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Full Length Research Paper

Ameliorative effect of 10-gingerol on drug induced hepatotoxicity in albino rats

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In the present study, the ameliorative hepatoprotective effect of 10-gingerol (5 and 10 mg/kg) was examined in albino rats using the model of acute hepatotoxicity induced by diclofenac sodium (DCFS). Hepatotoxicity was induced in rats by an intraperitoneal (i.p) injection of DCFS in a dose of 150 mg/kg. Rats received 10-gingerol by i.p. injection for 6 consecutive days before induction of hepatotoxicity. Animals were sacrificed at 6 h post intoxication and blood and liver samples were obtained. Liver injury was assessed biochemically and histologically. It was found that injection of DCFS to rats induced hepatic damage that was manifested by a significant increase in the activities of marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin in serum. The liver homogenate of intoxicated animals had increased levels of the malondialdehyde (MDA). Histological data presented marked damaged in sections of liver form DCFS-treated rats. Intraperitoneal dosing of 10-gingerol (10 mg/kg) to rats for 6-days before DCFS-intoxication reversed the altered serum parameter near to normal and silymarin control values. The elevation of MDA level in liver homogenate was significantly inhibited by 10-gingerol. In addition, 10-gingerol attenuated DCFS-induced hepatic histological alterations.

Key words: Diclofenac sodium, 10-gingerol, lipid peroxidation, hepatotoxicity, histopathology.

INTRODUCTION

10 gingerol a novel compound isolated from *Zingeber officinalis* (Family Zingiberaceae) plant has comparatively potent anti-oxidant property among all gingerols due to long C-Chain length (Lantz et al., 2007; Dugasani et al., 2010). The anti-inflammatory activity has shown by a potent COX enzymes inhibitor due to lipophilic alkyl side chain at around 14 carbons chain length with additional PGE2 and COX-2 mRNA inhibition among gingerol family (Tjendraputra et al., 2001). The other activities shown by 10-gingerol are, synergistic antibacterial effect (Nagoshi et al., 2006), inhibition of periodontal pathogens (Park et al., 2008), protect colon cancerous (Sarkar et al., 2011), as well as colorectal cancer cells (Chen et al., 2009), antiemetic (Toshiyasu et al., 1994) and anti-tuberculosis (Hiserodt et al., 1998).

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) of the acetic acid chemical class. As with other NSAIDs, diclofenac also possesses analgesic and antipyretic properties. Diclofenac is used for musculoskeletal complaints, especially arthritics, rheumatoid arthritis, polymyositis, dermatomyositis, osteoarthritis, dental pain, gout attacks and pain management in cases of kidney stones and gallstones (Laine, 2001; Paul and Chauhan, 2005). Normal therapeutic dose of DCFS are safe, effective and widely used analgesic–antipyretic drug; however, overdoses can induce severe hepatotoxicity in human and known to be major idiosyncratic hepatotoxic drugs (Kaplowitz, 2005). The exact mechanism of action is not entirely known, but it is thought that the primary mechanism responsible for its anti-inflammatory and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). The hepatotoxicity due to DCFS involves covalent protein modification (Gill et al., 1995), oxidative stress

generation (Bort et al., 1999) and mitochondrial damage (Gomez-Lechon et al., 2003). The hepatotoxicity induced by DCFS can be inverted by antioxidants drugs (Cantoni et al., 2003).

MATERIALS AND METHODS

Chemicals

Standard 10-Gingerol (CAS No. 23513-15-7) and *Z. officinalis* oleoresin (Batch no ZO/09005) were procured from Natural Remedies, Bangalore, India. aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin kits were supplied by Roche diagnostics, Germany. The trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Diclofenac Sodium (Votrex® 50) was purchased from King Khalid Hospital Pharmacy, KSU Riyadh, Saudi Arabia. 10-gingerol and DCFS solution were prepared by dissolving desired amounts in normal saline and was intraperitoneally injected in a volume of 0.1 ml /100 g body weight.

Isolation of 10-gingerol from *Z. officinale* oleoresin

The oleoresin (2 g) was weighed in a 50 ml round bottom flask and mixed in methanol. The 0.3 g silica gel was added in solution and complete dry using rotary evaporator. The dried oleoresin sample was fractionated by column chromatography (4.5 × 65 cm, i.d) containing silica gel (150 g, 70 to 230 mesh, Merck, Darmstadt, Germany) and eluted with 250 ml hexanes: ethyl acetate (80:20→50:50) solvent system. The column was connected with Foxy Jr. ® Fraction Collector (Teledyne Isco, Inc 4700 Superior Street, Lincoln, NE 68504) containing test tubes. 10 ml fraction of elute was collected in test tube. The total fraction was pulled in 4 major fractions G1-G4 on the basis of Thin-layer chromatography (TLC) (Aluminum Sheet, Silica 60 F254, Merck). The fraction G3 (80 mg) was found to contain 10-gingerol on the basis of spotting with reference on TLC plate. The isolated 10-gingerol was further purified by, LiChroprep RP-18 (25 to 40 µm, Merck) using 100 ml acetonitrile and water (6:4→4:6). 5 ml fraction of elute was collected in test tube. The total fraction was pulled in 3 major fractions G3a, G3b and G3c on the basis of TLC. G3b (38 mg) containing single spot was authenticated by comparing with standard by TLC method (Ficker et al., 2003).

