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TOXICOLOGICAL EVALUATION OF SOME NOVEL OXAZOLONE DERIVATIVES

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ABSTRACT

All new pharmaceutical drugs are tested for their safety prior to their use in human volunteers and patients. A key stage in ensuring the safety of drugs is to conduct toxicity study in appropriate animal models. In the present study the toxicological profile of some novel oxazolone derivatives was evaluated. Three derivatives viz: OXZ-1,OXZ-2,OXZ-3 were screened for their Acute and sub acute toxicity. Seed bioassay was also used as an alternative to animal experimentation. Various parameters were observed such as Body weight, Organ weight, haematology and biochemical analysis during the study. Histopathological studies of liver, kidney and heart were also performed to determine the toxicity of the derivatives. All the three derivatives were found to be non toxic having LD_{50} value above 5000mg/kg. The hematological parameters of oxazolone treated rats at the dose of 1000 mg/kg p.o. shown increase in total WBC count and increase in Platelets count. The results of histopathology of heart, liver and kidney were intermediary. Altogether it can be concluded that the Oxazolone derivatives were nontoxic upto a dose of 5000mg/kg.

Keyword: Oxazolone, Acute toxicity, OECD, Biochemical, Histopathology.

INTRODUCTION

Today, most developed countries have enacted laws and regulations to control the marketing of drugs, vaccines, food additives, pesticides, industrial chemicals, and other substances of potential toxicological concern. Such regulations often prescribe a specific regime of toxicity

testing to generate information that will enable government regulators to determine whether the benefits of a particular substance outweigh its risks to human health and/or the environment. This process of regulatory risk assessment can be broken down into three main phases: *Hazard identification:* Determination of a substance's intrinsic toxicity (e.g., eye irritation, birth defects, or cancer) through the use of toxicity tests. Test results are then analyzed to determine what, if any, adverse effects occur at different exposure levels (known as a "dose-response" assessment) and, where possible, to identify the lowest exposure level at which no adverse effects are observed (known as the "no observed adverse effect level" or "NOAEL").*Exposure assessment:* Determination of the extent of human and/or environmental exposure to a substance, including the identification of specific populations exposed, their composition and size, and the types, magnitudes, frequencies, and durations of exposure. *Risk characterization:* A composite analysis of the hazard and exposure assessment results to arrive at a "real world" estimate of health and/or ecological risk.

Heterocyclic compound are acquiring more importance in recent years due to the pharmacological activities. Nitrogen, sulphur, oxygen containing five/six member heterocyclic compound has occupied enormous significance in the field of medical chemistry. Oxazolone plays very important role in the manufacturing of various biologically active drugs as analgesic, anti-inflammatory, antidepressant, anticancer, anti-microbial, anti-diabetic and anti-obesity. In view of this it was of considerable interest to synthesize the derivatives of the title compound with a hope to obtain potent biologically active compound. Oxazolone is a class of small heterocyclic compounds. This heterocyclic compounds have acquired more importance in recent years due to their pharmacological activities. It plays a very pivotal role in the manufacture of various biologically active drugs with analgesic, anti-inflammatory, anti-depressant, anti-cancer, anti-microbial, anti-diabetic and anti-obesity properties.[1,2,3]. Its IUPAC name is 3H-Oxazole-2-one. The present study is to evaluate the toxicity associated with the various novel oxazolone derivatives.

MATERIALS AND METHODS

Animals

Male albino mice weighing 25-30g were selected and kept to be acclimatized for the period of ten days at room temperature. They were housed in a standard cage and maintained under standard environmental conditions 12:12 hr light: dark cycle. They have free access to food

and water *ad libitum*. All the procedures were performed in accordance to the Institutional Animal Ethics Committee.

Test Substances

Three powdered test substances OXZ-1,OXZ-2,OXZ-3 were provided by the Chemistry laboratory of Himalayan Pharmacy Institute.

