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ORIGNAL ARTICLE

Acute and Sub Chronic Oral Toxicity Study of Linkus (EMA), Plantain Syrup and AltheaSH Tablet in Mice

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ABSTRACT

Objectives: The present preclinical investigation was conducted with the aim to acknowledge the safety of three polyherbal formulations Linkus syrup (EMA), Plantain syrup and AltheaSH tablet extract by evaluating the acute and sub chronic oral toxicity studies in mice.

Methods: To determine acute toxicity mice were given individually the doses of 1 or 5 g/kg orally of Linkus syrup (EMA), Plantain syrup and AltheaSH tablet extract. For executing sub chronic oral toxicity**,** the formulations were given in doses of 50, 100 and 200 mg/kg of body weight for 28 days period.

Results: All three polyherbal formulations under test were not found to be the reason of mortality in animals at the administered doses of 1 or 5 g/kg. Further toxicity signs including loss of hairs, lacrimation, weight reduction, gait, tremors and drowsiness were not detected.

Conclusion: The current investigation provided confirmation of good tolerance of formulations under test and lack of harmful effects on the functions of the vital body organs of mice in acute and sub chronic oral toxicity analysis. Future prospects will be the conduction of clinical trials of the formulations as the clinical efficiency is verified in preclinical studies.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that is found to be the reason of chronic inflammation of the joints. Typical features of RA includes inflammation of the tissue surrounding the joints; sometimes the inflammation can also occur in other body organs [1]. Hence the disease is referred to as a systemic illness. RA begins in people who are below the age of 16 years is known as juvenile idiopathic arthritis (JIA). RA has significant morbidity rate and it also reduces the life expectancy rate [2]. The global prevalence of RA is 0.5 - 1% and is threefold more prevailing in females as compared to males [3].

The etiology of RA is unidentified and is a dynamic area of global research. Despite the fact that infectious agents for instance bacteria, viruses and fungi have long been assumed to be the cause, but nothing has been proven. Genetic factor is also considered important as certain genes may increase the risk for the development of RA[4]. It is also believed that some infections or environmental factors might activate the immune system in vulnerable persons that in turns show aggression against the body's own tissues that directs the inflammation in the joints and in different organs of the body[5]. Immune cells, known as lymphocytes, are stimulated and chemical messengers (cytokines, including tumor necrosis factor/TNF, interleukin-1/IL-1, and interleukin-6/IL-6) are expressed in the inflamed part of the body[6].

In spite of early recognition, existing treatment medications are inadequate in their effectiveness and are often toxic. Several patients switch to complementary and alternative medicine (CAM) selection in coping with the unbearable disease. Studies have shown that people suffering from chronic pain, like in RA, and those discontented with modern approaches of treatment are probable to look for alternative treatments, and as predictable 60–90% of arthritis patients utilize CAM. Amongst the most commonly used treatments are chiropractic and herbal therapies. This rising concern in herbal treatment practices undoubtedly indicates the call for more comprehensive analysis into the safety and efficacy of herbal drugs[7]. Hence the present study was executed to assess the acute and sub chronic oral toxicity of three polyherbal formulations Linkus syrup (EMA), Plantain syrup and AltheaSH tablet extract in the NMRI strain of mice.

MATERIALS & METHODS

Plant Materials

Herbs employed in formulations were kept in dark at 23℃. Each herb was examined for its macro and microscopic description.

Drugs

Linkus syrup (EMA), Plantain syrup and AltheaSH tablet extract – Herbion Pak. Pvt. Ltd. (Table 1)

Table 1: Composition of Linkus syrup, Plantain syrup and AltheaSH tablet

Linkus	*Syrup; Oral; Adhatoda Vasica 600 mg; Althaea 100 mg; Cordia Latifolia 100 mg; Hyssopus Officinalis 50 mg; Liquorice 75 mg; Onosma Bracteatum 50 mg; Piper Longum 100 mg; Tormentil; Viola Odorata 25 mg; Zizyphus Jujuba 100 mg / 10 ml*
Plantain	*Syrup; Oral; Mallow Flowers; Ribwort Plantain; Vitamin C (Ascorbic Acid)*
AltheaSH tablet	*Tablets 1 tab.* *Root extract of Althea officinalis 50 mg*

Extract preparation

Herbs were washed, dried in air and extracted by means of continuous extraction using Soxhlet apparatus and ethanol as a solvent in concentration of 96 %. Following exhaustive extraction, the collected extract was dried in reduced pressure by using rotavapor and dried at water bath.

