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ORIGINAL ARTICLE

Acute and Sub Chronic Oral Toxicity Studies of Weight Loss Formulation in Experimental Animal Models

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ABSTRACT

Objective: The current preclinical study aimed at appraising the claim of safety of the polyherbal weight reducing tablet formulation by evaluating its acute and sub chronic oral toxicity.

Methodology: The acute oral toxicity of weight loss tablets was evaluated as per the guidelines of Organization for Economic Co-operation and Development (OECD). Animals were divided into four groups (n=6) Group I, II and III were treated as test groups and received 7mg/kg (one tablet), 14mg/kg (2 tablets) and 21mg/kg (03 tablets) according to body weight respectively. Group IV received only distilled water and served as control group. An under-test drug was administered at doses of 50, 100, 250 and 500 mg/kg as per the body weight for 28 days for determining sub chronic oral toxicity.

Result: Polyherbal weight reducing tablets formulation were not found to be the reason of any mortality in albino mice at the specified doses. Other signs of toxicity like hair loss, mucus membrane (nasal), lacrimation, drowsiness, gait and tremors were also not observed.

Conclusion: The present study gave evidence of good tolerance of formulation and the absence of detrimental effects on the functional state of the vital organs of the experimental animals in acute and sub chronic oral toxicity test. Future prospects include the clinical trials of the finished product as the clinical efficacy is proven in animal studies.

INTRODUCTION

Obesity is a renowned social hitch, allied with several health risks and increased rate of mortality. Overweight and obesity is generally defined as 'a weight that is greater than what is healthy for a specific height' [1]. Numerous health problems for instance cardiac disease, gallbladder disease, diabetes mellitus, osteoarthritis, and numerous other complications are related to obesity and it is considered as a major health concern equally in developing and developed part of the world. Since weight perturbations are widespread and detrimental, several treatment options have been developed to triumph over the obese condition [2]. In recent times, very potent drugs have turn out to be a trendy means to overcome excessive weight [3]. However, severe adverse toxicities may restrict their effectiveness. The utilization of natural products as medicine has been recognized for hundreds of years in different traditional systems

of medicines all over the world. Natural substances less probable to produce severe toxicity are successful in reducing appetite and promoting considerable weight loss. It is a common perception that herbal medicines are harmless, gentle, unadulterated for self-treatment of mild disorders [4]. Since thousands of years in traditional Chinese medicine safe herbal formulas intended for weight loss have been used. Numerous trials have been conducted to discover innovative anti-obesity drugs through herbal sources to reduce adverse reactions associated with the current anti-obesity drugs. According to the World Health Organization, the use of herbal remedies exceeds to that of the conventional drugs by two to three folds all over the world [5].

The current investigation aims to determine the acute and sub chronic oral toxicity of herbal weight reducing tablets formulation (Table **1**).

Table 1. Tablet Composition Each 500mg tablet contains.

Foeniculum vulgare	10 mg
Trigonella foenum-graceum-seed	10 mg
Thea sinensis-leave	10 mg
Ephedera vulgaris	10 mg
Althaea officinalis	10 mg
Zingiber officinale	10 mg
Apium greveolens	10 mg
Moringa olefera	10 mg
Malotus philipinensis	10 mg

that integrates an outstanding combination of herbs including Foeniculum vulgare, Trigonella foenum-graceum-seed, Thea sinensis-leave, Ephedera vulgaris, Althaea officinalis, Zingiber officinale, Apium greveolens, Moringa olefera and Malotus Philippines's.

METHODOLOGY

Collection of herbs

The herbs utilized in formulation were procured from the market place of local vicinity and evaluated for their prescribed part, microscopic and macroscopic descriptions and compared morphologically with the samples available at Quality Control department of Herbion Pakistan Pvt. Ltd. All the herbs were also verified and authenticated by Prof. Dr. Iqbal Azhar, Dean, Faculty of Pharmacy, University of Karachi. The herbs were dried and coarsely powdered in electronic mixer, sieved through mesh no. 40 and they were then stored in air tight, well closed container till further use.

Extract preparation

The herbs used in the preparation were sieved through mesh #60. Each grinded herb was taken into extractor and water was added as solvent in the proportion of 1:10 (herb: solvent). The decoction was obtained by heating the extractors with steam for 2-3 hours. Filtration was done and the filtered decoction was shifted to evaporators to eradicate the additional solvent.

Toxicity studies

Animal selection

The acute oral toxicity was conducted in NMRI albino mice of either sex weight 150-200g acquired from the Animal House, Herbion Pakistan Pvt. Ltd. The experimental procedures relating to the animals were approved by ethical committee of Herbion Pakistan Pvt. Ltd. before starting the study. The animals used in the test were chosen randomly and marked on the tails for individual recognition. Every cage was placed in a room with constant humidity at temperature approximately 23 °C. The room was regulated with cycles of 12 h of light and 12 h of darkness. The animals were familiarized to the lab setting for a week earlier prior to conducting

the tests. Drinking water and food were provided ad libitum during the experiment, excluding the short fasting period where the drinking water was still in free access however no food was given 12 h preceding treatment.

