Original Article

Zincovit Drop Reduces Oxidative Stress Induced by Carbon Tetrachloride in Rats

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ABSTRACT

Zincovit drop is a combined formulation of multivitamins-minerals, flax seed oil and lysine. This study has focused on the *in vivo* antioxidant potential of Zincovit drop against carbon tetrachloride induced oxidative stress in rats. Rats divided into six groups containing six rats in each group. Zincovit drop at the dose of 25 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day was investigated in carbon tetrachloride intoxicated rats. Biochemical analysis of malondialdehyde, glutathione-S-transferase and catalase in liver tissue was performed. Oral treatment with Zincovit drop mostly at all the three doses 25, 50 and 100 mg/kg/day for 7 days reversed CCl4-induced alterations in malondialdehyde ($p<0.01$), glutathione-S- ($p<0.001$) and catalase ($p<0.05$) compared to carbon tetrachloride intoxicated control (untreated) animals. The present study demonstrated that Zincovit drop may be of great importance in oxidative stress associated diseases as nutritional food supplement.

Keywords: Zincovit drop, Flaxseed oil, Lysine, Zinc, Oxidative stress, Carbon tetrachloride.

INTRODUCTION

The imbalance of reactive metabolites production and antioxidant defense usually results in oxidative stress, which regulates the cellular functions leading to various pathological conditions. One of the studies suggest that oxidative stress plays an important role in the etiology and pathogenesis of many chronic diseases such as hepatotoxicity, nephrotoxicity, atherosclerosis, hypertension, diabetes mellitus and cancers.[1,2] Dietary supplementation of antioxidants can arrest or delay the oxidation of susceptible cellular substrates to prevent oxidative stress. One of the studies suggests the necessity to enrich diet with antioxidants to protect against many chronic diseases related to oxidative damage.[3]Previous studies revealed that dietary intake of vitamin E can normalize the damaging effect of oxidative stress

induced by oxygen free radicals.[4-7] Flaxseed has emerged as one of the nutritive and functional ingredient in food products. Scientific findings are growing in support of flaxseed consumption. Flaxseed contains good amount of α-Linolenic Acid (ALA), a omega-3 fatty acid, protein, dietary fiber, lignan, specifically Secoisolariciresinol diglucoside (SDG). One of the studies suggests that the antioxidant effect of flaxseed oil was amplified by its coadministration with vitamin E in potassium bromate induced oxidative stress in rats.[8] In rat colon carcinogenesis induced by 1, 2-dimethyl hydrazine (DMH), dietary supplementation of pronyl-lysine has been reported to significantly reverse the levels of lipid peroxidation products and antioxidants.[9] Zincovit drop is a combined formulation of high concentration of vitamins, minerals, flaxseed oil and lysine. Zincovit drop might release a stream of anti-oxidant benefits because of synergistic antioxidant potential of vitamins, minerals, flaxseed oil and lysine. Earlier, we had reported the antioxidant potential of combined formulation of grape seed extract and Zincovit tablets where flaxseed oil and lysine were not the ingredients.[10,11] Henceforth, the aim of the present study was to investigate the protective effect of Zincovit drop on oxidative stress induced by carbon tetrachloride in Wistar rats.

METHODS

Drugs and reagents

Zincovit drop was obtained from Apex Laboratories Private Ltd., Chennai (India). Thiobarbituric acid (TBA) and trichloroacetic acid (TCA), 1-Chloro-2, 4-dinitrobenzene (CDNB), 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) and reduced glutathione (GSH) were procured from Sigma Chemical Inc. (USA). Catalase and glutathione-S-transferase colorimetric assay kit was purchased from Bioassay Systems, Hayward (USA). Carbon tetrachloride (CCl_4), potassium chloride, sodium chloride, sodium hydroxide, ethylene-di-amine-tetra-acetic acid (EDTA) and all other chemicals were obtained from Merck Chemicals, Mumbai (India).

Animals

A total of 36 adult male albino Wistar rats weighing 150-200 g were housed in separate polypropylene cages maintained under standard conditions with temperature (22–24^{0}C), 12-h light/12-h dark cycle and relative air humidity 40-60%. The animals were acclimatized to the Standard laboratory conditions for seven days before the start of the experiment. Normal pellet diet (VRK Nutritional Solutions, Pune, India) and water ad libitum was provided to animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/39/2013). The experiment was conducted according to the ethical norms of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental design

In the experiment, 36 adult male Wistar rats (150-200 g) were used. The rats were divided into 6 groups containing 6 rats in each group. Treatment was done for seven days as follow[10].

Group I

Normal control rats were given 2% gum acacia (1 ml/kg/day; *p.o*).

Group II

CCl4 intoxicated control rats + 2% gum acacia (1 ml/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Group III

CCl4 intoxicated rats + Silymarin (50 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Group IV

CCl4 intoxicated rats + Zincovit drop (25 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Group V

CCl4 intoxicated rats + Zincovit drop (50 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Group VI

CCl4 intoxicated rats + Zincovit drop (100 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

At the end of 7th day treatment, all the experimental rats were kept overnight fasting and sacrificed by administering overdose of ketamine, i.p. At the end of the treatment, following autopsy liver was excised immediately and washed with ice-cold saline. Liver homogenates (10% w/v) was prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10000 rpm for 30 minutes using a Remi C-24 refrigerated centrifuge. The resulting supernatant was stored at -20˚C. All the following biochemical antioxidant parameters were estimated in triplicate manner and optical density was read for reagent and sample blank.

