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Acute and long term safety evaluation of Zincovit drop (Nutritional food supplement) in Wistar rats

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ABSTRACT

The aim of the present study was to investigate the acute and sub-chronic toxicity associated with Zincovit drop (Nutritional food supplement) in Wistar rats. Acute toxicity class method (OECD 423 guideline) was employed to determine acute toxicity in Wistar rats. Animals were observed individually after dosing daily for a total of 14 days. Sub chronic toxicity was investigated in normal control (2% gum acacia, 1 ml/kg/day) and Zincovit drop at 25, 100 and 400 mg/kg/day individually for 3 months in adult female Wistar rats (4 groups, n= 6). Clinical signs, hematological and biochemical parameters were assessed. During the acute toxicity study, according to annex 2a of OECD 423 guidelines, Zincovit drop falls under Category 4 (>300-2000) of globally harmonized classification system (GHS). For Zincovit drops, LD50 cut-off among Wistar rats was observed at 1000 mg/kg. There was no significant change in their body weight. During the 90 days of sub-chronic toxicity study, treatment with Zincovit drop among Wistar rats, the lowest-observed-adverse-effect level (LOAEL) and no-observed-adverse-effect level (NOAEL) was observed at 400 mg/kg/day and 100 mg/kg/day respectively. The present study revealed the long term safety ofZincovit drop especially at 25 mg/kg/day and 100 mg/kg/day in Wistar rats.

KEYWORDS : Zincovit drop, Flax seed oil, Docosahexaenoic acid, Lysine, Multivitamin-multimineral nutritional food supplement, Acute toxicity, Sub chronic toxicity, Antioxidants.

INTRODUCTION

The safety assessment of nutrients is an issue that presents achallenge different from that posed by the assessment of other chemicals in food, such as additives or contaminants. Because nutrients are essential, a dose-response relation exists at both ends of the intake range and in many cases the available database is limited. Now days, the huge concern is raised for the role of the dietary intake of antioxidant nutrients in the possible prevention of major diseases that affect humans worldwide. But, the long term safety of these supplements is still questionable. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems. In general, multivitamin and mineral supplements are used to ensure adequate intake and to prevent or mitigate diseases. The U. S. Food and Nutrition Board have established tolerable upper intake levels for several nutrients. The strength of the evidence used to determine an upper intake level of multivitamin-mineral depends on data availability. Hence, an update of the data on adverse effects will help researchers to evaluate the appropriateness of upper intake levels. Zincovit drop is a combined formulation of vitamins, minerals, lysine and flaxseed oil.Lysine, or L-lysine, is an essential amino acid. Lysine is important for proper growth, and it plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acids into energy and helping to lower cholesterol. Lysine appears to help the body to absorb calcium, and it plays an important role in the formation of collagen, a substance important for bones and connective tissues including skin, tendon, and cartilage. Flaxseed oil isrich source of the essential fatty acid alpha-linolenic acid (ALA), which is a biologic precursor to omega-3 fatty acids. Zincovit drop releases a stream of anti-oxidant benefits [1].Earlier, we have reported the acute and long term safety of combined formulation of grape seed extract and Zincovit tablets where flaxseed oil and lysine were not the ingredients [2]. Henceforth, the aim of the present study was to evaluate the acute and long term safety of orally administered Zincovit drop in Wistar rats.

MATERIALS AND METHODS

Drugs and Reagents

Zincovit drop was procured from Apex Laboratories Private Ltd., Chennai (India). The diagnostic kits for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and creatinine were obtained from Aspen Laboratories, New Delhi (India). Sodium chloride and all other chemicals were obtained from Merck Chemicals, Mumbai (India). The reagents were equilibrated at room temperature for 30 minutes before use, either at the start of analysis or when reagent containers were refilled.

Animals

Adult female Wistar rats (4 to 6-weeks-old), nulliparous and non-pregnant were selected for the study, which were bred locally in the central animal house of Manipal University, Manipal. They were housed in separate polypropylene cages (3 animals in each cage), maintained under standard conditions with temperature (22–24°C), 12-h light/12-h dark cycle and relative air humidity 40–60%. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The animals were provided with a normal pellet diet (VRK Nutritional Solutions, Pune, India) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/38/2013) and experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental design

Acute toxicity study

For acute toxicity study, both rats were divided individually into four groups (n= 3).