Animals

The research and animal care were approved by the Ethical Committee of the College of Pharmacy, King Saud University. Twenty Wister rats (≈200 g) obtained from the experimental animal care centre and housed in polyacrylic cage with five animals per cage and maintained under standard laboratory conditions (Constant temperature (22±2°C), humidity (55%) and a 12 h light/dark) and supplied with commercial food pellets and tap water.

Experimental design for hepatoprotective activity

Twenty rats were randomly divided into 5 groups of 5 animals each. The hepatoprotective activity of 10-gingerol was tested using DCFS model. Groups I (normal control) and II (intoxicated control) received saline solution only. Groups III and IV were given 10 gingerol in dose of 5 and 10 mg/kg, respectively, while group V was given silymarin (hepatoprotective control) (10 mg/kg). All treatments were given by ip injection for 6 consecutive days. After 6 h, hepatotoxicity was induced in all groups (except normal control) by i.p. injection at a dose of 150 mg/kg.

Assessment of hepatoprotective activity

The hepatoprotective activity was evaluated biochemically and histopathologically. After 24 h of DCFS-intoxication, the animals were dissected under ether anesthesia. Blood from each was withdrawn from heart puncture and collected in previously labeled centrifuging tubes and allowed to clot for 40 min at room temperature. Serum was separated by centrifugation at 600×g for 15 min.

The enzymatic activities of AST, ALT and ALP in serum were assay by using automatic analyzer apparatus (Reflotron plus, Roche Germany) and commercially available reflotron Kits (Roche diagnostics, Germany).

Hepatic tissues examination

The ventral portion of the left lateral hepatic lobe was collected from each rat, fixed in 10% formalin and store at -80°C for subsequent lipid peroxidation and histopathological examinations.

Lipid peroxidation

The frozen liver samples were thawed, weighed and cut into small pieces by sterilized scissors, then homogenized in ice-cold 100 mM sodium phosphate buffer (10% w/v, 500 ml, pH 7.4) and centrifuged at 10000 × g for 10 min at 4°C. The supernatants were used in the same day for all biochemical assays. Lipid peroxidation (nmol/mg) in tissue was estimated by the thiobarbituric acid test for malondialdehyde.

The quantitative measurement of lipid peroxidation was determined in liver tissues according to the previous method (Ljubuncic et al., 2005). Liver homogenates tissues were deproteinized with 500 ml of 40% trichloroacetic acid and then 1 ml of homogenate was mixed with 2 ml 0.67% 2-thiobarbituric acid in tubes heated for 20 min at 100°C. After cooling on ice, the samples were centrifuged at 840×*g* for 15 min and the absorbance of the supernatant was read at 532 nm.

Histopathological examination

Small fragments (approximately 0.2 x 0.2 cm) of liver were taken and fixed in 10% formalin solution (Abdel-Kader and Alqasoumi, 2008). They were dehydrated through placed (3 time, 2 h each) in graded solutions of alcohol (70 to 100%, respectively). They were cleared in 2 changes of xylene, infiltrated in 2 changes of paraffin wax and then transfer into paraffin waxed filled moulds. The sections of liver prepared by rotary microtome (Leitz 1512) were placed on clean slides and stained with Mayer's hematoxylin solution for 15 min, washed with water and 80% alcohol then counterstained with eosin-phloxine solution. The tissues were mounted on slides and examined under light microscope.

Statistical analysis

Statistical analysis was performed using Prism 5 for windows version 5.04 trails (Graphpad software Inc.). All comparisons of parameter values between control and experimental groups were made using the paired Student's *t*-test. Differences between means were considered to be significant at $p < 0.05$.

