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Full Length Research Paper

Role of ginger extract and N-acetylcysteine in acute renal tubular necrosis: Histological, immunohistochemical and gene expression study in rats

Mohamed M. Yousef[1], Naglaa F. Alhusseini[2*], Hamdy A. Mohamed[1], Rania Eldesoky[1] and Mohamed M. Zaki[1]

[1]Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Egypt.
[2]Department of Medical Biochemistry and Molecular Biology Unit, Faculty of Medicine, Benha University, Egypt.

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Acute renal failure (ARF) is a syndrome associated with high mortality rate in humans. ARF now known as acute kidney injury (AKI) represents a significant and devastating problem in clinical medicine. This work was performed on adult male albino rats to study the possible protective effects of ginger which is considered as one of the promising medicinal plants and N-acetylcystiene on acute tubular necrosis induced by glycerol. Sixty adult male Albino rats divided into 5 groups each were included in this study. Kidney tissue specimens were processed for histological study; hematoxylin and eosin (H & E), immunohistochemical staining for caspase3 and mRNA gene expression study by real time polymerase chain reaction (RT-PCR) through the assessment of catalase (CAT) and glutathione peroxidase (GPx1) genes. The renal CAT gene expression was found to be decreased in the glycerol group (affected group). Administration of ginger extract and N-acetylcystiene separately or combined before glycerol significantly increased the CAT and GPx1 gene expression. We concluded that the administration of oral ginger extract and N-acetylcystiene separately and combined had prophylactic role in ARF. Also the combination of both had synergistic effect.

Key words: Ginger, N-acetylcysteine, renal failure, glutathione peroxidase, catalase gene.

INTRODUCTION

Natural products and their active principles are sources for new drug discovery and treatment of diseases have attracted attention in recent years. Medicinal use of spices/herbs has been gradually increasing in developed countries (Ajith et al., 2008). Ginger, (*Zingiber officinale* Rosc.), a herbaceous perennial of the family Zingiberaceae

*Corresponding author. E-mail: NAGLA.ALHUSSEINI@fmed.bu.edu.eg.

is an important export-oriented spice crop with high medicinal value. Ginger is completely sterile and is propagated exclusively by vegetative means using rhizome (Nair and Thomas, 2013). Ginger has been recognized to have potent antioxidant properties being an effective forager of superoxide radicals which has been regarded as a promising protective mechanism against stress (Talpur et al., 2013). Ginger is reported to be a carminative, diaphoretic, antispasmodic, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent in addition to being useful in treating cold, headaches, arthritis, rheumatological conditions, and muscular discomfort. Studies have shown that ginger possesses antimicrobial, antischistosomal, anti-inflammatory, antipyretic, hypoglycemic hepatoprotective, diuretic and hypocholesterolemic effects (Haniadka et al., 2012).

N-acetylcysteine (NAC) is an N-acetylated derivative of the naturally occurring amino acid cysteine (Gass and Olive, 2008). NAC is a powerful antioxidant and a scavenger of hydroxyl radicals. Recently, NAC has also been shown to have anti-inflammatory activities in tissues (Uraz et al., 2013). The administration of NAC, as antioxidants, has been shown to provide partial protection against myoglobinuric-induced acute renal failure (Meeran et al., 2012). NAC has also been used to ameliorate inflammatory response in lipopolysaccharide-induced acute lung injury in rats and in an acute pan-creatitis experimental model (Luo et al., 2008). ARF is a syndrome associated with high mortality in humans. ARF now known as AKI represents a significant and devastating problem in clinical medicine (Adedapo and Oyekan, 2013).

Current therapy is limited to supportive measures and preventive strategies, none of which have been definitively shown to alter mortality (Bonventre and Weinberg, 2003). Oxidative stress as the result of increased free radical generation has been implicated in the pathogenesis of many diseases including several renal diseases, such as ARF induced by glycerol, gentamicin, cisplatin and cyclosporine. Free radical generation and the resulting oxidative stress have been reported as one mechanism leading to the development of ARF and organ injury. Therefore, interventions favoring the scavenging and/or depuration of reactive oxygen species (ROS) by dietary and pharmacological antioxidants should attenuate or prevent oxidative stress, thereby protecting against subsequent renal damage. Also, it has been reported that several antioxidants have protective effects in renal damage against oxidative stress (Yousefipour et al., 2010). Glycerol is used for the induction of ARF in vivo. Intramuscular administration of hypertonic glycerol is the most common used animal model of myoglobinuric ARF, characterized by a considerable reduction in renal blood flow and GFR. It is reported that the acute volume depletion models of glycerol-induced ARF are more closely related to the syndrome of ARF in human beings than the chronic dehydration model (Wang et al., 2011). This work was performed on adult male albino rats to study the possible protective effects of ginger which is considered as one of the promising medicinal plants and N-acetylcystiene on acute tubular necrosis induced by glycerol. We clarified the mechanisms of these effects through the assessment of catalase (CAT) and glutathione peroxidase (GPx1) genes expression

MATERIALS AND METHODS

The present study was performed on 60 adult male Albino rats, aged 8 to 10 weeks and with an average weight of 180 to 200 g obtained from the animal house, Moshtohor Faculty of Veterinary Medicine, Benha University. Strict care and cleaning measures were utilized to keep the animal in a normal healthy state; the animals were kept in animal cages under the prevailing atmospheric conditions and on normal balanced diet and tap water. Animals were divided into 5 groups, each 12 animals as follows:

Group I (Control group): Without any treatment.
Group II (Affected group): Glycerol (50%, 8 ml/kg, IM) was administered to rats intramuscular distributed equally in both hind limbs in one dose (Kim et al., 2010).
Group III: Each animal was given oral daily dose of ginger extract (400 mg/kg/day) for two weeks, then single injection of glycerol intramuscular distributed equally in both hind limbs in one dose.
Group IV: Each animal was given oral daily dose of N-acetylcysteine (10 mg/kg/day) for two weeks then single injection of glycerol intramuscular distributed equally in both hind limbs in one dose. (Meeran et al., 2012).
Group V: Each animal was given oral daily dose of ginger extract (400 mg/kg/day) (Shanmugam et al., 2011) and oral daily dose of N-acetylcysteine (10 mg/kg/day) for two weeks, then single injection of glycerol intramuscular distributed equally in both hind limbs in one dose.

After 24 h from the glycerol injection, the rats of all groups were scarified; kidney specimens and blood samples were taken. The kidney specimens were processed for histological study; H & E (Gamble, 2008), Immunohistochemical staining for caspase3 (Silverberg et al., 2006) and gene expression study by real time polymerase chain reaction (RT-PCR) through the assessment of CAT and glutathione peroxidase (GPx1) genes expression**.** Blood samples were obtained from the heart for measurement of serum levels of urea (mg dl) and creatinine (mg dl) using the commercially available kits (Park et al., 2012).

