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ORIGINAL ARTICLE

Chemoprotective Potential of Zingerone (Vanillyl Acetone) in Cyclophosphamide-induced Hepatic Toxicity

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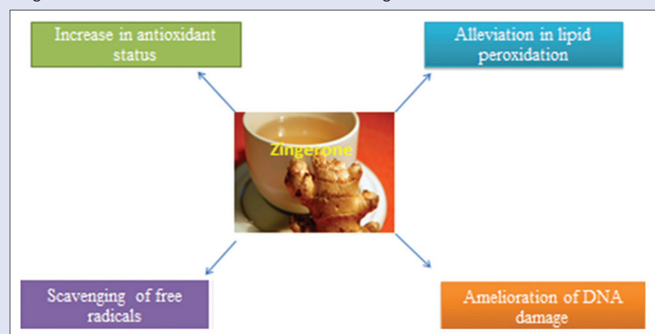
ABSTRACT

Introduction: Cancer is one of the lethal diseases in the global world. Proliferation of cancer cells is commonly inhibited by chemotherapeutics. Cyclophosphamide (CP) is an alkylating chemotherapeutic agent often used for treatment of various types of cancers, but it is full of side effects which in turn lead to organ toxicity. Zingerone, a polyphenolic alkanone found in ginger, has strong antioxidant potential and causes extensive scavenging of free radicals and offers defense against oxidative stress. Twenty-four adult male Wistar rats were divided into four groups, six rats in each group. **Materials and Methods:** Group I (control), Group II (CP, 2 mg/kg bwt), Group III (cotreatment with zingerone at the dose of 50 mg/kg bwt and CP at the dose of 2 mg/kg bwt), and Group IV (pretreatment of zingerone at the dose of 50 mg/kg bwt for 7 days and CP at the dose of 2 mg/kg bwt for next 7 days). **Results:** CP significantly increased the level of hepatic marker enzymes such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, drastically caused alteration in lipid profile and deficiency in antioxidant defense mechanism by decreasing the activities of antioxidant enzymes such as catalase, glutathione, glutathione-S-transferase, and glutathione peroxidase. This was accompanied by subsequent increase in lipid peroxidation, nitrite production, and marked DNA damage. **Conclusion:** The restoration of hepatic markers, amelioration of lipid profile, and improvement of antioxidant status and DNA damage by pre- and co-treatment with zingerone clearly indicate the ameliorative potential of zingerone against CP-induced organ toxicity and oxidative stress. The protective potential of zingerone may be attributed to its strong antioxidant activity.

Key words: Cyclophosphamide, oxidative stress and hepatic injury, zingerone

SUMMARY

- Zingerone ameliorated the oxidative stress induced by cyclophosphamide
- Zingerone improved the hepatic functions deteriorated by cyclophosphamide
- Zingerone treatment reduced the lipid peroxidation
- Zingerone treatment improved the lipid profile
- Zingerone treatment reversed the DNA damage.



Abbreviations used: CP: Cyclophosphamide; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; CAT: Catalase; GSH: Reduced glutathione; ROS: Reactive oxygen species; SOD: Superoxide dismutase; LPO: lipid peroxidation; MDA: Malonaldehyde; GPX: Glutathione peroxidase.

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INTRODUCTION

Cyclophosphamide (CP) is a widely used synthetic anticancer drug in chemotherapy.[1] Chemically, it is an alkylating agent that belongs to nitrogen mustard.[2] In the world of cancer, it has been found quite effective against broad spectrum of malignancies such as leukemia, ovarian cancer, prostate cancer, and lung and breast cancer.[3,4] CP is metabolized in the liver and gets transformed from inactive to active state. Oxidative stress generated in cancer cells by free radicals, reactive oxygen species (ROS), and lipid peroxidation (LPO) is the main mechanism associated with many of the anticancer drugs.[5] The ROS-mediated oxidative stress not only causes the physiological and biochemical alteration but also leads to drastic damage to DNA of cells.[6,7] The biotransformation of CP generates ROS not only in cancer cells but also in normal healthy cells. The injury of healthy normal tissues is the major limitation associated with CP which in turn leads to severe side effects in patients. CP has been reported to produce severe oxidative stress and genotoxicity in experimental mice.[8] The redox imbalance resulted in normal tissues due to CP exposure leads to various physiological and biochemical alterations, which in turn results disorders such as hemorrhagic cystitis, androgenic disorders, and liver and kidney disorders.[9] CP reacts with DNA and leads to the defective DNA and alteration in normal cellular functions and ultimately cell