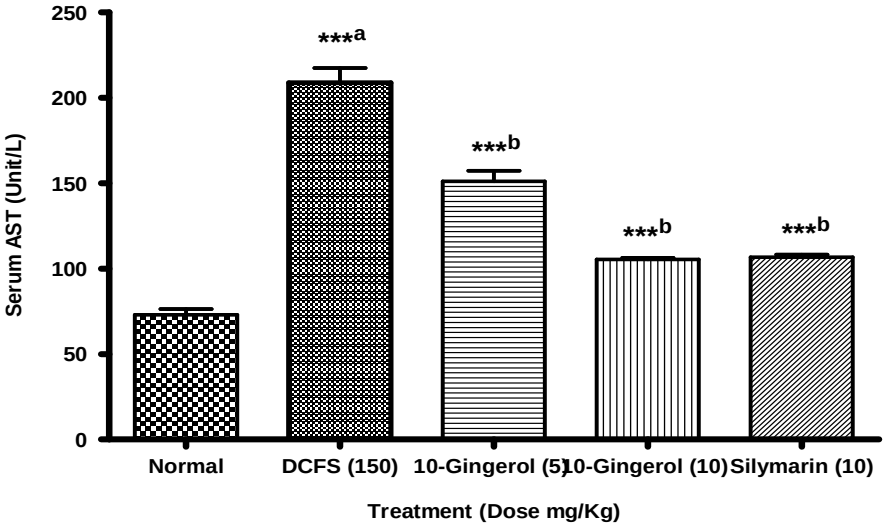

Figure 1. Effect of pretreatment with 10-gingerol (5 and 10 mg/kg) and silymarin (10 mg/kg) on serum activity of AST in DCFS-intoxicated rats. Each bar represent the mean ± SD, n = 5; ***P < 0.001. [a] = statistically significant compare to normal control group. [b] = statistically significant compare to DCFS-intoxicated control group.

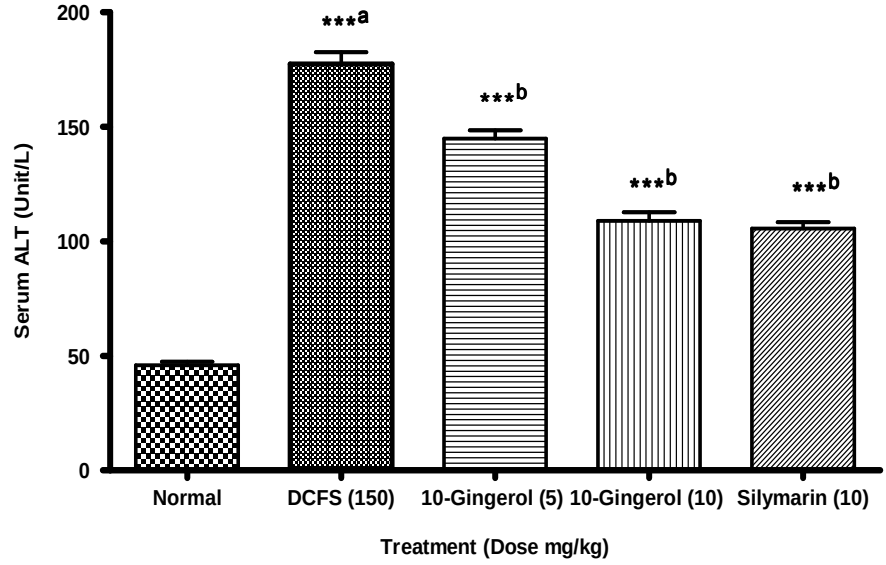

Figure 2. Effect of pretreatment with 10-gingerol (5 and 10 mg/kg) and silymarin (10 mg/kg) on serum activity of ALT in DCFS-intoxicated rats. Each bar represent the mean ± SD, n = 5; ***P < 0.001. [a] = statistically significant compare to normal control group. [b] = statistically significant compare to DCFS-intoxicated control group.

RESULTS

Effect on serum parameters

The present study had been attempted to demonstrate the role of hepatoprotective activity of 10-gingerol in DCFS-induced hepatotoxicity at dose of 5 and 10 mg/kg. The results of 10-gingerol against DCFS-induced hepatotoxicity are shown in Figures 1 to 6. The administration of DCFS to rats induced severe liver damage as there was a significant increase in the activities of marker enzymes: AST (208.8 ± 19.10 U/l),