Assessment catalase (CAT) and glutathione peroxidase (GPx1) genes expressions

Tissue handling

Kidney biopsies were taken and immediately placed in Cryo tubes and stored in RNA later solution (Qiagen, GmbH) 10 µl / mg of tissue at -80 °C for further processing. Total RNA extraction was done by using total RNA Purification Kit from Jena Bioscience GmbH, according to the manufacturer instructions with about 30 mg tissue collected in a micro centrifuge tube and then 300 ml of lysis buffer containing 2ME (2 Mercapto Ethanol) which was homogenized using rotor Tissue Rupror (Qiagen, GmbH).

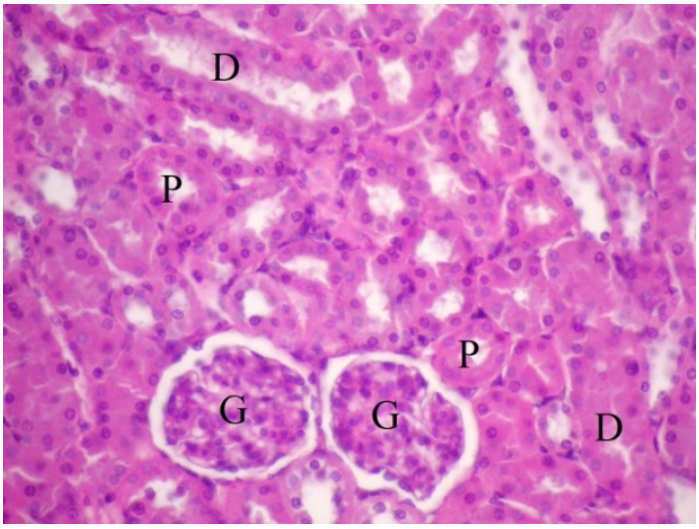

Figure 1. Photomicrograph of a section in renal cortecomedullary region of an adult male albino rat from group I (control group) showing renal glomeruli (G), proximal convoluted tubule (P) and distal convoluted tubules (D) (H & E × 400).

Spectrophotometric quantification of RNA

The absorbance of Nanodrop spectrophotometer (USA) was measured at A_{260} and A_{280}. Concentration of RNA sample was measured at 44ug/ml A_{260} (Wilfinger et al., 1997). The ratio of the reading at A_{260}/ A_{280} provides am estimate of the purity of RNA. Pure RNA has an A_{260}/A_{280} ratio of 1.9 to 1.3.

Two steps RT-PCR

1st step: Template RNA (5ul) and distilled water (15 ul) were added to Maxine RT pre mix tube. cDNA synthesis (Reverse transcription) reaction using G-storm thermalcycler (Ingland) was performed at a temperature of 45°C for 60 min followed by RTase inactivation step at 95°C for 5 min . This reactant was diluted by adding 30 ml nuclease free water.

2nd step: RT-PCR was done using ABI 7900HT fast real time PCR (applied Biosystem USA), the prepared reaction components were done in 96 well PCR plate (micro Amp ® 90 well optical reaction plate with Barcode, code 128). Single plex reaction was done using qPCR Green Master from (Jena Bioscience GmbH), using real time cycler conditions of 95°C and 5 min (Initial denaturation), followed by 35 cycles of 95°C, 30 s, 55°C, 1 min and 72°C, with 30 s for denaturation, annealing and extension steps, respectively. The primer sequences were from (5'-3') for all genes, CAT gene forward 5' – CCG ACC AGG GCA TCA AAA - 3' reverse 5'- CCC TGA GTT TAG CCT TCC TTT TG - 3', GPx1 forward 5'- ATG TCT GCT GCT CGG CTC TC -3' reverse 5'- GTT GCT AGG CTG CTT GGA CAG -3'and β-actin as endogenous control forward 5'- CCC ATT GAA CAC GGC ATT G -3' reverse- GTA CGA CCA GAG GCA TAC A -3'.

Data analysis

According to the RQ manager program 1.2 ABI SDS software (ABI 7900HT), the data are produced as sigmoid shaped amplification plots in which the number of cycle is plotted against fluorescence (when using linear scale). Because the samples of control group are used as calibrators, the expression levels are set to 1. But because the gene expression levels were plotted as log10 values (log10 of 1 is 0), the expression level of the calibrator samples appear as 0 in the graph. Because the relative quantities of the CAT or GPx1 genes are normalized against the relative quantities of the endogenous control β-actin gene fold expression changes are calculated using the equation $2^{-\Delta\Delta CT.}$

Statistical analyses

The mean area percentage of caspase-3 expression was quantified in 12 images for each group using Image- Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA). Differences in caspase-3 expression between the ginger and N-acetylcystiene separately or combined groups (III, IV and V) and the affected group (II) were assessed using the *F*-test, with $P < 0.05$ as the level of statistical significance. The differences in results of serum creatinine and urea between the ginger and N-acetylcystiene separately or combined groups (III, IV and V) and the affected group (II) were assessed using the *F*-test, with $P < 0.05$ as the level of statistical significance. Statistical analyses were carried out using Microsoft excel 2010 (Microsoft Egypt, Cairo, Egypt).

RESULTS

Biochemical results

Serum creatinine and urea levels in all groups are shown in Table 1. The levels of serum creatinine and urea were highly elevated significantly in the group II (affected group). Administration of ginger extract and N-acetylcystiene separately or combined before glycerol challenge significantly lowered the elevated levels of serum creatinine and urea ($p < 0.05$) compared to the affected group.

Table 1. Mean ± SD of serum urea (mg/dl) and creatinine (mg/dl) in all groups.

Parameter	Group I	Group II	Group III	Group IV	Group V	P value
	Mean ± SD					
Urea	21.3 ± 1.88	65.9 ± 5.19	30 ± 2.05	33.5 ± 2.46	26.5 ± 1.58	III: 0.0108* IV: 0.0363* V: 0.0015*
Creatinine	0.69 ± 0.11	2.17 ± 0.2	0.9 ± 0.08	1.19 ± 0.099	0.89 ± 0.073	III: 0.0134* IV: 0.0489* V: 0.0065*

*P<0.05, SD: standard deviation.

Table 2. Mean ± SD of the mean area percentage of caspase-3 expression in all groups.

Parameter	Group I	Group II	Group III	Group IV	Group V	P value
	Mean ± SD					
caspase-3 expression	0	0.1413 ± 0.005	0.0409 ± 0.0118	0.0841 ± 0.001	0.0233 ± 0.0019	III:0.088* IV:0.0636 V:0.00 8*

*P<0.05, SD: standard deviation.

