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ACUTE ORAL TOXICITY STUDY OF POLYHERBAL FORMULATION NIA/DG/2015/01

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ABSTRACT

Hypertension is a multifactorial clinical condition and therefore can be managed in multiple pathways. *Convolvulus pluricaulis (Shankhpushpi), Nordostachys jatamansi (Jatamansi), Terminalia Arjuna (Arjuna), Withania somnifera (Ashwagandha), Boerhavia diffusa (Punarnava)* are effectively used to manage hypertension, but each of them has different pharmacological properties and actions mitigating some pathway of hypertension. Therefore a combination of these herbs should fulfill the need of developing a broadspectrum antihypertensive. Each of these herbs is proven to be safe and effective individually. But the safety of the combination (NIA/DG/2015/01) is yet to be established. It is mandatory to do acute toxicity study for new formulations having proven safe ingredients. Therefore a toxicity study was conducted of NIA/DG/2015/01 as per OECD guideline 423. Six albino rats were used for this study in two groups having 3 animals each group, Group1 was given a single oral dose of 2000mg/kg body wt. and Group2 was given 300mg/kg body wt. The animals were observed for OECD prescribes parameters and duration. There was no lethal or toxic effect seen in any of the animals. The present study showed that the test substance NIA/DG/2015/01 does not show any toxicity at 300mg/kg body wt. and 2000mg/kg body wt.

KEY WORDS- *Convolvulus pluricaulis (Shankhapushpi), Nordostachys jatamansi (Jatamansi), Terminalia arjuna (Arjuna), Withania somnifera (Ashwagandha), Boerhavia diffusa (Punarnava)*, acute toxicity study, antihypertensive, OECD guideline

1. INTRODUCTION-

Cardiovascular disease is the leading cause of death worldwide. Hypertension is the most common cardiovascular disease and a major public health problem in both developed and developing countries. Hypertension is a multifactorial clinical condition and possesses different etiological and pathological origin. Treatment of hypertension involves the use of many allopathic drugs like as vasodilators, α, β adrenergic blockers, ACE inhibitors calcium channel blockers, diuretics etc. These drugs show that there are incidences of relapses, adverse effects and danger of drug interaction during hypertension therapy. Hence alternative therapy is need of current cardiovascular diseases. There are many plants which are traditionally used and reported to possess anti-hypertensive effects, but as hypertension possess different etiological and pathological origin, no single drug can able to effectively control the condition.[i] Hence in the proposed study, poly herbal formulation (NIA/DG/2015/01) is a hypothetical Ayurvedic formulation which contains aqueous extract of *Convolvulus pluricaulis (shankhpushpi), Nordostachys jatamansi (Jatamansi),Terminalia arjuna(Arjun),Withania somnifera (Ashwagandha), Boerhaavia diffusa(Punarnava)* which can give synergistic effect in hypertension. Above all these ingredients of poly herbal formulation have a classical basis to be used in hypertension, are proved to be safe for human use and have some proven efficacy in experimental /clinical models.[ii],[iii],[iv],[v],[vi] Single herbs are found effective in management of hypertension. However no evidence regarding safety and efficacy of the formulation is available. Hence the present study was undertaken to investigate overall safety of polyherbal formulation NIA/DG/2015/01.

2. MATERIALS AND METHODS:[vii]

Acute Oral Toxicity Study-

Acute Toxicity study of NIA/DG/2015/01 was performed following OECD Guideline 423 ANNEX 2c in the Institute of biomedical and industrial research, Jaipur, Rajasthan (Ref.no. ibir/iaec/2015/1-01) after due ethical clearance was obtained from its Institutional Animal Ethical Committee approval no. 1737/PO/RC/S/14/CPCSEA.

