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Research Article
Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) Protects Against Acetaminophen Induced Hepatotoxicity in Wistar Rats via Alleviation of Oxidative Stress and Inflammation

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Abstract

Background: Liver disorders continue to be a major challenge to global public health and makes a major contribution in the world mortality statistics. To combat the challenge of current liver disease in world is outmost importance. Zingerone a phenolic alkanone is one of the active constituent present in ginger have broad range of multiple pharmacological activities including anti-oxidant, anti-inflammatory, anticancer and antimicrobial properties. **Methodology:** The current study was designed to evaluate the preventive effect of zingerone against acetaminophen induced hepatotoxicity in Wistar rats. Animals were randomly divided into five groups (I-V) of six animals in each group. Group I served as normal control and received normal saline orally. Group II was treated with acetaminophen alone at a dose of 800 mg kg^{-1} b.wt. Group III and IV received oral treatment of zingerone at a dose of 25 and 50 mg kg^{-1} b.wt., respectively for 14 days. To determine the effect of zingerone rats were pretreated orally with zingerone for 14 days before acetaminophen suspension. **Results:** Pretreatment with zingerone revealed attenuation of serum activities of AST, ALT, ALP, total bilirubin, total protein, zingerone pre-treatment attenuated oxidative stress test (Lipid peroxidation), improved antioxidant parameter (GSH, SOD, catalase and GPX) lipid profile and suppressed the NF-κB activation. Zingerone treatment suppressed the gene activation of pro-inflammatory cytokine, TNF-α which was up-regulated with acetaminophen administration through NF-κB activation. **Conclusion:** These results suggests that zingerone may be a promising drug candidate for therapeutic interventions of liver diseases.

Key words: Acetaminophen, zingerone, ROS, SOD, GPX, catalase, GPX

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver is a vital organ in the body having a significant role in metabolism of nutrients and drugs. Its significant role in catabolic and anabolic reactions is remarkable and diverse. Metabolic processes including glycogenesis, glycogenolysis, lipid biosynthesis, synthesis of plasma proteins, detoxification of xenobiotics and different biochemical reactions are mediated by this vital organ. As a result, liver comes across a wide range of drugs and toxins. Liver injuries are induced due to many classes of drugs which lead to hepatic disorders and to about 50% of acute liver failures[1]. Many synthetic drugs are in use for treating various liver diseases. But they have many ill effects on liver and the body as a whole. Besides, the side effects faced due to the use of these drugs, the scarce availability is becoming the new issue. To combat these health challenges, there is a dire need to find a safe, efficient and easily available drug substitute for the treatment of liver disorders. In this regard herbal medicinal plants can come to the rescue and prove beneficial. The medicinal plants which find a mention in the ancient medicinal treatment methodology can be considered as an alternative therapeutic source. The ancient homemade treatment methods for liver disorders should be reconsidered[2]. Medicines derived from herbal plants have been in use since thousands of years[3]. India possess a treasure of several medicinal plants with hepatoprotective, antioxidant, anticancer and antimicrobial properties. Ginger (*Zingiber officinale*, family: Roscoe Zingiberaceae) is one of the medicinal plants, commonly used in the world. Originated in South-East Asia, it is one of the most commonly used spice, all over the world. Its strong pungent aroma and refreshing flavor gives a strong stimulus to the taste buds and is used for culinary purpose throughout. Besides giving taste to the food, it has a beauty of acting as the best therapeutic agent for treating many diseases. Its bulk of medicinal properties lies in its rich phytochemistry which helps in curing many metabolic and pathological disorders. It is a rich source of many minerals, vitamins and enzymes like zingibain, a protease. The rich phytochemistry of ginger attributes its medicinal characteristics it contains about 60 well known active constituents which have been mainly classified into two categories based on volatile and non volatile nature. Volatile compounds include hydrocarbons of monoterpenoids and sesquiterpene whereas non-volatile compounds include gingerols, paradols, shogals and zingerone. Zingerone is mostly found in dry ginger. By cooking ginger, gingerol gets converted into zingerone by retro-aldol reaction. Zingerone is present in significant quantity about 9.25% in ginger. Zingerone is a polyphenolicalkanone commonly known as vanillyl acetone. This phytocompound has strong pharmacological properties like anti-inflammatory, antioxidant[4], anticancer[5] and antimicrobial activities and thus, can be used as a potent drug for treatment of liver ailments. Oxidative stress is a major cause of liver injuries and pathogenesis[6]. Hence, phytocompounds like zingerone, having significant antioxidant properties, should be investigated and explored for treating liver diseases. Acetaminophene, a non-steroid anti-inflammatory drug, has been used as an analgesic, antipyretic and potent anti-inflammatory drug since long time. But an end product, N-acetyl-p-benzoquinoneimine (NAPQI), left after metabolism of acetaminophen is hepatotoxic and causes tremendous oxidative stress to the liver cells. The NAPQI overdose depletes glutathione levels and there is high production of reactive oxygen species which are detrimental to liver[7]. Acetaminophen-induced liver injury model is a reliable model employed by clinical researchers to explore the hepatoprotective effect of drugs[8]. Taking in consideration the above mentioned tremendous anti-oxidant properties of zingerone, the current study was designed to evaluate the hepatoprotective potential of zingerone in liver injury induced by acetaminophen overdose. However, the currently available scientific literature regarding the zingerone as a hepatoprotective drug in acetaminophen induced injury is limited.

