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Research Article

GRAPE SEED EXTRACT AND ZINC CONTAINING NUTRITIONAL FOOD SUPPLEMENT DECREASES THE OXIDATIVE STRESS INDUCED BY CARBON TETRACHLORIDE IN RATS

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ABSTRACT

Objective: Current study was designed to investigate in vitro erythrocyte membrane stabilizing activity and *in vivo* antioxidant potential of combined formulation of grape seed extract and Zincovit tablets in carbon tetrachloride intoxicated rats. Methods: Erythrocyte membrane stabilizing activity was assessed by hypotonic solution-induced rabbit erythrocyte haemolysis. All the rats intoxicated with carbon tetrachloride were treated with combined formulation of grape seed extract and Zincovit tablets (40mg/kg, 80mg/kg and 160mg/kg; *p.o*) for 7 days except the toxic and positive control group. The extent of carbon tetrachloride induced oxidative stress was studied by enzymatic, non-enzymatic antioxidant parameters and liver histopathology in experimental rats. Results: Combined formulation of grape seed extract and Zincovit tablets showed 97.37% inhibition of hemolysis at the concentration of 1mg/ml. Rats treated with this test drug had significant antioxidant potential in comparison with toxic control group. The hepatic architecture of toxic control rats was found to acquire near-normalcy in Zincovit treated rats. Conclusions: Single combined formulation of grape seed extract and Zincovit tablets may be of great value in oxidative stress associated complications of human subjects as nutritional food supplement.

Keywords: Grape seed extract, Membrane stabilizing, Nutritional food supplement, Oxidative stress, Zincovit tablets.

INTRODUCTION

There is increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular, hepatic, neuro- degenerative diseases, autoimmune disorders, aging etc [1]. Oxidation processes are one of the most important routes for producing free radicals in food, drugs and even living systems [2]. 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 [3]. A potent scavenger of these free radical species may serve as possible preventive intervention for free radical mediated diseases [4].

Carbon tetrachloride (CCl_4) is a potent, lipid-soluble hepatotoxic agent that, when bound to lipid and protein, produces peroxidative degeneration of many tissues [5-7]. Studies have demonstrated that CCl_4 can cause generation of reactive oxygen species (ROS) in tissues other than liver, such as kidneys, heart, lung, testis, brain and blood [8,9]. It is well documented that treatment with antioxidants such as vitamins C and E can ameliorate the toxic effects of CCl_4 on liver and kidneys [10]. Silymarin is well known to reduce the oxidative stress by its ability to prevent lipid peroxidation and replenishing the GSH levels.

Zincovit tablet is an advanced combined formulation of vitamins, minerals and grape seed extract (Table 1). Long-term daily administration of grape seed extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation [11]. The biologically active constituents of grape seed extracts are proanthocyanidins, which represent a variety of polymers of flavan-3-ol, such as catechin and epicatechin and have a strong antioxidative effect in aqueous systems [12]. Increased oxygen free-radical production lowers the intracellular magnesium concentration and in light of such evidence, vitamin E administration might also regulate the intracellular magnesium concentration [13]. A synergistic effect of vitamins C and E along with zinc could be expected based on the different environments in which they act [13]. Vitamin C acts in the hydrophilic milieu, scavenging reactive oxygen species, zinc located in the interphase of the bilayer prevents iron or copper binding to the membrane and alpha-tocopherol in the hydrophobic domains of the bilayer inhibits the lipid oxidation free-radical chain reaction [13]. Magnesium inhibits Malondialdehyde (MDA) formation in endothelial cells and low Magnesium oxide induced lipid peroxidation [13].

Table 1: Composition of Zincovit tablet

Ingredients	Per tablet contains
Vitamin C	75 mg
Vitamin B_3	50 mg
Vitamin E	15 mg
Vitamin B_1	10 mg
Vitamin B_2	10 mg
Vitamin B_5	10 mg
Vitamin B_6	2 mg
Folic acid	1 mg
Vitamin A	5000 IU
Vitamin D_3	400 IU
Biotin	150 mcg
Vitamin B_{12}	7.5 mcg
Zinc	22 mg
Magnesium	18 mg
Silica	1 mg
Manganese	0.9 mg
Copper	0.5 mg
Iodine	150 mcg
Boron	150 mcg
Selenium	50 mcg
Chromium	25 mcg
Molybdenum	25 mcg
Grape Seed Extract	50 mg

In previous studies, we found the strong *in vitro* antioxidant and antidiabetic potential of the combined formulation of grape seed extract and Zincovit tablets [1,14]. Therefore, the present study was undertaken to investigate effects of combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) on enzymatic, non-enzymatic antioxidant biochemical parameters and liver architecture against carbon tetrachloride induced oxidative stress in Wistar rats.