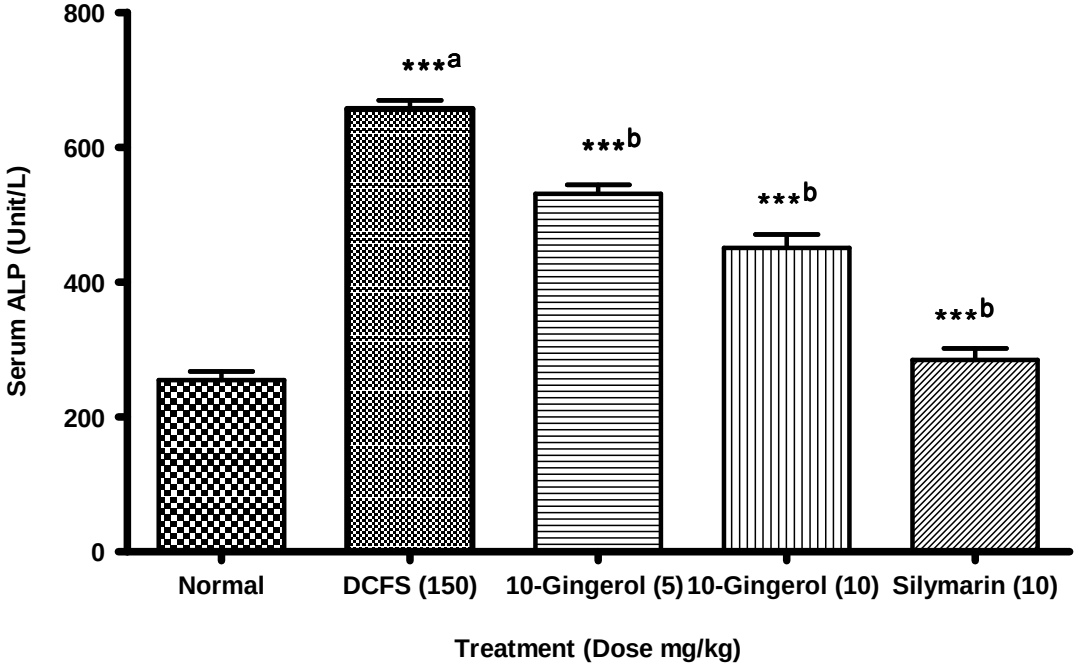

Figure 3. Effect of pretreatment with 10-gingerol (5 and 10 mg/kg) and silymarin (10 mg/kg) on serum activity of ALT in DCFS-intoxicated rats. Each bar represent the mean ± SD, n = 5; ***P < 0.001. [a] = statistically significant compare to normal control group. [b] = statistically significant compare to DCFS-intoxicated control group.

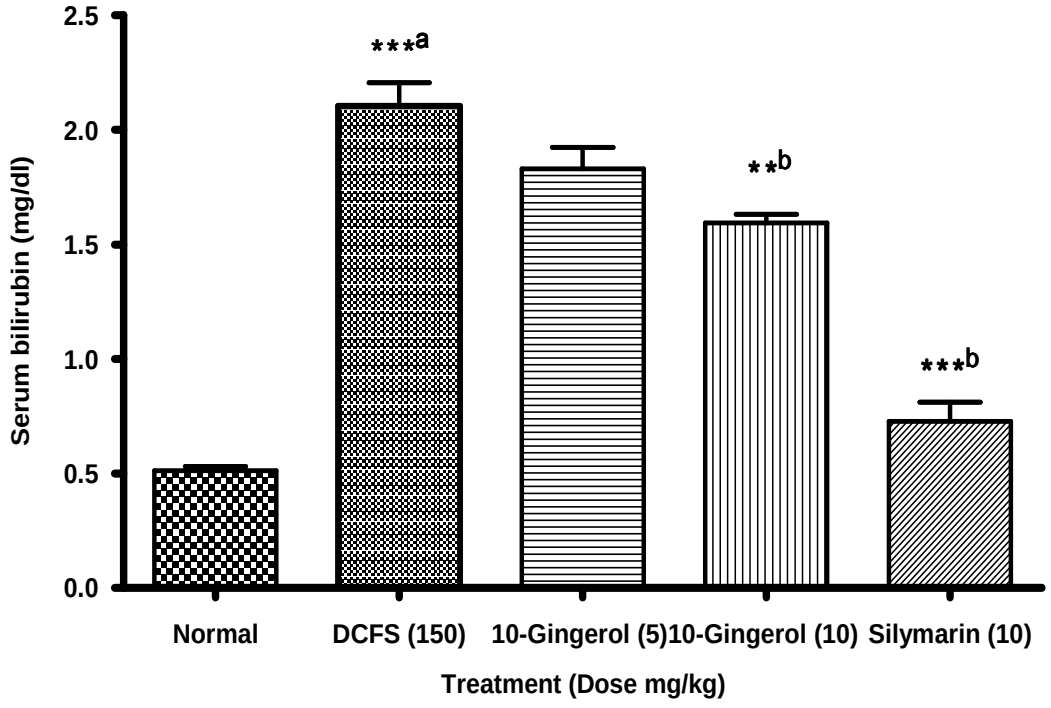

Figure 4. Effect of pretreatment with 10-gingerol (5 and 10 mg/kg) and silymarin (10 mg/kg) on serum activity of total bilirubin in DCFS-intoxicated rats. Each bar represent the mean ± SD, n = 5; **P < 0.01, ***P < 0.001. [a] = statistically significant compare to normal control group. [b] = statistically significant compare to DCFS-intoxicated control group.

