Research Article

Antidiabetic and Toxicity Studies of Some Novel Oxazolone Derivatives

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ABSTRACT

In this research work, 2, 4-substituted oxazolone derivatives OXZ-1 to OXZ-5 were screened for anti-diabetic activity at 200 and 400 mg/kg b.w. p.o. for 15 days in streptozotocin induced diabetic rats. Acute and sub-acute toxicity studies were performed as per OECD guideline Up and Down Procedure-425 and 407. Clinical signs, body weight, hematological, biochemical and histopathological examinations were carried out in sub-acute toxicity studies. The compounds OXZ-1, OXZ-3 & OXZ-5 at 400 mg/kg p.o reduced fasting blood glucose levels in streptozotocin induced diabetic rat on 15th day. In acute toxicity study no lethality was found up to 5000 mg/kg p.o. Sub-acute toxicity study revealed the elevated level of AST and ALT. The toxic signs in liver and kidney were appeared in histopathological studies at 1000 mg/kg p.o. of OXZ-1. It was concluded that the studied derivatives possess significant antidiabetic activity with mild toxicity in liver and kidney.

Keywords: Antidiabetic, Streptozotocin, Oxazolone, Acute and Sub-acute toxicity.

1. INTRODUCTION

Oxazolone is a class of five membered heterocyclic ring system, received more attention in recent years due to its diverse pharmacological activities (Lednicer and Mitscher, 2005). It plays vital role in the manufacturing of various biologically active drugs as analgesic, anti-inflammatory, anti-depressant, anti-cancer, anti-microbial, anti-diabetic and anti-obesity drugs (Seebach et al., 1997). 2, 5-substituted oxazolone derivatives like Thozalinone, Pemoline, Fenozolone, Cyclazodone and 4-methylaminorex are used as psychoactive drugs. In 2005, FDA withdrew approval of Pemoline due to hepatotoxicity (Yen-Koo and Balazs, 1980; Marotta and Roberts, 1998). Toxicity study provides information on the possible health hazards likely to arise from single and repeated exposures over a limited period of time. It also provides information on the target organs and the possibilities of cumulative effects. More over synthetic molecules are more prone to cause toxicities, so there is a great need to analyze such toxicities of the compounds during the drug development (Silverman, 2004). Keeping these in mind, the present work is designed to evaluate the pharmacological & toxicological effect of oxazolone derivatives.

2. MATERIALS AND METHODS

2.1. Oxazolone derivatives

In the present studies, we have undertaken to screen antidiabetic and toxicity profile of five 2, 4-substituted oxazolone derivatives (Figure 1) , which were synthesized and characterized from Department of Pharmaceutical Chemistry, Himalayan Pharmacy Institute, East Sikkim, India.

2.2. Animals

Healthy adult male Wistar albino rats (200-250 g) were used for the study. They were housed hygienically under standard conditions in the animal house of Himalayan Pharmacy Institute, East Sikkim, at temperature (24±1ºc), relative humidity (65±10%). They were allowed to acclimatize with free access to food and water *ad libitum* and 12 light/dark cycle environment. The procedure involved in this research work was approved by the Institute Animal Ethics Committee (IAEC) No: HPI/09/60/IAEC/0076.

2.3. Induction of experimental diabetes mellitus

The rats were rendered diabetic by a single intraperitoneal dose of 55 mg/kg b.w. streptozotocin (STZ), freshly dissolved in ice-cold 0.1M citrate buffer (pH 4.5). 72 hours after STZ injection, blood samples were collected from the tail of rat snipped by a fine

blade, the plasma glucose concentrations were measured with a portable glucometer (Accu Sure blood glucose monitoring system) and glucose oxidase-peroxidase reactive strips (Banik et al., 2008). Only those animals with blood glucose level higher than 250 mg/dl were selected as diabetic for the following experiment. The day on which hyperglycemia had been confirmed was designated as day 0 (Pushparaj et al., 2006).

2.4. Experimental Design

Rats were divided into thirteen groups (N=6): non-diabetic control group (I) received the vehicle only, diabetic control group (II) received the vehicle only, standard group (III) received glibenclamide 10 mg/kg b.w. diabetic test group (IV-XIII) received (200 and 400 mg/kg b.w.) of compounds OXZ-1 to OXZ-5. The change in body weight, food/water intake was recorded regularly during 15 days treatment and blood glucose was measured after 10 and 15 day of drug administration (Lia et al., 2006; Jong-Yuh and Mei-Fen, 2005).