Determination of Malondialdehyde (MDA) level

20 µl liver homogenate was added into an eppendorf tube containing 200 µl of 0.67% thiobarbituric acid and 100 µl of 20% trichloroacetic acid. Thereafter, the mixture was incubated at 100˚C for 20 minutes. Then, it was centrifuged at 12000 rpm for 5 minutes and 100 µl of supernatant was transferred to 96- wells of micro test plate. Optical density of supernatant was read at 540 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).[10]

Determination of Catalase (CAT) activity

Catalase activity was measured according to the standard protocol given along with the Catalase assay kit of Bioassay Systems, Hayward (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Glutathione-S-transferase (GST) activity

Glutathione-S-transferase (GST) activity was measured according to the standard protocol given along with the glutathione-S-transferase assay kit of Bioassay Systems, Hayward (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Statistical analysis

Using Statistical Package for Social Sciences (SPSS version 20.0; SPSS Inc., Chicago, USA), data were expressed as Mean ± SEM (Standard Error of Mean) 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 as statistically significant.

RESULTS

Effect on biochemical parameters

In the present study, CCl4 caused significant increase in malondialdehyde ($p<0.05$) and decrease in catalase ($p<0.01$) as well as glutathione-S-transferase ($p<0.001$) when compared to normal control rats. Oral treatment with Zincovit drop at the

dose of 50 and 100 mg/kg/day reversed CCl4-induced alterations in malondial-dehyde (p<0.01), glutathione-S-transferase (p<0.001) and catalase (p<0.05) in comparison with carbon tetrachloride intoxicated control (untreated) animals (Table 1). There was also a significant increase in glutathione-S-transferase (GST) level in CCl_4 intoxicated rats treated with 50 and 100 mg/kg/day of Zincovit drop when compared with positive control group (Silymarin, 50 mg/kg/day; p.o) (p<0.05) (Table 1).

DISCUSSION

The results of the present study showed that treatment of rats with Zincovit drop effectively protected the animals against CCl_4-induced oxidative stress. The results obtained indicate increased MDA levels in the liver in response to CCl_4 treatment, implying increased oxidative damage to the liver. CCl_4 also caused an increase in glutathione-S-transferase, catalase activity and decreased MDA content in the liver over those of the toxic control group. In this work, Zincovit drop treatment returned the increased MDA, decreased glutathione-S-transferase and catalase antioxidant enzymes levels back to their control levels, implying that Zincovit drop may prevent the oxidative stress produced by CCl_4. Glutathione-S-transferase catalyses the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione (γ-glu-cys-gly).[12] In this study, the significant increase in the activity of glutathione-s-transferase following the administration of Zincovit drop may be adduced to the presence of elements such as zinc that might have enhanced the synthesis of the enzyme. Catalases protect the cells from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen.[13]The ability of Zincovit drop to revert the reduced catalase activity buttresses its antioxidant potential. Based on the experimental results reported here, we hypothesize that Zincovit drop may play an important role in medicine by scavenging free radicals, stimulating activities of antioxidant enzymes subsequently protecting the liver against CCl4-induced damage. We supposed that the components (Vitamin C, Vitamin E, Zinc, Magnesium, Selenium) in combination with other components (lysine, flax seed oil) present in the Zincovit drop might be responsible for the reduction of oxidative stress in treatment groups.

CONCLUSION

The present study clearly demonstrates the ameliorating effects of Zincovit drop on oxidative stress caused by CCl_4 administration in Wistar rats. The dietary supplement of Zincovit drop may prove to be beneficial in oxidative stress associated diseases.

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Table 1. Effect of Zincovit drop oral treatment on malondialdehyde (nmoles/mg), glutathione-S-transferase (µmoles of CDNB conjugates/min/mg) and catalase (units of hydrogen peroxide oxidized/min/mg) in liver tissue homogenate

Groups (n=6)	MDA	GST	CAT
I- Normal control (2% gum acacia; 1 ml/kg/day)	27.94±3.70	100.14±6.79	0.92±0.05
II- CCl_4 intoxicated control (2% gum acacia; 1 ml/kg/day)	40.6±2.21^{*a}	33.00±1.92^{***a}	0.30±0.09^{**a}
III- CCl_4 intoxicated + Silymarin (50 mg/kg/day)	30.01±2.49^{*b}	66.43±10.63^{**b}	0.76±0.08^{*b}
IV- CCl_4 intoxicated + ZVT drop (25 mg/kg/day)	26.06±2.88^{*b}	44.18±4.06	0.47±0.13
V- CCl_4 intoxicated + ZVT drop (50 mg/kg/day)	22.47±2.16^{**b}	92.44±11.2$^{***b, *c}$	0.61±0.05^{*b}
VI- CCl_4 intoxicated + ZVT drop (100 mg/kg/day)	18.97±1.95^{**b}	100.60±0.85$^{***b, *c}$	0.95±0.04^{**b}

n, number of rats in each group; MDA-malondialdehyde; GST-glutathione-S-transferase; CAT-catalase, CCl_4- carbon tetrachloride; ZVT- Zincovit. Values are expressed as mean ± standard error of mean a, b, c - Significant compared to normal control, CCl_4 intoxicated negative control (untreated), CCl_4 intoxicated positive control-treated with Silymarin; level of significance- ***p<0.001, **p<0.01, *p<0.05.