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Original Article

Biological Evaluation of New Oxadiazole-Based Synthetic α-glycosidase Inhibitors for Hyperglycemia Management: A Research Study

Arefeh Khosravi[a], Nazanin Pirooznia[b], Gholamhassan Vaezi[a], Vida Hojati[a], Khosrou Abdi[b, c*]

[a]Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran, [b]Department of Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, [c]Iranian National Center for Addiction Studies (INCAS), Tehran University of Medical Sciences, Tehran, Iran.

Abstract

The present study was conducted to investigate the hypoglycemic activity of 3,4,5-triphenyl-oxadiazole derivatives and its effects on liver, lung, and kidney function in streptozotocin (STZ)-induced diabetic rats. For this purpose, male Wistar rats were divided into four groups (n = 4). Diabetes was induced in four groups by a single dose of STZ at 65 mg/kg body weight, administrated intraperitoneal. After 28 days of treatment, fasting blood sugar (FBS) levels and other biochemical parameters such as cholesterol, triglycerides phosphorous, urea creatinine, etc. were measured. Also, the markers of liver and kidney function, such as urea, serum creatinine, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase levels were determined. The study showed that the 3,4,5-triphenyl-oxadiazole derivatives at 100 mg/kg body weight had a significant antidiabetic activity after 28 days of treatment as the FBS levels decreased significantly while the serum insulin levels increased. Moreover, a significant decrease in the liver and kidney function markers in treated rats indicated the protective effect of the 3,4,5-triphenyl-oxadiazole derivatives against liver and kidney damage. The serum concentrations were normal in the control and the healthy group treated with these derivatives. The results of this study showed that both derivatives can regulate hyperglycemia and complications of diabetes.

*Keywords***:** 3,4,5-triphenyl-oxadiazole derivatives; Diabetes mellitus; Hyperglycemia; Streptozotocin; hypoglycemic activity

1. Introduction

Type 2 diabetes is one of the global public health concerns in the 21st century [1]; both developed and the developing countries are experiencing increasing rates of diabetes [2]. One strategy to manage type 2 diabetes is to delay glucose uptake by inhibiting α-glucosidase enzymes, which can reduce postprandial hyperglycemia [3-7]. Hyperglycemia impairs endogenous antioxidant defenses due to the induction of oxidative stress, which induces the destruction of pancreatic beta cells by the uncontrolled production of free radicals such as ROS, leading to multiple micro and macrovascular disorders [8-11]. Oxadiaole-derivatives are a

Corresponding Author: Khosrou Abdi, Department of Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran and Iranian National Center for Addiction Studies (INCAS), Tehran University of Medical Sciences, Tehran, Iran.
Tel: +982164122111
Email: khmabdi@tums.ac.ir

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group of pharmacophores with biological importance and have antibacterial [12], antifungal [13], anti-inflammatory [14], antihypertensive [15], antiviral [16], antidiabetic [17], anticonvulsant [18] and anticancer activities [19]. In our previous research, after synthesizing different derivatives of 3,4,5-triphenyl-oxadiazole derivatives and confirming their α-glucosidase inhibitory activity, conducting kinetic studies and molecular docking that confirmed a strong association with the active site of the enzyme, these compounds were considered as potential new anti-diabetic agents [7].

In this study, additional biological experiments were carried out on male Wistar rats with two derivatives with the highest inhibitory activity (3,4-bis(4-chlorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (derivative No. 1) and 3-(4-chlorophenyl)-4-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (derivative No. 2).

2. Materials and Methods

2.1 Ethical considerations

The ethical approval for the study protocol was obtained from the Animal Care and Ethics Committee (ACEC) of the Tehran University of Medical Sciences. According to ACEC recommendations, we tried our best to minimize research animal pain and suffering.

2.2 Materials

Biochemical parameters were measured using commercially available kits (Pars Azmoon, Tehran, Iran). Streptozotocin was purchased from Sigma-Aldrich (USA). All the solvents used were of high purity and HPLC grade. All other chemicals and reagents used in the whole study were of analytical grade. All animals were anesthetized by using the combination of ketamine and xylazine intraperitoneally. Then, blood samples were collected from the heart, and serum was separated. All serum biochemical parameters including fasting blood sugar (FBS), urea, creatinine, cholesterol, triglycerides, calcium, phosphorus, sodium, potassium, chlorine, bilirubin, aspartate amino-transferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), phosphorous, protein, albumin and globulins were analyzed. The mortality rate of the animal and the side effects of different doses were investigated. Then histological studies of kidney, liver, and lung tissues were carried out. Herein, we investigated the effects of new tri-aryl-oxadiazol-based synthetic α-glycosidase inhibitors on the biochemical and histological parameters in STZ-induced diabetic rats.

