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Protective role of *Zingiber officinale* Roscoe on Aceclofenac induced oxidative stress in rat liver

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ABSTRACT: Oxidative stress due to abnormal production of reactive oxygen molecules (ROM) is believed to be involved in the etiology of toxicities of many xenobiotics. In this study protective effect of *Zingiber officinale* (ZO) against Aceclofenac (ACE) induced oxidative stress in rat liver was evaluated. Twenty four young wistar rats were divided into four groups as follows: control group, ACE (single dose of 10 mg/kg body wt., i.p), ACE plus ZO (250 mg/kg) and ACE plus ZO (500mg/kg). The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione S-transferase (GST) ($p<0.001$) as well as the concentration of malondialdehyde, as an indicator of lipid peroxidation, were measured to evaluate oxidative stress in homogenates of the liver. ACE administration increased malondialdehyde levels ($p<0.001$) and decreased SOD, CAT, GSH, GPx and GST activities. Co-administration of ZO as a dose dependent manner (250 & 500 mg/kg) with ACE injections caused significantly decreased malondialdehyde levels as well as elevates SOD, CAT, GSH, GPx and GST ($p<0.05$ & $p<0.001$) activities in liver tissue when compared with ACE alone. It can be concluded that *Zingiber officinale* may prevent ACE-induced oxidative changes in liver by reducing reactive oxygen species.
Key words: Aceclofenac, Oxidative stress, *Zingiber officinale*

INTRODUCTION

Reactive oxygen species (ROS) are inevitably generated, due to the incomplete reduction of O_2 in electron transfer reactions, as byproducts of biological reactions. When ROS production is greater than the detoxification capacity of the cell, excessively generated ROS causes extensive damage to DNA, proteins and lipids and acts as a mediator of pro-inflammatory and carcinogenic events.[1] To avoid redox imbalance and oxidative damage, aerobic organisms possess efficient biochemical defense systems such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)[2], though it cannot completely protect them from severe oxidative stress. In this context, many scientists have tried to obtain dietary antioxidants such as ascorbate, tocopherol and carotenoids from fruits and vegetables, because they could help protect cells from cellular damage caused by oxidative stress.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the centerpiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases (OCT).[3] They are the most frequently used medications for the treatment of a variety of common chronic and acute inflammatory conditions, and continue to be important for the palliation of pain and in decreasing inflammation and fever.[4-8] Nearly all the NSAIDs have been implicated in causing liver injury, and tend to be hepatocellular in nature: the mechanism is thought to be immunological idiosyncrasy.[9,10] Recently, a number of in vitro animal models have been used to investigate the NSAID-related hepatotoxicity.[11,12]

Aceclofanac is a common and well established NSAID which is chemically designated as [{2-(2', 6'-dichlorophenyl) amino } phenylacetoxycertic acid], a phenyl acetic acid derivative. The mode of action of aceclofenac has been recently clarified in that the compound was shown to elicit preferential inhibition of COX2 as a result of limited but sustained

biotransformation to diclofenac.[13] Aceclofenac induced hepatotoxixity is almost always dangerous due to the diagnostic confusion.[14-17] It has been well known that aceclofenac have sever toxic effect on GI tract but no extensive research done till now.

Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. *Zingiber officinale* Roscoe, commonly known as ginger, is one of the commonly used spices in India and around the world. Ginger is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally and wlinary useful part.[18] Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platlet, antioxidant, anti-tumour, anti- rhinoviral, anti-hepatotoxicity and anti arthritic effect.[19-21]

The aim of this work was to establish the protective role of *Zingiber officinale* Roscoe on aceclofenac induced oxidative stress in rat liver.

MATERIALS AND METHODS

Drugs and Chemicals

Aceclofenac was obtained from Dey's Medical Stores (Mfg.) Ltd., 62, Bondel road, Kolkata-700019, India. All other reagents used for the experiments were of analytical grade.

Animals

Twenty four adult male white Wister albino rats, weighing 180-200g were used as experimental animals in this study. The animals were housed in the animals care centre of faculty of Pharmacy, Jadavpur University. They were kept in wire-floored cages under standard laboratory conditions of 12h/12h light/dark cycle, 25 ±2ºC with free access to food and water *ad libitum*. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India and approved by the Institutional Animal Ethics Committee (IAEC) of Jadavpur University.