Concise description of procedure of preparation of Linkus syrup (EMA) and Plantain syrup:

- Herb Extraction
- Syrup manufacturing
- Addition of excipients
- Addition of extract

- Cooling of Syrup
- Addition of flavors
- Volume makeup
- Final mixing
- Packaging
- One batch of syrup was prepared.
- Individual extracts were obtained via extraction process.
- A sugar syrup was made. The excipients and product extract were mixed into the syrup with continuous stirring. Then syrup was cooled down upto the room temperarture and then flavors were added.The volume of syrup was made up and final mixing was made.
- After that the syrup was packed into the bottles and then packed in to unit carton.
- All manufacturing steps and processes, and also the used manufacturing apparatus, are commonly used in the phyto pharmaceutical industry and in the phyto pharmaceutical practice and they are in compliance with the up-to-date GMP requirements.
- Regarding the chosen technological process, no problems are expected in the scale up of the manufacturing method and transfer of the results of the development phase in the production phase.

Animals

NMRI mice (n= 5/sex) were obtained from the facility of Animal house in Herbion Pak. Pvt. Ltd. They were housed under standard environmental conditions i.e. 25 ± 1 °C, relative humidity 52 - 61% and 12 h dark / light cycle. Food and water were available ad *libitum.*

Age at beginning of Treatment

6-8 weeks

Body Weight Range at the beginning of study

The body weights of each mice was taken following initial stabilization phase. Their weights were in between 25 – 35 g.

Identification

Mice per sex were housed and suitable recognition on the cages was marked by using cage cards

Experimentation Details

After taking the initial weights, the mice were disseminated at random into appropriate groups for the conduction of study. The investigation was carried out at the site of the conventional animal house facility of the Herbion Pak. Pvt. Ltd.

Toxicity study

Lethal Dose 50 (LD_{50})

Acute toxicity study was performed for Linkus syrup (EMA), Plantain syrup and AltheaSH tablet extract according to the acute toxic classic method as per OECD guidelines [8]. Mice were alienated into two groups i.e. Control and treatment group (n=5 per sex). Animals were treated orally using gastric gavage with doses (1 or 5g/kg) of test products and observe for 7 days.

The following parameters were noted in the study on daily basis:

Observations

- Physical examination
- Behavioral examination

- Mortality & Morbidity
- Food Intake
- Body Weight Changes

Sub chronic toxicity

Healthy NMRI albino mice (n=40) of either sex were alienated into 4 different groups having 10 animals in each group. Group I was provided with water and standard diet. Group II was administered standard diet, water and 50 mg/kg body weight of Linkus syrup (EMA)extract, group III was provided with standard feed, water and 100 mg/kg body weight of Linkus syrup (EMA) extract whereas group IV was provided with standard feed, water and 200 mg/kg body weight of Linkus syrup (EMA). The drugs were administered for duration of 28 days. The treated animals were weighed up at 0, 7, 14 and 28 day of the doses. Similar procedure was repeated for determining the sub chronic toxicity of Plantain syrup and AltheaSH tablet extract.

RESULTS

Physical Examination

Linkus syrup (EMA), Plantain and AltheaSH tablet extract did not cause any indication of ill health or overt toxicity in mice at the given doses of 1 or 5 g/kg. There was no hair or color loss; heart rate (chest expansion and retraction), writhing, stretching and fecal abnormality was observed normal.

Behavioral Examination

There was no significant change in the behavior was observed. Each and every treated animal retain normal locomotive and socialization activities.

Mortality & Morbidity

There was no mortality observed at the doses of 1 and 5 g/kg of Linkus syrup (EMA) extract, Plantain extract and AltheaSH tablet extract (Table 2).

Food Intake

There was not any considerable difference between food intake of treated and control groups noted.