2.3 Methods

2.3.1 Preparation of new oxadiazole-based synthetic α-glycosidase inhibitors

Synthesis of different triphenyl-oxadiazole-based synthetic α-glycosidase inhibitors was previously discussed [7]. Two derivatives with the highest α-glycosidase inhibitory activity were selected for further investigations (Figure 1).

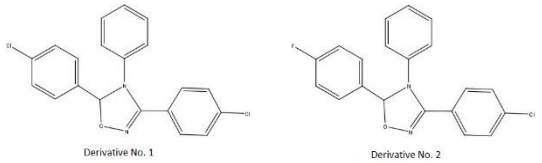

Figure 1. Chemical structure of (3,4-bis(4-chlorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (derivative No. 1) and 3-(4-chlorophenyl)-4-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (derivative No. 2).

2.3.2 Animals and Induction of Diabetes

Adult male Wistar rats with a weight range of 220-280 g were purchased from the research center and experimental animal house, Tehran University of Medical Sciences, Tehran, Iran. Animals were divided into four groups with four rats in each group and treated as follows: group I: healthy control rats treated with normal saline (HC); group II: diabetic control rats treated with normal saline (DC); group III: diabetic rats treated with oxadiazole-based synthetic α-glycosidase inhibitors derivative No. 1 (orally and intraperitoneally); group IV: diabetic rats treated with oxadiazole-based synthetic α-glycosidase inhibitors derivative No. 2 (orally and intraperitoneally). Diabetes was induced by a single intraperitoneal injection of 60 mg/kg streptozotocin (Sigma-Aldrich, USA) dissolved in 0.1 M citrate buffer (pH: 4.5) [20]. Fasted rats with blood glucose level ≥ 350 mg/dL were considered as diabetic.

2.3.3 Investigation of acute toxicity on rats

The acute toxicity studies were conducted over Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guidelines 423 [21]. Dose progression was used to determine the LD50 (Lethal Dose 50%). The first animal receives a dose a step below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased by a factor of 2-3, and if the animal dies, the dose for the next animal will be reduced accordingly.

The decision is based on the 48-hour survival pattern of all the animals up to that time. During this time, an estimate of the LD50 and a confidence interval is calculated based on the status of all the animals at termination. The LD50 is calculated using the method of maximum likelihood. Each group consisted of 6 male rats. The dose range was from 50 to 2000 mg/kg intraperitoneally and orally, and rat deaths were monitored for further 14 days.

2.3.4 Assessment of animal clinical condition

To check the clinical status of rats, increasing doses (50 to 2000 mg per kg of animal weight) are administered. All the animals were monitored once daily for skin, eye and respiratory symptoms, bloating, diarrhea, cardiac arrhythmias, changes in appearance, hypo and hyperthermia, motor problems, paralysis, and signs of pain.

2.3.5 Blood parameter analysis in rats

Following administration of derivative No. 1 and 2 in doses of 100 and 1500 mg/kg body weight, biochemical parameters were evaluated with respect to control group that received 0.9% normal saline solution. Blood serum was obtained by centrifuging the blood samples (without anticoagulant) at 1500×g for 15 min. The serum obtained was stored at − 20 °C for later use. The evaluated parameters include: fasting blood sugar (FBS), urea, creatinine, cholesterol, triglycerides, calcium, phosphorus, sodium, potassium, chloride, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALMA), phosphate, gamma-glutamyl transferase (GGT), serum protein, serum albumin, and globulin. These parameters were evaluated using an automated biochemistry analyzer (ADVIA 2400, Siemens Healthcare) and standard diagnostic test kits.