MATERIALS AND METHODS

Drugs and Reagents

Single combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) was obtained as kind gift from Apex Laboratories, Chennai (India). Thiobarbituric acid (TBA) and trichloroacetic acid (TCA), 1-Chloro-2,4dinitrobenzene (CDNB), 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) and reduced glutathione (GSH) were procured from Sigma Chemical Inc. (USA). Catalase 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). 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 minutes before use, either at the start of analysis or when reagent containers were refilled. Phosphate buffers were stable at +4˚C for one month.

Animals

Male Wistar albino rats weighing 150-300 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%. Rats had continuous access to normal calorie standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and to tap water. After randomization into various groups, the rats were acclimatized to the laboratory conditions for one week before the start of the experiment. Animals described as fasted were deprived of food for 16-h but had allowed free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/87/2012) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

In vitro erythrocyte membrane stabilizing activity

Blood was obtained from a healthy untreated rabbit through marginal ear vein using heparinized vacutainer The blood was washed three times with isotonic buffered solution (154mM NaCl) in 10mM sodium phosphate buffer (pH 7.4). The blood was centrifuged each time for 10 minutes at 3000 rpm.

Membrane stabilizing activity of the single combined formulation of grape seed extract and Zincovit tablets was assessed using hypotonic solution-induced erythrocyte haemolysis. The test sample consisted of 37.5 µl stock erythrocyte suspension mixed with 375 µl of hypotonic solution (50mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) containing 510 µl of combined formulation of grape seed extract and Zincovit tablets (0.25, 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 mg/ml) and 510 µl of Indomethacin as reference drug (0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 mg/ml). The control sample consisted of 37.5 µl of erythrocyte mixed with either 375 µl of hypotonic solution (50mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) solution or 375 µl of methanol alone. The mixture was incubated for 10 minutes at room temperature and centrifuged for 10 min at 3000 rpm and 200 µl of supernatant was transferred separately in 96 wells of micro test plate. Then, absorbance of supernatant was read at 540 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA). The experiment was carried out in triplicate manner. The percentage inhibition of haemolysis was calculated according to following formula-

% inhibition of haemolysis= (OD_1-OD_2 / OD_1) X100 OD_1= Optical density of hypotonic-buffered saline solution OD_2= Optical density of test sample in hyotonic solution

***In vivo* experimental design**

In the experiment, 48 adult male Wistar rats (150-300 g) were used. The rats were divided into 6 groups containing 8 rats in each group. Silymarin was considered as reference drug. Treatment was done for seven days as follows- Group I: Normal control rats were given 2% gum acacia (1ml/kg/day; p.o). Group II: CCl_4 intoxicated control rats + 2% gum acacia (1ml/kg/day; p.o) and simultaneously administered CCl_4: olive oil (1:1); (1ml/kg; i.p. every 72 h).

Group III: CCl4 intoxicated rats + Silymarin (50mg/kg/day; p.o) and simultaneously administered CCl4: olive oil (1:1); (1ml/kg; i.p. every 72 h). Group IV: CCl4 intoxicated rats + Zincovit with grape seed extract (40mg/kg/day; p.o) and simultaneously administered CCl4: olive oil (1:1); (1ml/kg; i.p. every 72 h). Group V: CCl4 intoxicated rats + Zincovit tablets with grape seed extract (80mg/kg/day; p.o) and simultaneously administered CCl4: olive oil (1:1); (1ml/kg; i.p. every 72 h). Group VI: CCl4 intoxicated rats + Zincovit tablets with grape seed extract (160mg/kg/day; p.o.) and simultaneously administered CCl4: olive oil (1:1); (1ml/kg; i.p. every 72 h). After seven days of treatment, the rats were kept overnight fasting and sacrificed by administering overdose of ketamine, i.p. according to the annexure-6 of euthanasia of laboratory animals in the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility. At the end of the treatment, 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 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 also read for reagent and sample blank:

Determination of Malondialdehyde (MDA) level

To 20 µl liver homogenate sample, 200 µl 0.67% thiobarbituric acid and 100 µl 20% trichloroacetic acid were added and 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).

