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RESEARCH ARTICLE

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Study of Toxic Effects of Oxothiazole Derivative as a New Antibacterial Agent

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ABSTRACT

The spread of antibiotic-resistant bacteria in many humans and animals has driven researches to identify and design novel antibacterial agents. *In vitro* inhibitory activity of (2*E*)-2-(4,5-dihydro-4-oxothiazol-2-yl)-2-(thiazolidin-2-ylidene) acetonitrile against many bacterial pathogens has been proven in both veterinary and human medicine. In this study, its *in vivo* toxic effects was studied in mice. The median lethal dose (LD50) value of 239.88 mg/kg was estimated using intraperitoneal injection in 8 groups of mice after 48 h treatment. Then, intraperitoneal injections of LD50 of oxothiazole solution into 4 other mice were done to evaluate histopathological changes in their liver and kidney tissues. The histopathological studies were identified as fatty change, hepatitis, necrosis and regeneration in liver, and fibrosis, necrosis, nephritis, hyaline cast and hyperaemia in kidney. In conclusion, the synthesized oxothiazole derivative causes renal and hepatic toxicity in mice at medium concentrations. The change of thiazole substituents and complexation may reduce its toxicity

Keywords

Toxic effects, Histopathological study, Oxothiazole derivative

Abbreviations

LD50: Median Lethal Dose

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Introduction

Species of pathogenic bacteria have developed resistance to current antibiotics such as penicillin, amoxicillin, clindamycin, tetracycline, erythromycin and sulfamethoxazole due to overuse of drugs in humans and animals [1]. This health problem increases costs of medical care [2]. In recent years, many researchers were encouraged to discover new antibacterial agents including herbal extracts, metal nanoparticles and heterocyclic derivatives [3].

Thiazoles are a major class of heterocyclic compounds, which have been used in the treatment of diseases such as cancer, hypertension and lipid disorders [4]. Significant antibacterial effects were observed by thiazole derivatives. It was found that they can inhibit the activity of DNA gyrase B and ecKAS III enzymes, and were effective against the replication of DNA and the synthesis of fatty acids, in bacteria [5].

In vitro inhibitory activities of thiazoles have been assessed against a variety of pathogenic bacteria including both Gram-positive and Gram-negative strains [6-8]. These compounds inhibit the growth of bacteria by blocking ion channels as well as inhibiting enzymatic and cellular activities [9-12]. However, there are a few *in vivo* studies on body and organ of animals.

Oxothiazole derivative is a recently synthesized heterocyclic compound that its *in vitro* inhibitory activities were evaluated against some Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Streptococcus agalactiae, Listeria monocytogenes, Bacillus cereus, Escherichia coli and Salmonella typhimurium* [13-16]. The present study was performed to assess the function and histology of the liver and kidney in mice exposed to the synthetic oxothiazole.

Results

The toxic and therapeutic effects of oxothiazole derivative were evaluated in 8 groups of 8 mice each. The 48 h LD50 value of compound was 239.88 mg/kg (Tables 1 and 2, Figure 1). Histopathological changes including fatty change, hepatitis, necrosis and regeneration in liver, and fibrosis, necrosis, nephritis, hyaline cast and hyperaemia in kidney were observed as illustrated in Figures 2 and 3.

Table 1
Mortality percent in each group after IP injection of oxothiazole.