MATERIALS AND METHODS

Zingerone was purchased from Sigma Aldrich, sodium dihydrogen phosphate, sodim hydrogen phosphate, sodium potassium tartarate 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 weeks old male Albino Wistar rats (130-180 g) were obtained from 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 SKUAST-Kashmir and were kept in controlled environment conditions under 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 (CPCSEA) and the study was approved by the animal ethics committee of FVSC&AH, SKUAST-Kashmir, J and K.

Experimental design: After the acclimatization period of 14 days animals were divided into five groups, six rats in each group. All the rats were kept on fast for 24 h prior to experiment:

- The first group was kept as control received normal saline
- The second group was given single dose of intraperitoneal injection of acetaminophen at the dose of 800 mg kg^{-1} b.wt.
- The third group was given zingerone at the dose of 25 mg kg^{-1} b.wt., by oral gavage for 14 days followed single dose of intraperitoneal injection of acetaminophen (800 mg kg^{-1} b.wt.) on 14th day after 1 h of the last treatment with zingerone
- The fourth group was given zingerone at the dose of 50 mg kg^{-1} b.wt., by oral gavage for 14 days followed by single dose of intraperitoneal injection of acetaminophen (800 mg kg^{-1} b.wt.) on 14th day after 1 h of the last treatment with zingerone

Biochemical parameters: The activities of AST, ALT, ALP and bilirubin was estimated by using Accurex diagnostic skits. Analysis were performed according to the manufacturer's instruction.

Post-mitochondrial 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 Elvehjenhomogenizer. The homogenate was filtered through muslin cloth and centrifuged at 3000 rpm for 10 min at 48°C in Remi cooling centrifuge to separate the nuclear debris. The aliquot so obtained was centrifuged at 12000 rpm for 20 min at 48°C to obtain Post Mitochondrial Supernatant (PMS), which was used as a source of various enzymes.

Lipid peroxidation level: The assay for membrane lipid peroxidation (LPO) was done by the method of Wright *et al.*[9].

Antioxidant enzyme assay

Measurement of SOD activity: The SOD activity was measured by the method of Marklund and Marklund[10].

Assay for glutathione reductase: It was determined by the method of Carlberg and Mannervik[11].

Assay for catalase activity: The catalase activity was assessed by the method of Clairborne[12].

Immunoassay estimation of NF-κB: The NF-κB content translocated to nucleus was estimated by using an ELISA kit (NF-κB p65 ELISA, Invitrogen Corporation, CA, USA) in nuclear fraction of liver tissue according to protocol provided by the manufacturer.

Assay for TNF-α levels: Serum levels of TNF-α was estimated by using an ELISA kit (NF-κB p65 ELISA, Invitrogen Corporation, CA, USA) according to protocol provided by the manufacturer.

Statistical analysis: The data from individual groups are presented as the mean+standard error of the mean (SEM). Difference between groups were analysed by using analysis of variance (ANOVA) 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 AST ALT and ALP levels: The levels of AST, ALT and ALP in serum were significantly increased ($p<0.001$) in acetaminophen alone treated positive control group II as compared to normal group. Zingerone pretreatment significantly decreased the levels of AST, ALT and ALP in group III ($p<0.01$) and group IV ($p<0.001$) acetaminophen induced rats (Table 1).