2.5. Toxicity studies

2.5.1. Acute toxicity study

Acute Oral Toxicity- Up and Down Procedure-425 (Main test) was used to study the acute toxicity of all the compounds (Ramirez et al., 2007). The main test consists of a single ordered dose progression in which animals were dosed, once at a time at a minimum of 48-hour intervals. All the animals were fasted overnight before starting the dosing and the dosing was initiated with 175 mg/kg. The test compounds OXZ-1 to OXZ-5 were administered orally in the form of suspension with 0.5 % CMC. The first animal received 175 mg/kg b.w. dose and then doses were increased by a factor of 3.2 up to 5000 mg/kg b.w. The animals were observed individually during the first 30 min, followed by the first 24 hour and daily thereafter for a total of 14 days. All the signs of toxicity were recorded during the period of study (OECD 425, 2001).

2.5.2. Sub-acute toxicity study

OECD TG 407 [Repeated Dose 28-day oral toxicity study in rodents] was used to study the sub-acute toxicity of all the compounds (OECD 407, 2007). Wistar rats were randomly assigned into three groups (n=10), five male and five female in each group. Group of five rats were housed together in stainless steel cages (males separated from females). The first group of animals, serving as control, received normal saline (5 ml/kg b.w.); the II and III group received the 500 and 1000 mg/kg b.w. of the oxazolone derivatives (OXZ-1 to OXZ-5) respectively. All animals were supplied with standard diet and water during the study periods. The clinical signs were observed at least once a day throughout 28 days of dosing. The body weight, water, food intake were measured once a week. On 29th day all surviving animals were fasted overnight, anesthetized and blood was collected by direct cardiac puncture method. Blood analysis such as hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count and clotting time were measured (Auletta, 2002). The non-heparinized blood was allowed to coagulate and the serum was analyzed for ALP, SGOT, SGPT, Glucose, HDL, Triglycerides, Cholesterol and Total bilirubin with the help of Assay kits (Merck chemicals) and Auto-analyzer (Merck ML-300). Histopathological examination was performed on the preserved organs of all animals (Mohan H., 2000).

2.5.3 Statistical analysis

The results were statistically analyzed using one way analysis of variance (ANOVA) followed by Dunnett's t-test.

3. RESULTS

3.1. Effect of oxazolone derivatives on body weight

Diabetic rats showed a decrease in body weight during the experimental period, and this was significantly antagonized by glibenclamide as well as all oxazolone treated groups [Table-1].

3.2. Effect of oxazolone derivatives on fasting blood glucose level

Normal control rats did not show any significant variation in the blood glucose level throughout the experimental period. STZ leads to several fold elevation of blood glucose level indicating stable diabetes during the experimental period. Oxazolone (OXZ-1, OXZ-3 and OXZ-5) treated groups showed significant (**P<0.01) reduction of blood glucose level after 15 days of study period [Table-1].

3.3 Acute toxicity study of oxazolone derivatives

Acute toxicity study revealed that the substituted oxazolone derivatives were non toxic up to 5000 mg/kg. No death was recorded within the observation period [Table-2].

3.4 Sub-acute toxicity study of oxazolone derivatives

No death, no significant changes in general behavior and other physiological activities were observed during the study period. Other parameters like body weight, food and water intake did not show any significant differences in either the control or treated groups of both sexes.

3.4.1 Effect of oxazolone (OXZ-1) on hematological parameters

Hematological analysis showed no significant changes of RBC, Hemoglobin, Hematocrit, WBC, lymphocyte, monocyte, leukocyte and platelets in male and female treated groups [Table-3].

3.4.2 Effect of oxazolone (OXZ-1) on Biochemical parameters

Biochemical analysis showed no significant differences in any of the parameters examined. The level of AST, ALT and serum glucose were slightly elevated in 1000 mg/kg treated rats, but not significantly in either male and female rats [Table-4].

3.4.3 Effect of oxazol-5-one (OXZ-1) on organ weight

No significant differences were seen in heart and kidney weight between the control and treated groups of male and female rats but there was slight increase in weight of the liver. [Table-5].