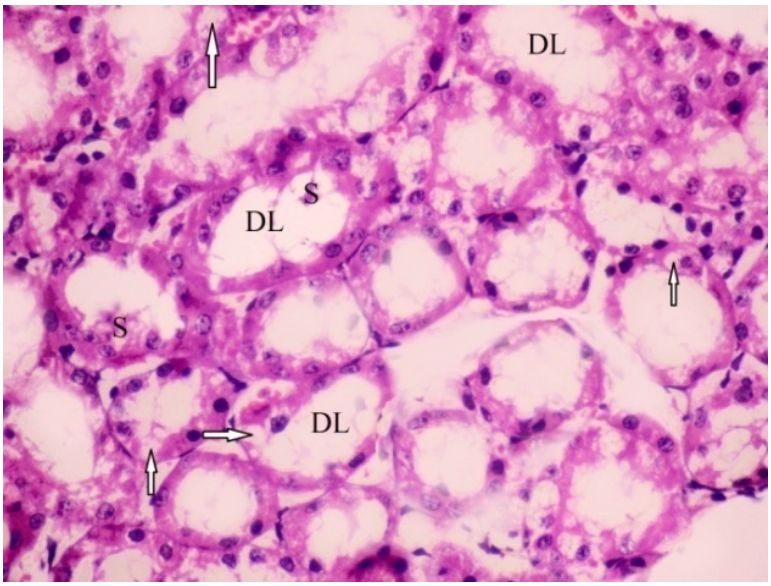

Figure 2. A photomicrograph of a section in renal cortecomedullary region of an adult male albino rat of Group II (Glycerol group) showing marked vacuolations (white arrow) in renal tubular cytoplasm with apparent dilatation (DL) of their lumen. Cellular debris (S) were observed in some renal tubules (H&E ×400).

Hematoxylin and eosin stain

The control group (group I), showed normal histological structure of both renal corpuscles and tubules; proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) (Figure 1). In glycerol group (group II), most of

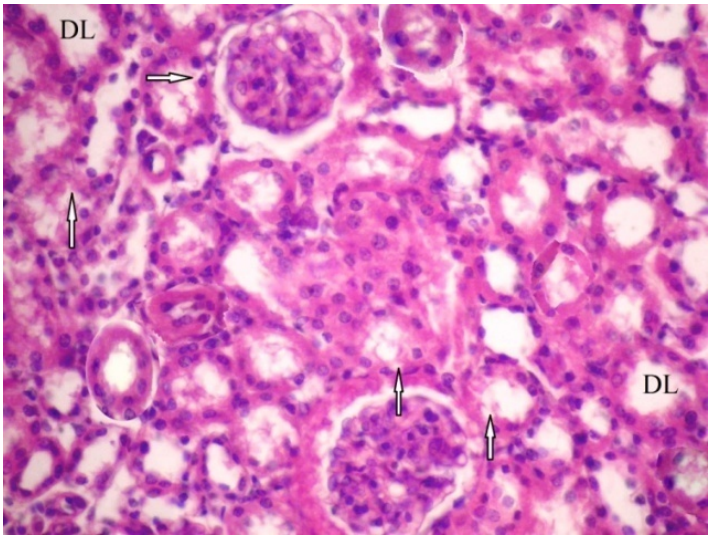

Figure 3. A photomicrograph of a section in renal cortecomedullary region of an adult male albino rat in Group III (Ginger group) showing mild vacuolations (white arrow) in tubular cell cytoplasm with slight dilatation (DL) in some tubular lumen. (H&E X 400)

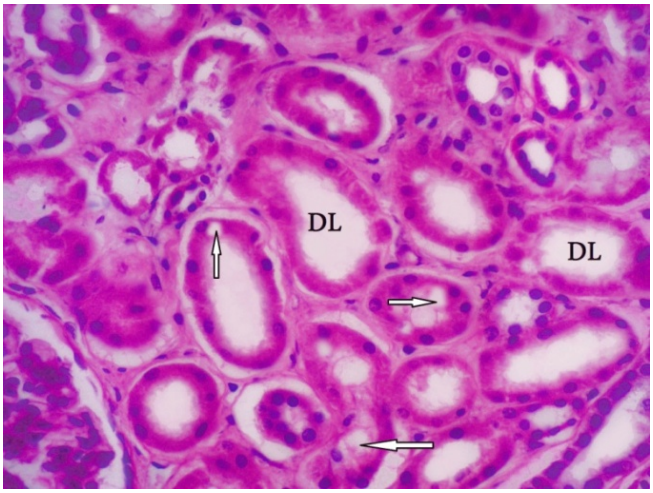

Figure 4. A photomicrograph of a section in renal cortecomedullary region of an adult male albino rat in Group IV (N-acetylcystiene group) showing moderate vacuolations (white arrow) in tubular cell cytoplasm with dilatation (DL) in some tubular lumen (H&E ×400)

the cells of renal tubules showed marked vacuolations in their cytoplasm with marked dilation of their lumens and there were desquamation of some cells and presence of cellular debris in lumen of some tubules (Figure 2). The ginger group (group III) showed mild vacuolations with slight dilatation of some renal tubules (Figure 3). N-acetyl cycteine group (group IV) showed moderate vacuolations with dilatation of some renal tubules (Figure 4). Ginger and N-acetyl cycteine group (group V) showed minimal vacuolations in few tubules (Figure 5).

Immunohistochemical staining for caspase3

It demonstrated brown cytoplasmic staining (index for degree of nuclear apoptosis). The control group showed negative cytoplasmic reaction for caspase-3 (Figure 6). Glycerol group showed highly (++++) expressed caspase-3 reaction in cytoplasm of the cell lining of PCT

