Protective effect of ginger against alcohol-induced renal damage and antioxidant enzymes in male albino rats

K R Shanmugam, CH Ramakrishna, K Mallikarjuna, & K Sathyavelu Reddy*
Division of Molecular Biology and Exercise Physiology, Department of Zoology,
Sri Venkateswara University, Tirupati 517 502, India
\(^a\)Laboratory of Exercise Biochemistry, Taipei Physical Education College, Taipei 11153, Taiwan, ROC

Received 16 April 2009; revised 17 November 2009

Superoxide dismutase, ascorbic acid, glutathione and uric acid levels were decreased and xanthine oxidase, glutathione-s-transferase activities were increased in alcohol treated (2 g/kg body weight, once daily for 30 days) group. However, treatment with ethanolic extract of ginger (100 mg/kg, 200 mg/kg body weight, po, once daily for 30 days) these parameters came to normalcy showing the antioxidant effect of ginger. The antioxidant compounds of ginger may modulate the oxidative stress parameters. The biochemical findings were supplemented by histopathological examination of the kidney. Severe congestion and degenerative changes in tubules in alcohol treated rats were restored by ginger extract treatment. The results confirm the renal protective effect of ginger in alcohol treated rats.

**Keywords**: Alcohol, Antioxidants, Oxidative stress, Histopathology, Kidney, Rats

Alcohol, the most commonly consumed xenobiotic, generated reactive oxygen species (ROS) whether used over a long period of time or it is taken acutely in a single large dose in rats\(^1\). Liver is the primary organ responsible for the oxidation of ingested alcohol, but other tissues, including the kidney, may contribute to alcohol metabolism as well\(^2\). The biochemical changes induced by alcohol consumption in the kidney are not well understood, though some clinical and experimental studies have been focused on the effects of alcohol feeding on renal function including gross and microscopic morphology\(^3\).

The frequent association between chronic alcoholism and diverse hepatic lesions, grouped under the term alcoholic liver disease (ALD). A possible pathogenic role of free radicals and related oxidant species in ALD of human beings has become an area of intense research. Evidences are emerging in support of the hypothesis that habitual consumption of large amounts of alcohol has a variety of deleterious effects on the kidney that are independent of chronic liver disease\(^4\). Das and Vasudevan\(^5\) reported that not only kidney but liver was also affected in chronic alcoholic condition. However, the hepatorenal syndrome and the other abnormalities of renal function associated with advanced liver disease, as well as the mechanisms of alcohol associated-hypertension, including the possible role of the kidney in determining water and sodium retention, have already been reviewed\(^6,7\).

The alcohol oxidation by kidney is favored in alcohol-treated rats, thereby suggesting a pathogenic role for acetaldehyde in the nephrotoxic effect of alcohol ingestion. Regular alcohol consumption raises the blood pressure, which per se is a risk factor for renal damage\(^8\). Also, increased ROS, partly generated from acetaldehyde oxidation, may contribute to the occurrence of oxidative stress in kidney tissue\(^9\). Oxidative stress and ROS-mediated toxicity have been considered as the primary routes of alcohol-induced ultra structural changes in kidney\(^10\) and play a central role in the development of alcoholic related diseases\(^11\).

However, treatment of alcohol-induced renal oxidative injuries by antioxidant drugs has not received a wide recognition. In spite of the tremendous advances made in allopathic medicine, no effective renal protective medicine is yet available. Plant derived products have been used for medicinal purposes for centuries and also being used in our daily

---

*Correspondent author
Telephone: +91 877-2240265 (R) +91 877-2289304 Ext: 104 (O)
+91 98490 23339 (Mobile)
Fax: +91 877-2260531
E-mail: (KSR): sathyakreddy@hotmail.com
(KRS): krshanmugam@yahoo.co.in
food intake. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. Drugs of plant origin are known to play a vital role in the management of kidney diseases, and have protective effect against oxidative stress in rats\textsuperscript{12,13}. Focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems\textsuperscript{14}.

Ginger is one of the world’s best known spices, used since time immemorial for its health benefits. In Ayurveda, ginger has been recommended for use as carminative, diaphoretic, antispasmodic, expectorant, circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent and diuretic and digestive aid\textsuperscript{15,16}. The dried rhizomes of the herb are also used traditionally for a variety of human ailments. It is used to cure diarrhoea, dysentery, fever, cough, ulcers, boils, and wounds\textsuperscript{17}. Ginger has been used extensively in folklore medicine to treat common ailments\textsuperscript{16}.

