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An Exploratory Toxicity Studies of homeoprotein DLX4 in Wistar Albino Rats

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ABSTRACT

Exploratory study of test compound DLX4 was conducted in male wistar albino rats. The study was carried out as per exploratory guidelines. Three animals were used for each step. DLX4 was administered by oral gavages as a single dose at a dose rate of 500 mg/kg body weight to I rat, 250 mg/kg body weight to rat II, 250mg/kg body weight to rat III. Rat I animals tested at 500 mg/kg showed mortality. There was no mortality in 250mg/kg treated groups. The results of the study concluded that LD50 for DLX4 was at 500 mg/kg body weight in male rats and 250mg/kg dose is the safest dose with no mortality or clinical sign and there is no significant difference between biochemical and haematological parameters.

Keywords: - Exploratory, Toxicity, DLX4

INTRODUCTION

Exploratory IND Study is conducted very early in Phase 1 (often referred to as "Phase Zero") is conducted prior to the traditional dose escalation, safety, and tolerance studies involves very limited human exposure (7 days) has no therapeutic intent. The ExpIND studies are condected to obtain:- information on pharmacokinetics, including biodistribution data, exploring a product's biodistribution characteristics using imaging technologies, selecting the most promising lead product from a group of candidates designed to interact with a particular therapeutic target, understanding the relationship between a MOA and the treatment of a disease. (*e.g.,* binding to target or enzyme inhibition).

The various contents of exploratory studies are described below:-

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1. Pharmacology & Toxicology Information

Pharmacological & toxicological informations depends on three type of studies i.e. Pharmacokinetic or imaging studies ("Microdose"), Pharmacological Studies, Mechanism of action related to efficacy.

Pharmacokinetic or imaging studies ("Microdose")Most drug candidates are dropped during development due to PK properties or ADME characteristics. Consequently, attrition rates can be increased by evaluating the PK properties of the compounds at the earliest possible stage.

In-vitro Animals, Human, Insilco (high-throughput screening emerging for very early prediction of PK properties):

- Microdosing allows the evaluation of PK properties in humans, prior to setting up the standard Phase 1 single and repeated escalating dose studies.
- Abbreviated pre-clinical:
  - ✓ NOAEL/Dose-Response
- Single Dose Toxicity (14 d, single species)

  Repeat Dose Toxicity (repeat dosing "ok" if separated by 1 week)

  Safety Pharmacology

  Genotoxitiy

  Carcinogenicity

  Local Tolerance

2. CMC Information

CMC for ExpIND very similar to Traditional IND CMC updated in "graded" fashion corresponding to the development phase as IND progresses include a summary report of the specific compound or group of compounds to be studied that includes, for example, a physical and chemical description of the compound, the grade, and quality of manufacturing excipients and product components, and stability data.

3. If drug from a particular batch that was used in nonclinical toxicology studies is to be used in the clinical setting, FDA will consider the material to be qualified for human use based on the general CMC info included in ExpIND.
4. However, if the compound used in the nonclinical studies is not the same material that will be used in humans, applicants should provide certain analytical testing data demonstrating that material is representative of drug from batches used in nonclinical toxicology studies.

5. Clinical Development Plan Information

These studies to be conducted under and ExpIND focus on a limited study or group of studies necessary to identify and further develop a promising compound, applicants must articulate a rationale for selecting the

compound rather than providing a more detailed development plan.Single-and multiple-dose studies are two potentially useful study designsSingle-dose studies could include the administration of a sub-pharmacologic of a compound to a limited number of subjects to collect pharmacokinetic info and/or perform imaging studies, or pharmacologic dose to collect formation on pharmacological effects.

6. GLP & cGMP for ExpIND

FDA expects that all preclinical safety studies supporting an ExpIND will be performed with Good Laboratory Practices (GLP)

- FDA final rule on cGMP is intended to streamline and accelerate the drug development process while ensuring the safety and quality of the earliest stage investigational drug products.
- The production of investigational new drug products for use in Phase 1 studies conducted under an IND is exempt from cGMP 21 CFR 211.
- FDA Guidance provides for the flexibility to allow producers to implement controls appropriate for their specific situation and application… based on a risk assessment for the product and manufacturing process.
- FDA will exercise oversight of the production of investigational Phase 1 drugs under the agencies general statutory cGMP authority to ensure that these drugs are produced under conditions sufficient to ensure their safety, identity, strength, quality and purity [1, 2].

MATERIALS AND METHODS

Experimental Animals-

Male Wistar Rats (130–180gm) were procured from animal house of Deshpande Laboratories, Bhopal. All animals were kept in propylene cages with sterile husk as bedding material. They were housed in an environmentally regulated room on a 12 hours light: 12 hours dark cycle with 25 ± 2° C and had free access to food and water. The experimental protocol was approved by the Institutional Animal Ethical Committee and experiments were conducted according to the CPCSEA, India guidelines on the use and care of experimental animals. All animals were transferred to Experimental room 7 days prior to experimentation for acclimatization.