Name	IUPAC name
OXZ-1	4-[benzylidene]-2-(4-methoxy-phenyl) oxazole-5-one
OXZ-2	4-[methoxy benzylidene]-2-(4-methoxy-phenyl) oxazole-5-one
OXZ-3	4-[furan-3-ylmethylene]-2-(4-methoxy-phenyl) oxazole-5-(4H)-one

Acute Toxicity Study

OECD guideline for testing of chemicals, Acute Oral Toxicity- Up and down Procedure-425 has been used to study the acute toxicity of all the compounds.[1]

Description of the Method

Selection of animal species

Wistar rats of both sex and approximately the same weights (150-200 g) were selected for the study. All the animals were falling under age group of 8-12 weeks.

Procedure for main test

All the animals were fasted overnight before starting the dosing. Single animals were dosed at 48 h intervals. Dosing was initiated with 175 mg/kg. All the compounds OXZ1-OXZ3 were administered orally in the form of suspension with 0.5 % carboxy methyl cellulose (CMC). A 23 gauze needle was used to administer the doses. First animal received 175 mg/kg dose and then doses were increased by a factor of 3.2. Animals were observed individually once during first 30 min. after dosing, periodically during the first 24 h. and daily thereafter for a total of 14 days. All the signs of toxicity were recorded during the period of study.[1]

Subacute Toxicity Study

OECD guideline for testing of chemicals OECD TG 407 [Repeated Dose 28-day oral toxicity study in rodents] has been used to study the subacute toxicity of the oxazolone derivatives.[3]

Experimental animals

Wistar rats (weight: 150-200 g; age: 6-8 weeks old) were randomly assigned into three groups (n=10), five females and five males in each group. Group of five rats were housed together in stainless steel cages (males separated from females) with 12 h light/dark cycle in a temperature and humidity controlled environment.[2,4]

Procedure

Treatments were administrated orally by gavage once a day for 4 weeks. The first group of animals, serving as control, received normal saline (5 ml/kg p.o.); second and third group received the oral doses of 500 mg/kg and 1000 mg/kg of the oxazolone derivative OXZ1 respectively. All animals were supplied with standard diet and water *ad libitum* during the testing periods. All rats were weighed and observed weekly for physiological and behavioral changes. Clinical signs were observed at least once a day throughout the 28 days of dosing. Any rat that died during the test period was tested pathologically, and all animals were examined at the end of the test period.[3,5]

Haematology

On the day 29th all surviving animals were fasted overnight and anesthetized afterwards by Phenobarbitone (100 mg/kg i.p.) for blood collection by cardiac puncture. Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count and blood clotting time were measured using the standard haematological examination techniques.[6]

Biochemical analysis

From a portion of blood collected, serum was separated by centrifugation (6000 rpm for 15 min.). It was undertaken for the study of biochemical parameters like ALP, ALT, AST, Triglycerides, Total Cholesterol, Blood Glucose, HDL and Total bilirubin were measured using biochemical diagnostic kits (Span Diagnostics Ltd, Surat).

Histopathology

The vital organs like heart, liver and kidneys were surgically removed from all the groups of animals. Organs were weighed and undergone for histopathological studies.

Tissue fixation

The purpose of tissue fixation is to prevent the decomposition of tissue due to loss of blood supply and oxygen, accumulation of products of metabolism, action of autolytic enzymes and putrefaction by bacteria. Fixatives act by denaturation or precipitation of cell proteins or by making soluble components of the cells insoluble.

Liver, heart and kidneys of animals from control and treated groups were sliced into small sections.

All the tissue samples were rinsed with cold saline. Then tissues were washed with 10% buffered formalin and preserved overnight in 10% buffered formalin.

Dehydration

This is a process in which water from cells and tissues is removed so that this space is subsequently taken up by wax. Dehydration is carried out by passing the tissues through a series of ascending grades of alcohol 30%, 50%, 70%, 90% and absolute alcohol.

Firstly, samples were washed repeated with 30% alcohol, and then washed with 50% alcohol for 4 hrs. Then washed with 70% alcohol and samples were preserved in 70% alcohol for overnight.

Again, next day samples were washed with 90% alcohol for 4 hrs. and with absolute alcohol for 1 hr. Then all samples were preserved in cedar Wood oil.

Clearing

This is the process in which alcohol from the tissues and cells is removed and is replaced by a fluid in which wax is soluble and it also makes the tissue transparent. Xylene is most commonly used clearing agent.

The tissue samples were taken up from cedar wood oil and were dipped in xylene for the removal of water.