2.3.6 Histological studies

To investigate any changes, including acute and chronic inflammatory responses, liver fat change, coagulative necrosis or hypertension, etc., histological studies were performed on 5 groups of 3 adult male Wistar rats (200-250g). Rats in group I (normal control group) received a normal saline solution. The second group (group II) received high-dose of derivative No. 1 while the third group (group III) received low-dose of derivative No. 1. Group IV received high-dose of derivative No. 2, and the fifth group (group V) received low-dose of derivative No. 2. Histopathological studies were performed on organ samples of liver, kidney, and lungs. The above-mentioned organs were surgically taken off and fixed in 10% buffered formalin (pH 7.4). Then, fixed tissue samples were dehydrated, washed, and enclosed in paraffin. Then, thin tissue sections of 5 μm were obtained on a rotary microtome and then the material was stained with hematoxylin-eosin (HE) [22]. The sections were then analyzed microscopically (Olympus BX51, Japan) for pathological examinations, and photomicrographs were recorded.

2.4 Statistical analysis

The results were calculated and expressed as Mean ± Standard deviation. The data obtained in the studies were subjected to a one-way analysis of variance (ANOVA) for determining the significant difference. The intergroup significance was analyzed using Dunnet's t-test. A p-value < 0.01 was significant.

3. Results and Discussion

3.1 Investigation of acute toxicity in rats

For the two derivatives No 1 and 2, the mortality rate following peritoneal and oral administration days has been studied daily for fourteen days (Table 1 and Table 2). Following intraperitoneal administration of drug derivative No. 1 at 50-1500 mg per kg of animal weight, fetal mortality was zero. When the dosage level goes beyond 1500 mg per kg signs of toxicity appear. No lethality was recorded in the animals that received derivatives number 1 and 2 up to the dose of 1500 mg/kg during the 14 days period of treatment.

Table 1. The mortality rate of rats treated intraperitoneally with derivative No. 1 for 2 weeks.

	50 (mg/kg)	100 (mg/kg)	500 (mg/kg)	1000 (mg/kg)	1500 (mg/kg)	2000 (mg/kg)
Day 1	Nil	Nil	Nil	Nil	1	1
Day 2	Nil	Nil	Nil	Nil	Nil	1
Day 3	Nil	Nil	Nil	Nil	1	1
Day 4	Nil	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil	Nil
Day 7	Nil	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	2	3

Each group consists of six male rats

Table 2. The mortality rate of rats treated intraperitoneally with derivative No. 2 for 2 weeks.

	50 (mg/kg)	100 (mg/kg)	500 (mg/kg)	1000 (mg/kg)	1500 (mg/kg)	1800 (mg/kg)	2000 (mg/kg)
Day 1	Nil	Nil	Nil	Nil	Nil	1	2
Day 2	Nil	Nil	Nil	Nil	Nil	1	1
Day 3	Nil	Nil	Nil	Nil	Nil	Nil	Nill
Day 4	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 7	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	2	3

Each group consists of six male rats

In another test, following the oral administration of drug derivatives No. 1 and 2, the mortality rate was investigated. In this experiment, death occurs in rats that received more than 1000 and 1500 mg/kg oral doses of derivatives No.1 and 2, respectively (Figure 2). For derivative No. 1 when administered orally, the mortality was higher.

Therefore, according to the above tables, the lethal dose or LD50 for drug derivatives No. 1 and No. 2 were estimated 2000 mg/kg.

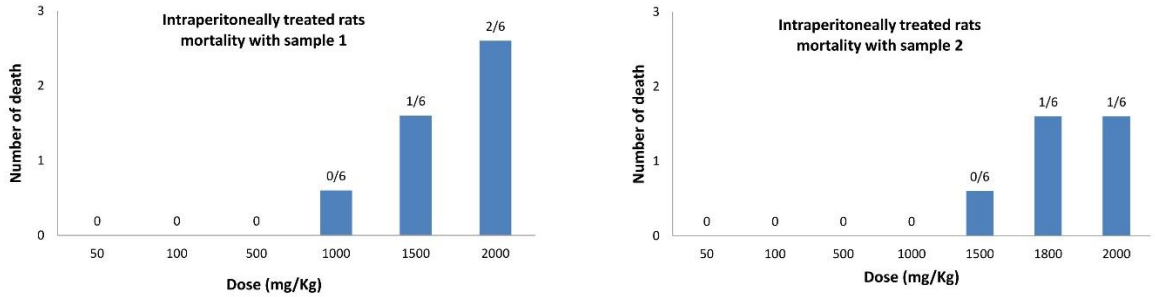

Figure 2. The mortality rate of rats treated orally with derivative No. 1 and No. 2 for 2 weeks.