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death.[10] The CP toxicity leads to search for a novel agent that could reduce the organ toxicity associated with CP.[11] Numerous studies have suggested that supplementation of antioxidants can influence the response to chemotherapy and side effects development resulted from anticancer drugs.[12] Among antioxidants, natural antioxidants are gaining tremendous attention. Zingerone is a pure natural compound present mostly in dry ginger. It has tremendous antioxidant potential more than ascorbic acid.[13] Zingerone is a polyphenolic alkanone commonly known as vanillyl acetone. This phytocompound has strong pharmacological properties such as anti-inflammatory,[14] anticancer,[15] and antimicrobial properties.[16] Zingerone is a also a potent include hepatoprotective,[17] Immuno-stimulating effect and lipolytic effect in on fat storage in ovariectomised rats.[18] Cancer chemoprevention is the use of different agents natural, synthetic, and semisynthetic compounds which prevent or inhibit the proliferation of cancer cells. Ginger has rich phytochemical constituents that have potent potential to inhibit the progression of cancer cells. Zingerone is one of the the main compounds of ginger phytochemistry and has been reported to inhibit colon carcinogenesis induced by 1,2-dimethylhydrazine by its strong antioxidant nature.[15] Besides zingerone, other phytoconstituents of ginger such as 6-gingerol and 6-shogaol also have significant ability against cancer cell progression by arresting cell cycle and promoting apoptosis of cancer cells.[19,20] Recently, 6-shogaol present in low amount in ginger has been reported as a strong anticancer agent of future.[21] Zingerone has been reported to prevent aging by downregulation of age-related-specific pro-inflammatory cytokines.[14] It also has been reported to provide protection to human lymphocytes against radiation-induced damage[22] and prevented mutation in *Escherichia coli* induced by ultraviolet rays.[23] To the best of our knowledge, no many clinical studies studied the protective effect of zingerone against various anticancer drugs Keeping this into consideration the present study was designed to study the ameliorative potential of zingerone against the oxidative insults induced by CP. This study was therefore designed to evaluate the protective effect of zingerone against CP-induced toxicity in Wistar rats.

MATERIALS AND METHODS

Zingerone was purchased from Sigma-Aldrich; sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium potassium tartrate, and sodium hydroxide were purchased from E. Merck Limited. All other chemicals and reagents were of analytical and highest purity grade commercially available.

Animals

For the current study, 4–6 week-old male albino Wistar rats (130–180 g) were obtained from the Central Animal House of University. The animals were housed in the polypropylene cages in groups of four rats per cage in animal house of faculty of veterinary science, Sher-e-Kashmir University of Agricultural Science and Technology-Kashmir (SKUAST-K), and were kept in controlled environment conditions under the standard conditions of temperature and humidity with an alternating 12 h light and dark cycle. The animals were fed *ad libitum* food and water.

The animals were maintained and managed in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals, and the study was approved by the Animal Ethics Committee of Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K, Jammu and Kashmir.

Experimental design

After the acclimatization period of 14 days, animals were divided into four groups, six rats in each group. All the rats were kept on fast for 24 h before experiment. The first group was kept as control received normal saline. The second group animals were treated with CP only at the dose of 2 mg/kg bwt orally dissolved in for 14 days. The third group was given zingerone at the dose of 50 mg/kg bwt and cotreatment of CP at the dose of 2 mg/kg bwt orally for 14 days. The Fourth group was pretreated with zingerone at the dose of 50 mg/kg bwt by oral gavage for 7 days after which CP commences for another 7 days. The dose for CP was given based on recommended adult dose for leukemia treatment while the dose for zingerone was decided from information available in literature. All the animals were euthanized with 24 h after the last treatment.

Biochemical parameters

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin were estimated using Accurex Diagnostic Skits. Analyses were performed according to the manufacturer's instruction.

Postmitochondrial supernatant preparation and estimation of different parameters

Livers were removed readily, cleaned free of debris and irrelevant material, and immediately perfused with ice cold saline (0.85% NaCl). The livers were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) using a Potter-Elvehjem homogenizer.