Infiltration

This is the process in which empty spaces in the tissue and cells after removal of water are taken up by paraffin wax. This process is done with molten paraffin wax at 59-65ºC.

For this purpose the tissues were first dipped in xylene: paraffin wax in 1:1 ratio for one hr. at 60ºC. Then tissues were dipped in molten paraffin for one hr. at 60ºC.

During this process paraffin wax was impregnated into the empty spaces between tissues.

This hardens the tissue which helps in section cutting.

Embedding and blocking

In this process the processed tissues are embedded in the molten wax for section cutting. Moulds are used for this purpose. We had used L-shaped moulds for our studies.

Molten wax was poured in the spaces between L-shaped moulds .The processed tissue pieces were put into wax with number tag and examining surface facing downward. Then wax was allowed to solidify. After solidification, L shaped moulds were removed and each block contained a tissue piece carrying a label.

Microtomy

We had used the rotary microtome (ACMAS Tech Pvt. Ltd., Delhi) for the purpose. In this microtome knife is fixed while the tissue block is movable. Paraffin blocks containing tissue in it were put in the rotary microtome and sections were cut down by operating the microtome manually after adjusting the thickness at 5-6 µm. Then sections were picked with the help of forceps and put on to the glass slides and glass slides were placed over a heating plate for a moment for the purpose of stretching the tissue and removing any air between the section and slide. These sections were then subjected for staining.[6,7]

RESULTS

Single dose 14 day oral toxicity study in rat

According to OECD guidelines for testing of chemicals, Acute Oral Toxicity- Up and down Procedure-425 all the oxazolone derivatives were screened for acute toxicity. According to stopping criteria, 3 consecutive animals survived at the upper bound. No death was recorded during the 14 days of observation period in the male and female animals given up to 5000 mg/kg p.o. of the compounds orally.

Limit test at 2000 mg/kg: We have administered 2000 mg/kg p.o. individually to one animal then after four animals and watched for lethal effect and signs of toxicity for 14 days

Serial no.	Animals	Dose	Log Dose	X-response O-nonresponce	LD_{50}
1	1	2000mg/kg p.o	3.3010	O	Maximum likelihood calculation cannot be completed. LD50 is Greater than 2000mg/kg.
2	2	2000mg/kg p.o	3.3010	O	
3	3	2000mg/kg p.o	3.3010	O	
4	4	2000mg/kg p.o	3.3010	O	
5	5	2000mg/kg p.o	3.3010	O	

Limit test at 5000 mg/kg: From the observation of limit test at 2000 mg/kg encouraged me to perform limit test at 5000 mg/kg. Here the same procedure was adopted as like limit test at 2000 mg/kg.

Limit test at 5000 mg/kg

Serial no.	animals	dose	Log dose	X-response O-nonresponce	Ld_{50}
1	1	5000mg/kg p.o	3.6990	O	Maximum likelihood calculation cannot be completed. LD_{50} is Greater than 5000mg/kg.
2	2	5000mg/kg p.o	3.6990	O	
3	3	5000mg/kg p.o	3.6990	O	
4	4	5000mg/kg p.o	3.6990	O	
5	5	5000mg/kg p.o	3.6990	O	

According to OECD guidelines for testing of chemicals, Acute Oral Toxicity- Up and down Procedure-425 all the oxazolone derivatives were screened for acute toxicity. According to stopping criteria, 3 consecutive animals survived at the upper bound. No death was recorded

during the 14 days of observation period in the male and female animals given up to 5000 mg/kg p.o. of the compounds-OXZ1, OXZ2, and OXZ3 .