Test Drug (Dlx-4)

DLX-4 was provided by the Deshpande Laboratories Bhopal.The physical properties of the given test compound is dark green in colour, sticky, odorless and is a liquid preparation.

Experimental Design

Three male healthy rats were selected. Individual dose will be calculated according to initial body weight. Animal-A was received a dose of 500mg/kg, animal-B and animal-C were treated with daily dose of 250 mg/kg. Body weight of Individual animal was record after the administeration of dose daily. The animals were observed daily for mortality; signs of gross Toxicity and behavioral changes after administer a test drug. The blood sample of each animal was collected from orbital sinus before and after the 7 days of drug admenistration for determination of various hematological and biochemical parameters [3-5].

Hematology

Different haematological parameters were investigated before initiation of tretment and after completion of treatment. The hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocytes count, platelet count, prothrombin time and activated partial thromboplastin time were investigated [6-8].

Biochemical Parameters

Different biochemical parameters were investigated before initiation of tretment and after completion of treatment. Serum Sodium, potassium, glucose, total cholesterol, urea, Creatinine, total protein and albumin, AST, ALT, ALP, total bilirubin were investigated by autoanalyzer with the help of specific kits.

Necropsy

After the completion of 7 days treatment and investigation all animals were euthanised by over dose of anaesthesis and were sacrificed for investigation of different organs weight like as Brain, Heart, Kidney, Liver, Spleen, Thymus, Testes.

RESULTS

In the present study, oral administration of DLX-4 in wistar rats at 250 mg/kg had no effect on mortality, clinical signs, body weight change, or gross observation but the animal treated with 500 mg/kg of test drug showed the motrtality on 7^{th} day so the LD-50 of DLX-4 is 500 mg/kg.

Body Weight and Clinical Observations

The body weight of all three animals was found to be decreased after the treatment of DLX-4.

The body wt. of Animal-A was found to be decreased 14 gm. after the 7 days of post treatment and the body wt. of animal-B and C were found to be decreased 8 gm and 12 gm.

The clinical observations and signs were indicated in table-1.

Biochemical Parameters

The change in biochemical paramenters after the 7-days treatment of DLX-4 was indicated in table-2. The serum albumin, ALT and potasium levels were found to be decreased significantly when compared to untreated level.

Haematological Parameters

The change in haematological paramenters were indicated in table-3. Except WBC all blood cells count were found to be reduced when compared to untreated level.

Effect of DLX4 on Necroscopy

The effect of DLX-4 on change in weight of different organ was represented in table-4, the wt. of brain, heart and liver was found to be decreased significantly when compared to untreated level.

Urinary Parameters

There was no change in urinary specific gravity and pH observed after the treatment of DLX-4. Fig.1.

DISCUSSION AND CONCLUSION

The DLX-4 is a glibinclamide derivative which is used in pro-insulin secretion in the body pharmacological actions is to maintain the blood glucose level but we would find out safest dose and LD-50 dose of this derivative.

This study has shown that the improperly used, could serve as a source of harm to animals.

behavioral changes, and mortality in the test groups as compared to the controls when observed during 7 days of the toxicity experimental period. This is because of the dose at 500 mg/kg produced significant decreases in the level of body weight and one animals shows mortality on 7^{th} day and the clinical signs and observation shows the toxicity at 500 mg/kg dose such as, fast breathing and redness on nose area the biochemical and haematological changes are significant decreases the function the organs at 500mg/kg dose on rat.

So, then we decreases the dose and give 250mg/kg then we observed that there are no harmful effects in clinical signs and reduction of body weight is decreases then haematological and biochemical changes not showing the significant reason to produce toxicity dose at 250mg/kg. These results showed that the extract showed no mortality of these rats at 250mg/kg dosage levels indicating the high margin of safety of this extract. In exploratory toxicity test the DLX-4 was found to be non toxic at the dose level of 250mg/ kg body weight.

During the dosing period and in the last day, the quantity of food and water intake by different dose groups was found to be comparable with control group. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal

No physical changes were observed throughout the dosing period except body weight compared to their initial values. However there was no significant difference between the different biological and laboratory limits. The weights of organs recorded did not show any significant differences in the treatment and the control group test compound was not toxic to heart, kidney or may be produce mild toxicity in liver.There was some significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), in all the treated groups as compared to respective control groups. Results of biochemical studies showed that there was no significant increase in the levels of the parameters at different doses ALP, AST, ALT in the different groups of animals treated with of the extract compared with control. This implies that the extract at the doses tested had mild effects on the liver.The effect of the DLX4 on protein, urea, uric acid, creatinine, glucose, in serum and urine of control and experimental rats respectively. This result showed that the DLX-4 at different levels tested did not produce considerable change in the levels of the different parameters tested.

So, we concluded that the LD-50 dose of DLX-4 is toxic at 500mg/kg then we decreases the safest dose (NOAEL) at 250mg/kg in the other means the test compound of DLX4 is used to be at 250mg/kg dose at no mortality sign in rat.

