

Effect of 6-shogaol and 6-gingerol on Diclofenac Sodium Induced Liver Injury

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Abstract: This study was designed to investigate the protective effect of 6-shogaol and 6-gingerol against hepatotoxicity induced by diclofenac sodium (DFS). Compounds 6-shogaol and 6-gingerol were isolated from 5% gingerol oleoresin and their potential hepatoprotective activity was evaluated. Hepatotoxicity was induced in rats by an intra-peritoneal (i.p.) injection of DFS (150 mg kg⁻¹). Rats were i.p. injected with 6-shogaol and 6-gingerol (10 mg kg⁻¹) for 6 days before induction of hepatotoxicity. Blood and liver sample were taken from each rat at 24 h post intoxication. Serum activity of liver marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed. Levels of alkaline phosphates enzymes (ALP) and total bilirubin in serum and malondialdehyde (MDA) in the liver homogenate were also estimated. Moreover, liver injury was assayed histologically. Results of present study revealed that i.p. injection of DFS to rats induced hepatic damaged that was manifested by significant (p<0.001) increase in the AST, ALT, ALP, total bilirubin in serum and MDA in liver homogenate. Histological data presented marked damaged in section of liver from DFS treated rats. I.p. injection of 6-shogaol to rats for 6 days before DFS-intoxication reversed these altered parameters near to normal control values. On the other hand, 6-gingerol had comparatively low hepatoprotective efficacy.

Key words: Shogaol, gingerol, diclofenac sodium, biochemical parameter, malondialdehyde, anti-hepatotoxicity

INTRODUCTION

NSAIDs are commonly used by more than 30% of developed countries (Laine, 2001). The common prescribed NSAIDs is ibuprofen; aspirin, diclofenac, paracetamol and piroxicam are mostly prescribed by orthopedic surgeon (76%) and least by general surgeon (39%) (Paul and Chauhan, 2005). NSAIDs are a leading cause of drug induced liver disease, for instance in Denmark between 1978 and 1987, approximately 9% of total liver grievance is related to these drugs (Lewis, 1989).

Recently cyclooxygenase-2 (COX-2) inhibitors were approved by the USA Food and Drug Administration (FDA) but due to liver dysfunction, some are already withdrawn from the market (Manov *et al.*, 2006). Hence it is recommended that people with liver disease avoiding using all NSAIDs (Shati and Elsaid, 2009). Diclofenac sodium is eminent to be foremost idiosyncratic hepatotoxic drugs (Kaplowitz, 2005) and is also

documented in experimental studies (Aydin *et al.*, 2003; Cantoni *et al.*, 2005; Amin and Hamza, 2005). The mechanism of DFS hepatotoxicity involves alteration of covalent protein by reactive metabolites (Gill *et al.*, 1995; Tang *et al.*, 1999), oxidative stress generation (Galati *et al.*, 2002) and mitochondrial injury (Masubuchi *et al.*, 2002). The drug-induced liver toxicity is reverted by antioxidants (Bort *et al.*, 1999). The effect of ginger has already reported against acetaminophen, mercuric chloride-induced hepatotoxicity (Ezeuko *et al.*, 2007; Verma and Asnani, 2007). Ginger has strong anti-oxidant components and may either mitigate or prevent generation of free radicals (Mallikarjuna *et al.*, 2008) and thus it is recommended for alcohol (Shati and Elsaid, 2009), drugs (Ezeuko *et al.*, 2007), induced liver injury and anti-diabetics (Karim *et al.*, 2011). 6-shogaol and 6-gingerol are similar to NSAIDs drug like properties (Mascolo *et al.*, 1989), with prominent COX enzymes inhibitor (Kim *et al.*, 2005, 2007) and dissimilar due to have antioxidant properties (Dugasami *et al.*, 2010).

6-shogaol is also additionally a, 5-lipoxygenase inhibitors (Flynn *et al.*, 1986) and may contribute to the increased intrahepatic vascular resistance of cirrhotic rat livers and prevented hepatotoxin induced necroinflammatory injury (Graupera *et al.*, 2002; Titos *et al.*, 2005). The carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes is proved that, the gingerols and shogaols wielded an intense antihepatotoxic activity (Hikino *et al.*, 1985).