3.2 Assessment of animal clinical condition

No clinical manifestations were observed following administration of drug derivative No. 1 at doses up to 1500 mg/kg (Table 3). The side effects caused following administration of these three doses (1500, and 2000 mg/kg) include skin problems, bloating, and pain. Locomotor impairments, diarrhea, and changes in fur appearance were seen at higher doses of 1500 and 2000 mg/kg. In fact, derivative No. 1 did not affect vision ability, breathing, heart rate, body temperature, and movements (Table 3).

Table 3. Clinical findings with derivative No. 1 treated intraperitoneally during toxicity study

Clinical manifestations	**50 (mg/kg)**	**100 (mg/kg)**	**500 (mg/kg)**	**1000 (mg/kg)**	**1500 (mg/kg)**	**2000 (mg/kg)**
Cutaneous	NS	NS	NS	NS	S	S
Ocular	NS	NS	NS	NS	NS	NS
Abdominal distension	NS	NS	NS	NS	S	S
Diarrhea	NS	NS	NS	NS	S	S
Respiratory	NS	NS	NS	NS	NS	NS
Cardiac arrhythmias	NS	NS	NS	NS	NS	NS
Changes in fur appearance	NS	NS	NS	NS	S	S
Hyperthermia/Hypothermia	NS	NS	NS	NS	NS	NS
Locomotor	NS	NS	NS	NS	S	S
Paralysis	NS	NS	NS	NS	NS	NS
Pain	NS	NS	NS	S	S	S

NS, Not Seen; S, Seen

Following administration of derivative No. 2, at doses of 1800 and 2000 mg/kg, no vision, respiration, cardiac arrhythmia, body temperature change, and paralysis were seen. Administration of these two doses had some adverse effects such as diarrhea, changes in fur appearance, movement complications, and pain. At 2000 mg/kg, skin complications, and bloating were seen (Table 4).

Table 4. Clinical findings with derivative No. 2 treated intraperitoneally during toxicity study.

Clinical manifestations	50 (mg/kg)	100 (mg/kg)	500 (mg/kg)	1000 (mg/kg)	1500 (mg/kg)	1800 (mg/kg)	2000 (mg/kg)
Cutaneous	NS	NS	NS	NS	NS	NS	S
Ocular	NS	NS	NS	NS	NS	NS	NS
Abdominal distension	NS	NS	NS	NS	NS	NS	S
Diarrhea	NS	NS	NS	NS	Ns	S	S
Respiratory	NS	NS	NS	NS	NS	NS	NS
Cardiac arrhythmias	NS	NS	NS	NS	NS	NS	NS
Changes in fur appearance	NS	NS	NS	NS	NS	S	S
Hyperthermia/Hypo thermia	NS	NS	NS	NS	NS	NS	NS
Locomotor	NS	NS	NS	NS	NS	S	S
Paralysis	NS	NS	NS	NS	NS	NS	NS
Pain	NS	NS	NS	NS	NS	S	S

NS, Not Seen; S, Seen

3.3 Blood parameter analysis in rats

In rats receiving drug derivative No.1 at doses 100 and 1500 mg/kg, blood parameters (fast blood sugar, liver enzymes, calcium, urea, globulins, chloride, total protein, and alkaline phosphate) were analyzed and compared with the control group (receiving normal saline). The results are summarized in Table 5.

The rats received derivative No 1 at doses of 100 and 1500 mg/kg showed reduced fasting blood sugar, liver enzymes ALT, AST, and alkaline phosphatase levels while insulin levels increased. Other parameters did not change significantly. Derivative No. 2 at doses of 100 and 1800 mg/kg showed higher antidiabetic and liver protective activity (Table 6).

Table 5. Analysis of blood parameters in male rats treated with derivative No. 1 (Mean±SD).