Determination of Superoxide dismutase (SOD) activity

To 25 µl of liver tissue homogenate sample, 925 µl sodium carbonate buffer (0.1M, pH 10-11) and 50 µl of adrenaline bitartarate (1mM) was added and absorbance (A_{0s}-A_{60s},) was read at 480 nm by using UV-2450 spectrophotometer, Shimadzu Corporation, Tokyo (Japan).

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 Reduced glutathione (GSH) level

Mixture of 100 µl of liver tissue homogenate and 100 µl of 5% trichloro acetic acid (TCA) solution was centrifuged at 5000 rpm for 5 minutes. Then, 25 µl of tissue supernatant, 150 µl sodium phosphate buffer (PBS 0.2 M, pH 8.0) and 25 µl DTNB (0.6mM) was added together in 96-wells of micro test plate and incubated for 10 minutes at room temperature and absorbance was read at 412 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Glutathione-S-transferase (GST) activity

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

Determination of Protein thiol (PT) level

20 µl of liver tissue homogenate sample was added in the mixture of 180 µl disodium edetate (2mM disodium edetate in 0.2 M disodium hydrogen phosphate) buffer solution and 4 µl DTNB solution (10mM DTNB in 0.2 M disodium hydrogen phosphate) in 96-wells of micro test plate. Then, optical density was read at 412 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Histopathological examination

The liver tissue samples taken from the 1-2 rats of each group were fixed in 10% phosphate buffered formalin. A small part of liver was cut and dehydrated in ascending grades of alcohol, defatted in xylene, and embedded in paraffin. 24 hours after block preparation, 6 micron thick paraffin sections were obtained using microtome and mounted on albumenized glass slides followed by their respective labeling. Tissues were then de-waxed in xylene for 10 minutes and further hydrated through descending grades of alcohol to water. The

sections were stained with hematoxylin and eosin (H&E). At the end 2-3 drops of DPX mountant was put on the glass slides and the cover slips were placed gently to avoid drying of tissue and then observed for any morphological changes under a light microscope (Magnus, Olympus Pvt. Ltd., New Delhi (India) 40 X. Later the microscopic slides of the liver cells were photographed.

Statistical analysis

Using Statistical Package for the Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, USA), normally distributed data were expressed as mean ± standard error of mean and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. Data with non-uniform distribution were expressed as median, Quartile (Q_1, Q_3) and analyzed by non-parametric K Independent samples test followed by Kruskal-Wallis H test. A level for $P \leq 0{\cdot}05$ was considered to be statistically significant (two-sided).

RESULTS

Effect on erythrocyte membrane stabilizing activity

Combined formulation of grape seed extract and Zincovit tablets (1 mg/ml) showed 97.37% inhibition of hemolysis where as reference drug Indomethacin showed 89.45% inhibition of hemolysis at the concentration of 0.4 mg/ml.

Effect on biochemical antioxidant parameters

Treatment with combination of grape seed extract and Zincovit tablets (Nutritional food supplement) reversed CCl_4-induced alterations in protein thiol (p=0.002), reduced form glutathione levels (p=0.021). Further there was significant increase in the activity of catalase (p<0.001), superoxide dismutase (p=0.031) and glutathione-s-transferase (p<0.001) enzyme activity in the Zincovit with grape seed extract treatment group when compared with the toxic control group (Table 2 and 3). There was a significant increase in the liver tissue malondialdehyde levels in CCl_4 treated control group when compared with the normal control group (p<0.001), which was prevented with treatment of combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) in a dose dependent manner (p<0.001). There was a significant decrease in malondialdehyde level in CCl_4 intoxicated rats treated with 80 mg/kg (p=0.016), 160 mg/kg (p<0.001) of combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) in comparison with Silymarin (50 mg/kg) treated rats (Figure 1).