Effect of pretreatment of zingerone on total bilirubin and total protein: The level of total bilirubin in serum on day 14 was significantly increased ($p<0.001$) in acetaminophen alone treated control group (Group II) as compared to the total bilirubin of normal group (Group I). However, zingerone pretreatment administration significantly reduced the levels of bilirubin in group III ($p<0.01$)and group IV ($p<0.00$). The levels of total protein were significantly reduced in acetaminophen alone treated group I ($p<0.001$) compared to the normal control group (Group I). Pretreatment with zingerone significantly increased the levels of protein in Group IV ($p<0.01$) (Table 2, 3).

Effect of zingerone on the activities of catalse (CAT) in liver: The CAT activities in acetaminophen alone treated group showed a significant decrease in activities of CAT as compared to normal group. However, pre-treatment with zingerone significantly ($p<0.01$) ameliorated

Table 1: Result effect of treatment of zingerone on alt, ast, total protein and total bilirubin

Treatment regimen (groups)	ALT	AST	ALP	Total protein	Total bilirubin
I	57.21±2.86	115.21±4.57	95.26±1.32	4.41±0.30	0.49±0.06
II	119.20±9.05***	234.11±15***	194.92±2.37***	3.36±0.21***	2.25±3.31***
III	89.01±6.05#	154.61±8.62##	164.65±1.84#	4.68±0.37#	0.71±0.15##
IV	71.78±4.89##	131.0±3.74##	132.58±1.28##	5.07±0.42##	0.58±0.13###

Results represent Mean±SE of six animals per group, ***p<0.001 toxic versus normal, #p<0.05 toxic versus pretreated with zingerone, ##p<0.01 toxic versus pretreated with zingerone, ###p<0.001toxic versus pretreated with zingerone

Table 2: Effect of treatment of zingerone on antioxidant enzymes like GSH, catalase, SOD and GPX

Treatment regimen (groups)	GSH (nmol GSH g^{-1} tissue)	Catlase (nmol H_2O_2 consumed min^{-1} mg^{-1} protein)	SOD (IU L^{-1})	GPX (nmol NADPH oxidized min^{-1} mg^{-1} protein)
I	84.73±8.79	664.4±40.16	4.38±0.81	7.42±0.17
II	31.76±8.79***	321.12±22.11***	2.73±0.15***	2.05±0.15***
III	54.24±7.73##	487.15±21.74##	3.46±0.13##	4.22±0.31***
IV	78.54±8.15###	670.14±16.72###	4.14±0.17##	5.97±0.15###

Results represent Mean±SE of six animals per group, ***p<0.001 toxic versus normal, #p<0.05 toxic versus pretreated with zingerone, ##p<0.01 toxic versus pretreated with zingerone, ###p<0.001 toxic versus pretreated with zingerone

Table 3: Effect of zingerone on total cholesterol (TC), high density, lipoprotein (HDL-C), low density lipoprotein (LDL-C) and triglycerides (TG) in different experimental groups

Treatment regimen (groups)	TC (mg dL^{-1})	HDL-c (mg dL^{-1})	LDL-c (mg dL^{-1})	TG (mg dL^{-1})
I	87.65±5.32	31.00±3.4	51.14±1.85	86.26±6.73
II	211.6±14.6***	14.56±0.79***	91.22±5.87***	171.3±12.5***
III	179.1±6.69#	24.81±1.21ns	94.44±7.75ns	136.5±7.61ns
IV	165.4±11.5##	23.12±2.10#	66.36±3.12##	119.41±14.0#

Results represent Mean±SE of six animals per group, ***p<0.001 toxic versus normal, #p<0.05 toxic versus pretreated with zingerone, ##p<0.01 toxic versus pretreated with zingerone

against the decrease in CAT activities when compared to acetaminophen treated animals.

Effect of zingerone on glutathione reduced (GSH) activity in liver: The GSH levels of animals treated with acetaminophen alone activities showed a significant (p<0.001) decrease as compared to control group. However, pre-treatment with zingerone significantly (p<0.01) increased the activities of GSH.

Effect of zingerone on liver glutathione-S-transferase (GST) activity: The activities of GST in acetaminophen alone treated animals was significantly reduced as compared to control group. However, pre-treatment with zingerone significantly (p<0.01) increased the activities of GST relative to acetaminophen alone treated group.

Effect of zingerone on liver glutathione peroxidase (GPX) activity: Acetaminophen alone treated group showed a significant decrease in GPX activity as compared to control group. However, pre-treatment with zingerone significantly (p<0.01) increased the activities of GST relative to acetaminophen alone treated group.