Dose (mg/kg)	FBS (mg/dL)	Insulin (Ug/L)	AST (IU/L)	ALT (IU/L)	ALP (U/L)
Control	218±13.49	0.89±0.218	79±2.828	47±4.243	621.5±21.1
100	183±11.48	1.6±0.315	60±1.414	42.3±3.657	561.5±33.3
1500	165±15.56	1.9±0.211	69.5±1.345	44.5±2.506	577.5±30.9

Dose (mg/kg)	Cholesterol (mg/dL)	TG (mg/dL)	Blood Urea (mg/dl)	Creatinine (mg/dL)	Bilirubin (mg/dL)	Serum albumin (mg/dL)	Globulin S (g/dl)	Total Protein (g/dl)	Calcium (mg/dL)	Sodium (mg/dL)	Potassium (mg/dL)	Chloride (mg/dL)	Phosphorous (mg/dL)
Control	80±4.8	71±4.22	45.5±4.95	0.6±0.02	0.2±0.018	3.7±0.31	2.9±0.565	6.15±1.202	11.4±1.98	141±11.66	4.7±0.452	92±6.36	8.6±0.501
100	74±3.46	67±3.12	45.5±24.12	0.7±0.022	0.2±0.014	3.2±0.223	3.15±0.353	6.4±0.424	11.6±0.848	140±10.12	5.3±0.364	80.5±13.43	8.3±0.53
1500	70±4.2	53±4.46	47.5±4.70	0.7±0.014	0.2±0.016	3.3±0.224	3.5±0.282	6.7±0.282	10.65±0.35	140±12.58	4.9±0.444	89.5±3.53	8.5±0.488

Table 6. Analysis of blood parameters in male rats treated with derivative No. 2 (Mean±SD).

Dose (mg/kg)	FBS (mg/dl)	Insulin (Ug/L)	AST (IU/L)	ALT (IU/L)	ALP (U/L)
Control	218±83.439	0.89±0.214	79±2.828	43±4.243	620±22.627
100	162±36.77	2.1±0.484	57±14.849	33.5±0.707	560±124.45
1800	151.5±14.849	2.7±0.349	65.5±9.192	39±2.828	541.5±272.89

Dose (mg/kg)	Cholesterol (mg/dL)	TG (mg/dL)	Blood Urea (mg/dl)	Creatinine (mg/dL)	Bilirubin (mg/dL)	Serum albumin (mg/dL)	Globulin S (g/dl)	Total Protein (g/dl)	Calcium (mg/dl)	Sodium (mg/dL)	Potassium (mg/dL)	Chloride (mmol/L)	Phosphorous (mg/dL)
Control	74±4.1	71±3.88	40±4.243	0.8±0.08	0.2±0.02	2.8±0.22	3.05±0.212	6.3±0.000	11.5±0.353	141±10.84	4.7±0.55	88±2.828	9.2±0.82
100	82±5.46	70±3.62	44±4.071	0.7±0.042	0.2±0.024	3.2±0.24	3.21±0.975	6.65±0.070	11.05±0.070	139±11.11	4.9±0.446	84±12.021	8.7±0.66
1800	76±4.82	56±3.94	45.5±4.950	0.7±0.024	0.2±0.018	3.2±0.222	2.9±0.565	6.15±1.202	11.4±1.980	138±10.38	5.1±0.502	82±6.364	8.6±0.628

3.4 Histological studies

Figure 3 shows the micrographs of the liver, kidneys, and lungs following the administration of derivatives No. 1 and 2. The results indicate a normal tissue morphology and shape with no specific histological changes.

After 24 hours of derivatives administration at low doses of 1000 mg/kg, micrographs of the liver, kidneys, and lungs do not show any significant histological changes. Following the administration of derivative No. 1 at a dose of 2000 mg/kg, mild proximal changes, and distal tubular cell swelling (cell degeneration) were observed. Structural necrosis is also seen in the kidney. Lung's inflammation is checked by the presence of inflammatory cells (major share), high blood pressure, and bleeding (metal-tipped arrow). Besides, parts of the liver showed mild hydropic degeneration of hepatocytes.

After 24 hours of administration of derivative No. 2 at 2000 mg/kg, mild hydropic degeneration of hepatocytes (ballooning degeneration) and high-density blood collection in hepatic sinus were seen. In addition, renal tubular cell necrosis, pulmonary hemorrhage, and inflammatory lesions within the alveoli were observed in the harvested specimens. Therefore, at 2000 mg/kg, both drug derivatives are considered toxic but nontoxic up to 1500 mg/kg.

Diabetes is one of the most serious, debilitating, and high-prevalence metabolic diseases in the world and is characterized by chronic hyperglycemia along with other disorders [1-2]. This disease is a serious health problem and requires careful examination and management [8-10]. One way to treat and control the disease is to delay glucose uptake by inhibiting the enzyme α-glucosidase. In recent years, much research has been done on the natural and synthetic inhibitors of the α-glucosidase enzyme [3-7]. The cytotoxicity of tri-aryl-1,2,4-oxadiazole derivatives were studied [23].