Lipid peroxidation level

The assay for membrane LPO was done by the method of Wright *et al.*[24] with some modifications. The reaction mixture in a total volume of 3.0 ml contained 1.0 ml tissue homogenate, 1.0 ml of trichloroacetic acid (10%), and 1.0 ml thiobarbituric acid (0.67%). All the test tubes were placed in a boiling water bath for 45 min. The tubes were then shifted to an ice bath and centrifuged at 2500 g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm. The results were expressed as the nmol MDA formed/g tissue using a molar extinction coefficient of $1{\cdot}56 \times 10^5$/M/cm.

Nitrite estimation

Nitrite (NO) assay was done using Griess reagent by the method of Green *et al.*, 1982.[25] In brief, 100 µl of Griess reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in water) was added to 100 µl of PMS incubate for 5–10 min at room temperature protected from light. Purple/magenta color began to form immediately. Absorbance was measured at 546 nm, NO concentration was calculated using a standard curve for sodium NO, and NO levels were expressed as l mol/mg protein.

Antioxidant enzyme assay

- Measurement of SOD activity: The SOD activity was measured by the method of Marklund and Marklund, 1974[26]
- Glutathione reductase (GR) activity: The GR activity was determined by the method of Carlberg and Mannervik, 1975[27]
- Assay for catalase (CAT) activity: The CAT activity was assessed by the method of Claiborne, 1985[28]
- Assay for glutathione peroxidase (GPX) activity: The activity of GPX was calculated by the method of Mohandas *et al.*, 1984[29]
- Detection of DNA fragmentation: DNA fragmentation was carried out in liver tissue by diphenylamine assay according to Donya *et al.*, 2012.[30]

Statistical analysis

The data from individual groups are presented as the mean ± standard error of the mean. Difference between groups were analyzed using

analysis of variance followed by Tukey–Kramer multiple comparison test and minimum criteria for statistical significance was set at $P \leq 0.05$ for all comparisons.

RESULTS

Effect of pretreatment of zingerone on aspartate aminotransferase alanine aminotransferase and alkaline phosphatase levels

Administration of CP alone caused significant increase in the plasma activities of AST, ALT, and ALP. However, pretreatment and cotreatment with zingerone attenuated the CP induced increase in the activities of AST, ALT, and ALP relative to CP-treated rats [Table 1].

Effect of zingerone on the activities of catalase in liver

CAT activities in CP alone-treated group showed a significant decrease in activities of CAT as compared to normal group. However, pretreatment and cotreatment with zingerone significantly ($P < 0.01$) ameliorated against the decrease in CAT activities when compared to CP-treated animals [Table 2].

Table 1: Effect of treatment of zingerone on alanine transaminase, aspartate aminotransferase, and alkaline phosphatase in cyclophosphamide-induced toxicity

Treatment regimen per group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group I	25.29±0.80	132.4283±9.41	254.15±5.23
Group II	63.13±1.28***	240.9817±12.91***	676.43±12.67***
Group III	41.53±1.63##	180.6767±5.15#	486.56±8.43#
Group IV	32.06±1.01###	162.6567±8.53##	353.27±9.25##

Results represent mean±SE of six animals per group. ***$P<0.001$, toxic versus normal; #$P<0.05$, toxic versus zingerone-treated group; ##$P<0.01$, toxic versus zingerone-treated group; ###$P<0.001$, toxic versus zingerone-treated group. ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; SE: Standard error

Effect of zingerone on glutathione reduced activity in liver

The glutathione (GSH) levels of animals treated with CP alone activities showed a significant ($P < 0.001$) decrease as compared to control group. However, pretreatment and cotreatment with zingerone significantly ($P < 0.01$) increased the activities of GSH [Table 2].

Effect of zingerone on glutathione-S-transferase activity in liver

The activities of glutathione-S-transferase (GST) in CP alone-treated animals were significantly reduced as compared to control group. However, pretreatment and cotreatment with zingerone significantly ($P < 0.01$) increased the activities of GST relative to CP alone-treated group [Table 2].

Effect of zingerone on glutathione peroxidase activity in liver

CP alone-treated group showed a significant decrease in GPX activity as compared to control group. However, pretreatment and cotreatment with zingerone significantly ($P < 0.01$) increased the activities of GST relative to CP alone-treated group [Table 2].