Parameters observed during acute toxicity study

Parameters	OXZ-1	OXZ-2	OXZ-3
Diarrhea	_	_	_
Muscle Relaxation	_	_	_
Paw Licking	_	_	_
Sedation	+	_	_
Muscle Spasm	_	_	_

Where, (+) =positive response, (-) =no response

Table No.1 Effects of OXZ on Haematological parameters in subchronic toxicity study (Male)

Oxazolone derivatives	**Dose(mg/kg)**	**Haematological Parameters**			
		RBCx 106	Hb(g/dl)	PVC(%)	WBCx 103
OXZ-1	**control**	4.6±0.5	10.8±0.2	30.6 ± 1.4	3.7±0.5
	500	6.1±0.7*	12.3±0.8*	41.0±1.1*	4.0±0.1
	1000	6.8±0.3*	14.7±1.3*	43.7±0.5*	5.1±0.6*
OXZ-2	**control**	8.3±0.5	11.7±0.6	35.6±1.2	4.2±0.5
	500	11.5±0.8*	14.2±0.3**	48.2±1.7*	4.9±0.8*
	1000	11.9±0.6*	15.8±0.9*	51.6±0.9*	5.3±0.4*
OXZ-3	**control**	3.9±0.4	10.4±0.4	33.8±0.8	3.2±0.5
	500	5.6±0.5*	13.1±0.2*	45.6±1.0*	3.5±0.3*
	1000	6.16.1±0.7*	13.7±0.8*	49.4±1.3**	4.1±0.9*

Data expressed as mean ±SEM. Evaluation by one way ANOVA followed by Dunnett's multiple comparison tests. *P<0.05 and **P<0.01 as compared to control.

Table no. 2Effects of OXZ on Haematological parameters in subchronic toxicity study (Male)

OXZ	Dose (mgkg)	Biochemical parameters					
		SGOT	SGPT	Total Protein	Albumin	Cholesterol	Triglycerides
OXZ-1	control	113.7±0.76	103.8± 0.81	7.32 ± 0.01	4.13± 0.01	472 ± 1.05	451 ± 1.05
	500	134.8±1.07**	119.4±0.72**	7.69±0.01*	4.28±0.01*	429 ± 0.83**	436 ± 0.93*
	1000	156.4±0.83**	142.7±0.83**	8.13±0.01**	4.39±0.01**	418 ± 1.02*	395 ± 0.87**
OXZ-2	control	119.6±1.05	107.2±0.93	8.01±0.01	4.17±.04	468 ± 1.21	446 ± 1.04
	500	149.8±0.71**	125.1±1.15**	8.39±0.03*	4.32±0.01*	421 ± 0.92*	441 ± 0.71*
	1000	162.6±0.83**	155.3±1.03**	8.68±0.01*	4.49±0.01*	409 ± 1.04*	422 ± 0.93*
OXZ-3	control	110.3±1.01	105±0.78	7.48 ± 1.01	4.09± 1.05	482±0.72	439±1.06
	500	139.4±0.93**	111±0.83*	7.67 ± 0.91	4.16± 0.84	437±0.81*	392±0.93**
	1000	168.7±1.02**	147±1.02**	7.89±1.13*	4.45±1.31*	403±1.03**	379±0.83**

Data expressed as mean ±SEM. Evaluation by one way ANOVA followed by Dunnett's multiple comparison tests. *P<0.05 and **P<0.01 as compared to control.

Table No. 3 Effects of OXZ derivatives on organ weight of animals in subchronic toxicity

Oxazolone derivative	Dose (mgkg)	Organ weight		
		LIVER	HEART	KIDNEY
OXZ-1	Control	5537±1.64	548±1.61	1121±1.82
	500 mg/kg	5592±3.01*	561±1.93*	1129±1.12
	1000 mg/kg	5783±1.38**	623±0.98**	1171±0.71**
OXZ-2	Control	5549±2.71	539±1.04	11021.95

	500 mg/kg	5557±1.93	543±1.15	1143±2.74**
	1000 mg/kg	5573±1.06**	604±0.97**	115±1.33**
OXZ-3	Control	5612±1.82	553±2.15	1113±2.75
	500 mg/kg	5671±1.91*	579±1.73**	1121±2.66
	1000 mg/kg	5894±2.04**	639±1.59**	1164±1.09**

Data expressed as mean ±SEM. Evaluation by one way ANOVA followed by Dunnett's multiple comparison tests. *P<0.05 and **P<0.01 as compared to control.