Group IA: 5 mg/kg of Zincovit drop
Group IB: 5 mg/kg of Zincovit drop
Group IIA: 50 mg/kg of Zincovit drop
Group IIB: 50 mg/kg of Zincovit drop
Group IIIA: 300 mg/kg of Zincovit drop
Group IIIB: 300 mg/kg of Zincovit drop
Group IVA: 2000 mg/kg of Zincovit drop
Group IVB: 2000 mg/kg of Zincovit drop

The acute toxicity class method (OECD 423) was employed for the acute toxicity study of Zincovit drop [3]. In this method, Zincovit drop was administered through oral route as a single dose to a group of experimental animals and a sequential testing procedure with three animals per group was used. Animals were fasted prior to dosing (food was withheld over-night but water ad libitum). Following the period of fasting, the body weight of animals was taken and the respective dose of drug was administered. After the administration of Zincovit drop, food was withheld for 3-4 hours. The time interval between treatment groups was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose was delayed until one was confident of survival of the previously dosed animals. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special

attention given during the first 4 hours and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included were changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activities and behavior patterns. Prime focus was for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. At the end of the test surviving animals were weighed and rehabilitated.

Sub chronic toxicity study

For sub chronic toxicity study, rats were divided into 4 groups (n=6). The corresponding doses of drugs were administered orally till 90 days as follow-

Group I: Normal control (2% gum acacia, 1 ml/kg)
Group II: 25 mg/kg/day of Zincovit drop
Group III: 100 mg/kg/day of Zincovit drop
Group IV: 400 mg/kg/day of Zincovit drop

Collection of blood samples

At the end of the experimental period, the animals were anesthetized with ketamine (80 mg/kg; *i.p.*) following a 12 h fast. Blood was collected from retro-orbital plexus through capillary tube. Serum was obtained by centrifugation of blood at 3,000 rpm for 20 min at 4°C using a refrigerated centrifuge (MIKRO 22R, Andreas Hettich GmbH & Co. KG, Germany).

Hematological parameters

0.5 ml of blood from each animal was collected in an EDTA containing vacutainer. Further RBC, WBC, differential leukocytes, platelet count and amount of hemoglobin was measured with the help of veterinary automatic blood cell counter.

Biochemical parameters

Blood glucose level was estimated in the fasting blood samples by glucose oxidase-peroxidase

reactive strips (Accu-chek, Roche Diagnostics, USA). Serum was analyzed further for assay of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine, triglyceride (TG), total cholesterol (T-CHO) and high density lipoprotein cholesterol (HDL-C) according to the standard protocols given along with respective kits (Aspen Laboratories, New Delhi, India). Low-density lipoprotein cholesterol (LDL-C) and Very low-density lipoprotein cholesterol (VLDL-C) was calculated by using Friedewald's equation:

VLDL-C = Triglycerides (TG)/5
LDL-C = Total cholesterol − (HDL-C+VLDL-C)

Statistical analysis

Using Statistical Package for the Social Sciences (SPSS version 20.0; SPSS Inc., Chicago, USA), normally distributed data were expressed as mean ± standard deviation and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. A level for $p \leq 0{\cdot}05$ was considered to be statistically significant.

RESULTS

During acute toxicity study, a total of 2 rats died at the dose of 2000 mg/kg (Group IV A). According to annex 2a of OECD 423 guidelines, Zincovit drop falls under Category 4 (>300-2000) of Globally Harmonized Classification System (GHS). For Zincovit drop, LD50 cut-off among Wistar rats was observed at 1000 mg/kg.