Effect of zingerone on lipid profile

CP induced significant increase in levels of total cholesterol (TC), triglycerides (TGs), and low-density lipoprotein (LDL) but decrease in high-density lipoprotein (HDL) in CP alone-treated group as compared to normal control group. In pre- and co-treated groups, zingerone markedly improved the altered levels of TC, TG, LDL, and HDL. Pretreatment with zingerone exhibited more significant amelioration in lipid profile than cotreatment [Table 3].

Effect of zingerone on nitrite production

Administration of CP resulted elevation of hepatic NO production in CP alone-treated animals ($P < 0.001$) as compared with control. We observed that pretreatment and cotreatment with zingerone lead to significant ($P < 0.01$, $P < 0.001$) decreasing in NO production

Table 2: Effect of treatment of zingerone on antioxidant enzymes such as catalase, glutathione reduced, glutathione-S-transferase, and glutathione peroxidase in cyclophosphamide-induced toxicity

Treatment groups	Catalase (nmol H_2O_2 consumed/min/mg protein)	GSH (nmol GSH/g tissue)	GST (nmol CDNB conjugate formed/min/mg protein)	GPX (nmol NADPH oxidized/min/mg protein)
Group I	224.31±5.02	13.37±0.37	807.49±10.43	233.47±6.69
Group II	90.40±1.21***	8.27±0.37**	438.41±16.36***	138.97±13.03**
Group III	162.05±2.48#	10.30±0.22#	542.58±11.57#	174.31±3.34#
Group IV	204.90±4.34###	11.63±0.21##	672.80±14.02##	201.12±3.42##

Results represent mean±SE of six animals per group. ***$P<0.001$, toxic versus normal; #$P<0.05$, toxic versus zingerone-treated group; ##$P<0.01$, toxic versus zingerone-treated group; ###$P<0.001$, toxic versus zingerone-treated group. GSH: Glutathione reduced; GST: Glutathione-S-transferase; GPX: Glutathione peroxidase; SE: Standard error; NADPH: Nicotinamide adenine dinucleotide phosphate; CDNB: 1-chloro-2,4-dinitrobenzene

Table 3: Effect of treatment of zingerone on lipid profile in cyclophosphamide-induced toxicity

Treatment regimen	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)
Group I	87.65±5.32	31.00±3.4	52.14±1.85	86.26±6.73
Group II	215.6±14.6***	13.56±0.79***	93.22±5.87***	175.3±12.5***
Group III	177.1±6.69#	25.81±1.21 (NS)	92.44±7.75 (NS)	134.5±7.61 (NS)
Group IV	161.4±11.5##	22.12±2.10#	64.36±3.12##	117.41±14.0#

Results represent mean±SE of six animals per group. ***$P<0.001$, toxic versus normal; #$P<0.05$, toxic versus zingerone-treated group; ##$P<0.01$, toxic versus zingerone-treated group; ###$P<0.001$, toxic versus zingerone-treated group. NS: Not significant; SE: Standard error; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides

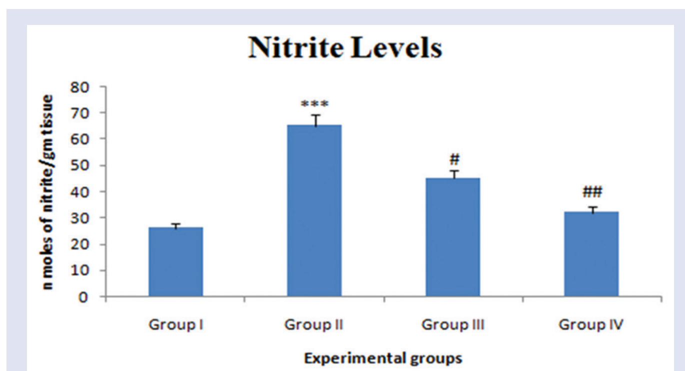

Figure 1: Effect of zingerone pretreatment and cotreatment on nitrite levels in cyclophosphamide-induced toxicity. Values are expressed as mean ± standard error of the mean (n = 6) results represent mean ± standard error of six animals per group. ***P < 0.001, toxic versus normal, #P < 0.05, toxic versus zingerone-treated group, ##P < 0.01, toxic versus zingerone-treated group

