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Research Article

Zincovit syrup ameliorates oxidative stress induced by carbon tetrachloride in rats

S. M. Satyam[1], K. L. Bairy[2]*

[1]Department of Pharmacology, Melaka Manipal Medical College, Manipal University, Manipal, Karnataka, India, [2]Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

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***Correspondence to:**
Dr. K. L. Bairy,
Email: kl.bairy@manipal.edu

ABSTRACT

Background: Zincovit (ZVT) syrup is a combined formulation of vitamins, minerals and lysine. The present study was aimed to investigate the *in vivo* antioxidant potential of ZVT syrup in carbon tetrachloride (CCl_4) intoxicated Wistar rats.
Methods: A total of 36 Wistar rats were divided into six groups of six rats each. Antioxidant potential of ZVT syrup at the dose of 15 mg/kg/day, 30 mg/kg/day and 60 mg/kg/day was evaluated in CCl_4 intoxicated rats. The extent of CCl_4 induced oxidative stress was studied by estimating malondialdehyde (MDA), glutathione-S-transferase (GST) and catalase (CAT).
Results: Oral treatment with ZVT syrup, especially at the dose of 30 mg/kg/day and 60 mg/kg/day reversed CCl_4 - induced alterations in MDA ($p<0.05$), GST ($p<0.01$) and CAT ($p<0.01$) compared to CCl_4 intoxicated control (untreated) animals.
Conclusion: The present findings revealed that ZVT syrup may be useful in oxidative stress associated tissue damage as a nutritional food supplement.

Keywords: Zincovit syrup, Lysine, Zinc, Oxidative stress, Antioxidant, Carbon tetrachloride

INTRODUCTION

Oxidative stress induced by reactive oxygen species (ROS) is caused by increased production of superoxide anion (O_2^{-1}) and its metabolites and/or by reduced bioavailability of antioxidant defenses. This imbalance between pro-oxidants and antioxidants gives rise to cellular oxidative stress, which plays an important role in the pathogenesis of many diseases. One of the studies suggests that antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and thereby prevent damage to lipids, proteins, enzymes, carbohydrates, DNA.[1] Few studies have reported that carbon tetrachloride (CCl_4) can cause generation of ROS in tissues other than liver, such as kidneys, heart, lung, testis, brain and blood.[2,3] One of the studies suggests that treatment with antioxidants such as vitamins C and E can ameliorate the toxic effects of CCl_4 on liver and kidneys.[4]

Zincovit (ZVT) syrup is an advanced formulation of high concentration of vitamins, minerals and lysine. ZVT syrup has a stream of antioxidant benefits. Nowadays, there has been increased interest in the use of antioxidant nutritional supplements. Earlier, we had reported the antioxidant potential of combined formulation of grape seed extract and ZVT tablets.[5,6] Consequently, the aim of the present study was to investigate the antioxidant potential of ZVT syrup against CCl_4 intoxicated Wistar rats.

METHODS

Drugs and reagents

ZVT syrup was procured from Apex Laboratories Private Ltd., Chennai (India). Thiobarbituric acid (TBA) and trichloroacetic acid (TCA), 1-chloro-2,4dinitrobenzene (CDNB), 5, 5'-dithiobis (2-nitrobenzoic acid) and reduced

glutathione (GSH) were obtained from Sigma Chemical Inc. (USA). Catalase (CAT) colorimetric assay kit was purchased from Bioassay Systems, Hayward (USA). CCl_4, potassium chloride, sodium chloride, sodium hydroxide, ethylene-di-amine-tetra-acetic acid and all other chemicals were obtained from Merck Chemicals, Mumbai (India). All reagents were analytical grade. All reagents except for the phosphate buffers were prepared every day and stored in a refrigerator at +4°C. The reagents were equilibrated at room temperature for 30 mins before use, either at the start of analysis or when reagent containers were refilled.

Animals

36 adult male albino Wistar rats weighing 150-200 g were housed in separate polypropylene cages. They were maintained under standard conditions with temperature (22-24°C), 12 hrs light/12 hrs dark cycle and relative air humidity 40-60%. The animals were acclimatized to the laboratory conditions for 1-week before the onset 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/43/2013) and experiments were 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, a total of 36 adult male Wistar rats (150-200 g) were used. The rats were divided into six groups containing six rats in each group. Treatment was done for 7 days as follow:[6]

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

Group II: CCl_4 intoxicated control rats + 2% gum acacia (1 ml/kg/day; p.o) and simultaneously administered CCl_4: olive oil (1:1); (1 ml/kg; i.p. every 72 hrs).

Group III: CCl_4 intoxicated rats + Silymarin (50 mg/kg/day; p.o) and simultaneously administered CCl_4: olive oil (1:1); (1 ml/kg; i.p. every 72 hrs).

Group IV: CCl_4 intoxicated rats + ZVT syrup (15 mg/kg/day; p.o) and simultaneously administered CCl_4: olive oil (1:1); (1 ml/kg; i.p. every 72 hrs).

Group V: CCl_4 intoxicated rats + ZVT syrup (30 mg/kg/day; p.o) and simultaneously administered CCl_4: olive oil (1:1); (1 ml/kg; i.p. every 72 hrs).

Group VI: CCl_4 intoxicated rats + ZVT syrup (60 mg/kg/day; p.o) and simultaneously administered CCl_4: olive oil (1:1); (1 ml/kg; i.p. every 72 hrs).

At the end of 7th day treatment, all the experimental rats were kept overnight fasting and sacrificed by administering an overdose of ketamine, i.p. according to the annexure-6 of euthanasia of laboratory animals in the CPCSEA guidelines for Laboratory Animal Facility. At the end of the treatment, following autopsy livers were excised immediately and washed with ice-cold saline to remove as much blood as possible. Liver homogenates (10% w/v) were 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 mins 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 also read for reagent and sample blank.