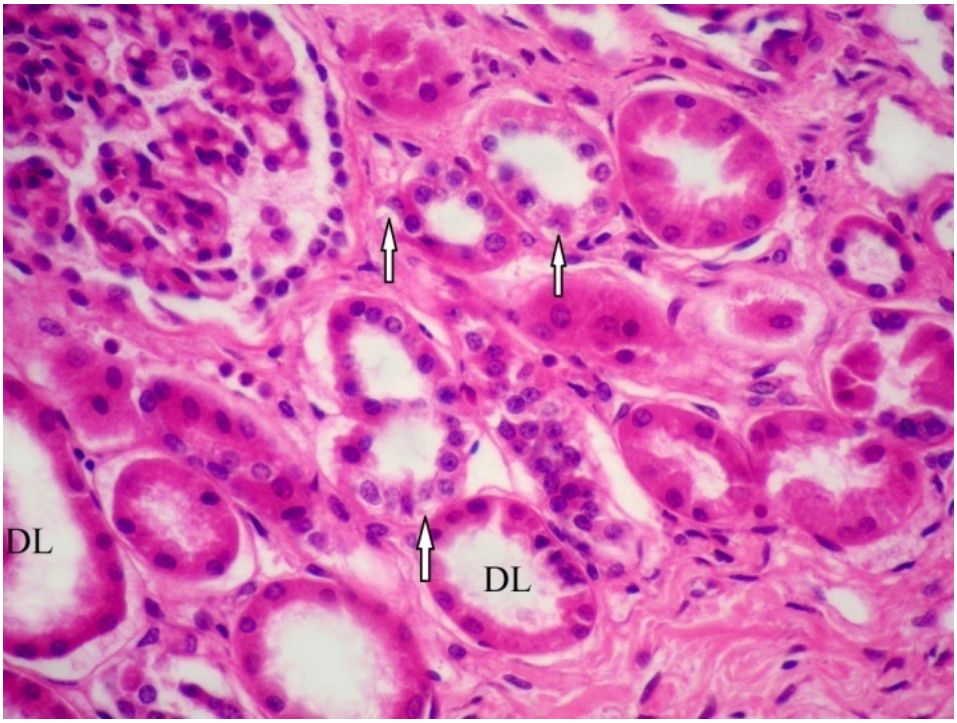

Figure 5. A photomicrograph of a section in renal cortecomedullary region of an adult male albino rat in Group V (Ginger and N-acetyl cycteine group) showing minimal vacuolations (white arrow) in few tubular cell cytoplasm with slight dilatation (DL) in some tubular lumen (H&E ×400).

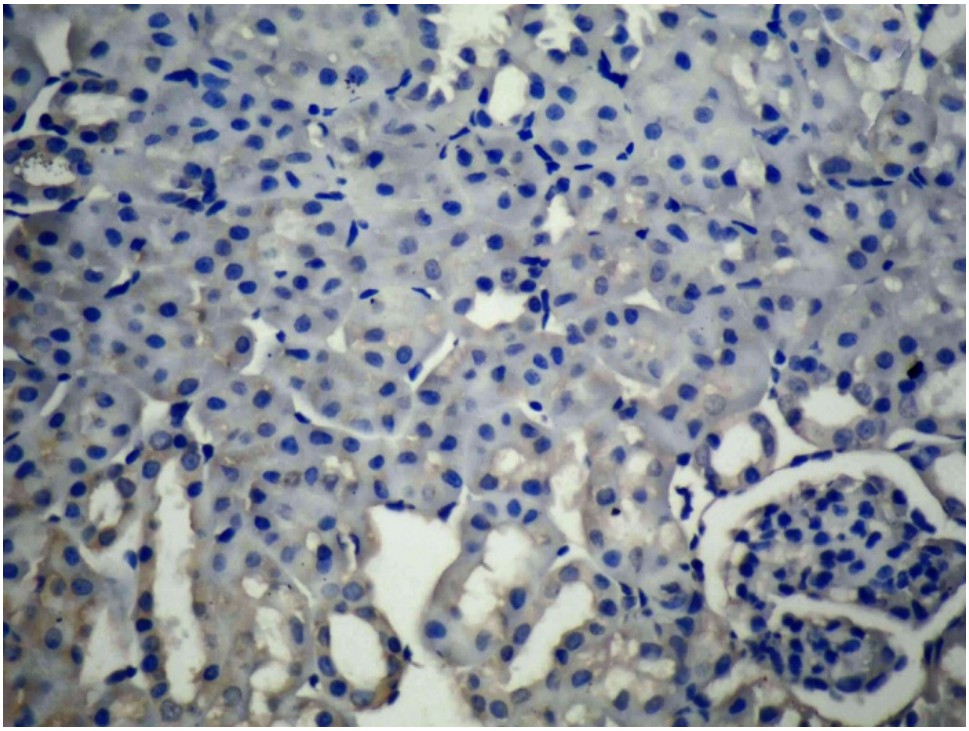

Figure 6. A photomicrograph section in renal cortecomedullary region of an adult male albino rat in group I (Control group) showing negative expression of Caspase-3 in the cytoplasm of cells of PCT and DCT (Avidin Biotin peroxidase for caspase-3 ×400).

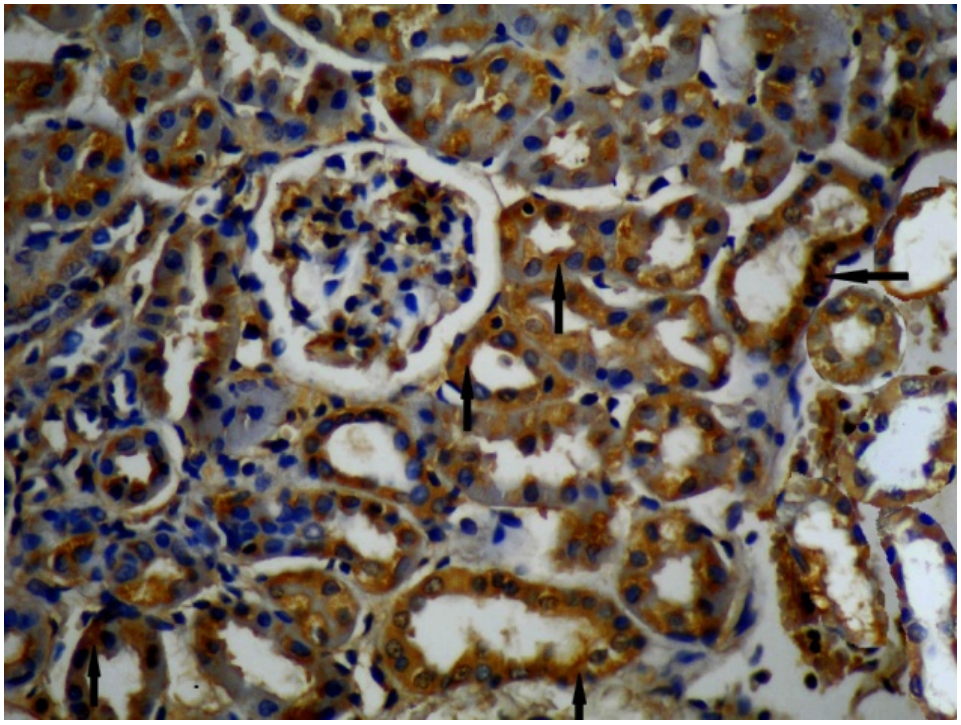

Figure 7. A photomicrograph section in renal cortecomedullary region of an adult male albino rat in group II (Glycerol group) showing high Caspase-3 expression (black arrow) in the cytoplasm of cells of PCT and DCT. (Avidin Biotin peroxidase for caspase-3 ×400).