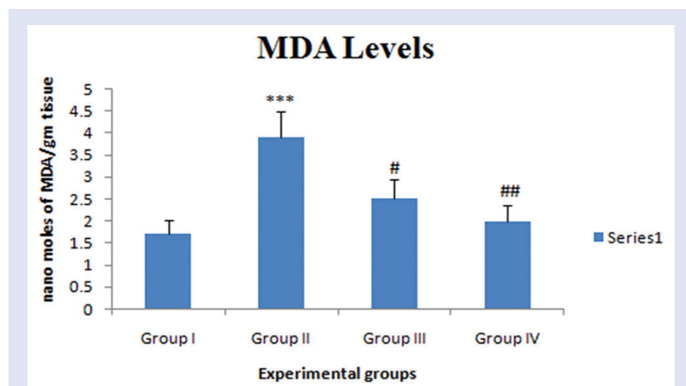

Figure 2: Effect of zingerone pretreatment and cotreatment on malondialdehyde levels in cyclophosphamide-induced toxicity. Values are expressed as mean ± standard error of the mean (n = 6). Results represent mean ± standard error of six animals per group. ***P < 0.001, toxic versus normal, #P < 0.05, toxic versus zingerone-treated group, ##P < 0.01, toxic versus zingerone-treated group

in Group III and IV when compared with CP alone-treated Group II [Figure 1].

Effect of zingerone on lipid peroxidation

LPO was measured in terms of MDA levels. The level of MDA was significantly increased (P < 0.001) in CP alone-treated animals as compared control group. Zingerone pretreatment and cotreatment markedly decreased the levels of MDA in Group III (P < 0.05) and Group IV (P < 0.01), respectively, as compared to Group II [Figure 2].

Effect of zingerone on DNA fragmentation

Marked DNA damage was observed in CP alone-treated animals due to tremendous oxidative stress. However, significant improvement in DNA repair was observed in Group III and IV by co- and pre-treatment with zingerone by its strong antioxidant action [Figure 3].

DISCUSSION

CP is an inactive cytostatic alkylating agent that is transformed into potent active metabolite phosphoramide and acrolein by hepatic microsomal enzymes.[31] It has been reported by several studies that tremendous oxidative stress is produced during bioactivation of CP by heavy production of ROS which in turn reduces the antioxidant potential.[4] The present investigation was demonstrated to evaluate the chemoprotective potential of zingerone against oxidative stress-mediated cellular toxicity induced by CP. Ginger is a common natural herbal medicinal plant, has strong antioxidant properties, and has gained tremendous attention in scientific research for the treatment of problems related to oxidative stress. Among the main constituents of ginger, zingerone is a key compound recognized for its strong antioxidant and antiinflammatory properties,[13] recently reported to have been associated with anticancer[15] and antimicrobial activities.[37] LPO induced by free radicals leads to alteration of the cell membrane structure and function. LPO status is indicated by increase in MDA production. Increase in LPO is therefore a reliable marker to show index of oxidative stress and tissue damage.[12] CP and its metabolites increased the levels of MDA in CP-treated animals because of free radical-mediated injury to cellular membranes. The attenuation of MDA levels by pretreatment and cotreatment with zingerone might be its strong potential to scavenge free radicals. Recent studies revealed that zingerone has scavenging effect against peroxynitrite formed from the reaction of superoxide and nitric oxide inducing cellular and tissue damage.[32] It has been found that there is a direct relationship between oxidative stress and hepatic injury.[33,34] CP results severe depletion of enzymes involved in detoxification and oxidant defense by generating ROS.[35] In the current study, the activity of detoxifying enzymes such as CAT, GSH, GPX, and GST was decreased in CP alone-treated groups. Reduction of activity in antioxidant enzymes such as CAT and GST by CP increases the susceptibility of liver for $O2^-$ and hydroxyl radicals-induced oxidative damage. GSH is the key nonenzyme intracellular antioxidant involved in detoxification of endogenous and exogenous toxins,[36] and GST is mainly involved in electrophile detoxification in conjugation with GSH.[37] Depletion of these detoxifying agents in caused oxidative stress mediated hepatic damage. However, pretreatment and cotreatment with zingerone improved the overall redox and antioxidant status of liver in rats. Our results are supported by previous findings.[38] Nitrite (NO) is a highly reactive and short-lived free radical synthesized from L-arginine. Peroxynitrite the potent and versatile ident is spontaneously formed when NO reacts with superoxide radical. Peroxynitrite in turn leads to membrane peroxidation,[39] nuclear DNA damage,[40] and depletion of GSH content.[41] Increase in NO production in CP-treated animals was found to be ameliorated in the current study by pretreatment and cotreatment with zingerone. Recently, studies have reported that zingerone has scavenging effect against peroxynitrite formed from the reaction of superoxide and nitric oxide inducing cellular and tissue damage.[32] DNA damage caused by increased oxidative stress generated by CP was found to be markedly improved by zingerone treatment; our result supported by the previous findings reported by Rajan *et al*., 2013[13] that zingerone has effective potential against DNA damage caused by oxidative stress. Free radicals induced by CP administration cause accumulation of cholesterol in cells (a) by significantly increasing the biosynthesis of cholesterol and its esterification, (b) by reducing ester hydrolysis of cholesterol, and (3) by decreasing efflux of cholesterol from cells.[9] Zingerone improved the oxidative stress induced by CP due to its strong antioxidant effects. The powerful antioxidative potential of zingerone is attributed to its highly scavenging power of zingerone against ROS. The more interesting fact regarding zingerone is that it has more antioxidant potential than ascorbic acid which makes it different from other herbs and herbal compounds.[13] Oxidative insults triggered by CP is improved by zingerone through its antioxidant effects. Hence, the marked alteration in lipid profile induced by CP in terms of increase in cholesterol,