MATERIALS AND METHODS

Chemicals: Standard 6-gingerol (CAS No. 23513-14-6) and 6-shogaol (CAS No. 555-66-8) were procured in June 2009, from Natural Remedies Pvt. Bangalore, India and maintained in refrigerator during 6 month study. 2-thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO). Diclofenac sodium tablets (Votrex®) was purchased from King Saud University, King Khalid Hospital, Pharmacy Centre, Riyadh Saudi Arabia. The i.p. injection samples were prepared in a saline solution. Sample solution was given in a volume of 1 mL 100 g⁻¹ body weight.

Isolation of 6-shogaol, 6-gingerol: The test compounds 6-Shogaol and 6-Gingerol were isolated from *Zingiber officinale* oleoresin (Batch No. ZO/09005, ≥30% Total gingerol) purchase from Natural Remedies Hosur road Bangalore, India-560100). Sample of 10 grams was proceeds in (110×4 cm) column using ethyl acetate and hexane solvent system. The extract was eluted with 500 mL of hexanes: ethyl acetate (90:20→70:30). Eluted fraction of 10 mL was collected in test tube using Foxy Jr.® Fraction Collector (Teledyne Isco, Inc 4700 Superior Street, Lincoln, NE 68504). By using preparative plate (Aluminum Sheet, Silica 60 F254, Merck) the shogaol and gingerol was further confirmed by standard. The all fraction were grouped in 1-6 according to TLC identification. Group 2 and group 5 were identified by TLC and pure compound was further achieved by packed reversed-phase absorbent (LiChroprep RP-18, 25-40 µm, Merck), columns (60×8 mm I.D.), using 100 mL of acetonitrile and water (90:10→10:90) solvent system. 5 mL of 60 fractions are further collected for 6-shogaol and 6-gingerol and identified using TLC plate on the basis of standard (Ficker *et al.*, 2003; Schwertner and Rios, 2007).

Animals: Wistar albino rats (≈150g) were obtained from the Animal House of experimental animal care centre, college of Pharmacy, King Saud University, Riyadh. The animals were maintained on Purina chow diet and water

adlibitum. Rats were housed in polycarbonate cages under constant temperature (22±2°C), humidity (55%) and 12 h light/dark condition. The experiments and procedures were performed according to the Ethical Committee of the College of Pharmacy, King Saud University, Riyadh.

Determination of hepatoprotective effect: The hepatoprotective effect was evaluated in rats using diclofenac sodium-induced liver injury (Hamza, 2007). Rats were randomly divided into five groups; each of five animals. Rats of the 1st (normal control) and 2nd (intoxicated control) groups received the vehicle in a dose of 5 mL kg⁻¹ body weight. Animals of the 3rd, 4th and 5th groups were treated with intraperitoneal injection of 6-shogaol, 6-gingerol and silymarin, respectively in a dose of 10 mL kg⁻¹ body weight for six consecutive days. After 6 h, of last treatments hepatotoxicity was induced in all groups (except normal groups) by i.p. injection of DFS at a dose of 150 mg kg⁻¹. After 24 h DFS injection all rats were killed under ether anesthesia, trunk blood was then collected and stored at -20°C until use. The ventral portion of the left lateral liver lobe were collected and fixed in 10% formalin and store at -80°C for subsequent malondialdehyde (MDA) and histopathological examinations.

Biochemical assays: The activity of Serum AST, ALT and serum alkaline phosphatase (ALP) were assay by using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE) while total bilirubin were estimated by reported methods (Thompson, 1969).

Malondialdehyde assays: The mean malondialdehyde (MDA) has been used as an indicator of lipid peroxidation of liver (nmol/mg), was determined in rats according to the previous method (Ibrahim *et al.*, 2009).