Dose mg/kg	log	Died	Percent	Lived	Total
160	2.20	0	0	8	8
200	2.30	1	12.5	7	8
220	2.34	2	25	6	8
240	2.38	4	50	4	8
265	2.42	6	75	2	8
300	2.47	8	100	0	8
350	2.54	8	100	0	8

Table 2
Different LD doses of oxotiazole

LD10	190.55
LD40	230.14
LD50	239.88
LD80	267.92
LD90	278.61

Discussion

Thiazole ring is an essential part of numerous biologically active compounds. Thiazole derivatives are recognized to affect cell cycle progression, protein production and DNA replication [17-19]. Sulfathiazole as an antibiotic containing thiazole ring was used in the treatment of gastrointestinal and respiratory infections. Its oral LD50 in mice is 4500 mg/kg. Although toxicity of the drug is low, it is prescribed less frequently in humans due to its side effects such as high fever, severe headache, stomatitis, conjunctivi-

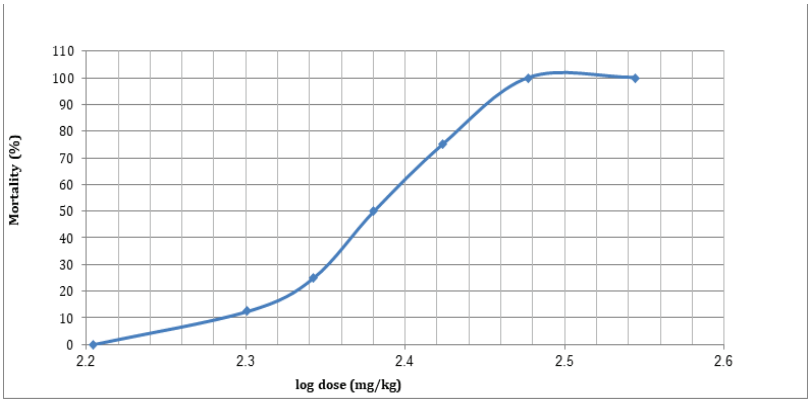

Figure 1
Mortality of mice infected with oxothiazole

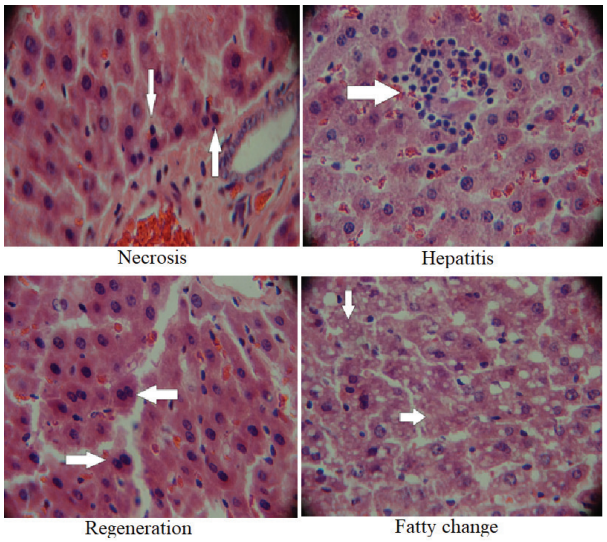

Figure 2
Histopathological changes of liver tissue in mice treated with oxothiazole

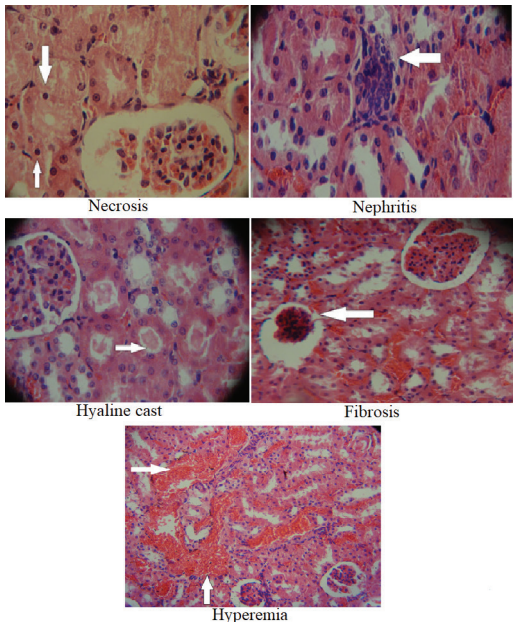

Figure 3
Histopathological changes of kidney tissue in mice treated with oxothiazole

tis, rhinitis, urethritis and balanitis [20]. Febuxostat is a xanthine oxidase inhibitor containing thiazole ring that lowers uric acid levels in blood. The LD50 of drug administered orally to mice is 300 mg/kg [21]. Sprycel (dasatinib) is a chemotherapy medication used to treat chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). It has a relatively high toxicity (oral LD50 = 100 mg/kg) in rat. Its consumption may cause anemia, swelling, pulmonary edema, heart failure, rash and diarrhea [22].

