kg body weight respectively. Significant difference was measured using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. Whereas, *p < 0.05, **p < 0.01, and***p < 0.001 and #p < 0.05, ##p < 0.01, and ##p < 0.001 significant increase vs. Control group. HDL: High-density lipoprotein, ALT: alanine amino transferase.

to 278.3 ± 4.1 within a week. Once it was confirmed that diabetes has been induced, the experiment was started. Mice were treated with the type and quantity of dosage daily for 28 days, as has already been mentioned for different groups in Section 2.3.1 of the study.

*2.4.0.3. Collection of blood samples and analysis of biochemical parameters*

Blood samples from mice in each group were collected (drawn from tail vein) at day 1, 3, 8, 14, 21 and 28. Serum was obtained after centrifugation and levels blood glucose, triacylglycerol, cholesterol, HDL etc. were determined through their respective kits.

### *2.5. Statistical analysis of experimental data*

The results obtained in each study are represented as mean SEM (Standard Error of Mean); data was statistically analyzed for determination of statistical significance of difference amongst the groups using ANOVA (one-way analysis of variance).

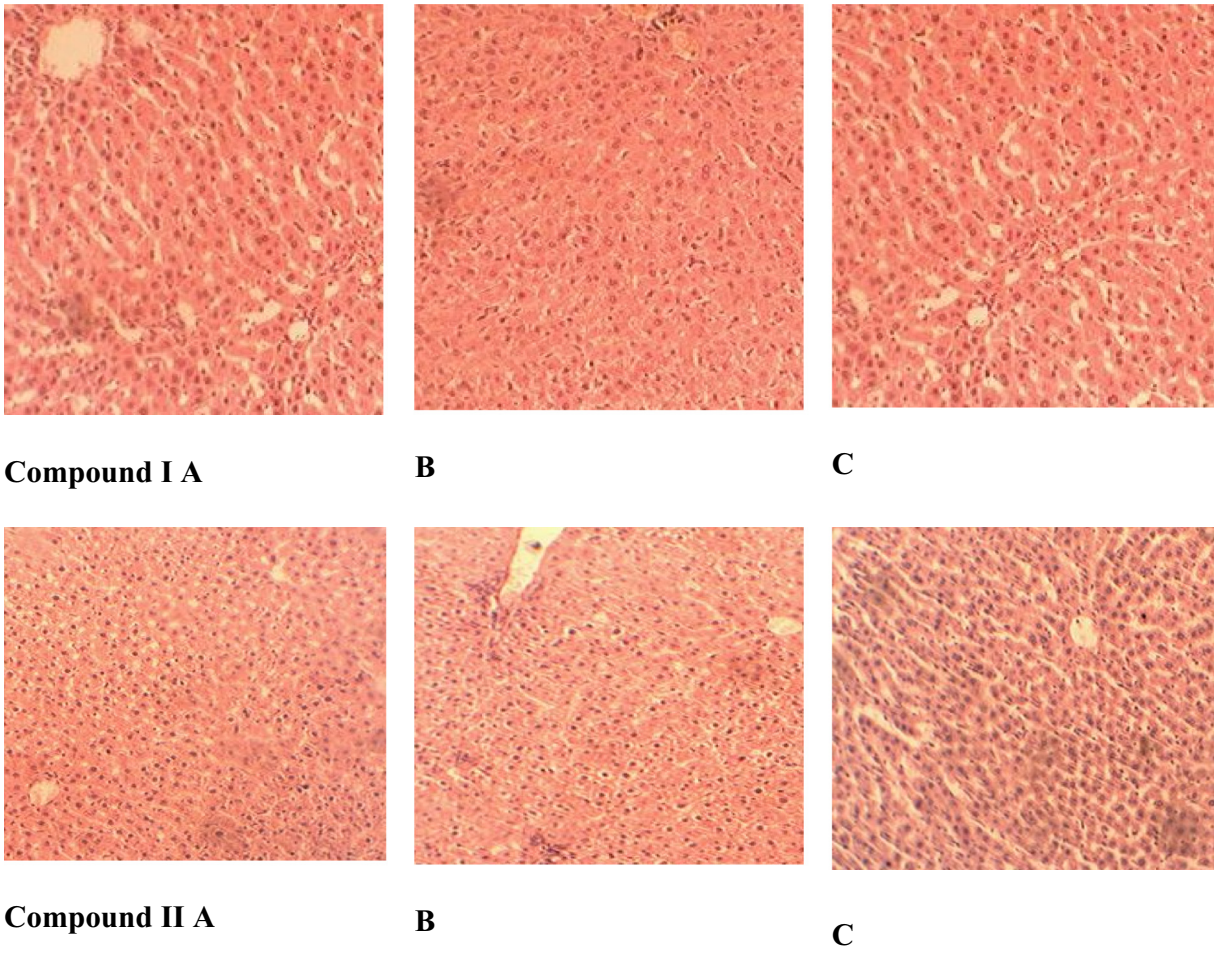



**Fig. 1.** Effect of the synthesized compounds on liver histology. I, II, III represent derivatives of thiourea, whereas, A, B and C represent dose rate of 0.5, 1.0 and 1.5 mg/kg body weight respectively.