During sub chronic study, there was no mortality/morbidity among the experimental animals during the 90 days treatment period. There was no significant change in blood cells count, hemoglobin content, fasting blood glucose, serum total triglyceride, total cholesterol, HDL, VLDL, and ALP level among experimental animal groups treated with Zincovit drop at all the three different doses (25, 100 and 400 mg/kg/day) when compared to normal control group of rats. Mostly, in rats treated with Zincovit drop at the dose of 25 and 100 mg/kg, different significant levels were observed for LDL (ZVT drop 25 mg/kg; p= 0.012, ZVT drop 100 mg/kg; p= 0.001), Creatinine (ZVT drop 25 mg/kg; p= 0.012, ZVT drop 100 mg/kg; p= 0.025, ZVT drop 400 mg/dg; p= 0.014), ALT (ZVT drop 25 mg/kg; p= 0.003, ZVT drop 100 mg/kg; p= 0.001, ZVT drop 400 mg/dg; p= 0.008) and AST (ZVT drop 25 mg/kg; p= 0.002, ZVT drop 100 mg/kg; p= 0.001) in comparison with normal control rats (Table 1-4). During the whole body gross necroscopy, there were no significant structural changes observed among normal control rats and rats treated with three different doses of Zincovit drops (25, 100 and 400 mg/kg/day). Also, at the end of the treatment, all the experimental animals were found healthy as shown by the normal appearance of respiratory pattern, color of body surfaces, frequency and nature of movement and absence of symptoms like seizures, loss of reflex.

DISCUSSION

In the present study, no significant changes were observed in the hematological parameters between the normal control and the zincovit drop treated groups which suggest that the Zincovit drop did not affect either the circulating red cells, or the hematopoiesis that could otherwise have caused a megaloblastic anemia, abnormal differential leukocyte count, platelets count and hemoglobin. Usually, an elevation in the plasma concentration of ALT and AST are indicators of liver and heart damage respectively [4, 5]. However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury [6]. The non-significant changes in the biochemical parameters of liver function, renal function, lipid profile and glucose level reveals absence of hepatotoxicity, nephrotoxicity, dyslipidemia and hyperglycemia respectively with the Zincovit drop treatment. All the surviving animals were healthy as shown by the normal appearance of respiratory pattern, color of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures of contraction of voluntary muscle, and loss of reflex. In the present study, Zincovit drop gives the indication of its cardio protective property by decreasing LDL and AST level in serum at the dose of 25 and 100 mg/kg/day in Wistar rats. This might be due to the presence of flaxseed oil as one of the constituent of zincovit drop in addition to other vitamins, minerals and lysine. One of the studies also suggests the cardio protective role of flaxseed oil [7]. Since toxicity in humans cannot always be entirely extrapolated from animal studies, further clinical evaluation should be performed to precisely define the safe dosage to advice Zincovit drops as nutritional food supplement in humans.

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Table 1- Effect of Zincovit drops on white blood corpuscles (x10^{3}cells/µl), lymphocytes (%), monocytes (%) and granulocytes (%)

Groups (n=6)	WBC	Lymphocyte	Monocyte	Granulocyte
I- Normal control (2% gum acacia)	9.63±1.32	78.65±2.04	10.45±0.73	10.90±1.36
II- ZVT drop (25 mg/kg/day)	8.85±1.61#	77.21±2.84#	11.11±1.04#	11.66±1.83#
III- ZVT drop (100 mg/kg/day)	9.53±0.85#	71.61±1.80#	12.36±0.56#	16.01±1.26#
IV- ZVT drop (400 mg/kg/day)	10.00±3.57#	74.38±1.42#	11.88±0.86#	13.73±0.85#

n, number of rats in each group; WBC, white blood corpuscles; ZVT, zincovit. Values are expressed as mean ± standard deviation. #indicates statistically non-significant difference compared to normal control group ($p > 0.05$).

Table 2- Effect of Zincovit drops on red blood corpuscles (x10^{6}cells/µl), hemoglobin (g/dl), platelets (x10^{3}cells/µl) and triglycerides (mg/dl)

Groups (n=6)	RBC	Hemoglobin	Platelets	Triglycerides
I- Normal control (2% gum acacia)	8.69±0.06	12.81±0.19	600.66±27.93	106.68±13.64
II- ZVT drop (25 mg/kg/day)	8.71±1.00#	12.80±1.61#	492.00±64.77#	130.18±3.63#
III- ZVT drop (100 mg/kg/day)	10.85±0.45#	16.40±0.73#	479.83±40.47#	127.73±2.70#
IV- ZVT drop (400 mg/kg/day)	8.38±0.68#	12.55±0.93#	656.50±53.38#	135.15±10.93#

n, number of rats in each group; RBC, red blood corpuscles; ZVT, zincovit. Values are expressed as mean ± standard deviation. #indicates statistically non-significant difference compared to normal control group ($p > 0.05$).