Body Weight Changes

Body weights of each animal recorded earlier to the administration of formulation under test (Day 0) and on Days 7, 14. There was not any important difference observed in the weight between groups exposed to Linkus syrup (EMA) (Table 3&4), Plantain syrup (Table 5&6) and AltheaSH tablet extract (Table 7&8) and those receiving the water (control)

Table 2: Observations of Mortality

S.No	Test t Substance			Dose Level (mg/kg)	Mortality (No. of death/total No. of animals tested)
1	Linkus Syrup (extract)	Plantain Syrup (extract)	AltheaSH tablet extract	1000	0/10
				5000	0/10
2	Control	Control	Control	Water	0/10

Table 3: Individual Body Weights and Body weight Changes (Linkus syrup)

Dose level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Body Weight Decreased/ increased (g) During 2 Weeks
		0	7	14	2
1000	1- Male	25	25	24	-1
	2- Male	28	28	28	0
	3- Male	28	28	28	0
	4- Male	28	28	29	+1
	5- Male	28	28	28	0
	6- Female	26	27	27	+1
	7- Female	24	24	24	0
	8- Female	28	28	29	+1
	9- Female	30	30	30	0
	10- Female	32	30	30	-2
5000	1- Male	28	28	28	0
	2- Male	30	28	28	0
	3- Male	28	28	28	0
	4- Male	29	29	29	0
	5- Male	26	26	26	0
	6- Female	28	28	28	0
	7- Female	30	29	29	-1
	8- Female	26	24	24	+2
	9- Female	24	24	24	0
	10- Female	20	18	18	+2
Control	1- Male	32	30	30	0
	2- Male	30	30	30	0
	3- Male	29	28	28	-1
	4- Male	31	31	31	0
	5- Male	28	29	29	+1
	6- Female	28	29	29	+1
	7- Female	28	28	26	+2
	8- Female	29	29	27	-2
	9- Female	31	31	31	0
	10- Female	28	28	28	0

Table 4: Percentage (%) of Body Weight

Test Substance	Dose Level (mg/kg)	Body weight decreased (%)	Body weight Increased (%)	Duration (Days)
Linkus syrup (EMA) (Aqueous extract)	1000	1.2	1.2	14
	5000	0.37	1.48	
Control	Water	1	1.36	

Table 5: Individual Body Weights and Body weight Changes (Plantain syrup)

Dose level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Body Weight Decreased (g) During 2 Weeks
		0	7	14	2
1000	1- Male	30	30	30	1
	2- Male	30	30	28	2
	3- Male	30	28	28	2
	4- Male	28	28	28	0
	5- Male	26	24	24	2
	6-Male	28	28	-	0
	7-Male	26	28	26	0
	8-Male	28	28	28	0
	9-Male	28	28	28	0
	10-Male	26	26	26	0
5000	1- Male	26	26	26	0
	2- Male	26	24	26	0
	3- Male	28	28	28	0
	4- Male	28	28	28	0
	5- Male	32	32	32	0
	6-Male	26	24	26	0
	7-Male	32	32	30	2
	8-Male	28	28	28	0
	9-Male	28	28	28	0
	10-Male	26	26	26	0
Control	1- Male	28	29	29	1
	2- Male	30	32	32	2
	3- Male	32	32	33	1
	4- Male	28	29	29	1
	5- Male	30	30	30	0
	6-Male	29	29	29	0
	7-Male	28	29	29	1
	8-Male	30	31	31	1
	9-Male	31	32	33	2
	10-Male	30	31	31	1

Table 6: Percentage (%) of Body Weight

Test Substance	Dose Level (mg/kg)	Body weight decreased (%)	Duration (Days)
Plantain Syrup (extract)	1000	2.5	14
	5000	0.7	
Control	Water	3.3	

Table 7: Individual Body Weights and Body weight Changes (AltheaSH tablet)

Dose level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Body Weight Decreased/ increased (g) During 2 Weeks
		0	7	14	2
1000	1- Male	25	25	24	1
	2- Male	28	28	28	0
	3- Male	28	28	28	0
	4- Male	28	28	28	0
	5- Male	32	30	28	4
	6- Female	20	22	22	+2
	7- Female	24	24	24	0
	8- Female	28	28	30	+2
	9- Female	30	30	30	0
	10- Female	32	30	30	2
5000	1- Male	30	30	30	0
	2- Male	30	28	28	0
	3- Male	28	28	28	0
	4- Male	28	28	28	0
	5- Male	26	26	26	0
	6- Female	28	28	28	0
	7- Female	22	20	18	4
	8- Female	26	24	24	2
	9- Female	24	24	24	0
	10- Female	20	18	18	2
Control	1- Male	32	30	29	2
	2- Male	30	31	31	+1
	3- Male	30	28	28	2
	4- Male	31	31	31	0
	5- Male	28	29	29	+1
	6- Female	28	29	29	+1
	7- Female	28	28	27	1
	8- Female	29	29	27	2
	9- Female	32	31	31	1
	10- Female	28	28	28	0