Histopathological studies: Small fragments of liver were fixed in 10% formalin solution (Alqasoumi *et al.*, 2009) and placed (3 time, 1 h each) in different concentration of ethanol (70 to 100%), xylene and finally to paraffin wax (4 time, 1 h each) and then transferred in to paraffin waxed filled moulds. The sections of liver prepared by rotary microtome (Leitz, 1512) were placed on clean slides, temperate (37-40°C), stained (Mayer's hematoxylin solution for 15 min), washed (lukewarm running tap water for 15 min, distilled water and 80% ethyl alcohol simultaneously for 2 min) then counterstained eosin-phloxine solution (2 min). The stained tissue slides were mounted and cover with cover slips and examined under light microscope.

Statistical Analysis: Data are expressed as Mean \pm SEM (n = 5). Data were analyzed using one-way analysis of variance (ANOVA) followed by dunnett's multiple comparison test and unpaired student's t-test. GraphPad Prism 5 software (San Diego, CA, USA) was used for all statistical analysis and significance (p<0.05) was considered statistically significance.

RESULTS

Serum biochemical's assays: Single dose of DFS significantly (p<0.001) elevated the AST and ALT activities when compared to the normal control animals. Treatment of 6-shogaol prior to DFS significantly protected the elevation of transaminases, ALP and bilirubin activities. The activities of AST and ALT in the 6-shogaol plus DFS treated group were 139.25 \pm 8.9 and 117.4 \pm 9.9 IU L⁻¹, respectively when compared with the intoxicated control 208.5 \pm 11.01 and 174.75 \pm 5.80 IU L⁻¹, respectively (Table 1). Similarly the activity of ALP and bilirubin were significantly (p<0.01) decreased in 6-shogaol plus DFS treated group (375.25 \pm 13.23 IU L⁻¹ and 1.26 \pm 0.04 mg dL⁻¹, respectively) than the intoxicated control group (518.5 \pm 20.06 IU L⁻¹ and 2.05 \pm 0.11 mg dL⁻¹, respectively). Silymarin treated animals also vetoed the induced AST, ALT, ALP and bilirubin with to 106.6 \pm 3.78,

105.5 \pm 6.44, 284.4 \pm 38.76 U L⁻¹ and 0.72 \pm 0.18 mg dL⁻¹, respectively. I.p. injection of 6-gingerol (10 mg kg⁻¹) once daily for 6 days prior to DFS, did not elicit any significant effect on the altered serum levels of AST, ALT, ALP and total bilirubin when compared with DFS-intoxicated group.

Malondialdehyde assays: The effect of 6-shogaol and 6-gingerol on the DFS-induced lipid peroxidation was examined through the observation of the levels of MDA in liver tissues. Hepatic MDA level was significantly (p<0.01) elevated in the intoxicated control group (294.87 \pm 7.40 nmol g⁻¹ tissue) than the normal animals (117.94 \pm 1.04 nmol g⁻¹ tissue). Silymarin (10 mg kg⁻¹, i.p.) prior treatment also prevented the DFS elevated MDA (160.2 \pm 14.20 nmol g⁻¹ tissue). Treatment of 6-shogaol and 6-gingerol were significantly (p<0.001 and p<0.05,

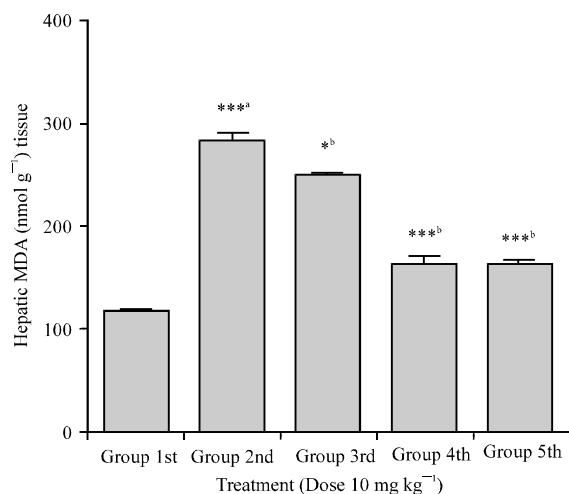


Fig. 1: Effect of 10 mg kg⁻¹ (i.p.) of 6-shogaol, 10-gingerol and silymarin on Level of MDA in DFS-intoxicated rats. Each column represent the Mean \pm SEM (n = 5). *p<0.05, ***p<0.001 compared to vehicle treated normal group (one-way ANOVA followed by dunnett's multiple comparison test), Where, ^a: Statistically significant compare to normal group and ^b: Statistically significant compare to DFS-intoxicated control group