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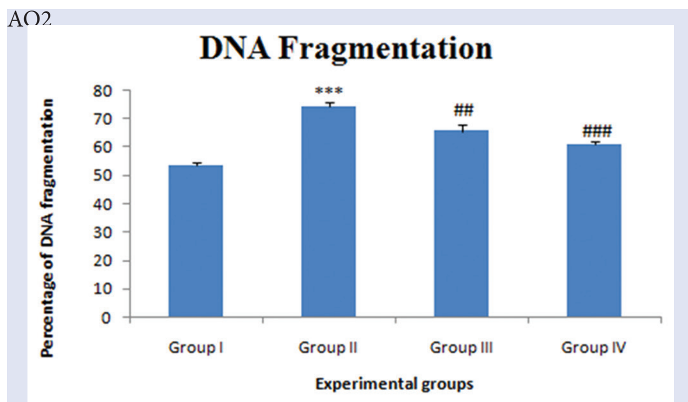

Figure 3: Effect of zingerone pretreatment and cotreatment on DNA fragmentation in cyclophosphamide-induced toxicity. Values are expressed as mean ± standard error of the mean ($n = 6$) results represent mean ± standard error of six animals per group. $^{***}P < 0.001$, toxic versus normal, $^{\#\#}P < 0.01$, toxic versus zingerone-treated group, $^{\#\#\#}P < 0.001$, toxic versus zingerone-treated group

TG, LDL and decrease in HDL may be attributed to its mechanistic effect on increased synthesis of cholesterol and reduction in its utilization. Zingerone pre- and co-treatment remarkably ameliorated the changes in lipid profile induced by CP. Zingerone has previously been reported to have hypolipidemic effect.[42] Han *et al.* (2008)[18] reported the antilipolytic effect of zingerone in ovariectomized rats. Zingerone has been found to be effective in enhancing basal lipolysis and isoprenaline-induced lipolysis in adipocytes.[35] ALT, AST, and ALP act as biomarker enzyme of hepatic damage.[17] Leakage of these enzymes in serum occur due to hepatocyte damage. CP activation in the liver leads to the formation of toxic metabolites which in turn causes hepatic damage as indicated by increase in ALT, AST, and ALP levels. Our results of the current study indicated that pretreatment and cotreatment with zingerone restored the activities of ALT, AST, and ALP. The hepatoprotective effects of zingerone observed in this study is supported by various previous studies.[43,44]

CONCLUSION

The biochemical investigations of the present study revealed the strong antioxidant potential of zingerone against CP-induced toxicity. However, the pretreatment of zingerone was found more effective than cotreatment against CP-induced organ toxicity and oxidative stress. The exact mechanism involved in protection of zingerone against CP is although unknown, the plausible mechanism found in the current study suggests that zingerones protection against the CP-induced toxicity may be through the scavenging of free radicals generated by CP reactive metabolites. In view of above-mentioned findings, we hypothesize its possible use in chemotherapy and other stress-associated disorders as a supplementary therapy, but before that, further studies are warranted to elucidate the exact protective mechanism of zingerone.