DISCUSSION

Up to 5000mg/kg oxazolone derivatives did not show any specific changes in the general appearance and toxic signs during the observation period. But OXZ-1 showed minor signs of sedation compared to others derivatives whereas the other derivatives were relative non-toxic in nature. The haematological parameters of oxazolone treated rats at the dose of 1000 mg/kg p.o. shown increase in total WBC count and increase in Platelets count. The biochemical parameters of oxazolone treated rats shown decrease in cholesterol and triglycerides levels which may be due increase HDL levels. At a dose of 1000mg/kg the derivatives showed a slight increase in SGOT and SGPT levels as compared to control group whereas at a dose of 500mg/kg the levels of albumin, SGPT, SGOT and total protein were insignificant which proves oxazolone to be non toxic substance. In case organ weight of oxazolone treated animals the weight of liver, kidney and heart does not shown significant change. After evaluation of the obtained results it may be concluded that the oxazolone derivatives were non toxic in nature at a dose of 500mg/kg.

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REFERENCE

1. M.J. Aaglawe, S.S. Dhule, S.S. Bahekar, P.S. Wakte, D.B. Shinde, Synthesis and antibacterial activity of some oxazolone derivatives, *Journal of Korean chemical society* 47(2), 2003,131-134.

2. Tri Murti Andayani, Izham Mohamed, Ibrahim Mohamed, Ahmad H Asdie, the safety of triple therapy with oral antidiabetics versus insulin in type 2 diabetes. *Asian journal of pharmaceutical and research* 3,2010,201-203.
3. George sonia, kumar manoj, acharjee parameswaran, chakraborty raja, ravi thengungal kochupappy. Synthesis and evaluation of the biological activities of some 3-_[5-(6-methyl-4-aryl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl]-imino_-1,3-dihydro-2*H*-indol-2-one derivatives . Acta pharma ,58, 2008,119-121.
4. Coffen *et al*. oxazolone derivetives and uses thereof, US6, 355,641B1 (2002).
5. Garg Neha, Chandra Trilok , Kumar Ashok, Department of Pharmacology, Medicinal Chemistry Division, L.L.R.M. Medical College, Meerut (U.P.)250004, India. Synthesis and anticonvulsant activity of substituted quinoline derivatives, *International Journal of Pharmacy and Pharmaceutical Sciences* 2, 2010, 88.
6. (Patent) Puig Charles Journal of medicinal chemistry 2000. 43,
7. Muhammad.A.Mesaik, Shagufta.Rahat,Khalid M. Khan, Zia-Ullah, Muhammad I.Choudhary, Shahnaz Murad, Zakiah Islami, Atta-urn-Rahaman, Aqeel Ahmed. Synthesis ad immunomodulatory properties of special oxazolone derivetives, Bioorganic and Medicinal Chemistry. 12,(2004), 2049-2075.
8. Khan Khalid Mohammed, Mughal Uzma Rasool, Taareq Mahmud, Khan Hassan, Zia-ullah, Perveen Shanaz, Choudhury Muhammad Iqbal. Oxazolone: New tyrosinase inhibitors; synthesis and their structure activity relationships, Bioorganic and Medicinal Chemistry. 14, (2006), 6027-6033.
9. Turki Saoussen, Jabloun Zeineb , Mrabet Ghada , Marouani Ammar , Thonart Philippe ,Diouani Fethi Mohammed, Abdallah Ben Fethi, Amara Abdelkader , Ahmed Rejeb , Kalle Héla, Preliminary safety assessment of Yarrowia lipolytica extracellular lipase: Results of acute and 28-day repeated dose oral toxicity studies in rats, *Food and Chemical Toxicology* 48 (2010) 2393–2400.
10. Antonelli-Ushirobira T.M., Kaneshima E.N. , Gabriel M. , Audi E.A. Marques L.C. , Mello J.C.P., Acute and subchronic toxicological evaluation of the semipurified extract of seeds of guaraná (Paullinia cupana) in rodents, *Food and Chemical Toxicology* 48 (2010) 1817–1820.
11. Jodynis-Liebert Jadwiga, Nowicki Michal , Murias Marek , Adamska Teresa , Ewertowska Małgorzata , Kujawska Małgorzata, Piotrowska Hanna , Konwerska Aneta , Ostalska-Nowicka Danuta, Pernak Juliusz, acute and subchronic toxicity of ionic liquid,

didecyldimethylammonium saccharinate, in rats, *Food and Chemical Toxicology*, Regulatory Toxicology and Pharmacology 57 (2010) 266–273.