Effect of pretreatment of zingerone on lipid profile: In the present study, acetaminophen alone treated rats showed considerable increase in serum Total Cholesterol (TC), triglycerides (TG), LDL-C levels accompanied by a marked decrease in serum HDL-C level when compared with control group (-ve). While pretreatment with zingerone, restored the lipid profile in rats, there was very remarkable improvement in TC, TG, LDL-C and HDL-C levels in group III and IV when compared with untreated acetaminophen alone treated group II. Group IV revealed a significant decrease (p<0.001) in the TC, TG and LDL-C levels and considerable increase in HDL-C.

Effect of pretreatment of zingerone against acetaminophen induced lipid peroxidation: The levels of malondialdehyde (MDA) was significantly enhanced (p<0.001) in acetaminophen alone treated group II as compared to normal group I. Zingerone pretreatment markedly reduced the levels of MDA in group III (p<0.05) and IV (p<0.01) (Fig. 1).

Effect of zingerone and acetaminophen on NF-κB: The NF-κB redox sensitive nuclear transcription factor levels was found to be significantly increased in acetaminophen alone treated group II (p<0.001) as compared to normal group I. However, pretreatment with zingerone attenuated the levels of NF-κB in group III (p<0.01) and IV (p<0.001) (Fig. 2).

Effect of treatment of zingerone against acetaminophen on TNF-α levels: The TNF-α levels was found to be highly increased in acetaminophen alone treated group II (p<0.001)

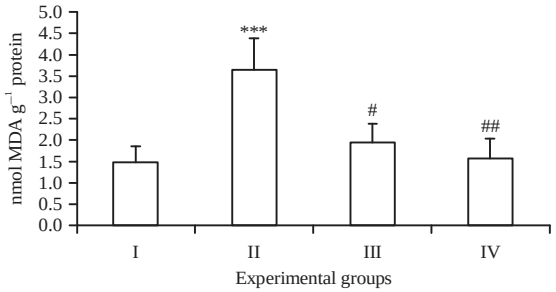

Fig. 1: Effect of zingerone pretreatment on acetaminophen induced MDA levels. Values are expressed as Mean±SEM (n = 6), results represent Mean±SE of six animals per group. ***p<0.001, toxic versus normal, #p<0.05, toxic versus pretreated with zingerone, ##p<0.01, toxic versus pretreated with zingerone

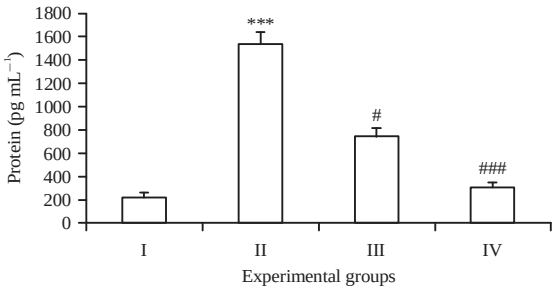

Fig. 2: Effect of zingerone pretreatment on acetaminophen induced NF-κB levels. Values are expressed as Mean±SEM (n = 6), results represent Mean±SE of six animals per group. ***p<0.001, toxic versus normal, #p<0.05, toxic versus pretreated with zingerone, ###p<0.001, toxic versus pretreated with zingerone

as compared to normal group I. However, pretreatment with zingerone moderately reduced the levels of TNF-α in group III (p<0.01) and significantly in group IV (Fig. 3-5).

DISCUSSION

The present investigation was an attempt to explore the prophylatic hepatoprotective effect of zingerone in acetaminophen induced liver injury. Overdosing of he antipyretic and analgesic drug acetaminophene, leads to hepatic injury and fatal hepatic necrosis both in animals and humans, though safer in optimum concentrations. The NAPQI a toxic metabolite of acetaminophene catabolism causes depletion of antioxidant agent glutathione and there is increase in levels of reactive oxygen species. These ROS cause

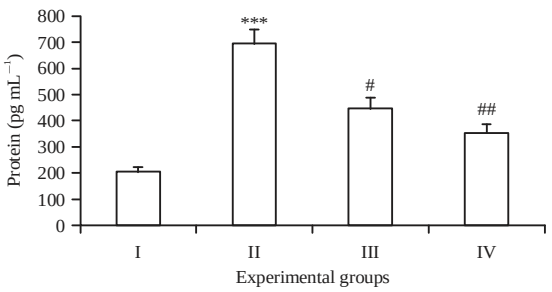

Fig. 3: Effect of zingerone pretreatment on acetaminophen induced TNF-α levels. Values are expressed as Mean±SEM (n = 6), results represent Mean±SE of six animals per group, ***p<0.001, toxic versus normal, #p<0.05, toxic versus pretreated with zingerone, ##p<0.01, toxic versus pretreated with zingerone