2.1 Preparation of Test sample –

Name of Formulation: NIA/DG/2015/01. Test formulation contains five *Ayurvedic* herbal drugs viz. *Shankhapushpi* (*Convolvulus pluricaulis* choisy.), *Jatamansi* (*Nardostachys jatamansi* DC.), *Arjuna* [*Terminalia arjuna* (Roxb.) W. & A], *Ashwagandha* (*Withania somnifera* Linn.), *Punarnava* (*Boerhaavia diffusa* Linn.). All the herbs were taken in equal proportion and were separately extracted in aqueous medium. 100gm powdered (20gm of each drug) drug was extracted with 2000ml. of water using Soxhlet apparatus (Hot extraction method) for 72 hrs. At the end of extraction the extract was concentrated and solvent was totally evaporated in a petri dish with the help of water bath. The thick and sticky paste thus obtained and it was

stored in air tight container at room temperature till further use and this extract was used as test drug.

2.2 Housing and feeding conditions

The temperature in the experimental animal room was maintained at 22ºC (±3ºC) and relative humidity was maintained at 50-60% during room cleaning. Lighting was artificial, the sequence of 12 hours light, 12 hours dark. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water. Animals were group caged by dose of Group 1 and Group2.

2.3 Preparation of animals

The animals were randomly selected, and each animal of respective group marked with Picric acid as H (mark on head), B (Mark on Back), T (mark on Tail) for individual identification and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

2.4 Number of animals and dose levels

A total of six albino rats were used in this study. These were divided into two groups of three rats each. The dose level was selected from one of two fixed levels 2000 and 300 mg/kg body weight.

Group 1 - 2000 mg/kg test sample

Group 2 - 300 mg/kg test sample

2.5 Administration of doses

Animals were fasted over night prior to dosing without withholding water. The animals weighed and the test formulation was administered in a single dose gavage by an oral feeding needle. Following the substance was administered; food was withheld for a further 3-4 hours.

3. OBSERVATIONS

Behavioral observations: Behavior observed individually after dosing at 30 min, 4 hr, 24 hr., 48 hr., 1week, and 2week. All observations were systematically recorded with individual record maintained for each animal. Changes in skin and fur, eyes, mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhea, morbidity, mortality were observed.

Hematological tests: Blood sample were withdrawn on 7th days and 14th days by orbital puncture technique.

Pathology: One test animal in each group was used to observe gross pathological changes. Vital organ Brain, Liver, Kidney, and Spleen were isolated after 14 days and observed pathological changes.

4. RESULTS

Acute Oral Toxicity (OECD GUIDELINE 423):- Observation Report of NIA/DG/2015/01

Table: 1 Observation for the Test at 2000mg/kg Body weight of an animal

Observation	30min.	4hr.	24hr.	48hr.
Skin and Fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal

Salivation	Normal	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil
Morbidity	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil

Table: 2 Observation for the Test at 300 mg/kg Body weight of an animal

Observation	30min.	4hr.	24hr.	48hr.	1week	2week
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil	Nil	Nil

Table: 3 Hematological tests:

Sr. No.	**Hematological Parameters**	**300 mg/kg**	**2000 mg/kg**	**Normal Range**
1.	Haemoglobin	14.5	13.7	11.5-16.1
2.	WBC	6.9	6.4	6.6-12.6 x 103/mm3
3.	RBC	8.4	6.98	6.76-9.75 x 106/ mm3
4.	Neutrophils	2.56	2.27	1.77-3.38 x103/ mm3
5.	Lymphocytes	9.1	8.4	4.78-9.12 x 103/ mm3
6.	Eosinophils	0.04	0.04	0.03-0.08 x 103/ mm3
7.	Monocytes	0.02	0.01	0.01-0.04 x 103/ mm3

8.	Basophiles	0.0	0.0	0.00-0.03 x 103/ mm3
9.	Platelets	396	421	150-460 x 103/mol

***** All parameters are given in mean values.

Pathological changes: In the end of the observation period, one test animals in each group was used to observe gross pathological changes. No pathological changes were seen in the internal organs.

5. CONCLUSION:

From the results of this study, it is observed that there was no mortality. There were no behavioral changes and hematological changes recorded at the dose level 300mg/kg body wt. and 2000mg/kg body wt. which proves that NIA/DG/2015/01 formulation has no significant toxic effect in rats. The present study confirms that this polyherbal formulation is practically non-toxic & safe. This is only a preliminary study; in the future this research will pave an opportunity to conduct clinical trials in human subjects.

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