So far, limited information exists concerning the beneficial effects of ginger against alcohol-induced oxidative injuries in kidney. Hence, the present study has been undertaken to evaluate the possible ameliorative effect of ethanolic ginger extract in alcohol-treated rats.

**Materials and Methods**

**Animals**—Wistar strain male albino rats weighing 180±20 g body weight were obtained from the Indian Institute of Sciences, Bangalore. The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (27°C± 2°C) with 12 : 12 h L:D photoperiod. The rats were given standard pellets diet (Lipton Rat Feed, Ltd., Pune) and water ad libitum throughout the experimental period.

The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CPCSEA/ dt.17.07.2001) in its resolution number 9/IAEC/SVU/2001/dt. 4.03.2002).

**Chemicals**—All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburgh, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

**Extraction of ginger**—THE fresh rhizomes of *Zingiber officinale* (Roscoe) were purchased locally, washed with water to make them free of soil and air dried. Air-dried rhizomes of the herb (2 kg) were milled into fine powder mechanically and extracted in cold percolation with 95% alcohol for 24 h. The extract was recovered and 95% ethanol was further added to the ginger powder and the extraction was continued. This process was repeated three times. The three extracts were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulted ethanolic extract was air-dried, finally measured to 80 g of dark brown, gelatinous matter. It was stored at -20°C and this crude ethanolic extract was used for the experiments. Dose equivalent to 100 mg and 200 mg of the crude drug per kg body weight of the animal was calculated and suspended in 2% Tween 80 (v/v) solution for the experiment as per Bhandari *et al*\textsuperscript{18}.

**Treatment**—The animals were divided into 6 groups of six rats each and treated as follows:

- **Group 1: Normal control (Nc)**: This group of rats received vehicle solution (2% of Tween 80).
- **Group 2: Ginger treatment (Gt\textsubscript{1})**: Rats received ethanolic extract of ginger (100 mg/kg body weight orally for 30 days).
- **Group 3: Ginger treatment (Gt\textsubscript{2})**: Rats received ethanolic extract of ginger (200 mg/kg body weight orally for 30 days).
- **Group 4: Alcohol treatment (At)**: Rats received alcohol orally at a dose of (2 g/kg body weight orally for 30 days as per the method of Mallikarjuna *et al*\textsuperscript{19}.
- **Group 5: Alcohol treatment + ginger treatment (At+Gt\textsubscript{1})**: This group of rats received both alcohol and ginger as described in group 2 and group 4 for 30 days.
- **Group 6: Alcohol treatment + ginger treatment (At+Gt\textsubscript{2})**: This group of rats received both alcohol and ginger as described in group 3 and group 4 for 30 days.

**Analytical procedures**—After completion of 30 days treatment the animals were sacrificed by cervical dislocation and the kidney tissue was excised at 4°C. The tissue was washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at -80°C for further biochemical analysis. Activities of superoxide dismutase (SOD)\textsuperscript{20}, glutathione-s-transferase (GST)\textsuperscript{21}, xanthine oxidase (XOD)\textsuperscript{22}, and levels of ascorbic acid (AA)\textsuperscript{23}, uric acid (UA)\textsuperscript{24}, tissue protein\textsuperscript{25}, glutathione (GSH)\textsuperscript{26} were measured.
Statistical analysis—The data were analyzed by using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel software for the significance of the main effects (factors), and treatments along with their interactions. The data were compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at $P < 0.001$.

Results
In alcohol treated rats, there was a significant reduction in SOD, GSH, AA, and UA levels (Fig. 1). The effects of ginger extract at doses of 100 and 200 mg/kg in alcohol treated group were significantly greater than that of control group. These parameters were reversed back to normal levels following ginger treatment. Activities of renal GST and XOD were elevated in alcohol treated group. However, with ginger treatment the activities came back to normal levels, indicating that treatment with ginger normalized the altered renal antioxidant enzyme levels as well.

Alcohol ingested rat kidney showed severe degenerative changes in tubules, diffused cellular infiltration, and severe congestion of blood vessels.