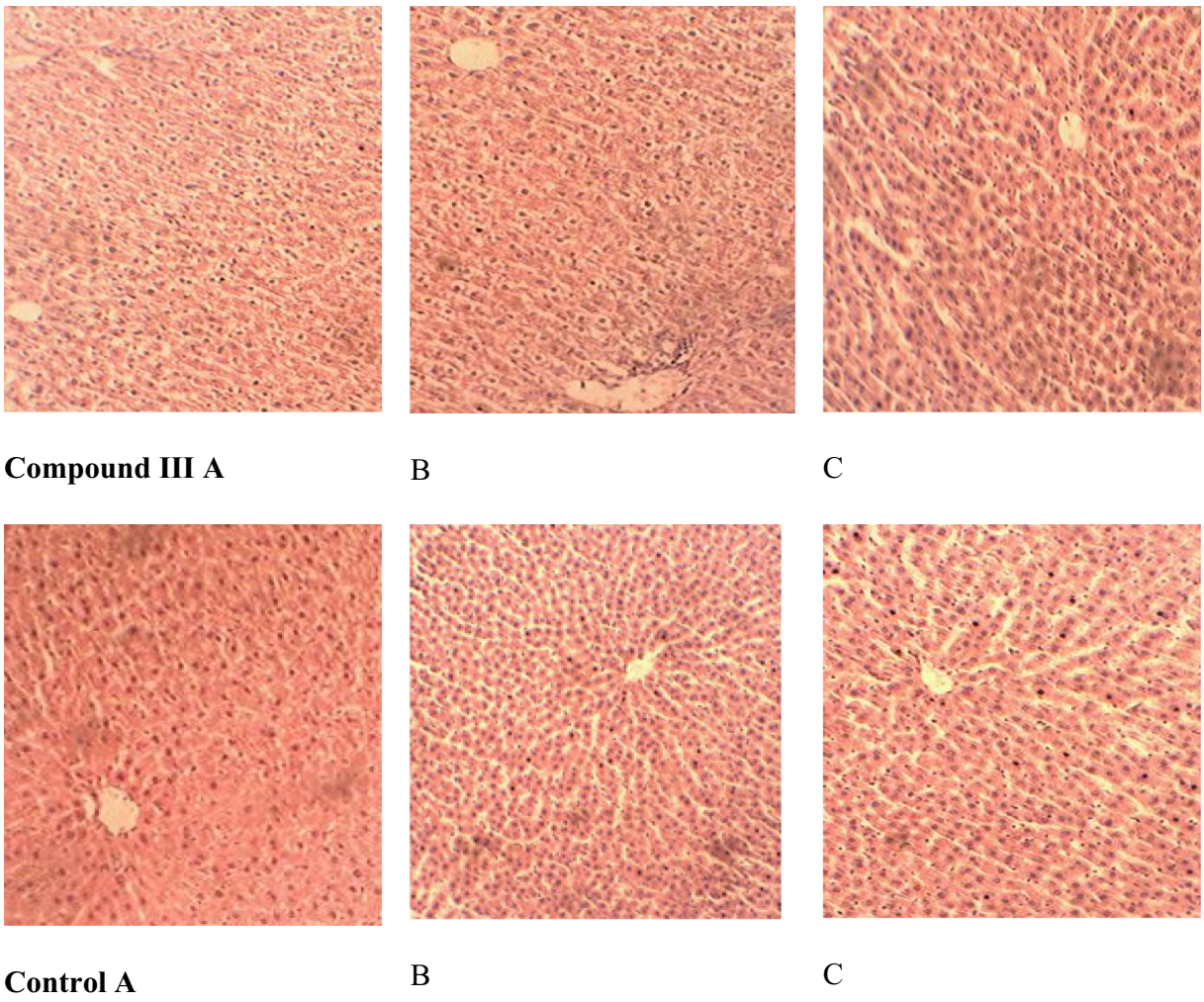



**Fig. 1** (*continued*)

## 3. Results

### 3.1. Effect of the thioureas on the activity of glucose-6-phosphatase

Table 1 shows the effect of each of the 3 compounds on activity of this enzyme *in vivo*. In general, a significant decrease in the activity of the enzyme in case of each derivative have been observed. However, a highest inhibition was seen in case of exposure to **I** at 1.5 mg/Kg body weight.

### 3.2. Effect of the derivatives on hematological parameters

Table 2 shows the effect of the compounds on different hematological parameters in mice when analyzed after 28th day of treatment with the tested derivatives. PCV was significantly decreased ($p < 0.001$) at all dose rates by **II** and **III** whereas, **I** had no significant effect on PCV. Erythrocyte count remain unaffected at all dose rates by all the derivatives. **II** exhibited a significant ($p < 0.01$) decrease in hemoglobin levels at the highest tested dose; a signif-

**Table 4**
Effect of safe compounds on the level of blood glucose measured at different days of the experiment after treatment with the safe compounds.

| *Group* | **Blood glucose (mg/dL)** | | | | | |
|---|---|---|---|---|---|---|
| | **1ST DAY** | **3RD DAY** | **8TH DAY** | **14TH DAY** | **21ST DAY** | **28TH DAY** |
| **NC** | 85.28 ± 3.46 | 76.69 ± 4.43 | 83.17 ± 2.83 | 95.36 ± 2.32 | 88.2 ± 3.76 | 91.57 ± 1.82 |