Liver histopathology

The qualitative histopathological study revealed fatty degeneration, necrosis, fibrosis, homogeneous cytoplasm of hepatocytes with absence of few nuclei in CCl_4 intoxicated control rat whereas such observations were not found in normal control and combination of grape seed extract and Zincovit tablets treated rats (40 mg/kg, 80 mg/kg, 160 mg/kg). Combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) at the dose of 160 mg/kg showed very uniform nuclei and hepatocyte distribution as compared to normal control, toxic control and Silymarin treated rats (Figure 2A-2F).

Table 2: Effect of combined formulation of grape seed extract and Zincovit tablets on catalase (units of hydrogen peroxide oxidized /min/mg), superoxide dismutase (units of adrenaline bitartarate protected from auto oxidation/min/mg) and protein thiol (µmoles/mg) in liver tissue homogenate

Groups (n=8)	CAT (Q_1, Q_3)	SOD (Q_1, Q_3)	PT (Q_1, Q_3)
I- Normal control (2% gum acacia)	0.24 (0.18, 0.39)	3.45 (1.93, 6.81)	7.95 (2.89, 22.05)
II- Toxic control (2% gum acacia)	0.18 (0.12, 0.34)	1.26 (0.46, 3.18)^{*a}	7.34 (5.76, 12.71)
III- Toxic + Silymarin (50 mg/kg/day)	0.19 (0.13, 0.25)	1.71 (0.84, 7.88)	11.77 (3.90,31.20)^{*b}
IV- Toxic + ZVT (40 mg/kg/day)	0.16 (0.14, 0.21)	2.07 (1.26, 3.18)	2.81 (2.11, 6.05)^{*b}
V- Toxic + ZVT (80 mg/kg/day)	0.32 (0.29, 0.44)^{**b}	5.95 (2.66, 10.28)^{**b}	4.34 (3.53,25.39)^{*b}
VI- Toxic + ZVT (160 mg/kg/day)	0.50 (0.47, 0.52)^{***b}	3.07 (2.33, 5.70)^{*b}	40.87 (2.03, 47.16)^{***b}

n- Number of rats in each group. CAT- Catalase, SOD- Superoxide dismutase and PT- Protein thiol. Data are expressed as the median (quartiles- Q_1, Q_3) and different treatments were analyzed by non-parametric test K Independent sample test followed by Kruskal-Wallis H test. ZVT- Zincovit tablets with grape seed extract. [a] when compared with normal control rats, [b] when compared with toxic control rats. $^{*}p < 0.05$ $^{**}p < 0.01$and $^{***}p < 0.001$

Table 3: Effect of combined formulation of grape seed extract and Zincovit tablets on reduced glutathione (µmoles/mg) and glutathione-s-transferase (µmoles of CDNB conjugates/min/mg) in liver tissue homogenate

Groups (n=8)	GSH (Q_1, Q_3)	GST (Q_1, Q_3)
I- Normal control (2% gum acacia)	0.14 (0.10, 0.25)	5.05 (1.36, 8.20)
II- Toxic control (2% gum acacia)	0.13 (0.12, 0.20)	0.64 (0.16, 1.89)^{***a}
III- Toxic + Silymarin (50 mg/kg/day)	0.20 (0.15, 4.53)	7.24 (4.18, 10.58)^{***b}
IV- Toxic + ZVT (40 mg/kg/day)	5.13 (0.16, 8.22)^{***b}	5.58 (4.10, 7.28)^{***b}
V- Toxic + ZVT (80 mg/kg/day)	0.20 (0.13, 0.27)	23.33 (18.45, 31.35)^{***b}
VI- Toxic + ZVT (160 mg/kg/day)	0.26 (0.22, 0.37)	12.44 (9.91, 26.18)^{***b}

n- Number of rats in each group. GSH- Reduced glutathione, GST- Glutathione-S-transferase. Data are expressed as the median (quartiles- Q_1, Q_3) and different treatments were analyzed by non-parametric test K Independent sample test followed by Kruskal-Wallis H test. ZVT- Zincovit tablets with grape seed extract. [a] when compared with normal control rats, [b] when compared with toxic control rats. $^{*}p < 0.05$, $^{**}p < 0.01$and $^{***}p < 0.001$