Fig. 1—Effect of feeding alcohol and alcohol+ginger extract on various biochemical parameters in male albino rats (a = superoxide dismutase, b = glutathione-s-transferase, c = xanthine oxidase, d= glutathione, e = ascorbic acid and f = uric acid. Values are mean±SD from 6 observations each. $P<0.001$ when compared with normal control (Dunnett's multiple comparison test)
Whereas, with ginger treatment in alcohol treated group, the kidney appears to be normal and all the renal cells which were damaged due to alcohol stress appeared to be regenerated (Figs 2–7).

**Discussion**

The etiology of free radicals in alcohol kidney disease is well established. The results of the present study show that supplementation of ginger may

![Photomicrograph of rat kidney following feeding of alcohol and alcohol+ginger extract](image)

Figs 2–7—Photomicrograph of rat kidney following feeding of alcohol and alcohol+ginger extract [2—Normal control (Nc) kidney showing, (a) renal parenchyma, (b) normal tubules, (c) normal glomeruli. 3—Ginger extract (Gt 1: 100 mg/kg) treated kidney showing, (a) normal glomeruli, (b) normal tubules, (c) normal glomeruli. 4—Ginger extract (Gt 2: 200 mg/kg) treated kidney showing, (a) Normal glomeruli, (b) normal tubules. 5—Alcohol treated (At) kidney showing, (a) severe congestion of blood vessels, (b) necrosis of the renal cell, (c) severe degenerative changes in tubules, (d) damaged glomeruli. 6—Alcohol treated +Ginger extract treated (At+Gt1) (100 mg/kg) kidney showing, (a) regeneration of glomeruli, (b) blood clotting, which shows that regeneration of renal cells, (c) regeneration of tubules. 7—Alcohol treated +ginger extract treated (At+Gt2) (200 mg/kg) kidney showing, (a) glomeruli appears to be restored, (b) congestion of blood showing regeneration of renal cells (c) regeneration of tubules]. (H×E 20×).
protect the renal cells and reduces the severity of damage due to alcohol toxicity.

SOD activity was decreased with alcohol consumption in liver, heart, brain, kidney, muscle and serum of rats. The reduced activity of SOD in presence of alcohol may cause the accumulation of O$_2^-$, H$_2$O$_2$ or the products of its decomposition. The SOD activity was elevated in rats dosed with ginger extract alone (groups 2 and 3) and also in ginger plus alcohol treated rats (groups 4 and 5). This elevation may be due to the presence of antioxidant bioactive compounds in ginger. The antioxidant compounds, like gingerols, shogals, ketone compounds and the phenolic compounds of ginger were responsible for scavenging the superoxide anion radicals.

The increase in the GST activity may be correlated with the observed decrease in GSH level of the kidney as a response to the alcohol consumption. The increased GSH participation in conjugation reaction mediated by increased GST activity seems to be a plausible model for the reduced GSH level due to the long term alcohol exposure. Increased GST activity suggests its activation due to oxidative stress. Das and Vasudevan, in their dose dependent alcohol studies observed increased GST activity. However, Ahmed et al. reported that GST activity was decreased with ginger treatment. The stimulation of GST due to ginger feeding in liver, lungs, and kidney indicates that ginger feeding can confer protection against the toxic effect of xenobiotics. The increase in GST activity in all these tissues further support the hypothesis that regular intake of ginger can enhance the activity of phase II detoxification enzymes.

The xanthine oxidase activity was significantly elevated with alcohol treatment in rats. Abbondanza et al. reported an increase in the XOD activity after repeated alcohol administration and an increase in the intermediate XDH/XOD after prolonged alcohol feeding. The acute administration of alcohol significantly increased the plasma xanthine oxidase activity in both rats and hamsters. An over-production of free radicals through the intervention of xanthine oxidase after alcohol administration could also result from substrates different from acetaldehyde and have greater affinities for xanthine oxidase. However, with ginger treatment in alcohol treated groups XOD activity was decreased. This may be due to the counter action of ginger compounds like gingerols, shogals and other pharmacological compounds of ginger as they have the capacity to reduce the free radical toxicity. Therefore in alcohol treated group with ginger treatment XOD activity was decreased by ginger supplementation.