Table 8: Percentage (%) of Body Weight

Test Substance	Dose Level (mg/kg)	Body weight decreased (%)	Body weight Increased (%)	Duration (Days)
AltheaSH tablet (Blend of actives)	1000	2.5	1.45	14
	5000	3	0	
Control	Water	2.7	1	

Sub chronic toxicity

The formulations under test were administered for duration of 28 days. An evaluation of sub chronic toxicity of under test formulations revealed that throughout the entire observation period no group of animals showed any unusual change in behavior or in locomotors activity and no signs of distress or toxicity/death were observed. All animals were found energetically moving, climbing, jumping over the cage cover. We did not observe any abnormality and change in activities of mice treated with all three formulations under test as compared with control group.

DISCUSSION

World Health Organization emphasizes particularly to the utilization of traditional medications in the treatment of chronic disorders, owing to the fact of its economic practicability, accessibility and experience of our intimates. As per World Health Organization, the use of herbal medicines has been exceeded than conventional medicines all over the world by two to three folds. Plant based drug is still in the use of about 75 - 80% of the worldwide population for accomplishing the primary health care needs [9].

Numerous plants are identified as toxic that necessitates additional investigation to execute the pharmacological activities and toxicity of remedial herbs [9]. Hence the toxicity evaluation of herbal extracts is vital to consider them a treatment that is safe. The mice used for experimental purpose are sensitive to toxic substances present in plants[10]. Therefore the extracts are administered in increasing quantities enables the estimation of the toxicity limits[11].

Hence, the present preclinical study was executed with the aim at appraising the claim of safety by evaluating the acute and sub chronic oral toxicity of the formulations under test. The current investigation was carried out on herbal formulations that integrate an excellent combination of herbs. Linkus syrup (EMA) extract is a combination of *Adhatoda Vasica, Althaea, Cordia Latifolia, Hyssopus Officinalis, Liquorice, Onosma Bracteatum, Piper Longum, Tormentil; Viola Odorata* and *Zizyphus Jujuba.* Plantain syrup extract contains Mallow Flowers, Ribwort Plantain and Vitamin C whereas AltheaSH tablet extract contains root extract of *Althea officinalis*.

Althea officinalis, universally recognized as marsh-mallow, have conventionally been used for the management of inflammatory diseases and chronic pain from the early time. In customary Persian medicine, this therapy possesses a broad range of remedial indications including joint pains. The flowers of *Althea officinalis* have anti-inflammatory activity and decrease capillaries permeability by attenuating discharge of PGE from inflammatory tissue. Scopoletin, a major component of the leaves of *Althea officinalis*, can improve RA by holding back the release of the pro-inflammatory cytokines PGE2, TNF-a,IL-1band IL-6 and restraining the expression of COX-2[12].

Adhatoda vasica L. is an aboriginal herb of family *Acanthaceae* and is used in the home-grown system of medicine globally as herbal

therapy for curing rheumatism and rheumatic painful

inflammatory swellings. Several important biomarkers including alkaloids, glycosides, tannins, flavnoids, sugars and terpenes are present in plant [13]. Other herbs used in the formulations under test also have proven anti-inflammatory activities and can be used as an alternative remedy for the osteoarthritis and RA [14].

In present investigation no mortality was observed in NMRI albino mice at the doses administered. Further toxicity signs like hair loss, mucus membrane (nasal), tremors, lacrimation, drowsiness and gait were not observed as well. An evaluation of sub chronic toxicity of under test formulations revealed that during the entire period of observation no group of animals showed any odd change in behavior or in locomotors activity and no signs of distress or toxicity/death were observed. The mice were found energetically moving, climbing, jumping over the cage cover.

CONCLUSION

The present study demonstrated that the formulations under test are potentially safe as they were not found to be the reason of apparent mortality and morbidity in mice. Hence the formulations provided confirmation of good tolerance and the lack of harmful effects on the vital organs of the experimental mice.

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