Anticoagulant and antiarrhythmic effects of some thiazole derivatives were studied by Abd El-Galil et al. in 2009 via determination of LD50 values of 292-736 mg/kg. Although oxothiazole is more toxic than these derivatives, the determination of median effective dose (ED50) and therapeutic index (TI) of this heterocycle in future researches is necessary for a better conclusion [23]. Siddiqui et al. in 2010 calculated the toxic effects of 3-[4-(substituted phenyl)-1,3-thiazol-2-ylamino]-4-(substituted phenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thiones as new anticonvulsant agents, their LD50 values (834 and 936 mg/mL) are higher than those in phenytoin (a common anticonvulsant drug with LD50 = 224 mg/mL) [24].

Efficiency of some synthesized imidazo[2,1-b] thiazole derivatives as diuretic agents was proven in comparison with those of hydrochlorothiazide and furosemide, LD50 values were reported in the range of 1000 to 1500 mg/mL [25].

Among 12 thiazolidines synthesized by Wang et al., only three derivatives showed significant anti-inflammatory activities. They can be used to treat hepatic injuries according to the results obtained by further Con A-induced acute liver injury of BALB/c mice [26]. Thiazole-Zn is a chinese-created systemic fungicide that had no pathological effects on the liver and kidney of female rats when used as oral gavage, while its destructive effects were observed in the thyroid gland [27].

The toxicity of oxothiazole derivative has been preliminarily studied in mice at doses of 350 and 160 mg/kg without determination of LD50. All mice died at dose of 350 mg/kg after 24 h. The liver of mice treated at dose 160 mg/kg was evaluated after 48 h [15]. To extend toxicological studies, some biochemical and physiological effects of this potential antibacterial agent were assessed via determination of LD50 value and evaluation of histopathological changes in the liver and kidney of mice. Renal and hepatic toxicity were observed in mice when medium doses of heterocyclic derivative were injected. New oxothiazole derivatives with low toxicity can be designed in future studies by changing thiazole substituents and complexation.

Material and methods

General procedure for the synthesis of oxothiazole compound

Oxothiazole (3) was synthesized according to a previous procedure [28] as follows: a suspension of 1 mmol of each (*E*)-2-cyano-2-(thiazolidin-2-ylidene) ethanethioamide (1), ethyl bromoacetate (2) and sodium bicarbonate ($NaHCO_3$) in 1 mL DMF as solvent was stirred at room temperature for 8 h. 5 mL of water was added to the reaction mixture. The precipitate was filtered out, and washed with water (1 × 5 mL) and ethanol (1 × 5 mL),

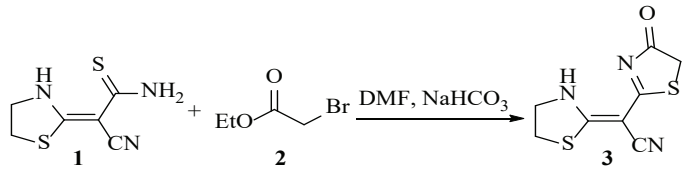

Figure 4
Synthesis of oxothiazole derivative 3 *(2E)-2-(4,5-Dihydro-4-oxothiazol-2-yl)-2-(thiazolidin-2-ylidene)acetonitrile.*

respectively. The pure thiazole 3 was obtained in 75% yield after recrystallization from acetonitrile, and its chemical structure was confirmed through the elemental analysis and spectroscopic data (Figure 4).