In the present study, low level of GSH was observed in alcohol treated group. The long-term alcohol exposure increases enzyme activities related to the recycling and utilization of glutathione in the kidney. Earlier reports also stated that GSH level was decreased in kidney tissue of alcohol treated rats. Administration of alcohol induces lipid peroxidation and depletes GSH reserves, but there are events that occur after the formation of alcohol metabolites. The reactive oxygen intermediates generated during the metabolism of alcohol leads to GSH oxidation, resulting in the depletion of GSH. However, with ginger treatment to alcohol ingested rats, GSH levels were increased. Ahmed et al. explained that ginger exerts an antioxidant effect by decreasing lipid peroxidation, increasing GSH level and maintaining normal levels of antioxidant enzymes. Treatment of ginger ethanolic extract (250 mg/kg) showed protective effect in cisplatin-induced nephropathy in rats by enhancing the antioxidant enzyme activities including GSH level. The increased GSH level with ginger treatment in alcohol treated rats are in agreement with the earlier reports.

As a scavenger of ROS, ascorbate has been shown to be effective against the superoxide anion radical, hydrogen peroxide, the hydroxyl radical, and singlet oxygen. The observed decrease in the level of kidney ascorbic acid in alcohol treated group only could be as a result of increased utilization of this antioxidant in scavenging the free radicals generated during acute alcohol intoxication. In the present study with ginger treatment in alcohol treated group, ascorbic acid level was increased. This may be due to the influence of ginger compounds on the ROS which were produced during alcohol metabolism. Thus, ginger may exert a beneficial effect in countering the toxic free radicals in the kidney.

Uric acid is the most abundant aqueous antioxidant, particularly effective in quenching the hydroxyl and superoxide anion radicals and its antioxidant property was first reported by Howell and Wyngaarden. In the present study, uric acid levels were decreased in the kidney tissue of alcohol ingested rats. This may be due to inhibition of adenine nucleotide turnover. Another possibility is, that high amount of uric acid may have been utilized for scavenging the free radicals which are...
generated during alcohol intoxication. Reactive oxygen species react with lipids and cause peroxidative changes that result in elevated lipid peroxidation. The increased lipid peroxidation with alcohol may be an indication of a decrease in non-enzymatic antioxidants of defense mechanisms. Pushpalatha\textsuperscript{45} reported that with alcohol treatment cardiac uric acid levels were decreased. The decreased uric acid levels in the alcohol treated group may be due to alterations in the catabolism of purines. Serum uric acid level is known to be increased by alcohol via alcohol induced activation of adenine nucleotide turnover, which was triggered by the acetate formed from alcohol\textsuperscript{46}. However, with ginger treatment to alcoholic group, uric acid level was increased. This may be due to the reduction of ROS and free radicals’ deleterious effect by ginger feeding in alcohol treated rats.

The present study was exclusively carried out to know the impact of ginger on kidney antioxidant defense system enzymes and protective role of ginger on kidney. Liver antioxidant enzymes and hepatic marker enzymes like AAT, ALAT were also estimated. The antioxidant enzymes like SOD, XOD activities and ascorbic acid, glutathione levels were decreased in the liver of alcohol treated group, and with ginger treatment these parameters were reversed back to normal levels. The hepatic markers like AAT and ALAT levels were increased in alcohol treated group, whereas with ginger treatment the same were decreased (unpublished data).

Histopathological analysis of group 1 (Nc) kidney tissue revealed appreciably normal kidney section and healthy renal cells. However, in alcohol treated (At) group the glomeruli and tubules were damaged. Renal cells and renal parenchyma were also damaged. Glomeruli and tubules appeared to be regenerated following ginger treatment in alcoholic groups 5 and 6 (At+Gt\textsubscript{1,2}). The administration of ginger mops up free radicals generation by alcohol metabolism, and is responsible for the healthy state of renal cells, suggesting ginger’s renal protective effect in alcohol treated rats.

To conclude, the results confirmed that alcohol-induced renal toxic effect may be due to free-radical mechanism and provide evidence that ginger significantly protects the renal cell and reduces the severity of damage caused by alcohol intoxication. However, further detailed studies are required to establish its clinical application.

Acknowledgement
KSR is thankful to UGC, New Delhi for financial support.

References