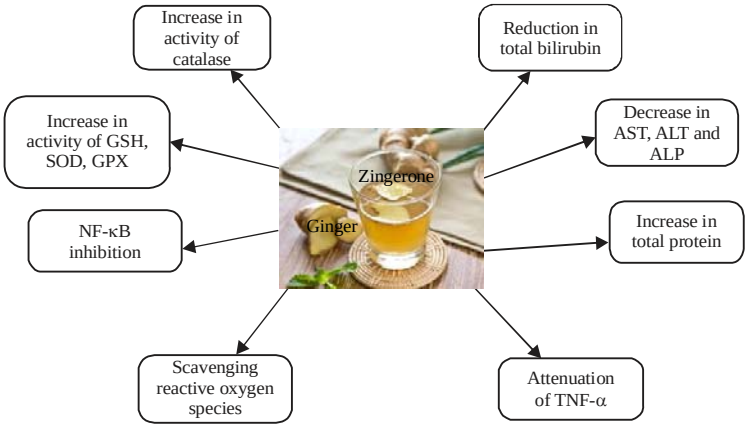

Fig. 4: Summary of effects of zingerone on various parameters in actemaminophen induced hepatotoxicity

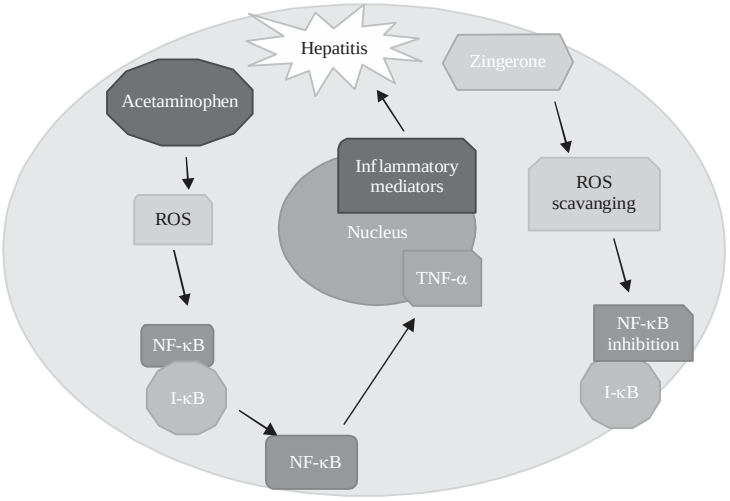

Fig. 5: Summary of molecular mechanism involved in hepatoprotective potential of zingerone in acetaminophen induced hepatotoxicity

degeneration of cell membranes and there is loss of important substrates from cell and cell death is the outcome. The cytosolic and mitochondrial GSH depletion by NAPQI leads to liver injury[13]. Liver injury caused by overdose of acetaminophen is a classical model for screening the hepatoprotective activity of compounds[14]. Hence, the use of such antiinflammatory drugs is detremental to liver and finding alternatives is the new goal. Therefore, the natural compounds with strong antioxidant properties are gaining a broad range of attention. The current study was carried to elucidate the effect of zingerone on acetaminophen induced liver injury and assess its role in modulation of oxidative stress and inflammatory process. Zingerone is present in significant amount in ginger and has been well recognized for its strong antioxidant and antiinflammatory properties[15] and recently reported to have been associated with anticancer[16] antimicrobial activities[17]. In this study, we observed that pretreatment with zingerone demonstrated protection against acetaminophen induced liver injury.

Liver specific bio-assays like AST and ALT enzymes were found to be elevated in acute liver injuries. Intrahepatic cholestasis, hepatitis and infilitrative diseases of liver are related to increase in levels of ALP[18]. The leakage of large quantities of enzymes into the blood stream was associated with centrilobular necrosis of the liver. Similarly in this study, increases in serum enzyme level of ALT, AST and ALP after exposed to acetaminophen was observed and thereby confirms the hepatic structural damage. The diminished levels of these enzymes by the prophylactic treatment with zingerone indicates it hepatoprotective action. The reliable criteria for judging the quality of any hepatoprotective drug are to preserve the normal hepatic physiological functions that have been disturbed by hepatotoxic agent[19]. The amelioration in levels of transaminases is attributed to healing and regeneration of hepatocytes. The levels of bilirubin and total protein in serum were related to the function of hepatic cell. A high concentration of bilirubin in serum is an indication for erythrocytes degradation rate caused due to liver injury[20]. Diminution of total protein is a further indication of liver damage. The level of total protein will be decreased in hepatotoxic condition due to defective protein biosynthesis in liver[21] in the current study the levels of total bilirubin and total protein in zingerone pretreated rats restored towards its normal values indicating its hepatoprotective potential.