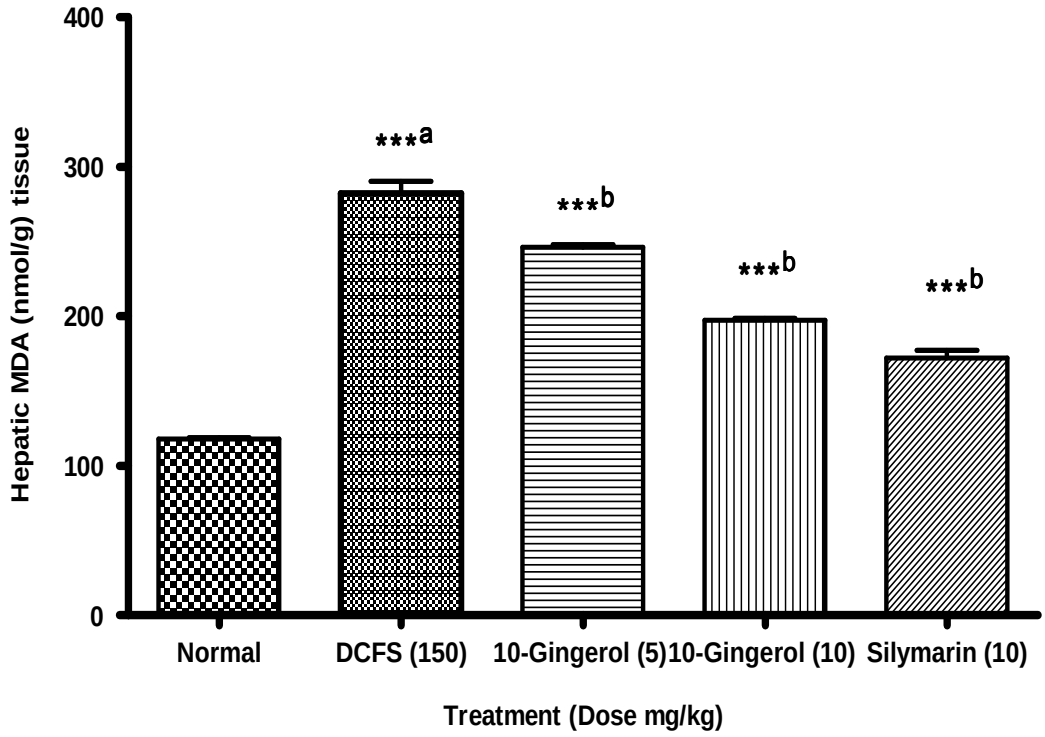

Figure 5. Effect of pretreatment with 10-gingerol (5 and 10 mg/kg) and silymarin (10 mg/kg) on liver tissues MDA level in DCFS-intoxicated rats. Each bar represent the mean ± SD, n = 5; ***P < 0.001. [a] = statistically significant compare to normal control group. [b] = statistically significant compare to DCFS-intoxicated control group.

ALT (177.4 ± 11.67 U/l) and ALP (657 ± 28.51 U/l) in addition to total bilirubin (2.10 ± 0.22 mg/dl) as compared to normal control rats (Figures 1 to 4) which may be due to acute hepatocellular damage and biliary obstruction. Pretreatment with 10-gingerol for 6-days in dose of 5 and 10 mg/kg exhibited a significant ($p < 0.001$) reduction in the DCFS-induced increase in the levels of AST (151.2 ± 13.92 and 105.4 ± 1.94 U/l), ALT (136.8 ± 5.02 and 108.9 ± 8.56 U/l), ALP (450.8 ± 44.45 and 324.2 ± 24.90 U/l) and total bilirubin (1.83 ± 0.20 and 159.4 ± 0.08 U/l). The hepatoprotective effect of 10-gingerol in a dose of 10 mg/kg was higher than that of 5 mg/kg.

Effect on lipid peroxidative products in liver homogenates

There was a significant increase in the concentration of malondialdehyde (MDA) (Figure 5) in the liver homogenate of DCFS-intoxicated rats (282.1 ± 18.13 nmol/g) when compare to normal control (117.9 ± 1.81 nmol/g) and silymarin control (172.2 ± 11.04) groups. Pretreatment with 10-gingerol in dose of 5 and 10 mg/kg for 6 days significantly prevented MDA elevation in liver tissues (246.2 ± 4.05 and 197.4 ± 2.56 nmol/g, respectively).