| **STZ-DC** | 321.61 ± 4.77$^{***}$ | 412.61 ± 3.81$^{***}$ | 456.39 ± 3.72$^{***}$ | 484.65 ± 2.19$^{***}$ | 493.42 ± 4.51$^{***}$ | 512.28 ± 3.32$^{***}$ |
| **STZ-GM** | 223.8 ± 2.54$^{***###}$ | 213.31 ± 3.58$^{***###}$ | 170.3 ± 3.14$^{***###}$ | 173.5 ± 4.55$^{***###}$ | 135.2 ± 2.18$^{***###}$ | 102.3 ± 3.73$^{***###}$ |
| | 288.16 ± 2.85$^{***###}$ | 263.88 ± 2.34$^{***###}$ | 242.21 ± 4.32$^{***###}$ | 224.06 ± 2.57$^{***###}$ | 203.91 ± 4.44$^{***###}$ | 162.29 ± 4.16$^{***###}$ |
| **STZ-SA** | 238.44 ± 4.92$^{***###}$ | 229.12 ± 3.82$^{***###}$ | 202.36 ± 2.66$^{***###}$ | 185.38 ± 4.28$^{***###}$ | 151.11 ± 4.21$^{***###}$ | 121.75 ± 3.74$^{***###}$ |
| **STZ-SB** | 224.35 ± 3.42$^{***###}$ | 202.32 ± 1.39$^{***###}$ | 180.18 ± 1.58$^{***###}$ | 165.19 ± 3.24$^{***###}$ | 129.41 ± 2.82$^{***###}$ | 108.56 ± 4.15$^{***###}$ |

Normal control (NC), Streptozotocin induced diabetic control (STZ-D), Streptozotocin induced diabetic mice treated with Glibenclamide (STZ-GM), Streptozotocin induced diabetic treated with the safe compound, A (0.8 mg/Kg BWT) B (1.6 mg/Kg BWT). Data is expressed as means ± standard deviation (SD) of n = 6.Significant difference was measured using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Whereas, $^{*}p < 0.05$, $^{**}p < 0.01$, and$^{***}p < 0.001$vs. NC group; ##p < 0.001 and ###p < 0.0001 vs. STZ-treated diabetic group, respectively.