Table 3- Effect of Zincovit drops on total cholesterol (mg/dl), high density lipoprotein (mg/dl), low density lipoprotein (mg/dl), very low density lipoprotein (mg/dl) and fasting blood glucose level (mg/dl)

Groups (n=6)	T-CHO	HDL	LDL	VLDL	FBG
I- Normal control (2% gum acacia)	81.94±4.32	37.79±4.99	22.76±5.13	21.33±2.73	103.83±2.79
II- ZVT drop (25 mg/kg/day)	82.96±1.42#	48.17±1.87#	8.75±2.00**	26.03±0.72#	105.16±3.62#
III- ZVT drop (100 mg/kg/day)	73.94±2.70#	44.43±1.48#	3.96±0.87***	25.54±0.54#	107.00±2.30#
IV- ZVT drop (400 mg/kg/day)	87.27±1.34#	43.33±1.98#	16.91±1.29#	27.02±2.18#	110.00±2.95#

n, number of rats in each group; RBC, red blood corpuscles; ZVT, zincovit; T-CHO, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; FBG, fasting blood glucose. Values are expressed as mean ± standard deviation. **#**indicates statistically non-significant difference compared to normal control group ($p > 0.05$), **indicates statistically significant difference compared to normal control group ($p < 0.01$), ***indicates statistically significant difference compared to normal control group ($p < 0.001$)

Table 4- Effect of Zincovit drops on alanine transaminase (U/L), aspartate transaminase (U/L), alkaline phosphatase (U/L) and very low density lipoprotein (mg/dl)

Groups (n=6)	ALT	AST	ALP	Creatinine
I- Normal control (2% gum acacia)	51.83±6.05	214.86±16.08	385.88±60.58	0.95±0.11
II- ZVT drop (25 mg/kg/day)	71.81±2.55**	135.10±12.29**	372.34±20.14#	0.60±0.04**
III- ZVT drop (100 mg/kg/day)	74.01±3.32***	129.24±5.28***	459.19±32.81#	0.63±0.04**
IV- ZVT drop (400 mg/kg/day)	78.32±2.49#	174.88±17.17#	403.13±57.09#	0.61±0.04**

n, number of rats in each group; RBC, red blood corpuscles; ZVT, zincovit; T-CHO, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein. Values are expressed as mean ± standard deviation. **#**indicates statistically non-significant difference compared to normal control group ($p > 0.05$), **indicates statistically significant difference compared to normal control group ($p < 0.01$), ***indicates statistically significant difference compared to normal control group ($p < 0.001$).

REFERENCES

1. Satyam SM, Bairy KL. Zincovit drop reduces oxidative stress induced by carbon tetrachloride in rats. AJPTT 2015;3(5):469-474. | 2. Satyam SM, Bairy KL, Pirasanthan R. Acute and long term safety evaluation of grape seed extract and zinc containing multivitamin-mineral nutritional food supplement in rodents. RJPBCS 2014;5(5):561-566. | 3. OECD guideline for testing of chemicals. Acute Oral Toxicity-Acute Toxic Class Method. 423: Adopted: 17th December 2001. | 4. Wasan KM, Najafi S, Wong J, Kwong M. J Pharm Sci 2001; 4: 228-34. | 5. Mythilypriya R, Shanthi P, Sachdanandam P. J Health Sci 2007; 53: 351-58. | 6. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. Toxicol 2008; 245: 194-05. | 7. Harper CR, Edwards MJ, DeFilipis AP, Jacobson TA. Flaxseed Oil Increases the Plasma Concentrations of Cardioprotective (n-3) Fatty Acids in Humans. J Nutr 2006;136:83-87. |