Histopathology finding

The histopathological examination of control and treated animals liver was summarized in Figure 6. Liver histopathological examination showed no histological abnormalities in livers of normal control rats, portal areas were clear, the hepatic lobular architecture was normal, did not see connective tissue proliferation (Figure 6A). The hepatocytes; in DFS-treated rats showed lymphocytic infiltrate and early bridging necrosis, extensive early fatty change (Figure 6B), in DFS + 10-gingerol (10 mg/kg) treated rats showed central fatty changes (Figure 6C), pretreated rats with 20 mg/kg of 10-gingerol showed normal pattern and mild congestion of hepatic cells (Figure 6D). The silymarin control group showed mild congestion of hepatic cells (Figure 6E).

DISCUSSION AND CONCLUSION

In this study, DCFS was used as a hepatotoxin in the experimental study of liver disease. The assessment of liver function can be made by estimating the activities of serum enzymes such as ALT, AST and ALP in addition to serum level of total bilirubin. Administration of DCFS to rats markedly increases serum activity of AST, ALT, ALP

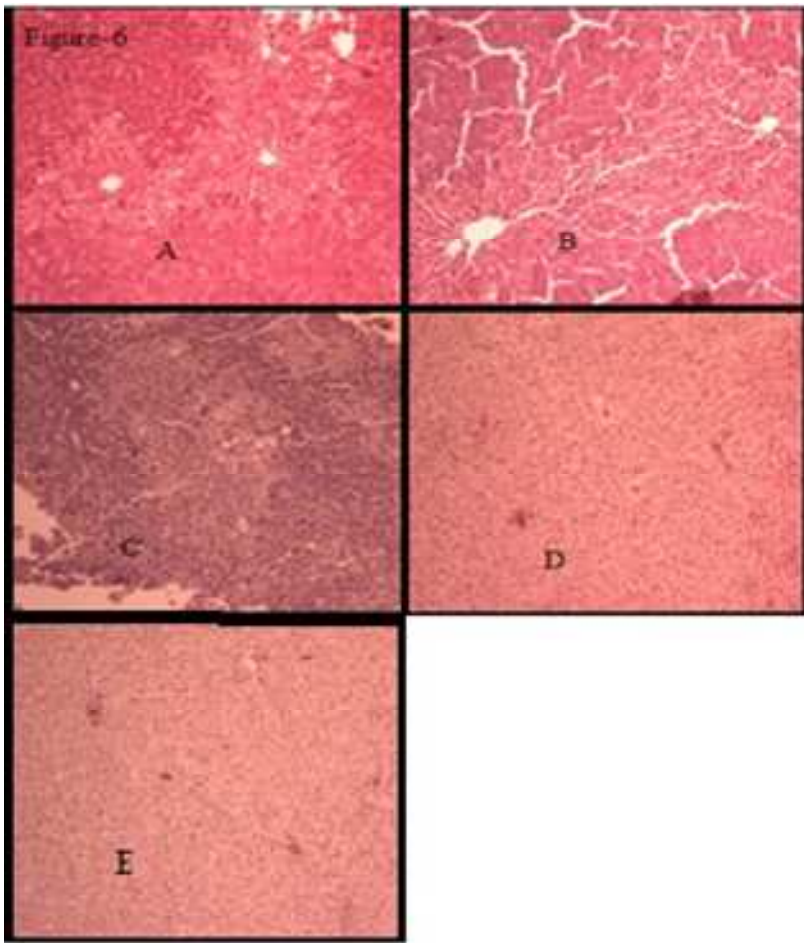

Figure 6. Light micrographs of liver sections: Liver section from normal rats was showing two normal lobules (A). Liver sections from DCFS group show lymphocytic infiltrate and early bridging necrosis, extensive early fatty change (B). Liver section of DCFS and 5 mg/kg of 10-gingerol was showing central and peripheral fatty changes (C). Liver section of rat treated with DIC and of 10 mg/kg 10-gingerol was showing normal pattern and mild congestion (D). While hepatoprotective control silymarin (10 mg/kg) showing mild congestion (E) (Haematoxylin and eosin stain H&E magnification × 400.

and serum level of total bilirubin which reflects the severity of liver injury. The present study has demonstrated that pretreatment with 10-gingerol at dose of 5 and 10 mg/kg for 6 days exhibited significant dose-dependent hepatoprotective activity against liver injury induced by DCFS. Diclofenac, like other NSAIDs, can cause hepatocellular damage (Schapira et al., 1986) and are previously documented for animals as well as in human liver toxicity and commonly used as an experimental method for investigation of hepatoprotective drugs (Aydin et al., 2003; Amin and Hamza, 2007). Liver enzymes are cytoplasmic in nature the hepatotoxin drugs alter the cell membrane function because of cellular leakage, the hepatoprotective enzymes are release into circulation indicates liver toxicity.