Estimation of MDA levels produced by lipid peroxidation provides a quantitative analysis of cellular damage occurring due to oxidative stress. Increased MDA levels in liver treated with acetaminophen alone suggest enhanced lipid peroxidation leading to tissue damage and failure to prevent formation of excess free radicals. Pretreatment with zingerone significantly restored the MDA levels. The hepatoprotective action of zingerone may be attributed to its membrane stabilizing and strong antioxidant action. In the present study acetaminophen significantly increased the lipid peroxidation and caused the depletion of antioxidant enzymes (GSH, catalse, SOD and GPX). However, pretreatment with zingerone reversed the acetaminophen induced oxidative stress by improving the activities of GSH, catalse, SOD and GPX and facilitating the rapid and efficient consumption of reactive oxygen species generated by bio-activation of acetaminophen. Therefore, the observed hepato-protection against acetaminophen induced liver injury may be due to enhanced GSH activities. Recently, it has been reported that ginger is gifted with strong antioxidants which provide

protection against acetaminophen induced liver injury[22]. The strong antioxidant action of zingerone observed in this study is in accordance with previous reports[4]. It has been found recently that zingerone is strong antioxidant as compared to vitamin C. Rajan *et al.*[4] study revealed that zingerone has scavenging effect against peroxynitrite formed from the reaction of superoxide and nitric oxide inducing cellular and tissue damage[15]. Recently a study found that zingerone by virtue of its antioxidant activity protects the heart of rats against the isoproterenol induced myocardial infarction[23]. Profound impairment of lipid profile was observed in acetaminophen alone treated rats, the significant increase in levels of cholesterol and triglycerides in acetaminophen alone treated rats than control and zingerone pretreated rats indicates that acetaminophen alters the lipoprotein metabolism. Zingerone pretreatment improved lipid profile by decreasing cholesterol, triglycerides, LDL and increasing HDL levels. The hypolipidemic effect of zingerone may be attributed to its enhanced esterification effect and hepatoprotective effect which in turn improves lipid profile. The mechanism responsible for its lipolytic action has been attributed to its increasing nor epinephrine mediated lipolysis associated with translocation of hormone sensitive lipase. Zingerone has been found to be effective in enhancing basal lipolysis and isoprenalineinduced lipolysis in adipocytes[24].

The NF-κB activation is a critical determinant in the expression of pro-inflammatory cytokines like TNF-α and other inflammatory mediators involved in acute inflammatory responses and other conditions related to increased ROS generation. Acetaminophen induced oxidative stress leads the activation of NF-κB which regulates the synthesis of inflammatory cytokine like TNF-α implicated in hepatotoxicity[25]. Pretreatment with zingerone in the present study was found to inhibit the NF-κB expression and also abrogated the acetaminophen induced TNF-α expression. The potential of zingerone to inhibit NF-κB expression may be due to decrease in levels of phosphorylated form of inhibitor of kappa B (I-κB) and suspended the nuclear translocation of NF-κB[65]. Inhibition of NF-κB is a valuable approach for the treatment of acute and chronic liver disorders. The strong anti-inflammatory activity of zingerone observed in the present study are in agreement with previous studies who have reported zingerone to suppress NF-κB activity[26]. Kumar *et al.*[17] reported that zingerone protects liver damage induced by lipolysacchride induced liver injury via down-regulation of inflammatory mediators like TNF-α.

CONCLUSION

Since oxidative stress induced inflammation cascade induced by acetaminophen was reversed by prophylactic treatment of zingerone. Findings of the present study have indicated that zingerone exhibits hepatoprotective effect by inhibiting ROS generation, controlling inflammatory cascade and decreasing lipid peroxidation. However, the exact molecular mechanism of zingerone involved in its hepatoprotective potential is not yet clear. Research is going in our laboratory to ascertain its molecular mechanism of action.

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