**Table 5**
Effect of thioureas on lipid profile in the diabetes induced mice.

| *Groups* | *HDL-cholesterol (mg/dl)* | *LDL-cholesterol (mg/dl)* | *TG (mg/dl)* |
|---|---|---|---|
| *NC* | 44.2 ± 4.6 | 86.3 ± 3.8 | 113.6 ± 4.6 |
| *STZ-DC* | 36.4 ± 3.1*** | 118.5 ± 4.7*** | 152.6 ± 2.6*** |
| *STZ-GM* | 45.4 ± 3.6### | 89.2 ± 3.9### | 117.4 ± 4.1### |
| *STZ-S2* | 46.1 ± 3.1### | 91.3 ± 4.5*### | 117.7 ± 3.9### |

Normal control (NC), Streptozotocin induced diabetic control (STZ-D), Streptozotocin induced diabetic mice treated with Glibenclamide (STZ-GM), Streptozotocin induced diabetic treated with the safe derivative, A (0.8 mg/Kg BWT) B (1.6 mg/Kg BWT). Data is expressed as means ± standard deviation (SD) of n = 6.Significant difference was measured using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Whereas, *p < 0.05, **p < 0.01, and***p < 0.001vs. NC group; ##p < 0.001 and ###p < 0.0001 vs. STZ-treated diabetic group, respectively.

icant decrease at all doses was observed for **III**, whereas **I** had no significant effect on hemoglobin levels. Platelet count was significantly decreased (p < 0.01) by **II** and **III** as compared to that of normal control mice. However, **I** had no significant effect on platelet count.

### 3.3. Effect of the derivatives on serum biochemical parameters

Table 3 shows effect of the test compounds on different biochemical parameters in mice when analyzed at 28th day of treatment with the test compounds. Blood glucose level was significantly (p < 0.001) reduced by **I** at all doses. An increase in TG levels was observed at all doses by **II**, and at higher doses by **III.** A significant decrease (p < 0.001) in TG levels was caused by **I** at all doses. Cholesterol level was increased (p < 0.001) at all doses by **II** and **III**; **I** at all doses significantly (p < 0.001) decreased its level. HDL level was decreased (p < 0.001) by **III**; while increased by compound **I** at all doses. ALT level was significantly increased (p < 0.001) by **II** and **III** at all doses.

### 3.4. Effect of the derivatives on histopathology of mice liver

Fig. 1 shows the effect of the derivatives on histology of the mice liver after 28th day. Compound **II** and **III** caused inflammation, necrosis and vacuolation at all doses as can be seen in slide A, B and C; **I** had no effect on at dose rate as shown in the slides A to C.

### 3.5. Effect of compound *I*, the safe derivative on the concentration of blood glucose in STZ induced diabetic mice

Table 4 demonstrates how the blood glucose level has affected by the safe derivative measured at different intervals in the total 28 days of exposure as compared to that of STZ induced diabetic mice (STZ-DC) and STZ-induced diabetic mice treated with Glibenclamide (STZ-GM). The STZ-DC mice were neither treated with Glibenclamide nor with any test compound showed the highest significant (p < 0.001) increase in glucose level as compared to that of normal control at all durations of exposure compared to that of normal control non-diabetic mice (NC). STZ-induced mice that were also given Glibenclamide (STZ-GM) and both the groups that are treated with the safe compound at two different doses showed a significant increase in blood glucose as compared to that in the NC group but there blood glucose was also significantly (p < 0.001) low as compared to that of STZ-DC at all exposure durations. The highest decrease in blood glucose level was observed at day 28 for both groups, STZ-SB had the highest decrease in glucose level with concentration of 108.56 ± 4.15 mg/dL, which was very close to 102.3 ± 3.73 108.56 ± 4.15 mg/dL (glibenclamide-medicated group).

Table 5 displays the effect of the test compounds on the serum lipid profile. A decrease in HDL-cholesterol and increase (p < 0.001) in LDL-cholesterol and TG levels in STZ-diabetic control group as compared to that of control group were observed. An increase (p < 0.001) in HDL-cholesterol and a decrease in LDL-cholesterol and TG levels in comparison to control group (STZ-DC) was observed for STZ-GM, STZ-S groups.