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Table-1 Clinical observations and sign on different days after the treatment of DLX-4

Days	Animal-A		Animal-A	
	Clinical Observation	Clinical Sign	Clinical Observation	Clinical Sign
2	Breathing increased	Redness of nose	Itching	Redness of nose
3	Muscle strength-↓ Motor activity-↓ Vertigo	Alopecia (Minor) Redness of nose	Body weight-↓	Redness recovered
4	Itching Breathing-↓	↓-Redness of nose	Body weight-↓	Redness Disappear
5	Improve in weight	No sign	No observation	No signs & symptoms
6	Body weight-↓	Muscle weakness	No observation	No signs & symptoms
7	Death	-	No observation	No sign & symptoms

Table-2 Effect of DLX4 on Biochemical Parameters

Treatment /Group	Alb. (g/dl)	ALP (U/L)	ALT (U/L)	AST (U/L)	Bilrubin (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Potassium (mEq/L)	Sodium (mEq/L)	Total Cholesterol (mg/dl)	Total Protein (g/dl)
Untreated	4.43 ± 0.20	94.6 ± 2.20	20.66 ± 0.98	56.4 ± 4.24	0.44 ± 0.03	0.49 ± 0.05	93.33± 6.65	6.62 ± 0.25	149.33 ± 4.04	101 ± 11.35	6.4 ± 0.2
Treated	4.00 ± 0.1**	92.3 ± 2.51 ns	18.6± 1.67**	52.3± 3.28ns	0.43 ± 0.04ns	0.51 ± 0.03ns	94 ± 9.84ns	5.87 ± 0.20***	149.33 ± 4.35ns	91 ±4 ns	6.06 ± 0.65ns

Values are mean ± SD of 3 animals
***P <0.0001, ** P<0.01, ns= not significant when compared to test group.

Table 3: Effect DLX4 on Hematological Parameters

Treatment	Basophils (x103 /mm3)	Eosinophils (x103 /mm3)	HB g/dl	Lymphocytes (x103 /mm3)	Monocytes (x103 /mm3)	Nuetrophils (x103 /mm3)	RBC(*106/mm3)	WBC(*103/mm^3)
Untreated	8.31 ± 0.17	0.05 ± 0.00	14.6 ± 0.87	8.65 ± 0.58	0.2 ± 0.00	3.08 ± 0.10	9.1 ± 0.45	11.63 ± 0.75
Treated	0 ± 0	0.04 ± 0.00***	12.43 ± 0.46*	7.79 ± 0.29*	0.13 ± 0.00***	2.39 ± 0.29**	8.33 ± 0.20**	10.66 ± 0.85ns

Values are mean ± SD of 3 animals
***P <0.0001, ** P<0.01, ns= not significant when compared to test group.

Table 4: Effect of DLX4 on Necroscopy

	Brain (gm)	Heart (gm)	Kidney (gm)	Liver (gm)	Spleen (gm)	Thymus (gm)	Testes (gm)
Untreated	1.17 ± 0.03	0.69 ± 0.03	0.55 ± 0.03	4.38 ± 0.06	0.50 ± 0.07	0.25 ± 0.03	0.96 ± 0.01
Treated	1.13 ± 0.12***	0.59 ± 0.09**	0.55 ± 0.02ns	4.57 ± 0.19**	0.45 ± 0.13ns	0.24 ± 0.01ns	0.89 ± 0.03ns

Values are mean ± SD of 3 animals

***P <0.0001, ** P<0.01, ns= not significant when compared to test group.

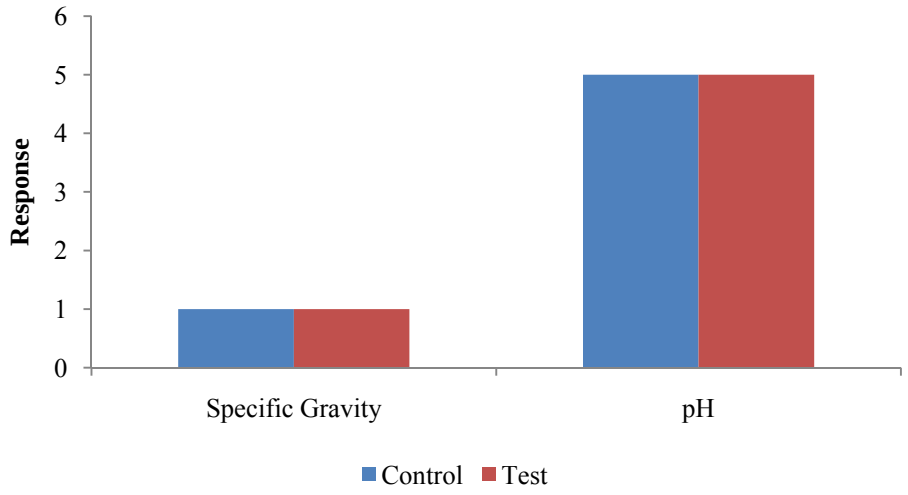

Figure1: Effect of DLX-4 on Urinary Parameters