Acute oral toxicity test

The acute oral toxicity of weight loss tablets was evaluated as per the guidelines of Organization for Economic Co-operation and Development (OECD) [6]. All animals were divided into four groups (n=6) Group I, II and III were treated as test groups and received 7mg/kg (one tablet), 14mg/kg (2 tablets) and 21mg/kg (03 tablets) according to body weight respectively. Group IV received only distilled water and served as control group. All drugs were administered through the oral route following the overnight fasting period. The animals were observed strictly after dosing for general behavioral changes, symptoms of toxicity and mortality during first 30 minutes followed by first six hours and daily further for 14 days. Every day changes in eyes and mucus membrane (nasal), respiratory rate, skin and fur, heart rate, autonomic effects (salivation, lacrimation, pilo erection, urinary incontinence, perspiration and defecation), blood pressure and central nervous system (lethargy, gait, ptosis, tremors and seizure) were observed.

All animals were alienated into four groups (n=6) Group I, II and III were treated as test groups. Drug was administered at doses of 50, 100, 250 and 500 mg/kg as per the body weight. Group IV received only distilled water and served as control group. All drugs were given through the oral route following the overnight fasting period. The doses were administered on a daily basis for 28 days at the similar time and observed at least two times for morbidity and mortality.

RESULTS

Acute toxicity studies

The drug under test was not found to be the reason of any mortality in NMRI albino mice at the given doses. Other signs of toxicity like hair loss, mucus membrane (nasal), lacrimation, drowsiness, gait and tremors were also not observed. The test product appeared to be safe on doses 7mg/kg (one tablet), 14mg/kg (2 tablets) and 21mg/kg (03 tablets) (Table **2**).

Table 2. Acute oral toxicity test of weight reducing formulation

Groups	No. of animals	No. of animals died	No. of animals survived	%	
				Mortality	Survival
I	6	Nil	6	0	100
II	6	Nil	6	0	100
III	6	Nil	6	0	100
IV	6	Nil	6	0	100

Sub chronic toxicity

The tested product was administered for a period of 28 days. An evaluation of sub chronic acute toxicity of under test weight reducing tablets formulation revealed that during the whole 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 actively moving, climbing, jumping over the cage cover.

We did not find any abnormality and change in behavior of rats treated with weight loss tablets as compared with control group.

DISCUSSION

An incidence of obesity is escalating globally ensuing in an association with main health problems. Pharmacologic management and surgical interference used in some conditions are not always acceptable. Furthermore, in spite of short-term benefits, its frequently associated with rebound weight gain following the cessation of drug use, adverse effects and the probability for drug misuse [7]. The majority of such treatments involve caloric restraint, based on the theory that stored up calories are consumed in the form of fats, if ingestion of food is less than energy

expenditure. Despite the fact that, in case the diet regime is stopped up, weight is rapidly got back. Further treatment options increases metabolism that burned calories thus declining body weight [8] Although there are splendid advancements in modern medicine, yet traditional medicine has always been accomplished for treating obesity. The traditional medicine sector has become a vital resource in health care, particularly in rural and tribal areas of the country. According to the World Health Organization (WHO), the utilization of plant-based medicines has exceeded to that of conventional medicines all over the globe by two to three times.

Plant derived drug is still the mainstay of about 75 - 80% of the global populace for primary health care; owing to the general belief that herbal drugs are devoid of any side effects besides being economical and easily accessible [9]. In cases if the conventional drugs not succeeded to treat chronic conditions for instance obesity efficaciously devoid of adverse events, several people look for alternative therapies include herbal medication [10,11]. Hence, the present preclinical study aimed at appraising the claim of safety by evaluating the acute and sub chronic oral toxicity of the formulation under test. The current investigation was carried out on herbal weight reducing tablets formulation that integrates an outstanding combination of herbs including Foeniculum vulgare, Trigonella foenum-graceum-seed, Thea sinensis-leave, Ephedera vulgaris, Althaea officinalis, Zingiber officinale, Apium greveolens, Moringa olefera and Malotus philipinensis.

Research has revealed that fenugreek seed extract supplementation reduces the body and adipose tissue weight. The likely means of fenugreek decreasing the total body and adipose tissue weight may be that fenugreek flushes out the carbohydrates from the body earlier than they come into the blood stream ensuing in weight loss. Fenugreek seeds have a high percentage (40%) of soluble fibers which forms a gelatinous structure that may have effects on slowing the digestion and absorption of food from the intestine and produce a sense of fullness in the abdomen, consequently suppresses appetite and encourages weight loss [12]. Ephedrine, an important alkaloid obtained from Ephedra vulgaris, is a sympathomimetic amine and used as appetite suppressors. Its chief mechanism of action depends on its direct and indirect actions on the adrenergic receptor system, a component of the sympathetic nervous system [13]. Mehta reported that Moringa oleifera was found to increase the excretion of faecal cholesterol. The ethanolic extract of Zingiber officinale proved outstanding protection from the high fat diet-induced metabolic disturbances by strongly repressing the body weight gain [14].

In current study no mortality was observed in NMRI albino mice at the given doses. Other signs of toxicity like hair loss, mucus membrane (nasal), lacrimation, drowsiness, gait and tremors were also not observed. An evaluation of sub chronic acute toxicity of under test weight reducing tablets formulation revealed that during the whole 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 noted. All animals were found actively moving, climbing, jumping over the cage cover. Any abnormality and change in behavior was not observed of rats treated with weight loss tablets as compared with control group.

Hence the weight reducing formulation gave evidence of good tolerance of and the absence of detrimental effects on the functional state of the vital organs of the experimental animals in acute and sub chronic oral toxicity test.

CONCLUSION

In the present study, the developed polyherbal weight reducing formulation incorporating the herbs in standardized form provide an opportunity to validate its traditional claim regarding its therapeutic efficacy. Future

prospects include the clinical trials of the finished product as the clinical efficacy is proven in animal studies.

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