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Nil.

Conflicts of interest

The authors declare that there is no conflict of interests among all the coauthors of this manuscript.

REFERENCES

1. Goldberg MA, Antin JH, Guinan EC, Rappeport JM. Cyclophosphamide cardiotoxicity: An analysis of dosing as a risk factor. Blood 1986;68:1114-8.
2. Takimoto CH, Calvo E. Principles of oncologic pharmacotherapy. In: Cancer Management: A Multidisciplinary Approach, 2008;11:1-9.
3. Khan TS, Sundin A, Juhlin C, Wilander E, Oberg K, Eriksson B. Vincristine, cisplatin, teniposide, and cyclophosphamide combination in the treatment of recurrent or metastatic adrenocortical cancer. Med Oncol 2004;21:167-77.
4. Shanafelt TD, Lin T, Geyer SM, Zent CS, Leung N, Kabat B, *et al.* Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia. Cancer 2007;109:2291-8.
5. Hanane A, Claire L, Caroline A, Fabrice M. Anticancer drug metabolism: Chemotherapy resistance and new therapeutic approaches. In: Topics on Drug Metabolism. In Tech 2012. p. 23-42.
6. Khandelwal N, Abraham SK. Protective effects of common anthocyanidins against genotoxic damage induced by chemotherapeutic drugs in mice. Planta Med 2014;80:1278-83.
7. Liu M, Hales BF, Robaire B. Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line. Biol Reprod 2014;90:72.
8. Premkumar K, Pachiappan A, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A. Effect of *Spirulina fusiformis* on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in mice. Fitoterapia 2001;72:906-11.
9. Abarikwu SO, Otuechere CA, Ekor M, Monwuba K, Osobu D. Rutin ameliorates cyclophosphamide-induced reproductive toxicity in male rats. Toxicol Int 2012;19:207-14.
10. Lee S, Schmitt CA. Chemotherapy response and resistance. Curr Opin Genet Dev 2003;13:90-6.
11. Premila A, Indirani K, Preethi K. Alterations in antioxidant enzyme activities and increased oxidative stress in cyclophosphamide-induced hemorrhagic cystitis in the rat. Cancer 2008;6:563-70.
12. Weijl NI, Cleton FJ, Osanto S. Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat Rev 1997;23:209-40.
13. Rajan I, Narayanan N, Rabindran R, Jayasree PR, Manish Kumar PR. Zingerone protects against stannous chloride-induced and hydrogen peroxide-induced oxidative DNA damage *in vitro*. Biol Trace Elem Res 2013;155:455-9.
14. Kim MK, Chung SW, Kim DH, Kim JM, Lee EK, Kim JY, *et al.* Modulation of age-related NF-kappaB activation by dietary zingerone via MAPK pathway. Exp Gerontol 2010;45:419-26.
15. Vinothkumar R, Vinothkumar R, Sudha M, Nalini N. Chemopreventive effect of zingerone against colon carcinogenesis induced by 1,2-dimethylhydrazine in rats. Eur J Cancer Prev 2014;23:361-71.
16. Kumar L, Chhibber S, Harjai K. Zingerone inhibit biofilm formation and improve antibiofilm efficacy of ciprofloxacin against *Pseudomonas aeruginosa* PAO1. Fitoterapia 2013;90:73-8.
17. Kumar G, Banu GS, Kannan V, Pandian MR. Antihepatotoxic effect of beta-carotene on paracetamol induced hepatic damage in rats. Indian J Exp Biol 2005;43:351-5.
18. Li-kun H, Morimoto C, Zheng YN, Lie W, Etsuko *et al.* Effect of zinge rone on fat storage in ovariectomized rats. YakungZasshi 2008;128:1195-201.
19. Yagihashi S, Miura Y, Yagasaki K. Inhibitory effect of gingerol on the proliferation and invasion of hepatoma cells in culture. Cytotechnology 2008;57:129-36.
20. Keum YS, Kim J, Lee KH, Park KK, Surh YJ, Lee JM, *et al.* Induction of apoptosis and caspase-3 activation by chemopreventive [6]-paradol and structurally related compounds in KB cells. Cancer Lett 2002;177:41-7.
21. Wang CZ, Qi LW, Yuan CS. Cancer chemoprevention effects of ginger and its active constituents: Potential for new drug discovery. Am J Chin Med 2015;43:1351-63.
22. Rao BN, Archana PR, Aithal BK, Rao BS. Protective effect of zingerone, a dietary compound against radiation induced genetic damage and apoptosis in human lymphocytes. Eur J Pharmacol 2011;657:59-66.