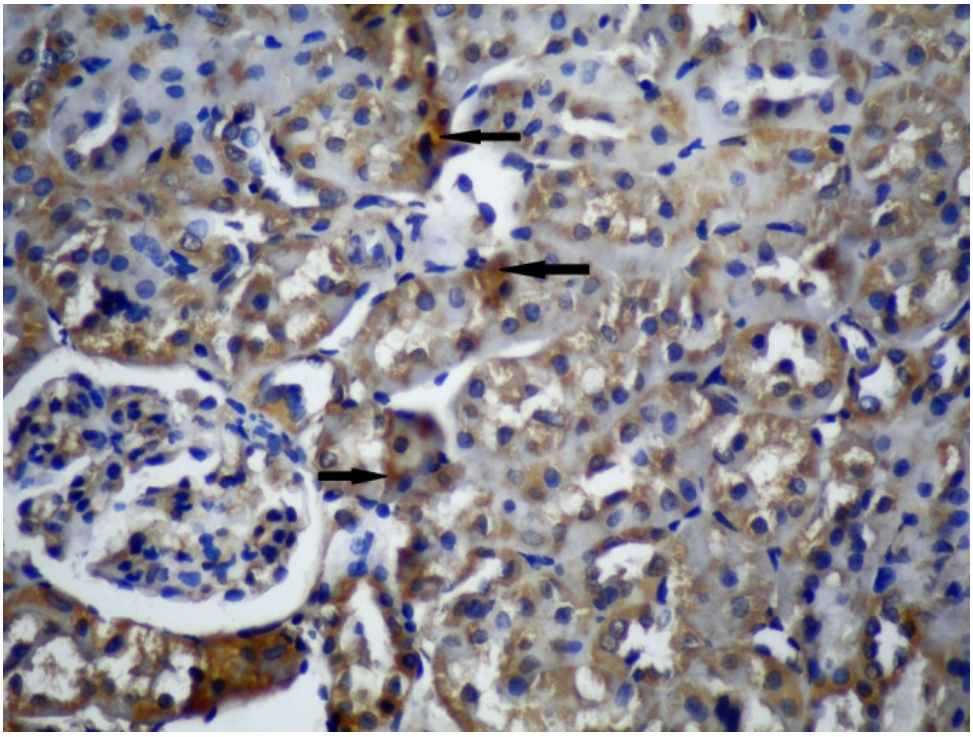

Figure 8. A photomicrograph section in renal cortecomedullary region of an adult male albino rat in group III (Ginger group) showing mild Caspase-3 expression (black arrow) in the cytoplasm of cells of PCT and DCT. (Avidin Biotin peroxidase for caspase-3 ×400).

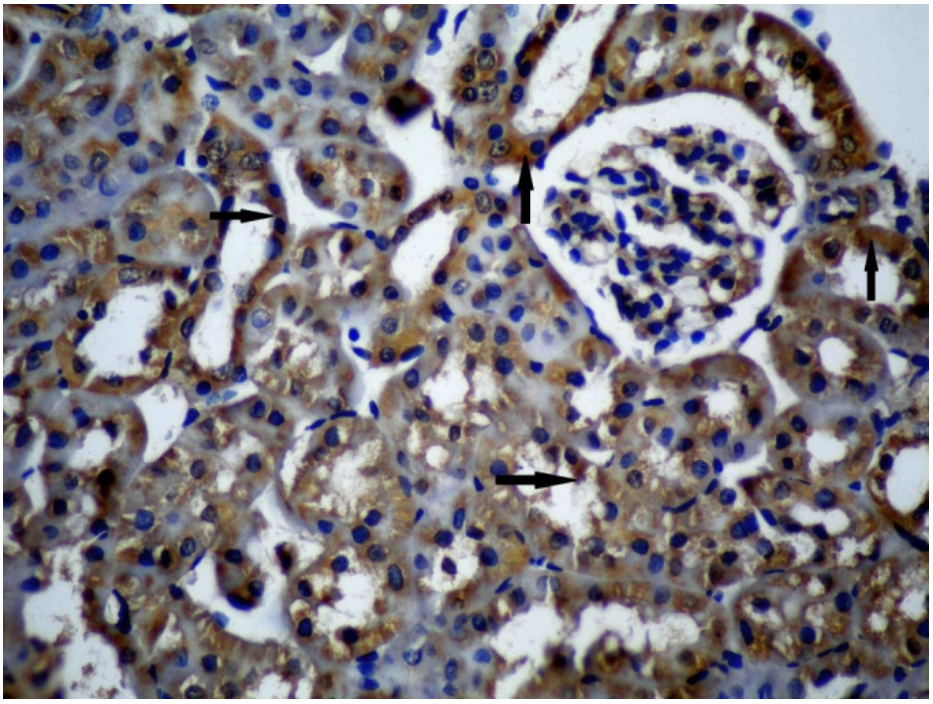

Figure 9. A photomicrograph section in renal cortecomedullary region of an adult male albino rat in group IV (N-acetylcysteine group) showing moderate Caspase-3 expression (black arrow) in the cytoplasm of cells of PCT and DCT. (Avidin Biotin peroxidase for caspase-3 ×400)

