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Acute Toxicity Studies of a newly developed anti-obesity polyherbal formulation in Zebrafish

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Abstract

Obesity is one of the major lifestyle diseases affecting more than 2 billion people worldwide. Currently, there are synthetic drugs to reverse the disease of obesity. These drugs are expensive and can cause several side effects. Therefore, researchers are looking for herbal solutions. In the present study, we have prepared a novel polyherbal formulation for the treatment of obesity. We have determined the toxicity of this polyherbal formulation on adult Zebra fish and its embryos. The lethal concentration LC_{50} of this polyherbal formulation was found to be 1371 mg/L in adult Zebra fish whereas 57.1 mg/L in Zebra fish embryos. This result will be taken into consideration for further studies on adult Zebra fish.

Keywords

Obesity, polyherbal formulation, Zebra fish, Adipose tissue, etc.

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INTRODUCTION:

Obesity is a modern-day lifestyle generated disease. According to World Health Organization (WHO), obesity has almost multiplied by three times from the year 1975 all around the world. As per the WHO reports published in 2016 close to 2 billion people are either obese or overweight. [1] WHO suggests that obesity can be controlled? In the current market scenario, obesity is treated with synthetic drugs such as Sibutramine, Orlistat, etc [2]. These synthetic drugs are costly and sometimes can cause severe side-effects. [3] Therefore, therefore is a need of herbal formulations for the treatment of obesity. [4]

Toxicity study is one of the most widely used methods to decide the range of tolerance of any drug. [5] Present day researchers widely use vertebrate organism Zebrafish for studying the toxicity of any drug. [6] Zebrafish is scientifically known as *Daniorerio* which is currently used by researchers all over the world for studying different developmental processes

as well as human diseases. There is a close genetic similarity between Zebrafish and humans. [7] In this present study we will determine the toxicity of a polyherbal formulation.

MATERIALS AND METHODS:

In this study, a new Poly Herbal Formulation (PHF) was prepared using *Linumu sitatissimum* (Flax seeds), *Piper Nigrum* (Black pepper), *Macrotylomauniflorum* (Horse Gram), *Nelumbonucifera* (Lotus seeds), *Glycine max* (Soyabean), *Curcuma longa* (Turmeric), *Avena sativa* (Oats), *Syzygiumaromaticum* (Clove), *Zingiber officinale* (Ginger), *Allium sativum*(Garlic), *Cucurbitapepo* (Pumpkin seeds) and *Cinnamomum cassia* (Cinnamon).These plant materials were collected from local market in Bangalore (India). These Materials were washed and properly dried. The dried plant materials were authenticated at 'Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore (Authentication / SMPU / RARIMD / BNG / 2017-18 / 1071). This PHF was prepared in accordance with the maximum dosage provided in the Ayurvedic Pharmacopeia of India (API).

Acute Toxicity Testing of The Newly Developed Polyherbal Formulation:

Toxicity study was performed according to the OECD test guideline 203(Fish Acute Toxicity Test), in order to decide Lethal Concentration (LC_{50}) of Polyherbal Formulation in Adult Zebrafish, for fish embryo Toxicity Test, OECD guideline 236 was used to determine Acute Toxicity of Polyherbal Formulation on developmental embryos of Zebrafish. [8,9]

Procedure for Acute Toxicity in Adult Zebrafish:

In this experiment, Adult Zebrafishes ranging between 0.5-1 grams were procured from the Local Suppliers in Bangalore. For acclimatization, all the Zebrafishes were treated with Antibiotic containing water for 24 hours. For further acclimatization, they were kept in 500litres water tank and tank was continuously aerated. During this time, 12hours light and 12hours dark light cycles was maintained. Zebrafishes were fed with Shrimp flakes during acclimatization. Total 42 fishes were divided into 6 groups; each group had 7 Zebrafishes. Five different concentrations of the PHF were prepared to determine the toxicity.

Procedure for Acute Toxicity in Zebrafish Embyros:

In this experiment, 6 Male and 15 Female Zebrafishes were allowed to breed in a Breeding Chamber overnight. Zebrafish embryos were collected in the early morning. Total 42 embryos were divided into 6 groups, each group had 7 embryos. Five different concentrations of the Polyherbal Formulation were prepared for toxicity studies on embryos. Both the experiments were repeated three times.

The mortalities of both Adult Zebrafish and embryos were recorded at 24, 48, 72, and 96 hours and toxicity of the Polyherbal Formulation was determined.

LC_{50} was calculated using the below mentioned formula.

$$\mathbf{LC_{50} = LC100 - \sum (a \times b)/n}$$

Where n = total number of animals in a group

a=the difference between two successive doses of administered substance

b=the average number of dead animals in two successive doses.

LC100 = Lethal dose causing 100% death of all the test animals

RESULTS AND DISCUSSION:

The maximum concentration at which 50% of the fishes and embryos died was recorded. The abnormal changes and mortality of the fish polyherbal formulation was recorded after 24, 48, 72 and 96hours. The present study was undertaken to determine the toxic dose for the polyherbal formulation. No abnormal changes in swimming behavior, pigmentation and survival were observed in the control group fishes. Changes in swimming behavior and pigmentation of Zebrafish treated with PHF were observed with minimal effect. For Zebrafish embryos, there were no mortalities in control group. There were no deformities observed during the four-day developmental process. From the result, the lethal concentration LC50 of the PHF was found to be1371 mg/L. A similar result was obtained for Zebrafish embryos. From the result, the lethal concentration LC50 of the PHF was found to be 57.1 mg/L.

Table 1: The cumulative mortalities and acute 96 h LC_{50} of ofpolyherbal formulation in Adult Zebrafish according to Behrens-Karber's method (Klassen, 1991).

PHF dose (mg/L)	No. of exposed fish	No of dead fish				Overall deaths within 96 h
		24	48	72	96	
0	7	0	0	0	0	0
200	7	0	0	0	1	1
400	7	1	2	2	3	3
800	7	2	4	5	5	5
1600	7	3	6	7	7	7
3200	7	5	7	7	7	7

Table 2: The cumulative mortalities and acute 96 h LC_{50} of polyherbal formulation in ZebrafishEmbroyos according to Behrens-Karber's method (Klassen, 1991).

PHF dose (mg/L)	No. of exposed embryos	No of dead fish embryos				Overall deaths within 96 h
		24	48	72	96	
0	7	0	0	0	0	0
20	7	0	1	3	3	3
40	7	2	2	4	6	6
80	7	3	3	5	7	7
160	7	6	7	7	7	7
320	7	7	7	7	7	7

CONCLUSION:

The present study predicts that the adult Zebra fish and its embryos shows the toxicity at a higher concentration. This may be due to the fact that all the components used in the polyherbal formulation are edible in nature. Therefore, it is expected to be safer for human consumption. Several researchers have used these components as a part of their formulations for the treatment of various diseases. These ingredients are also consumed by human beings almost daily. Most of these ingredients have the proven ability to break down the visceral and subcutaneous fats. On the basis of the present study these polyherbal formulation will be used for performing the antiobesity studies on adult Zebra fish.

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Conflict of interest: Nil

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