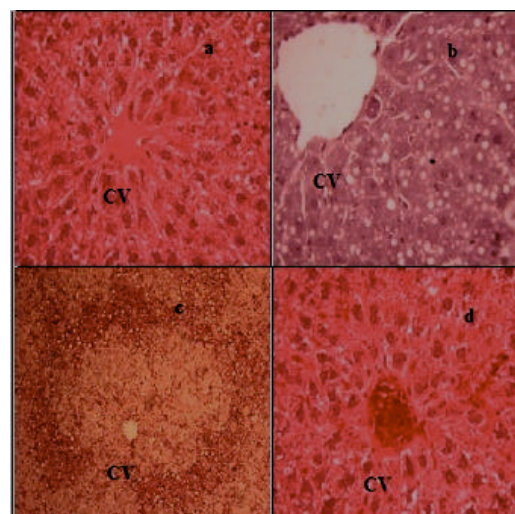


Fig. 2: Light micrographs of liver sections: Liver section from control rats appeared normal hepatic architecture in central area (Fig. 2a). Liver sections from DFS group in central area appeared necrosis and extensive early fatty change in the central parts, congested sinusoids with inflammatory cell infiltration (Fig. 2b). Liver section of DFS+6-gingerol group appeared peripheral fatty change and congestion appearance of hepatocytes (Fig. 2c). Liver section of rat treated with DFS+6-shogaol appeared normal appearance of hepatic cells with only some degrees of swelling and degeneration; CV (central area) (Fig. 2d). Haematoxylin and eosin stain H and E magnification x 400

Table 1: Effect of 10 mg kg⁻¹ (i.p.) of 6-shogaol, 10-gingerol and silymarin on serum activity of AST, ALT, ALP and bilirubin in DFS-intoxicated rats

| TREATMENT | AST(IU L ⁻¹) | ALT(IU L ⁻¹) | ALP(IU L ⁻¹) | Bilirubin (mg dL ⁻¹) |
|--------------------|-----------------------------|-----------------------------|------------------------------|----------------------------------|
| Normal | 73.03±4.39 | 26.4±2.45 | 225.00±16.83 | 0.51±0.02 |
| Control | 208.5±11.01 ^{a***} | 174.75±5.80 ^{a***} | 518.50±20.06 ^{a***} | 2.05±0.11 ^{a***} |
| DFS and 6-gingerol | 220.5±10.42 | 192.00±8.84 | 475.75±14.95 | 1.98±0.07 |
| DFS and 6-shogaol | 139.25±8.98 ^{a***} | 117.4±9.90 ^{b***} | 375.25±13.23 ^{b***} | 1.26±0.04 ^{b***} |
| DFS and Silymarin | 106.6±3.78 ^{a***} | 105.5±6.44 ^{b***} | 284.40±38.76 ^{b***} | 0.72±0.18 ^{b***} |

Values are expressed as Mean±SEM (n=5). **p<0.01, ***p<0.001 compared to vehicle treated normal group (one-way ANOVA followed by unpaired student's t-test), Where, ^astatistically significant compare to normal group and ^bstatistically significant compare to DFS-intoxicated control group

respectively) prevented the elevated MDA while compared with DFS-intoxicated group (Fig. 1).

Histopathological studies: The histopathological examination of liver of control and treated animals was summarized in Fig. 2. The liver showed central area necrosis, fatty change and sinusoids with inflammatory cell in DFS treated rats (Fig. 2b), peripheral fatty change and congestive appearance of hepatocytes in 6-gingerol treated group (Fig. 2c), while normal appearance of hepatic cells with some degree of swelling in 6-shogaol treated group (Fig. 2d).