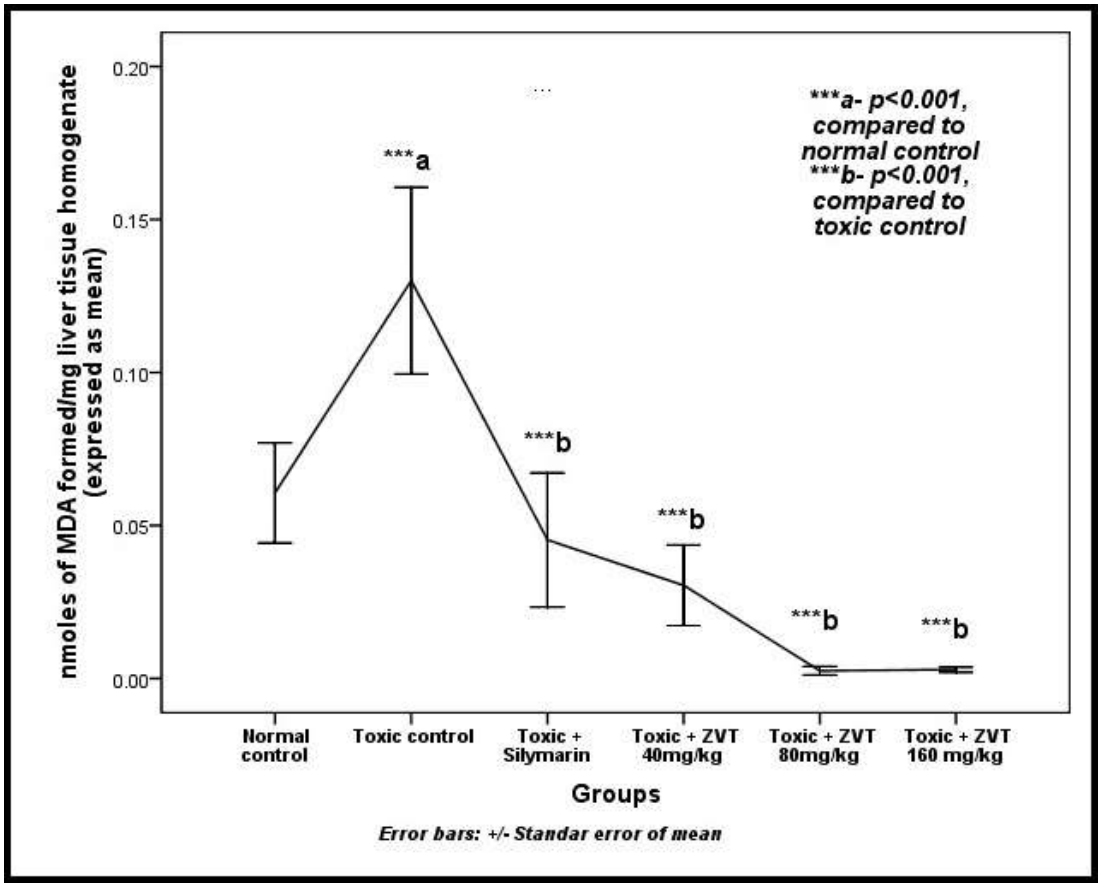

Fig. 1: Effect of combined formulation of grape seed extract and Zincovit tablets on malondialdehyde level

n= 8; number of rats in each group. MDA- Malondialdehyde. Data are expressed as mean ± standard error of mean and different treatments were analyzed by one way analysis of variance (ANOVA) followed by post-hoc Tukey test. ZVT- Zincovit tablets with grape seed extract.