Elevation serum AST/ALT >1 is consider the alcoholic fatty liver disease (AFLD) while AST/ALT < 1 is considered as non alcoholic fatty liver disease (NAFLD), metabolites syndrome (MS) and visceral fat accumulation marker waist circumference (WC) (Ohgo et al., 2009). Liver serum enzymes are predictable sign of liver injury, thus, the lowering of elevated serum enzymes are an unambiguous sign of hepatoprotective action of drug (Sadasivan et al., 2006). The amount of bilirubin in blood is measured by total bilirubin testing. Diclofenac sodium at higher dose induce hepatotoxicity in elderly human is usually accompanied by rise in bilirubin level (Schapira et al., 1986). Bilirubin is the conventional indicator of liver diseases and rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice

(Davies and Anderson, 1997).

A decrease of serum bilirubin after treatment with 10-gingerol indicates the effectiveness of the natural isolated compound in the normal functional status of the liver. An oxidative stress is generated when high dose of DCFS used due to reactive oxygen species (ROS) propagate mitochondrial injury and cytochrome P450-dependent monooxygenases inhibition (Gill et al., 1995; Masubuchi et al., 2002). The mechanism of antioxidant drugs reduce the hepatic injury may be due to release of oxidative stress (Cantoni et al., 2003; Bort et al., 1999). Silymarin, an antioxidant, has extensive been used in the treatment of liver diseases as well as investigation of livoprotective drugs. These properties of protecting liver cells seem to be due to their ability to scavenge free radicals and inhibiting lipid peroxidation (Mansour et al., 2006).

The DCFS induced liver lesion was associated with massive elevation in liver MDA level. The MDA elevation has been well accepted as a reliable marker of lipid peroxidation (Packer and Cadenas, 2002). 10-gingerol has leading antioxidant properties among their own gingerol family member due to long carbon chain (Lantz et al., 2007; Dugasani et al., 2010). MDA elevation is a result of oxidative stress has previously demonstrated through the decrease of total antioxidant capacity (Amin and Hamza, 2007). The result of the present study indicates the protective effects of 10-gingerol on DCFS induced liver toxicity exert an antioxidant effect against DCFS induced oxidative stress by attenuating the increased MDA of liver tissue. MDA elevation is a result of oxidative stress demonstrated here through the decrease of total antioxidant capacity.

The comparative lowering the serum enzyme, bilirubin and MDA of liver tissue on DCFS induced hepatotoxicity with silymarin support the hepatoprotective action of 10-gingerol due to antioxidant nature. This study was further conformed by histological changes in liver. Most of the hepatocytes in DCFS treated rats displayed cellular degeneration and loss of their characteristic configuration (Sadasivan et al., 2006). Histopathological changes in the liver included lymphocytic infiltrate and early bridging necrosis, extensive early fatty change. The changes is histological structure in 10-gingerol treated groups show the protective nature of this bioactive compounds on drug induced liver injury in concentrated dependent manner.

In conclusion, administration of 10 gingerol for 6 days effectively prevented DCFS-induced hepatotoxicity in rats. The antioxidant activity of 10-gingerol that has been identified (Lantz et al., 2007; Dugasani et al., 2010) could be responsible for the observed hepatoprotective effect. However; further detailed studies are required to establish its clinical application.

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REFERENCES

Abdel-Kader MS, Alqasoumi SI (2008). Evaluation of the hepatoprotective effect of the ethanol extracts of Solanum nigram, Cassia fistula, Balanites aegyptiaca and cathamus tinctorius against experimentally induced liver injury in rats. Alex. J. Pharm. Sci., 22: 47-50.

Amin A, Hamza AA (2007). Curcuma longa, Glycyrrhiza glabra and Moringa oleifera Ameliorate Diclofenac-induced Hepatoxicity in Rats. Am. J. Pharmacol. Toxicol., 2(2): 80-88.

Aydin G, Gokcimen A, Oncu M, Cicek E, Karahan N, Gokalp O (2003). Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. Turk. J. Vet. Anim. Sci., 27: 1131-1140.

Bort R, Ponsoda X, Jover R, Gomez-Lechon M, Castell JV (1999). Diclofenac toxicity to hepatocytes: a role for drug metabolism in cell toxicity. J. Pharm. Exp. Ther., 288: 65-72.

Cantoni L, Valaperta R, Ponsoda X, Castell JV, Barelli D, Rizzardini M, Mangolini A, Hauri L, Villa P (2003). Induction of hepatic heme oxygenase-1 by diclofenac in rodents: role of oxidative stress and cytochrome P-450 activity. J. Hepatol., 38: 776-783.