Determination of malondialdehyde (MDA) level

To 20 µl liver homogenate sample, 200 µl 0.67% TBA and 100 µl 20% TCA were added and incubated at 100°C for 20 mins. Then, it was centrifuged at 12,000 rpm for 5 mins, and 100 µl of supernatant was transferred to 96-wells of the micro test plate. Optical density of supernatant was read at 540 nm by using an ELISA reader Bio-Tek Instruments ELx800-MS, (USA).[6]

Determination of CAT activity

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

Determination of GSH-S-transferase (GST) activity

850 µl phosphate buffer (0.1 M, pH 6.5), 50 µl CDNB (20 mM) and reduced GSH (GSH, 20 mM) were added together and incubated at 37ºC for 10 mins. Then, 50 µl of liver tissue homogenate sample was added in the above mixture and optical density was read at 340 nm at 1 min interval for 5 mins by using UV-2450 spectrophotometer, Shimadzu Corporation, Tokyo (Japan).[6]

Statistical analysis

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

RESULTS

Effect on biochemical parameters

In the present study, CCl_4 caused significant increase in MDA ($p<0.05$) and decrease in CAT ($p<0.01$) as well as

Table 1: Effect of ZVT syrup on MDA (nmoles/mg), GST (µmoles of CDNB conjugates/minutes/mg) and CAT (units of hydrogen peroxide oxidized/minutes/mg) in liver tissue homogenate.

Groups (n=6)	MDA	GST	CAT
I - Normal control (2% gum acacia; 1 ml/kg/day)	26.78±1.81	98.22±4.12	0.90±0.04
II - CCl_4 intoxicated control (2% gum acacia; 1 ml/kg/day)	42.80±1.12*[a]	35.20±1.82***[a]	0.34±0.02**[a]
III - CCl_4 intoxicated+silymarin (50 mg/kg/day)	25.20±3.25*[b]	67.24±11.23**[b]	0.74±0.02*[b]
IV - CCl_4 intoxicated+ZVT syrup (15 mg/kg/day)	31.81±5.18	55.95±10.91**[b]	0.44±0.01
V - CCl_4 intoxicated+ZVT syrup (30 mg/kg/day)	26.06±2.88*[b]	73.31±6.98**[b]	0.58±0.03*[b]
VI- CCl_4 intoxicated+ZVT syrup (60 mg/kg/day)	20.68±4.56*[b]	47.29±7.18**[b,*c]	1.00±0.02**[b]

n: Number of rats in each group. Values are expressed as mean±standard error of mean. [a,b,c]Significant as 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, CDNB: 1-chloro-2,4dinitrobenzene, MDA: Malondialdehyde, GST: Glutathione-S-transferase, CAT: Catalase, CCl_4: Carbon tetrachloride, ZVT: Zincovit

GST (p<0.001) when compared to normal control rats. Oral treatment with ZVT syrup, especially at the dose of 30 mg/kg/day and 60 mg/kg/day reversed CCl_4-induced alterations in MDA (p<0.05), GST (p<0.01) and CAT (p<0.01) compared to CCl_4 intoxicated control (untreated) animals (Table 1). There was also a significant increase in GST level in CCl_4 intoxicated rats treated with 60 mg/kg of ZVT syrup 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 demonstrated antioxidant potential of ZVT syrup by ameliorating oxidative stress. CCl_4 is activated by cytochrome P-450 2E, 2B1or 2B2 and possibly CYP 3A, to form the trichloromethyl radical CCl_3• and trichloromethyl peroxy radical CCl_3OO•, which leads to lipid peroxidation and subsequent tissue damage.[7] In the present study, silymarin was used as standard drug (positive control) because studies suggest the protective role of silymarin in hepatic injury due oxidative stress.[8,9] MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage.[10] CCl_4 alters the ratio of polyunsaturated to other fatty acids, thus, leading to a decrease in the membrane fluidity, which may be sufficient to cause cell death.[11] It is possible that ZVT syrup has prevented the formation of free radicals by interfering with cytochrome P-450 or might have promoted its glucuronide conjugation. GST catalyses the conjugation of electrophilic xenobiotic substrates with the tripeptide GSH (GSH; γ-glu-cys-gly).[12] In this study, the significant increase in the activity of GST following the administration of ZVT syrup may be due to the presence of elements such as zinc that might have enhanced the synthesis of the enzyme. CAT protect the cells from toxic effects of ROS by converting hydrogen peroxide to water and molecular oxygen.[13] The ability of ZVT syrup to revert the reduced CAT activity buttresses its antioxidant potential. The antioxidant potential of ZVT syrup might be attributed to synergistic interplay of lysine, vitamins C, E, folic acid, biotin and minerals such as zinc, copper, selenium, magnesium, manganese, chromium and molybdenum mainly, which are promoters of antioxidant activity. The synergistic effect of vitamins C and E and zinc might be based on the different environments in which they act. Vitamin C acts in the hydrophilic milieu, scavenging ROS; Zn, located in the interphase of the bilayer, will prevent iron or copper binding to the membrane and alpha-tocopherol and in the hydrophobic domains of the bilayer, will inhibit the lipid oxidation free-radical chain reaction.[14]

CONCLUSIONS

From the present study, it can be concluded that ZVT syrup is the potential functional nutritional food supplement that can ameliorate the oxidative stress induced by CCl_4 in Wistar rats. Since, the therapeutic effect seen in animal studies cannot always be entirely extrapolated to humans, a clinical evaluation should be performed to define precisely the antioxidant role of ZVT syrup in humans. This may be of great value in oxidative stress associated complications of human subjects as a nutritional food supplement.

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Ethical approval: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/43/2013)

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