DISCUSSION

Diclofenac sodium is a well-known anti-inflammatory, antipyretic and analgesic drug which is safe in therapeutic doses, but can produce serious hepatic necrosis in man and experimental animals with toxic doses (Hamza, 2007; Schapira *et al.*, 1986). The present study confirm previous study of a single dose (150 mg kg⁻¹, i.p.) of DFS injection induce hepatic damage in animals (Hamza, 2007). The hepatic injury causes leaking of cellular enzymes into the plasma due to the disturbance of hepatocytes transport functions. A variety of enzymes to be found normally in cytosol is discharge into the blood, when liver cell plasma is damaged, thereby causing increased enzyme levels in the serum. The serum enzymes are useful quantitative marker of hepatocellular damage estimation (Sadasivan *et al.*, 2006). In the existing investigation, the rats treated with of DFS developed significant hepatic injury which was observed through a substantial increase in the concentration of serum parameters. The ginger plant is well-documented for reduction of elevated transaminase in previous study but not documented for 6-gingerol and 6-shogaol as per my knowledge (Ajith *et al.*, 2007). Pre-treatment with 6-shogaol (10 mg kg⁻¹ b.w.x.), for 6 days was exhibited a significant hepatoprotective activity in reduction in serum activities of AST, ALT and ALP when compared to DFS-intoxicated rats. The rise in the levels of serum bilirubin in DFS-intoxicated is the conservative indicator of liver diseases (Sallie *et al.*, 1991), due to the inhibition of cytochrome P450 and promotion of its glucuronidation

(Cavin *et al.*, 2001). The noticeable elevation of bilirubin in the serum of intoxicated control group was significantly decreased in rats pretreated with 6-shogaol. DFS at hepatotoxic dose, increase MDA level (Hamza, 2007) and it has been shown that protective agents wield their action either through decreased production of free radical derivatives or due to antioxidant activity of the protective agent itself (Cantoni *et al.*, 2005). Silymarin, an antioxidant, routinely used for livoprotective drugs investigation, possibly acts as a free radical scavenger, protecting membrane permeability, lipid peroxidation inhibitor and preservation of the activity of total serum antioxidants (Najafzadeh *et al.*, 2010; Mansour *et al.*, 2006). The result of present study indicated that 6-shogaol was comparatively more significantly decreased the hepatic MDA than 6-gigerol may be due to dominant antioxidant properties (Dugasani *et al.*, 2010). Earlier data have mentions that DFS-induced hepatotoxicity cause perceptible changes in histopathological of liver of central area showing necrosis and degeneration of hepatocytes, congested sinusoids with inflammatory cell infiltration (Hamza, 2007). Present study was showed that 6-shogaol treated rats showed normal appearance of hepatic cells while 6-gingerol treated rats showed central necrosis diffuse and congestion peripheral fatty change. Inflammation and 5-lipoxygenase products of arachidonic acid metabolism have played a key role during acute hepatitis (Pithayanukul *et al.*, 2009). The inhibitory activities of 6-shogaol against inflammation (Mascolo *et al.*, 1989) and 5-LOX (Flynn *et al.*, 1986), could be an ameliorative factor in the protective effect of 6-shogaol for DFS-induced hepatotoxicity in rat. Silymarin and 6-shogaol both inhibited the 5-LOX enzymes (Agarwal *et al.*, 2006; Flynn *et al.*, 1986) and inflammation (Mascolo *et al.*, 1989). The inhibitory activities of 6-shogaol against 5-LOX (Flynn *et al.*, 1986) and inflammation (Mascolo *et al.*, 1989), could be an ameliorative factor in the protective effect of 6-shogaol for DFS-induced hepatotoxicity in rats.

CONCLUSION

The results of the present study concluded that 6-shogaol prevented the DFS-induced acute

hepatotoxicity by protecting serum marker enzymes, bilirubin and antioxidant activity similar to silymarin and supported due to antioxidant mechanism of action. However, further detailed studies are required to establish its clinical application.

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