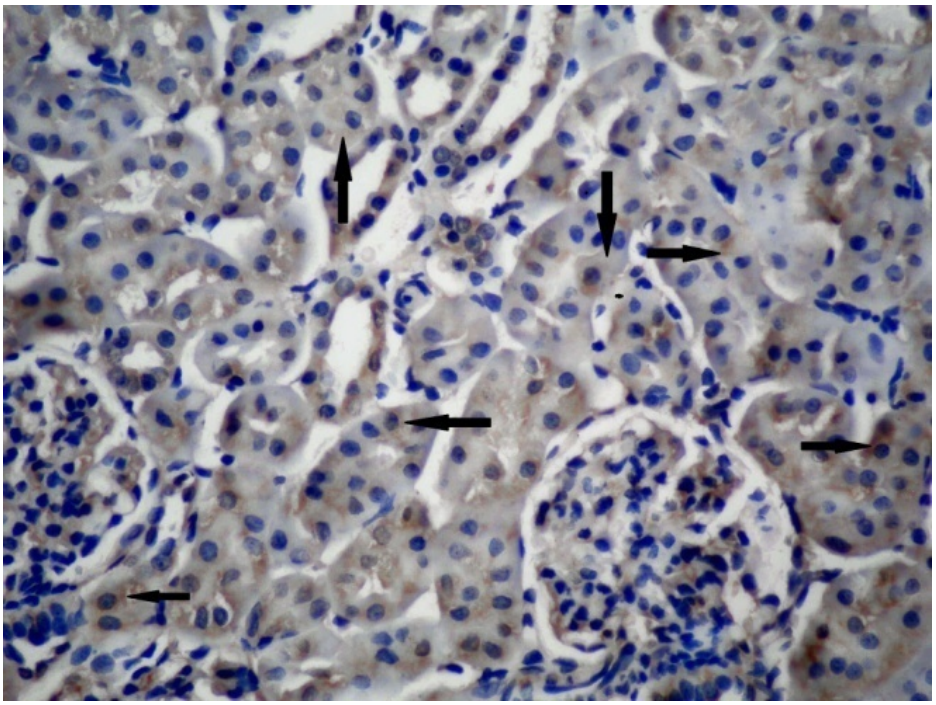

Figure 10. A photomicrograph section in renal cortecomedullary region of an adult male albino rat in group V (Ginger and N-acetylcysteine group) showing minimal Caspase-3 expression (black arrow) in the cytoplasm of cells of PCT and DCT (Avidin Biotin peroxidase for caspase-3 ×400).

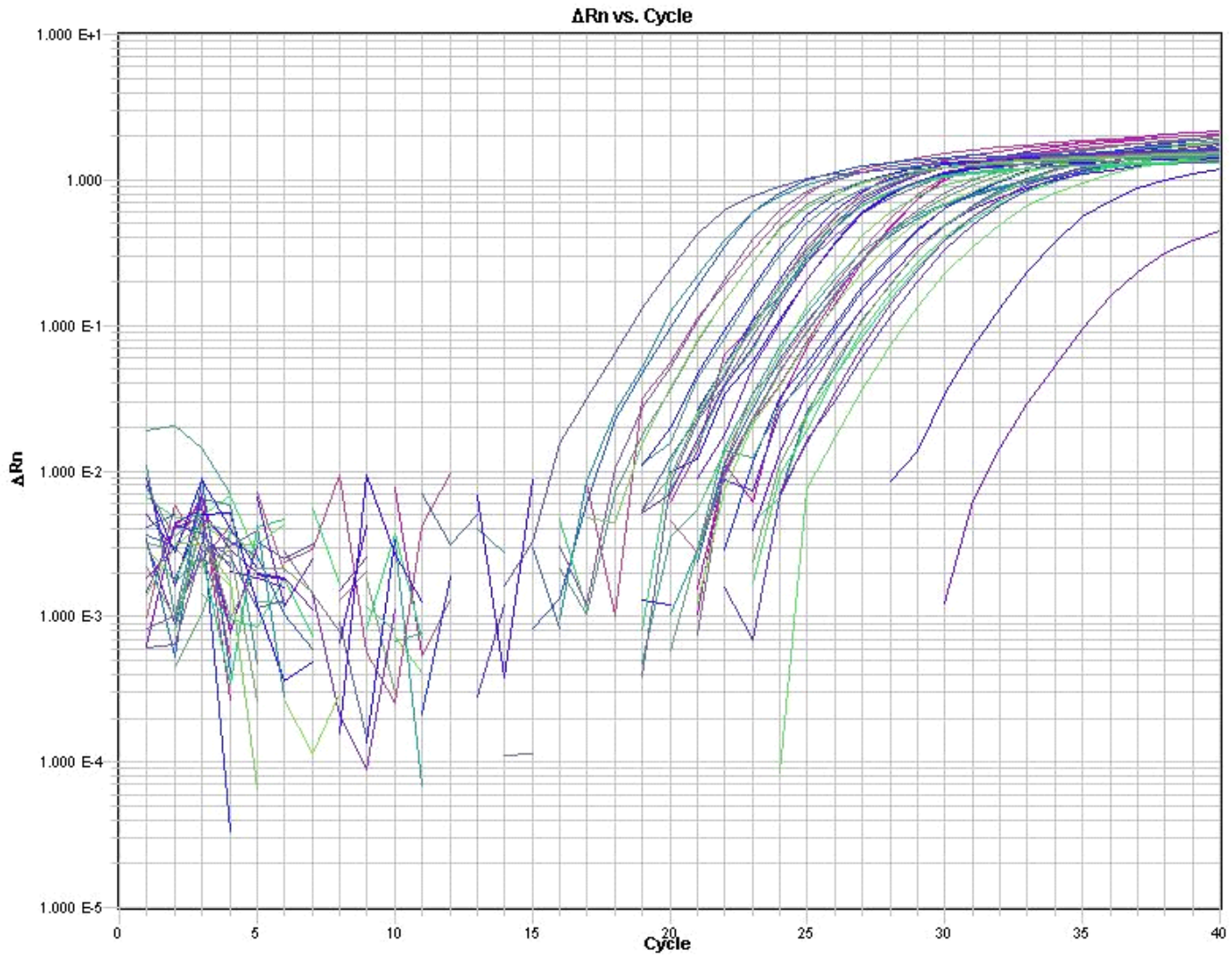

Figure 11. Amplification plot curves for detector CAT and GPx genes in the kidney of all groups.

and DCT (Figure 7). Ginger group showed mildly (++) expressed caspase-3 reaction in cytoplasm of the cell lining of PCT and DCT (Figure 8). N-acetyl cycteine group showed moderately (+++) expressed caspase-3 reaction in cytoplasm of the cell lining of PCT and DCT (Figure 9). Ginger and N-acetyl cycteine group showed minimally (+) expressed caspase-3 reaction in cytoplasm of the cell lining of PCT and DCT (Figure 10). The mean area % of casapase -3 expression for all groups was represented in Table 2 and Figure 12. There was a significant decrease (P> 0.05) in casapase -3 expression in groups III and V compared with group II (affected group).

Gene expression

The amplification curves of targeted genes among all samples printed from real time polymerase chain reaction of ABI 7900 real time machine. (Figure 11). The renal CAT gene expression was found to be decreased in the glycerol group (affected group). Administration of ginger extract and N-acetylcystiene separately or combined before glycerol significantly increased the CAT gene expression. The maximum increasing effect was obtained in ginger extract with N-acetylcystiene treated group (group V). The CAT gene expression in ginger extract group was higher than that of N-acetylcystiene administered group (Tables 3 and 4). The expression of GPx minimally increased in the glycerol treated group (affected group). Administration of ginger extract and N-acetylcystiene separately or combined before glycerol significantly increased the GPx activity. The maximum increasing effect was obtained in the combined treated group (Tables 3, 4, 5 and 6).