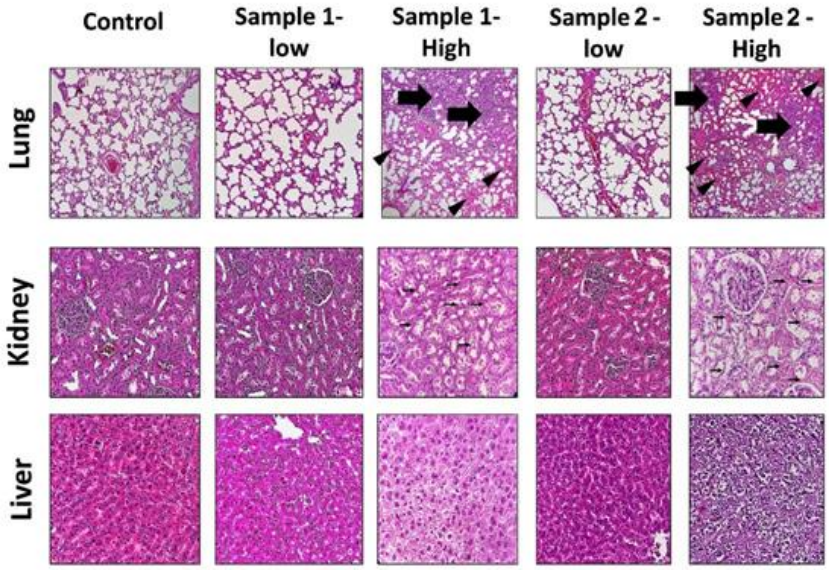

Figure 3. Histopathologic sections of the kidney (X400), liver (X400), and lung (X100) samples in different experimental groups, Thick arrows: infiltration of inflammatory cells, arrowheads: hemorrhage, Thin arrows: renal tubular cell necrosis. H&E stain.

Anti-proliferative activity against MCF-7 and K562 cell line using MTT assay were screened at 50 µM concentration on more than 35 number of the related structure. Some of them showed very low toxicity (IC_{50} more than 250 µM). Later two compounds (1,2) were selected for evaluation of anti-diabetic activity in-vitro model. Different synthesized derivatives of tri-aryl-oxadiazole were studied for α-glucosidase inhibitory activity, enzyme kinetic, and molecular docking studies into the active site of the enzyme [7]. These compounds were suggested as potential candidates to display antidiabetic properties. In this study, two derivatives with the highest inhibitory activity were selected and further biological experiments were performed on rats. Blood parameter analysis in rats was performed after prescribing. Evaluated parameters were fasting blood sugar (FBS), urea, creatinine, cholesterol, triglycerides, calcium, phosphorus, sodium, potassium, chlorine, bilirubin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), protein, albumin, and serum globulin. The mortality rate of the animal and the side effects of different doses of derivatives 1 and 2 and then histological studies of kidney, liver, and lung tissues were performed. The lethal dose or LD50 value for the derivative No 1 and 2 was 2000 mg/kg.

Derivatives No 1 and 2 had no adverse effects on vision and respiration function, neither cause cardiac arrhythmia, temperature disturbances, and disability with no side effects on the skin, gastrointestinal tract, appearance change, and pain up to 1500 mg/kg. Blood parameters (sugar, liver enzymes, calcium, urea, globulins, chloride, total protein, and alkaline phosphate) treated rats were compared with the control group. Following the administration of low doses, significant decrease in fast blood sugar and considerable increase in insulin level in respect to the control group was seen. Liver markers level showed noticeable changes which confirm the protective ability of both derivatives. The drug effect on other blood parameters such as creatinine, cholesterol, triglycerides, phosphorus, sodium, potassium, bilirubin, omega, and albumin were also tested and did not have a significant effect on any of these parameters.

4. Conclusion

Following the administration of an increased dosage of these derivatives, liver, kidney, and lung micrographs show normal tissue morphology with no histological changes. At higher doses (2000 mg/kg), some histopathological changes were observed. Therefore, both derivatives were considered nontoxic and safe up to 1500 mg/kg.

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Conflicts of interest

The authors declare that they have no conflicts of interest concerning this manuscript.

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