Preparation of the plant extract

Rhizome of *Z. officinale* was purchased from the local market. The rhizome (500 g) were cut into small pieces and homogenized in a kitchen mixer using 50% ethanol (w/v). The homogenate was kept on water bath at 70–80°C for 10-15 h with intermittent shaking. The homogenate was centrifuged at 1500g for 10 min and the supernatant was collected. Solvent in the pooled supernatant was completely evaporated at low temperature using a water bath. The residue thus obtained (6.5 g, w/w) was used for the experiment.[22]

Study design

Young healthy twenty four male wistar albino rats weighing 180-200 g were divided into 4 groups each containing 6 animals. Group 1 Normal (supply standard laboratory food and water *ad libitum*), Group 2 (Control) treated with ACE (single dose of 10 mg/kg, i.p.), Group 3, 4 received ZO 250 and 500 mg/kg/day orally along with ACE. Total

Preparation of tissue homogenate

After the experimental period all the animals were sacrificed and liver tissue was washed with ice-cold saline. The tissue were then cut into fragments and homogenized with 3 volumes (w/v) of the appropriate buffer using a Potter-Elvehjam homogenizer with a Teflon pestle and centrifuged at 12000 g for 20 min at 4º C. The supernatant was used for the biochemical estimations.

Determination of lipid peroxidation

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in the tissues using the method of Ohkawa et al.[23] The pink chromogen produced by the reaction of secondary products of lipid peroxidation such as malondialdehyde (MDA) with thiobarbituric acid was estimated at 532 nm.

Determination of antioxidant enzymes

SOD was assayed according to the method of Kakkar et al.[24] The assay was based on the 50% inhibition of the formation of NADH-phenazine methosulfate nitroblue tetrazolium formazan at 520 nm. The activity of catalase was assayed according to the method of Sinha based on the conversion of dichromate in acetic acid to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm.[25]

Reduced glutathione (GSH) in the tissue was assayed according to the method of Ellman.[26] GSH estimation was based on the development of yellow colour when 5.5-dithiobisnitro benzoic acid was added to compounds containing sulfydryl groups.

Hepatic GST activity was assayed according to the method of Habig et. al. (1984) with some modifications.[27] GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2 O_2 and NaN_3 (Hafemann et. al. 1974) [28] . Protein was estimated by the method of Lowry et al. using bovine serum albumin as the standard.[29]

Statistical analysis

Data were expressed as mean ± SD, and all statistical comparisons were made by means of two-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. SPSS 10.0 software (SPSS Inc, 1999) was used for statistical analysis. The Difference showing a *P* level of 0.05 or lower was considered to statistically significant.

RESULTS

Effect of ZO on hepatic lipid peroxidation

The lipid peroxidation (LPO) level in liver was significantly increased ($p<0.001$) in ACE-treated animals when compared to normal. Treatment with ZO at 500 mg/kg showed significant ($p<0.001$) decrease in LPO level when compared to ACE-treated groups. Treatment with ZO at 250 mg/kg showed less significant ($p<0.05$) decrease in LPO level when compared to ACE-treated groups (Fig. 1).

Effect of ZO on antioxidant enzymes

The SOD and CAT activity in the liver tissue of normal and experimental animals were given in Fig. 2 & 3. SOD and CAT activity in liver of rats treated with ACE (group 2) were significantly ($p<0.001$) lowered compared with normal rats (group 1), whereas administering ZO to ACE-treated rats (group 3 & 4) significantly elevated their activity compared with ACE treatment alone (group 2).

The activities of GSH and glutathione-related enzymes such as GPx, and GST in the liver tissue of both the normal and experimental animals are given in Fig. 4-6. The levels/activities of GSH, GPx, and GST were significantly lowered in the liver of animals treated with ACE (group 2) compared with the normal rats (group 1). ZO at a dose of 500 mg/kg together with ACE treatment significantly ($p<0.001$) elevated the activities of GSH, Gpx, and GST in liver compared with those of the unsupplemented ACE-treated rats (group 2). Treatment with ZO at 250 mg/kg showed less significant ($p<0.05$) increase in GSH, GPx, and GST level when compared to ACE-treated groups.

DISCUSSION

Liver is the main organ responsible for drug metabolism and appears to be sensitive target site for substances modulating biotransformation.[30] During the course of aerobic metabolism, considerable amounts of reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are generated[31], which undergo a variety of chain reactions and produce free radicals such as $OH^{\cdot}$. These highly reactive species attack polyunsaturated fatty acids and thereby initiate the process of lipid peroxidation.[32] Resulting in oxygen damage which can further be manifested to degradation and inactivation of various important biomolecules.