3.4.4 Histopathological study of oxazolone

OXZ-1 treated liver tissue showed slight elongation of sinusoids, nucleatic hypertrophy and nucleatic vacuolation [Figure-2a] Photomicrograph of OXZ-1 treated kidney tissue have shown slight destruction of tubular cells and atrophied glomeruli which may be due to toxicity produced by drug treatment [Figure-2b] OXZ-1 treated heart showed normal cellular structure of heart muscle cells without any damage [Figure-2c].

4. DISCUSSION

STZ-induced hyperglycemia has been described as a useful experimental model for studying both type-I and type-II diabetes. In this research work OXZ-1, OXZ-3 and OXZ-5 have reduced blood glucose level effectively as compared to other derivatives. Glibenclamide was used as standard drug which stimulates the pancreatic cells to control hyperglycemia (Larner, 1985). The present study revealed that insulin producing cells are functioning and the stimulation of insulin release could be responsible for most of the metabolic effects. Decrease in body weight of diabetic rats is possible due to catabolism of fats and protein, even though food intake is more in diabetic rats and control (Sharma et al., 2006). Due to insulin deficiency protein content is decreased in muscular tissue by proteolysis. Oral administration of oxazolone derivatives showed dose dependent improvement in body weight of diabetic rats. It can be explained that these derivatives may interfere with proteolysis to normalize the body weights. The oral administration of a single dose of synthesized compounds caused a significant reduction in blood glucose in diabetic rats. These results revealed that oxazolone derivatives may be effective in insulin-independent diabetes mellitus. The significant hypoglycemic effects of oxazolone derivatives in diabetic rats indicate that it can be mediated by stimulation of glucose utilization by peripheral tissues. Since the oxazolone derivatives are having structural resemblance with thiazolidinone with minor modification, it can be expected that the mode of action of the oxazolone derivatives may be similar to thiazolidinone derivatives (Robert and McDonald., 2003). Hence oxazolone derivatives may act as agonist upon binding to peroxisome proliferator activated receptor (PPAR *γ*) which preferentially binds to DNA activating transcription of a wide variety of metabolic regulators. The regulators increase expression of a number of insulin responsive genes involved in the regulation of glucose and lipid metabolism. In acute toxicity study, animals did not show any specific changes in the general appearance and toxic signs during the observation period up to 5000 mg/kg b.w. It ascertained the relative non-toxic nature of the 2, 4-substituted oxazolone derivatives. On the basis of antidiabetic activity OXZ-1 was chosen for sub-acute toxicity study. The hematological analysis showed no significant changes but in biochemical analysis there is a slight elevation of AST, ALT at 1000 mg/kg b.w. Histopathological study of liver revealed that there was mild toxic effect which was evidenced by elongation of sinusoids, nuclear vacuolation and hypertrophy. In a toxic environment, blood level of AST and ALT are known to significantly increase (Adam, 1998; Crook, 2006). These two classical enzymes are reliable indices of liver toxicity. From the subacute toxicity it can be assumed that the increased level of alkaline phosphatase, AST and ALT level may be responsible for the tissues damages in the liver and kidney. However the histopathological features of cardiac tissue was not affected. There was

slight destruction of tubular cells and glomeruli of kidney; it may be understood by excess nephrotic function for excretion of drug at 1000 mg/kg b.w. There was no remarkable change in weight of kidney and heart of OXZ-1 treated rats on either sex but mild variation in liver weight. It may be due to atrophy or hypertrophy of liver. Chronic toxicity study and further investigations are needed to elucidate the exact mechanism behind its effects to assure the safety of the molecule.

5. CONCLUSION

From the research finding, it is inferred that 2,4-substituted oxazolone derivatives has significantly antidiabetic activity as it lowers the fasting blood glucose level in diabetic rats. Moreover, in toxicity studies oxazolone derivatives seems to be less toxic. Hence, it can be concluded that it is worthwhile to modify a structure to obtain more potent and safe drug candidate.