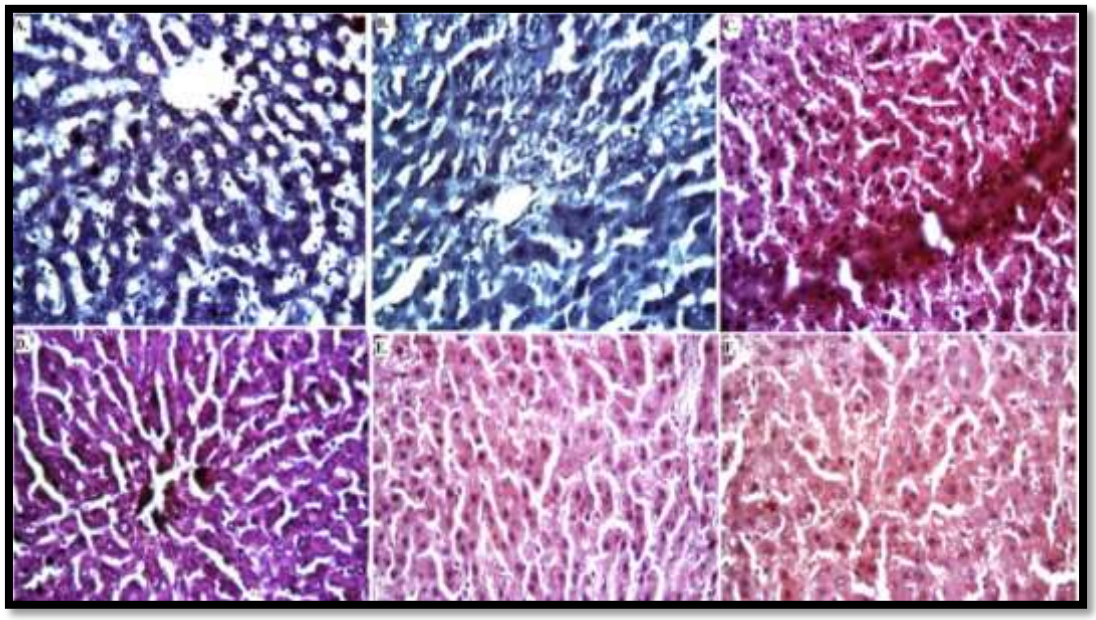

Fig. 2: Microphotographs of hematoxylin and eosin stained sections of liver seen under 400X

(A) Normal control (2% gum acacia; 1ml/kg)
(B) Toxic control (CCl_4 + 2% gum acacia; 1ml/kg)
(C) Positive control (CCl_4 + Silymarin; 50 mg/kg)
(D) CCl_4 + Zincovit tablets with grape seed extract; 40 mg/kg
(E) CCl_4 + Zincovit tablets with grape seed extract; 80 mg/kg
(F) CCl_4 + Zincovit tablets with grape seed extract; 160 mg/kg

DISCUSSION

The results of present study reveal antioxidant potential of combined formulation of grape seed extract and Zincovit tablets by ameliorating oxidative stress. The erythrocyte membrane protective activity in terms of percentage inhibition of hemolysis by the Zincovit tablets with grape seed extract may be mainly attributed to the membrane stabilizing property of procyanidins [subunits constituted of (+)-catechin (C) and (−)-epicatechin (EC)], present in the grape seed extract.

CCl_4 has been extensively used in experimental models to elucidate the cellular mechanisms behind oxidative damage [15]. CCl_4 is activated by cytochrome P-450 2E, 2B1or 2B2 and possibly CYP 3A, to form the trichloromethyl radical $CCl_3^{\bullet}$ and trichloromethyl peroxy radical $CCl_3OO^{\bullet}$, which leads to lipid peroxidation and subsequent tissue damage [16]. Enhanced lipid peroxidation associated with depletion of antioxidants in the tissues leads to change in the structures of endoplasmic reticulum and other membrane, loss of metabolic enzymes activation, reduction of protein synthesis and elevation of serum transaminase, total bilirubin and conjugated bilirubin leading to liver damage [17].

The significant increased level of non-enzymatic antioxidant that is protein thiol in Zincovit 160 mg/kg treatment group as compared to normal control and toxic control might be due to presence of higher amount of nutritional supplement at this dose.