Lipid peroxidation, a reactive oxygen species-mediated mechanism, has been implicated in the pathogenesis of various liver injuries and subsequent liver fibrogenesis in experimental animals and humans.[33] MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid (PUFA).[34] Therefore, the hepatic content of MDA is used as an indicator of liver tissue damage involving a series of chain reactions.[23] In the present study, a single dose of ACE at 10 mg/kg, resulted in a significant increase in the hepatic MDA concentration, indicating increased lipid peroxidation caused by administration of ACE (Fig. 1). The significant dose dependent decrease in the hepatic MDA concentration confirms that pretreatment with ZO could effectively protect against the hepatic lipid peroxidation induced by ACE.

SOD is a ubiquitous chain-breaking antioxidant found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays an important protective role against oxidative damage induced by reactive oxygen species. SOD converts superoxide ion (O_2^-) to hydrogen peroxide (H_2O_2) and the hydrogen peroxide thus formed is degraded by CAT and GPx. CAT is present in all major body organs of animals and humans and is especially concentrated in the liver.[35,36] The activities of SOD and CAT were significantly lowered in ACE treated rats as compared with those of the normal rats. ZO supplementation to the ACE-treated group elevated the SOD and CAT activity in the liver emphasizing the antioxidant and hepatoprotective activities of *Z. officinale*.

GSH is a tripeptide (L-γ-glutamylcysteinylglycine), an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification from reactive oxygen species, conjugation and excretion of toxic molecules, and control of the inflammatory cytokine cascade.[37] Depletion of GSH in tissues leads to impairment of the cellular defence against reactive oxygen species, and may result in peroxidative injury. The levels of GSH were significantly decreased in ACE-treated rats. Moreover, in addition to being a direct free radical scavenger, GSH is known to function as a substrate for GPx and GST. The activities of GPx and GST in this study were reduced on ACE treatment, which may be attributed to the unavailability of GSH. Administration of ZO to ACE-treated rats increased the levels of GSH and the activities of GPx and GST.

CONCLUSION

On the whole, it can be concluded that *Z. officinale* may protect from free radical induced oxidative injury caused by aceclofenac induced hepatotoxicity in animal model.

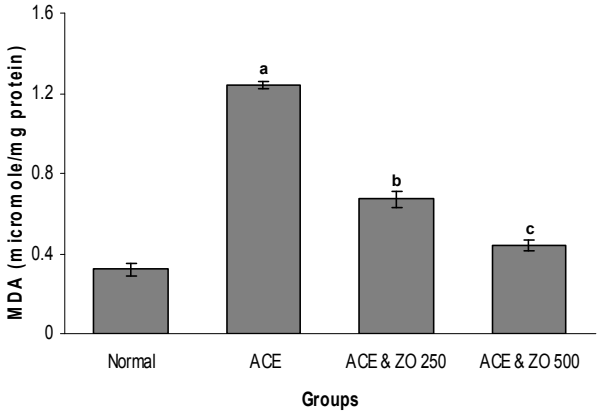

Fig. 1. Effect of ethanol extract Z. officinale (ZO) on hepatic MDA level in rat treated with Aceclofenac (ACE). Values are mean ± S.D., n=6 animals. [a]*p*< 0.001 significantly different from normal group. [b]*p*<0.05, [c]*p*<0.001 significantly different from ACE group.

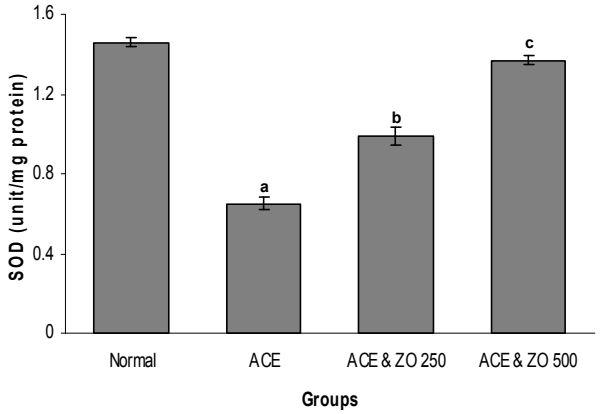

Fig. 2. Effect of ethanol extract Z. officinale (ZO) on hepatic SOD activity in rat treated with Aceclofenac (ACE). Values are mean ± S.D., n=6 animals. [a]*p*< 0.001 significantly different from normal group. [b]*p*<0.05, [c]*p*<0.001 significantly different from ACE group.