23. Motohashi N, Ashihara Y, Yamagami C, Saito Y. Antimutagenic effects of dehydrozingerone and its analogs on UV-induced mutagenesis in *Escherichia coli*. Mutat Res 1997;377:17-25.
24. Wright JR, Colby HD, Miles PR. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. Arch Biochem Biophys 1981;206:296-304.
25. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-8.
26. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
27. Carlberg I, Mannervik B. Glutathione level in rat brain. J Biol Chem1975;250:4480-4575.
28. Claiborne A. Catalase activity. In: Greenwald RA, editor. CRC Handbook of Methods for Oxygen Radical Research. Boca Raton, FL: CRC Press; 1985. p. 283-4.
29. Mohandas M, Marshall JJ, Duggin GG, Horvath JS, Tiller D. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Cancer Res 1984;44:5086-91.
30. Donya SM, Farghaly AA, Abo-Zeid MA, Aly HF, Ali SA, Hamed MA, *et al.* Malachite green induces genotoxic effect and biochemical disturbances in mice. Eur Rev Med Pharmacol Sci 2012;16:469-82.
31. de Jonge ME, Huitema AD, Rodenhuis S, Beijnen JH. Clinical pharmacokinetics of cyclophosphamide. Clin Pharmacokinet 2005;44:1135-64.
32. Shin SG, Kim JY, Chung HY, Jeong JC. Zingerone as an antioxidant against peroxynitrite. J Agric Food Chem 2005;53:7617-22.
33. Oh JM, Jung YS, Jeon BS, Yoon BI, Lee KS, Kim BH, *et al.* Evaluation of hepatotoxicity and oxidative stress in rats treated with tert-butyl hydroperoxide. Food Chem Toxicol 2012;50:1215-21.
34. Yamamoto T, Kikkawa R, Yamada H, Horii I. Identification of oxidative stress-related proteins for predictive screening of hepatotoxicity using a proteomic approach. J Toxicol Sci 2005;30:213-27.
35. King PD, Perry MC. Hepatotoxicity of chemotherapy. Oncologist 2001;6:162-76.
36. Ali-Osman F. Quenching of DNA cross-link precursors of chloro ethyl nitrosoureas and attenuation of DNA inter strand cross-linking by glutathione. Cancer Res 1989;49:5258-61.
37. Touliatos JS, Neitzel L, Whitworth C, Rybak LP, Malafa M. Effect of cisplatin on the expression of glutathione-S-transferase in the cochlea of the rat. Eur Arch Otorhinolaryngol 2000;257:6-9.
38. Hemalatha KL, Stanelymainzen. Preventive effect of zingerone on altered lipid peroxids and nonenzymatic antioxidants in the circulation of isoproterenol-induced myocardial infarcts. Biochem Mol Toxicol 2014;10:102-4.
39. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991;288:481-7.
40. Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG. The role of nitric oxide in cancer. Cell Res 2002;12:311-20.
41. Vander VA, Smith DO, Neill CA, Kaur H, Darley-Usmar V, Cross CE, *et al.* Interactions of peroxynitrite with human plasma and its constituents: Oxidative damage and antioxidant depletion. Biochem J 1994;303:295-301.
42. Thunchomnang K, Lawa K, Mangkhalathon A, Saenubol P, Rattanakiat S, Pulbutr P. Lipolytic effects of zingerone in isolated rat adipocytes, The 4th Annual Northeast Pharmacy Research Conference 2012.
43. Kumar L, Chhibber S, Harjai K. Hepatoprotective effect of zingerone (4-(4-hydroxy-3-methoxyphenyl) butan-2-one) in lipopolysaccharide induced liver injury mouse model through downregulation of inflammatory mediators. Int J Pharmacogn Phytochem Res 2014;6:308-17.
44. Cheong KO, Shin DS, Bak J, Lee C, Kim KW, Je NK, *et al.* Hepatoprotective effects of zingerone on carbon tetrachloride-and dimethylnitrosamine-induced liver injuries in rats. Archives of Pharmacal Research 2016;39:279-91.