GSH is necessary to maintain the normal reduced state of cells so as to counteract the inhibitory effects of ROS induced oxidative stress [18]. It is a potential scavenger of O_2^- (superoxide), H_2O_2 (hydrogen peroxide) [19,20] and most dangerous reactive oxygen species (ROS) like $OH^{\bullet}$ [21]. Additionally, GSH plays a key role in the antioxidative defense system by regenerating another potential water soluble antioxidant like ascorbate (ASH), via the ASH-GSH cycle [22]. It has been reported that when the intensity of a stress increases, GSH concentrations usually decline and redox state becomes more oxidized, leading to deterioration of the system [23]. There was decrease in reduced form glutathione (GSH) level in the toxic control group whereas Zincovit tablets with grape seed extract treated group at the dose of 40mg/kg restored the level of reduced glutathione but further decrease in the GSH level in higher dose (80mg/kg and 160mg/kg) treatment group of Zincovit tablets with grape seed extract might be due to presence of copper as one of the constituent of Zincovit tablets which may resulted into its more binding to membrane and finally decrease in GSH level.

Superoxide dismutase (SOD) is one of the chief cellular defense enzymes that dismutate superoxide radical to H_2O_2 and oxygen [24]. The reduction in the activities of SOD observed in this study following the administration of CCl_4 alone, suggests oxidative stress in the toxic control group with respect to normal control rats. The increase in SOD activities observed in co-administered Zincovit tablets with grape seed extract may be attributed to the presence of zinc which is required not only for the proper functioning of the enzyme but for its synthesis.

Catalases which are heme-containing proteins protect the cells from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen [24]. The reduction in the activity of catalase by CCl_4 administration is an indication of damage to the hepatic cells [24]. The ability of the combined formulation of grape seed extract and Zincovit tablets to revert the reduced catalase activity further buttresses its antioxidant potential.

Glutathione-S-transferases are a large and diverse group of enzymes which catalyze the conjugation of electrophilic xenobiotics substrates with the tripeptide glutathione (GSH; γ-glu−cys−gly) [25]. In fact, GSTs can reduce peroxides with the help of GSH and produce scavengers of cytotoxic and genotoxic compounds [25]. In this study, the significant increase in the activity of glutathione-s-transferase following the administration of the combined formulation of grape seed extract and Zincovit tablets may be adduced to the presence of elements such as zinc that might have enhanced the synthesis of the enzyme.

Malondialdehyde, a secondary product of lipid peroxidation, is used as an indicator of tissue damage [26]. Sipes et al. [27] reported that the trichloromethyl radical could abstract hydrogen atom from a fatty acid to form a lipid radical. These radicals may then react with oxygen to initiate lipid peroxidation. Elevated levels of MDA following CCl_4 administration have been well documented in various organs such as liver [28,29], liver and kidney [30,31], and liver, kidney and heart [32]. It is possible that the combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) has prevented the formation of free radicals by interfering with cytochrome P-450 or might have promoted its glucuronide conjugation. The ability of the combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) to ameliorate the increased levels of MDA might be attributed to synergistic interplay of chemical composition of Zincovit tablets, such as- grape seed extract proanthocyanidins which comprise only procyanidins [subunits constituted of (+)-catechin (C) and (−)-epicatechin (EC)], Vitamins C, E, folic acid, biotin and minerals like zinc, copper, selenium, magnesium, manganese, chromium and molybdenum mainly, which are promoters of antioxidant activity.

The Histopathological appearance of toxic control rat liver treated with CCl_4 alone showed homogenous hepatic cytoplasm with absence of few nuclei, necrosis, microvesicular fatty changes and ballooning degeneration. This appearance indicated poor protection of the hepatocyte against the hepatotoxic agent. Liver tissue of CCl_4 intoxicated rats treated with combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) displayed a good progress with the disappearance of fatty changes and necrosis. This appearance of hepatocyte indicated the effectiveness of combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) to express the significant protective effect of the liver cells against the oxidative stress mediated damage produced by CCl_4, which confirmed the results of biochemical studies.

From the present study it can be concluded that the combined formulation of grape seed extract and Zincovit tablet is the potential functional nutritional food supplement that can ameliorate the oxidative stress induced by carbon tetrachloride in Wistar rats. Since, the therapeutic effect seen in animal studies cannot always be entirely extrapolated to humans. Hence, clinical evaluation should be performed to precisely define the antioxidant role of single combined formulation of grape seed extract and Zincovit tablets in humans. This may be of great value in oxidative stress associated complications of human subjects as nutritional food supplement.

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