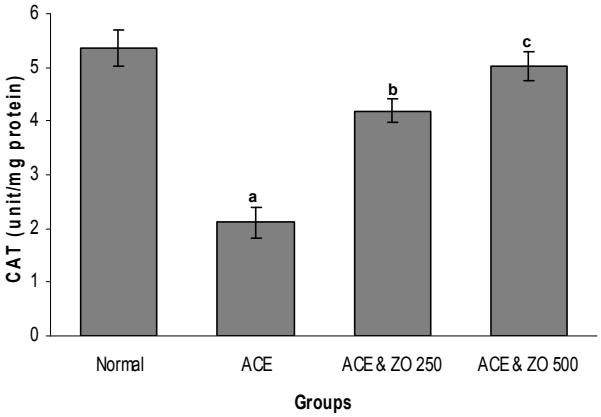

Fig. 3. Effect of ethanol extract Z. officinale (ZO) on hepatic CAT activity in rat treated with Aceclofenac (ACE). Values are mean ± S.D., n=6 animals. [a]*p*< 0.001 significantly different from normal group. [b]*p*<0.05, [c]*p*<0.001 significantly different from ACE group.

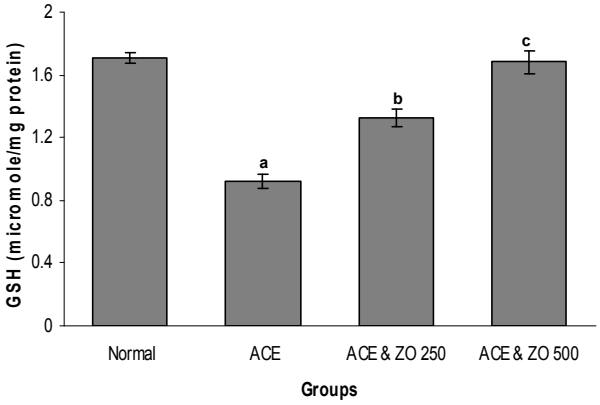

Fig. 4. Effect of ethanol extract Z. officinale (ZO) on hepatic GSH concentration in rat treated with Aceclofenac (ACE). Values are mean ± S.D., n=6 animals. [a]*p*< 0.001 significantly different from normal group. [b]*p*<0.05, [c]*p*<0.001 significantly different from ACE group.

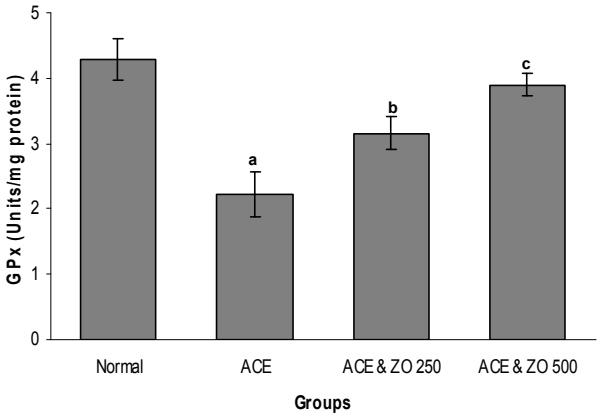

Fig. 5. Effect of ethanol extract Z. officinale (ZO) on hepatic GPx activity in rat treated with Aceclofenac (ACE). Values are mean ± S.D., n=6 animals. [a]*p*< 0.001 significantly different from normal group. [b]*p*<0.05, [c]*p*<0.001 significantly different from ACE group.

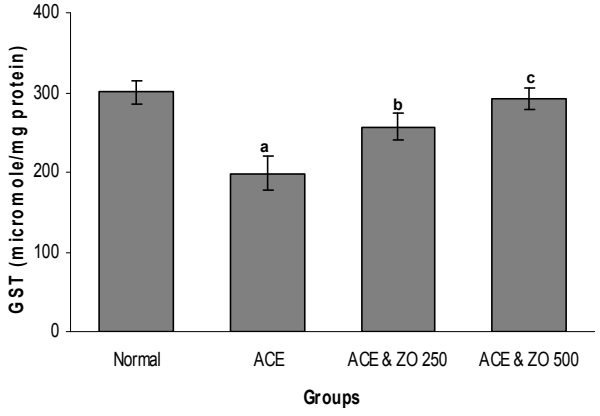

Fig. 6. Effect of ethanol extract Z. officinale (ZO) on hepatic GST activity in rat treated with Aceclofenac (ACE). Values are mean ± S.D., n=6 animals. [a]*p*< 0.001 significantly different from normal group. [b]*p*<0.05, [c]*p*<0.001 significantly different from ACE group.

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