DISCUSSION

Acute renal failure not only occurs with high frequency, but is also associated with significant morbidity and

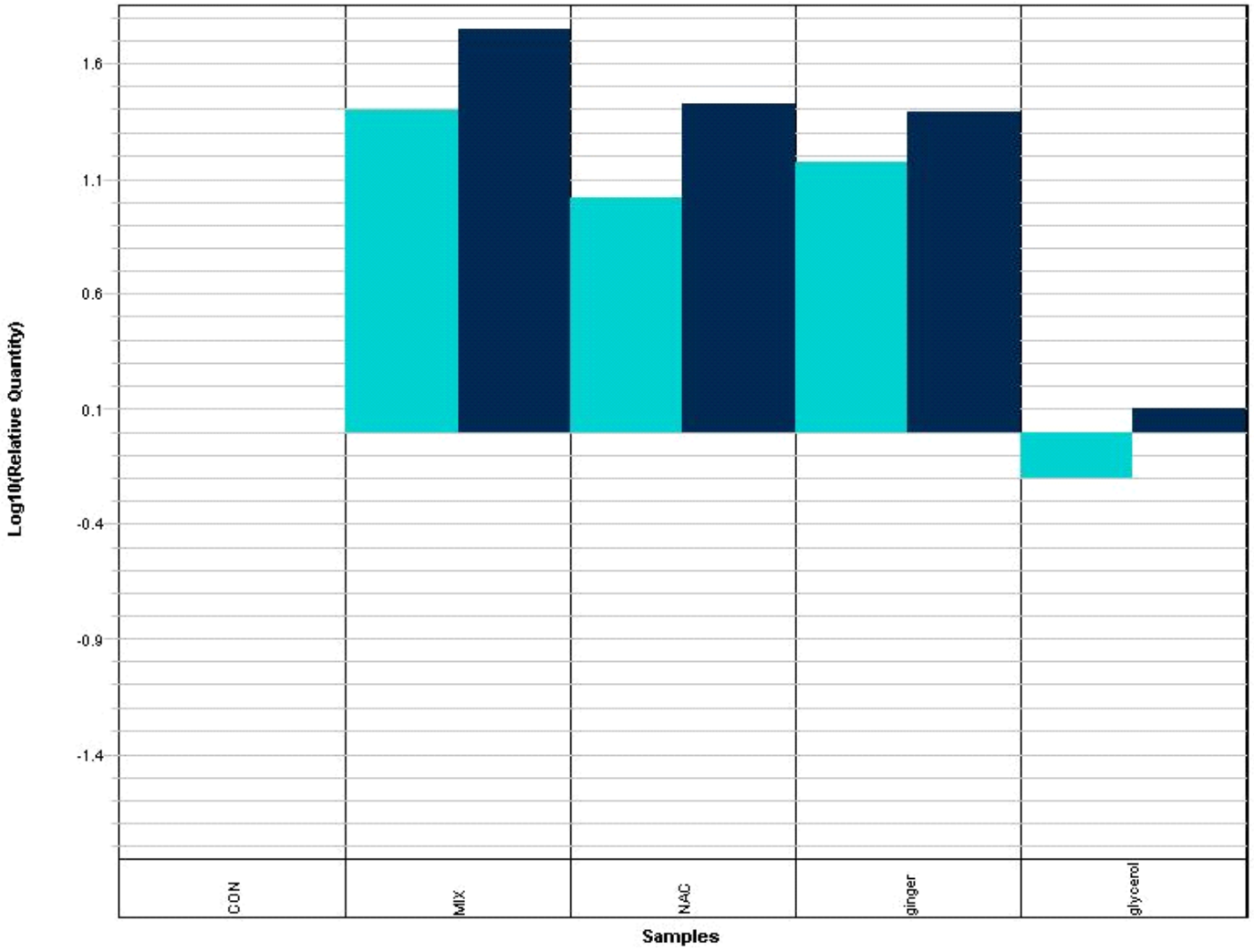

Figure 12. Showing gene expression bars of Log10 relative quantity levels of CAT and GPx m-RNA in kidney among groups as compared to control groups.

Table 3. Mean ± SD of LOG10 relative units of CAT and GPx1 mRNA expressions in kidney among all groups.

Parameter	Group I	Group II	Group III	Group IV	Group V	P value
	Mean ± SD					
CAT	7.2809 ± 0.0100	7.0843 ± 0.0100	8.4600 ± 0.0233	8.3042 ± 0.0193	8.6627 ± 0.0378	III: 0.0085* V: 0.0001*
GPx1	5.9171 ± 0.032	5.9585 ± 0.0311	7.3311 ± 0.0299	7.4237 ± 0.0321	7.6154 ± 0.0323	III: 0.0105* IV: 0.0093* V: 0.0053*

*P<0.05, SD: standard deviation.

mortality (Kaul and Ruhela, 2012). Alternative and indigenous systems of medicine are popular amongst the poorer sections of society in the developing world. Their use in the developed world has also increased in recent times. The source and composition of these medicines vary in different parts of the world, but herbs and other

Table 4. Fold changes in the CAT and GPx genes expression in kidney among groups as compared to control group I.

Group	CAT	GPx
Group II: Glycerol 0.8ml/kg/50%.	0.636	1.11
Group III: Ginger 100 mg /kg B.Wt	14.677	26.428
Group IV: N-acetylcystiene10 mg/kg B.Wt	10.435	26.799
Group V: N-acetylcystiene10 mg/kg B.Wt and Ginger 100 mg /kg B.Wt.	25.026	56.196

botanicals are central to these systems (Vivekanand, 2010). The levels of urea and creatinine were increased in the affected group (glycerol group) of the present study, 24 h after glycerol administration. H & E stained sections showed extensive vacuolations with dilatations in tubular cells of the PCT and DCT. PAS reaction was decreased in the apical borders and basement membrane of the PCT and DCT cells indicating their loss or desquamation. Casapase -3 highly expressed indicate increased apoptosis. These results are consistent with Mohammad et al. (2011) who reported that renal function was markedly damaged in glycerol induced ARF rats. Also Shaalan and Khalil (2009), reported that the renal tubules are the main site of renal damage induced by glycerol injection and the degenerative and necrotic changes in the forms of cloudy swelling and vacuolated cytoplasm with decreased PAS reaction in the brush borders and basement membrane. Similar findings have been reported by Brand et.al. (2009) who found that glycerol induced degeneration and necrosis of the cells of the proximal and distal convoluted tubules. Glycerol induced kidney tubular degeneration was explained by Pisoni et al. (2008) who reported that renal damage induced by glycerol is believed to result from decreased renal blood flow and consequently ischemia of the glomerular and tubular system as a result of increased circulating myoglobin. The tubular degradation of myoglobin results in the generation of reactive oxygen species which implicated renal damage and consequently induction of renal failure. Also Polo et al. (2004) and Vlahović et al. (2007) reported that myoglobinuric ARF has three pathogenic mechanisms: tubular obstruction, renal vasoconstriction and oxidative stress. The latter is generated through the iron released from the heme group of the myoglobin. Iron induces the formation of high-activity oxygen free radicals that increase oxidative stress and provoke lipid peroxidation and cellular death. Reactive oxygen species (ROS) include a mixture of highly reactive forms of oxygen, like hydrogen peroxide (H_2O_2) and oxygen molecules with an unpaired electron in their valence shell, termed free radicals, including superoxide (O_2•-) and hydroxyl (•OH) (da Silva et al., 2010).