Initial and final body weights of mice in the different group were also measured; the data is presented in table 6. Diabetic control mice showed a significant decrease in body weight as compared to their initial body weight and that of mice in the control group. A significant difference (p < 0.001) as compared to diabetic control group and no significant difference (p < 0.001) as compared to normal control group and to their initial body weights was recorded for the other two groups.

## 4. Discussion

With the advances in the use of enzyme inhibition as a treatment tool to treat type-2 diabetes, any of the several enzymes involved in carbohydrate metabolism could be considered as target for potential inhibition by a test substance. One of such enzymes is glucose-6-phosphatase; a favorite target because of two reasons *viz*., firstly, it is involved in two main pathways of carbohydrate metabolism i.e., glycogenolysis and gluconeogenesis and secondly its activity is chronically high in diabetic patients than in a normal non-diabetic person (Jiang et al., 2007; Naz et al., 2019). Decreasing/repressing its activity can effectively normalize blood glucose level in patients with type-2 diabetes. Because of the reported therapeutic effects of many alkaloids as well as of thioureas, this study was designed to take the benefit of both moieties by synthesizing some alkaloid based thiourea derivatives and to investigate their effects on the activity of the selected enzyme. For the purpose, 2-picolylamine was used as a simple and readily available analogue of natural alkaloids. The analogue (2-picolylamine), on one hand was used to form symmetrical thiourea (compound **I**) and on the other hand it was converted to its dibenzyl derivative, which was further used to synthesize a symmetrical (compound **II**) and an unsymmetrical thiourea derivative (compound **III**). These derivatives (to an appreciable degree) repressed the activity of enzyme in a 21-day *in vivo* study carried out in mice. However,

**Table 6**
Difference between initial and final weights of the mice in different groups.

| *Groups* | *NC* | *STZ-DC* | *STZ-GM* | *STZ-S2* |
|---|---|---|---|---|
| *INITIAL BWT (DAY 1ST)* | 30.6 ± 4.2 | 30.5 ± 1.4 | 30.4 ± 2.6 | ***32.0 ± 3.4*** |
| *FINAL BWT (DAY 28TH)* | *35.8 ± 2.1* | *26.1 ± 1.8**** | *34.9 ± 1.4###* | *33.2 ± 1.2###* |

Normal control (NC), Streptozotocin induced diabetic control (STZ-D), Streptozotocin induced diabetic mice treated with Glibenclamide (STZ-GM), Streptozotocin induced diabetic treated with the safe compound, A (0.8 mg/Kg BWT) B (1.6 mg/Kg BWT). Data is expressed as means ± standard deviation (SD) of n = 6. Significant difference was measured using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Whereas, *p < 0.05, **p < 0.01, and***p < 0.001vs. NC group; ##p < 0.001 and ###p < 0.0001 vs. STZ-treated diabetic group, respectively.

before considering them as new diabetic drug candidates, they were also assessed for their physiological effects on hematology, serum biochemistry and liver histology of the mice in sub-chronic toxicity studies. Only compound **I** had no toxic or ill effects on any of the parameters evaluated. In order to confirm the therapeutic effect of compound **I** in the treatment of diabetes, another experiment was performed where the mice were induced with diabetes through streptozotocin and were fed with **I** for 28 days. The derivative effectively reversed hyperglycemia and hyperlipidemia in the diabetic mice thus confirming its antidiabetic capability.

## 5. Conclusion

The synthesized thiourea derivatives showed good *in vivo* inhibition of glucose-6-phosphatase with compound **I** having the highest inhibitory activity. Toxicity studies were carried out to confirm the safety/toxicity of the synthesized derivatives and only compound **I** was found to be the safe one. The safe derivative i.e. **I** was used to treat induced type-2 diabetes in mice and it affectively treated the symptoms in a 28 days experiment. The derivative **I** can be considered as a new potential drug in the treatment of type-2 diabetes.

## Acknowledgments

The authors extend their appreciation to the Researchers supporting project number (RSP-2020/248) King Saud University, Riyadh, Saudi Arabia.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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