m.p. 248-252 °C; IR (KBr) ν: 3422 (NH), 2193 (C≡N), 1688 (C=O), 1573 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.63 (t, J = 7.8 Hz, 2H, SCH_2), 4.01 (s, 2H, $COCH_2$), 4.12 (t, J = 7.8 Hz, 2H, $NHCH_2$), 10.53 (s, 1H, NH) ppm; MS (EI, m/z): 225 (M+, 21), 176 (37), 150 (94), 109 (28), 46 (100); Anal. Calcd for $C_8H_7N_3OS_2$: C, 42.65; H, 3.13; N, 18.65. Found: C, 42.74; H, 3.00; N, 18.46.

In vivo study

68 white male BALB/c mice, weighing 26-34 g, were obtained from the Central Animal House, University of Zabol, Zabol, Iran. Mice were maintained at all stages with free access to standard food and refined water under 25 ± 2 °C temperature, 50% humidity, 12/12 light dark cycle [24].

Assessment of median lethal dose (LD50)

An up-and-down method was applied for acute toxicity testing. 64 mice were divided into 8 groups. Finally, doses including 160, 200, 220, 240, 265, 300 and 350 mg/kg of oxothiazole in 10% DMSO were selected for intraperitoneal injection (IP). 10% DMSO was also injected as negative control. Mice mortality was recorded in each group during 48 h [24].

Histopathological examination

4 BALB/c were injected intraperitoneally with the median lethal doses of oxothiazole. The abdominal walls of dead and live mice were excised immediately or after ether-euthanasia. The liver and kidney of mice were removed from their abdominal cavity, and stored in 10% formalin. All solutions were refreshed three times every 24 h. The histological sections were molded in 4- to 6-µm-thick paraffin according to the histopathological principles. The prepared slides were stained with hematoxylin and eosin, and examined under a light microscope [27].

Acknowledgment

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Author Contributions

All authors contributed to the design of study, data analysis and manuscript preparation.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Abstracts (in Persian)

بررسی اثرات سمیت مشتق اکسوتیازول به عنوان یک عامل ضد باکتریایی جدید

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چکیده

گسترش مقاومت باکتریایی در بسیاری از پاتوژن‌های مهم انسانی و دامی، سبب افزایش تمایل به شناسایی ترکیبات جدید ضد باکتریایی و اثرات دارویی و سمیت آنها به منظور مهار سویه‌های مقاوم شده¬است. مشتقات تیازول از ترکیبات جدید ضد باکتریایی هستند. مشتق جدید اکسو تیازول ترکیبی است که در شرایط آزمایشگاهی، بر بسیاری از باکتری‌های بیماری زا اثر مهارکننده داشته است، در این مطالعه اثرات سمی آن روی موش بررسی شده است. با تزریق داخل صفاقی محلول اکسو تیازول به ۶۴ موش سوری نژاد ویستار در ۸ گروه ۸ تایی، دوز کشنده متوسط (LD۵۰) محاسبه گردید. سپس با تزریق مجدد دوز معادل LD۵۰ به موش‌ها، تغییرات هیستوپاتولوژی بافت کبد و کلیه آنها بررسی شد. نتایج اثر سمیت اکسو تیازول را با LD۵۰= ۲۳۹/۸۸ mg/Kg و عوارض تغییرات چربی، رجنراسیون، هپاتیت و نکروز در بافت کبد و نفریت، نکروز، فیبروزی شدن و کست هیالن را در کلیه موش‌های سوری نشان داد. پس از تعیین میزان سمیت اکسو تیازول ، می‌توان در مورد این ترکیب با اندازه گیری دوز مؤثر (ED۵۰) و شاخص درمانی (TI) قضاوت کرد.

واژگان کلیدی

اثر سمیت، مطالعات هیستوپاتولوژی، مشتق اکسوتیازول