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Table1: Effect of oxazolone derivatives in body weight & blood glucose of experimental rats

Treatment mg/kg p.o	Body weight (g)			Blood glucose level (mg/dl)		
	0 day	10 day	15 day	0 day	10 day	15 day
Non diabetic control	166±1.62	180±1.3**	191±1.14**	98.4±8.1**	92.5±5.3**	6.1±7.5**
Diabetic control	158±1.20	151±0.96	140±0.9	360±19.8**	398±21.3**	372±21.9**
Glibenclamide -10	161±1.35	176±1.4**	188±1.32**	374±28.1	180±16.1**	107±19.7**
OXZ-1a	162±1.21	165±1.02**	162±1.3**	364±21.3	281±22.5*	260±18.4*
OXZ-1b	167±1.03	173±0.9**	176±1.2**	378±38.9	251±22.7**	226±17.5**
OXZ-2a	159±1.3	153±1.04	155±1.0**	370±28.1	328±24.1	305±20.5
OXZ-2b	165±0.8	156±1.2*	159±0.9**	358±24.9	297±24.8	253±24.0*
OXZ-3a	156±1.23	159±1.1**	163±1.1**	320±19.5	285±21.3*	264±20.1*
OXZ-3b	162±1.02	165±1.02**	169±1.0**	369±27.4	272±25.2**	231±19.7**
OXZ-4a	158±1.4	154±0.93	155±0.9**	376±24.8	340±22.6	315±22.3
OXZ-4b	160±1.2	156±1.0*	158±1.1**	381±25.9	310±24.5	284±21.7
OXZ-5a	161±1.21	163±1.3**	166±1.2**	368±23.7	321±22.9	301±18.6
OXZ-5b	164±1.04	169±0.98**	172±1.1**	379±28.5	283±23.8*	248±19.4**

Values are expressed as mean±SEM; (N=6), a -200 mg/kg b.w. and b- 400 mg/kg.*P<0.05, **P<0.01 compared with diabetic control.

Table 2: Effect of 2,4-substituted oxazolone in acute toxicity study

Step	Animals	Dose (mg/kg/p.o.)	Log dose	X(response) O(no response)	LD_{50}
1	1	175	2.2430	O	Maximum likelihood calculation cannot be completed since LD_{50} is greater than 5000 mg/kg
2	2	550	2.7404	O	
3	3	1750	3.2430	O	
4	4	5000	3.6990	O	
5	5	5000	3.6990	O	
6	6	5000	3.6990	O	

Table 3: Hematological evaluation of oxazolone (OXZ-1) treated rats in sub-acute toxicity study

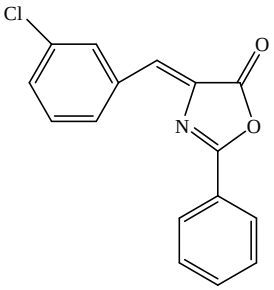
	Male			Female		
	Control	500 mg/kg	1000 mg/kg	Control	500 mg/kg	1000 mg/kg
RBC	8.6±0.23	8.4±0.57*	7.1±0.56	8.35±0.3	8.52±0.64*	8.22±0.78
Hb	15.2±0.9	15.62±0.7	14.34±0.7	13.9±0.5	13.88±0.7	13.47±0.4
Hr	45.5±2.0	44.67±1.3*	41.24±2.0*	43.56±2.5	43.13±1.9*	47.42±1.3*
WBC	6.3±2.01	5.37±1.22	4.6±2.34	5.31±1.5	5.76±2.78	5.81±2.48
Lymp	71.2±6.2	83.9±9.16	85.6±9.78	83.42±1.6	82.57±4.6	87.53±3.2
Mono	3.9±1.20	4.2±3.22*	5.1±4.7*	2.6±1.8	3.1±1.55*	4.03±1.28*
Eosi	0.6±0.03	3.4±0.87	3.7±0.67	0.7±0.4	0.8±0.5	1.2±0.8*
PLT	901±98	945.2±102	978.9±79*	901.6±78	954.6±48	947.3±50
ClT.	2.0±0.5	2.52±0.76	2.35±0.65	2.63±0.1	2.47±0.45	2.62±0.97*

Data are expressed as mean±SEM, (N=5), *P<0.05 compared with diabetic control. RBC- *Red blood cells* ($\times 10^6 mm^{-3}$), Hb- Hemoglobin (g/dl), Hr- Hematocrit (%), WBC- *White blood cells* ($\times 10^3 mm^{-3}$), Lymp- Lymphocyte(%), Mono- Monocyte(%), Eosi- Eosinophil (%), PLT- Platelets($\times 10^3 mm^{-3}$), ClT- *Clotting time (min).*

Table 4: Biochemical analysis of oxazolone (OXZ-1) treated rats in sub-acute toxicity study