Davies NM, Anderson KE (1997). Clinical pharmacokinetics of diclofenac: therapeutic insights and pitfalls. Clin. Pharmacokinet., 33: 184-213.

Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J. Ethnopharmacol., 127: 515-520.

Ficker C, Smith ML, Akpagana K, Gbeassor M, Zhang J, Durst T, Assabgui R, Arnason JT (2003). Bioassay-guided Isolation and Identification of Antifungal Compounds from Ginger. Phytother. Res., 17: 897-902.

Gill M, Ramirez MC, Terencio MC, Castell JV (1995). Immunochemical detection of protein adducts in cultured human hepatocytes exposed to diclofenac. Biochem. Biophysic. Acta, 1272(3): 140-146.

Gomez-Lechon M, Ponsoda X, Connor EO, Donato T, Castell JV, Jover R (2003). Diclofenac induces appoptosis in hepatocytes by alteration of mitochondrial function and generation of ROS. Biochem. Pharmacol., 66: 2155-2167.

Hiserodt RD, Franzblau SG, Rosen RT (1998). Isolation of 6-, 8-, and 10-Gingerol from Ginger Rhizome by HPLC and Preliminary Evaluation of Inhibition of Mycobacterium avium and Mycobacterium tuberculosis. J. Agric. Food. Chem., 46(7): 2504-2508.

Kaplowitz N (2005). Idiosyncratic drug hepatotoxicity. Nat. Rev. Drug Discov., 4: 489-499.

Laine L (2001). Approaches to NSAID use in the high-risk patient. Gastroenterology, 120: 594-606.

Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD, Timmermann BN (2007). The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine, 14(2-3): 123-128.

Ljubuncic P, Song H, Cogan U, Azaizeh H, Bomzona A (2005). The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental liver disease. J. Ethnopharmacol., 100: 198-204.

Mansour HH, Hafez HF, Fahmy NM (2006). Silymarin Modulates Cisplatin-Induced Oxidative Stress and Hepatotoxicity in Rats. J. Biochem. Mol. Biol., 39(6): 656-661.

Masubuchi Y, Nakayama S, Horie T (2002). Role of mitochondrial permeability transition in diclofenac-induced hepatocyte injury in rats. Hepatology, 35: 544-551.

Nagoshi C, Shiota S, Kuroda T, Hatano T, Yoshida T, Kariyama R, Tsuchiya T (2006). Synergistic effect of [10]-gingerol and aminoglycosides against vancomycin-resistant enterococci (VRE). Biol. Pharm., 29: 443-447.

Ohgo H, Yokoyama H, Hirose H, Kawabe H, Saito I, Tomita K, Hibi T (2009). Significance of ALT/AST ratio for specifying subjects with metabolic syndrome in its silent stage Diabetes and Metabolic Syndrome. Clin. Res. Rev., 3(1): 3-6.

Packer L, Cadenas E (2002). Oxidative stress and disease. In E. Cadenas and L. Packer, editors. Handbook of Antioxidants. Marcel Dekker,Inc., New ok, Basel, USA., pp. 5-8.

Park M, Jungdon B, Dae-Sil L (2008). Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria Phytother. Res., 22: 1446-1449.

Paul AD, Chauhan CK (2005). Study of usage pattern of nonsteroidal anti-inflammatory drugs (NSAIDs) among different practice categories in Indian clinical setting. Eur. J. Clin. Pharmacol., 60(12): 889-892.

Sadasivan S, Latha PG, Sasikumar JM, Rajashekaran S, Shyamal S, Shine VJ (2006). Hepatoprotective studies on *Hedyotis corymbosa* (L.) Lam. J. Ethnopharmacol., 106: 245-249.

Sarkar P, Mahmud MAK, Kaiyum-Mahmud MA (2011). Gingerol might be a sword to defeat colon cancer. Int. J. Pharm. Bio. Sci., 2(1): 816-827.

Schapira D, Bassan L, Nahir AM, Scharf Y (1986). Diclofenac-induced hepatotoxicity. Postgrad. Med. J., 62: 63-65.

Tjendraputra E, Tran VH, Liu-Brennan D, Roufogalis BD, Duke CC (2001). Effect of Ginger Constituents and Synthetic Analogues on Cyclooxygenase-2 Enzyme in Intact Cells. Bioorganic. Chem., 29: 156-163.

Toshiyasu K, Kaoru K, Kiyotaka K, Kunio T (1994). Anti-emetic principles of Magnolia obovata bark and Zingiber officinale rhizome. Planta Med., 60(1): 17-20.