The excessive accumulation of these toxic by-products causes serious damage to lipids, proteins and nucleic acids when oxidative stresses are induced by radiation, high temperature, malnutrition, chemicals or pathogens (Sachdev and Davies, 2008). In order to protect cells against the toxicity caused by ROS, animals have evolved protective antioxidant enzymatic systems, such as superoxide dismutase (SOD), CAT and GPX (da Silva et al., 2010). Catalase is one of the most important enzymes involved in ameliorating the effects of oxygen metabolism (Mandal et al., 2013). CAT catalyzes the breakage of toxic hydrogen peroxide produced in the cell to O_2 and H_2O (Linares et al., 2006). The glyceraldehydes produced may auto-oxidize in the presence of oxygen, yielding super oxide radical with accumulation of hydrogen peroxide (Mohammad et al., 2011). The glycerol group (group II) of the present study, showed reduction in renal CAT expression and this is in accordance with Linares et al. (2006) who stated that glycerol injected rats presented a reduction in renal CAT activity. Mohammad et al. (2011), explained the inhibition of CAT activity by glycerol as CAT inactivation by ROS, produced an auto-oxidation of glyceraldehydes (metabolite of glycerol). The decreased CAT expression predisposes the kidney of rats to oxidative stress, because this enzyme catalyzes the decomposition of ROS.

GPx, an important antioxidant enzyme represents a compensatory mechanism to degrade H_2O_2, its expression was increased minimally in glycerol group, which indicates impaired scavenging of H_2O_2 and lipid hydroperoxides and this was in accordance with Sachdev and Davies (2008) who stated that, CAT is the main gene that play a role in glycerol acute renal failure and GPx was a secondary gene in protection of this disease. The levels of urea and creatinine in the present study were significantly decreased in the ginger group (group III) compared with glycerol group (affected group) 24 h after glycerol administration, H & E stained sections showed mild vacuolations of tubular cells with slight dilatations of the PCT and DCT and significant decrease in caspase-3 expression. These results is in agreement with Masuda et al. (2004), who reported that renal protective activity of ginger extract might partially be due to its antioxidant property as in the ginger rhizome, the gingerols (polyphenols) were identified as the major active component. Presence of volatile oils with many mono and sesquiterpenes and flavonoids were also reported by Zhang et al. (2010) who reported that the presence of such active components might be responsible for the ex-hibited renal protective activity. Also, there was significant

increase in renal CAT expression in the ginger group (group III) of the present study and this is in accordance with Shanmugam et al. (2011) who reported that ginger was effective in protection against the inhibition of the enzymatic activity induced by glycerol as ginger increased CAT expression, suggesting that the protective effect of ginger against renal damage induced by glycerol involves the antioxidant effect of gingerols. The increased expression of CAT gene in ginger group was explained by Ajith et al. (2008) who reported that treatment of acute renal failure animals with ginger increased the antioxidant enzyme activities significantly. This may be due to the presence of many antioxidant compounds, such as gingerols, shogaols, phenolic ketone derivatives, volatile oils and flavonoids of ginger. These antioxidant compounds may modulate the antioxidant enzymes in rats. Indeed, the importance of hydroperoxides in the mechanism of renal failure caused by hemoproteins reported by Boutaud et al. (2010) demonstrated that scavenging hydrogen peroxide protects the kidney from injury after rhabdomyolysis induction in rats.

The ginger group (group III) in the present study showed significant increase in renal GPx gene expression and this is in accordance with Afshari et al. (2007) who reported that plasma antioxidant capacity is increased by ginger treatment in rats and glutathione serves as a sensitive marker of oxidative stress so it plays an important role in maintaining the integrity of the cell system. Glutathione (GSH) is involved in several reactions in the body and is one of the most prominent non-enzymatic antioxidants. Ajith et al. (2007), reported that the ginger had antioxidant effect by decreasing lipid peroxidation, increasing GSH level and maintaining normal levels of antioxidant enzymes.

The levels of urea and creatinine were significantly decreased in the NAC group (group IV) of the present study compared with glycerol group (affected group) 24 h after glycerol administration, H & E stained sections showed moderate vacuolations of tubular cells with slight dilatations of the PCT and DCT, insignificant decrease in caspase-3 expression and significant increase in renal CAT and GPx gene expression suggested that the protective effect of N-acetylcysteine against renal damage induced by glycerol was explained by Luo et al. (2008) who stated that NAC is directly associated with inhibition of apoptosis and promotion of cell growth. They showed that NAC can protect cells from apoptotic death through a mechanism other than the scavenging of radicals. Kim et al. (2005), proposed that NAC may have beneficial effects through multiple mechanisms including its anti-apoptotic capacity according to the cell types and NAC could ameliorate glycerol-induced kidney injury through inhibition of apoptotic cell death. However, most of the action of NAC has been focused on the role of antioxidants or scavenger of radicals. The increased gene expression in this group explained by Fishbane (2008) and Anderson et al. (2011) who reported that GSH present in NAC can react directly with reactive intermediates such as ROS, or act as a cofactor in biotransformation, such as GPx. This process results in the oxidation of GSH, which forms a disulfide bridge with another molecule of GSH to form glutathione disulfide (GSSG) and this is a cofactor in detoxification of H_2O_2 and other peroxides in the cytosol and mitochondria.

The levels of urea and creatinine were significantly decreased in ginger and N-acetylcysteine group (group V) of the present study. 24 h after glycerol administration, H & E stained sections showed minimal vacuolations of tubular cells with slight dilatations of the PCT and DCT, significant decrease in caspase-3 expression and synergistic effect on CAT and GPx genes expression with significant increase in their expression. This was explained by Boutaud and Roberts (2011) who reported that, a therapy combining chain-breaking antioxidants and a glutathione precursor, such as N-acetylcysteine, or hydrogen peroxide scavengers as ginger may provide a synergistic effect on acute kidney failure.

Conclusion

The present study showed that the administration of oral ginger extract and N-acetylcystiene separately and combined prior to glycerol induced injury had prophylactic role in ARF. Also, the combination of both had synergistic effect. Further studies are needed to determine if there is a therapeutic role of Ginger extract and N-acetylcystiene in ARF.

Conflict of interest

Authors declare no conflict of interests.

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