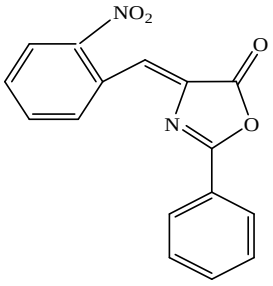
	Male			Female		
	Control	500 mg/kg	1000 mg/kg	Control	500 mg/kg	1000 mg/kg
TB	0.26±0.005	0.27±0.6*	0.26±0.9*	0.22±0.034	0.21±0.07*	0.24±0.02
AST	172.6±29.5	179±21.4	246.6±19	167.7±12.5	183.5±19.5	231.5±15*
ALT	47.2±3.9	49.7±3.5*	58.3±0.69*	48.08±0.92	50.6±0.45	56.3±0.89
HDL	37.1±4.28	38.4±5.1*	42.5±4.9	36.79±3.4	36.1±2.4*	35.8±2.1
Chol.	56.2±3.2	55.7±3.7	56.4±4.6*	55.8±3.9	56.5±5.9	55.6±4.8
TG	42.3±14.3	42.9±14.5	43.1±11.8	44.3±10.3	45.2±9.4	44.7±11.2*
Glu.	100.7±0.87	135.7±2.6	193.7±2.9*	118.2±2.67	129.7±1.7*	187.6±2.6
ALP	38.2±0.55	36.4±0.76*	38.6±0.89	37.4±0.46	38.4±0.65	38.3±0.83*

Data are expressed as mean±SEM, (N=5), *P<0.05 compared with diabetic control. TB- Total bilirubin (mg/dl), AST- Aspartate transaminase (U/L), ALT- Alanine transaminase (U/L), HDL- High density lipoprotein (mg/dl), Chol.- Cholesterol (mg/dl),TG- Triglycerides (mg/dl), Glu.- Glucose (mg/dl), ALP- Alkaline Phosphatase (U/L).



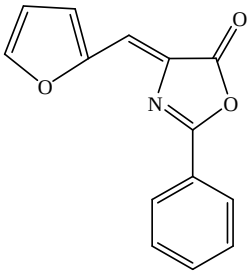
4-[3-chloro-benzylidine]-2-phenyl oxazol-5-one

OXZ-1



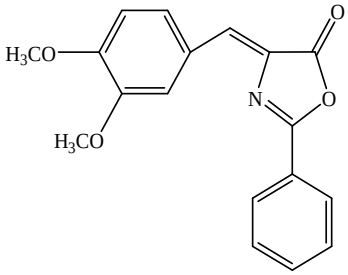
4-[2-nitro-benzylidine]-2-phenyl oxazol-5-one

OXZ-2



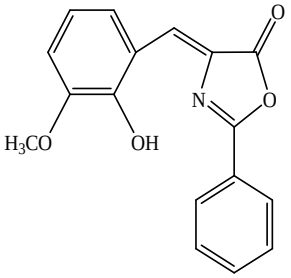
4-[furfurylidine]-2-phenyl oxazol-5-one

OXZ-3



4-[3,4-dimethoxy-benzylidine]-2-phenyl oxazol-5-one

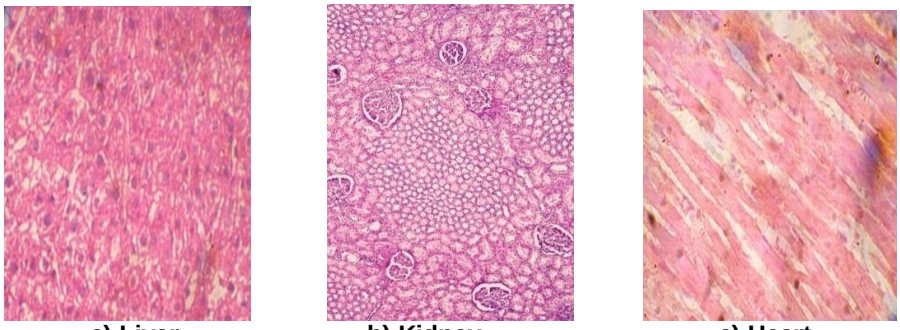
OXZ-4



4-[2-hydroxy-3-methoxy-benzylidine]-2-phenyl oxazol-5-one

OXZ-5

Fig.1: Structures of 2, 4 substituted oxazolone derivatives (OXZ-1 to OXZ-5)



a) Liver **b) Kidney** **c) Heart**

Fig. 2: Photomicrograph showing the effect of OXZ-1 on histopathological features of

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