N-acetyl-L-cysteine Prevents Bile Duct Ligation Induced Renal Injury by Modulating Oxidative Stress

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Abstract The aim of the present study was to evaluate the effect of N-acetyl-L-cysteine (NAC) on bile duct ligation (BDL) induced oxidative stress in kidneys. Male Wistar rats were randomly segregated into four groups; sham control (SC), SC + NAC, BDL and BDL + NAC group. Liver damage was induced following BDL and renal injury was assessed by kidney function tests along with lipid peroxidation, nitrite levels, thiols and antioxidant enzymes. Three weeks after BDL, rats developed renal dysfunction in terms of elevated serum creatinine levels. BDL animals exhibited an increase in lipid peroxidation, reduction in thiols and redox ratio in liver and kidney tissue along with altered antioxidant enzymes in kidneys. BDL animals that were orally administered NAC at a daily dose 100 mg/kg for duration of two weeks, showed significant reduction in serum creatinine levels. NAC was effective in lowering lipid peroxidation and was able to restore thiol levels along with GSH/GSSG ratio in both liver and kidneys along with the activity of antioxidant enzymes in the kidneys of BDL animals. The results clearly demonstrate the efficacy of NAC in attenuating oxidative stress in kidneys, suggesting a therapeutic role for NAC in individuals with renal dysfunction following BDL.

Keywords Bile duct ligation · Kidney · Liver damage · N-acetyl-L-cysteine · Oxidative stress

Abbreviations

BDL	Bile duct ligation
CAT	Catalase
CDNB	1-Chloro-2, 4-dinitrobenzene
DTNB	5,5-Dithiobis-2-nitrobenzoic acid
GR	Glutathione reductase
GSH	Non protein thiols
GSH-Px	Glutathione peroxidase
GSSG	Glutathione disulfide
GST	Glutathione-s-transferase
MDA	Malondialdehyde
NAC	N-acetyl-L-cysteine
NEDA	N-(1-Naphthyl) ethylenediamine dihydrochloride
NO	Nitric oxide
ONOO ⁻	Peroxynitrite anion
PBS	Phosphate buffer saline
P-SH	Protein thiols
ROS	Reactive oxygen species
SC	Sham control
SD	Standard deviation
SOD	Superoxide dismutase
T-SH	Total thiols

Introduction

Biliary obstruction is an injurious insult that results from retention of bile promoting liver cirrhosis [1]. In addition to liver, biliary obstruction also triggers a pathophysiological cascade of events that may affect other organs such as the kidneys, intestine, heart, blood and brain [2–4]. According to Garcia-Taso et al. [5] ~20% of patients admitted to

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hospitals with renal dysfunction are also diagnosed with hepatic damage. This may be attributed to the accumulation of hydrophobic bile acids [6] and ammonia [4] in systemic circulation involving intra organ generation of reactive oxygen species (ROS) that in turn evokes an inflammatory response [7]. A recent study by Silveira et al. [8] has demonstrated that liver cirrhosis mediated by bile duct ligation (BDL) induces apoptosis in renal tissue through intracellular oxidative stress. These observations support the hypothesis that oxygen free radicals might play an important role in the development of complications from liver dysfunction such as renal damage.

Experimental BDL is the model most frequently used to study obstructive cholestasis [9]. Kaler et al. [10] reported that bile acids are involved in alterations in renal function and structure following BDL. A report on temporal progression of BDL in young rats displayed higher creatinine levels, elevated tubulointerstitial injury scores and markers of renal damage [11]. In a study carried out by Pereira et al. [12] the duration of BDL positively correlated with renal dysfunction including reduction in creatinine clearance, water excretion and urinary sodium concentration. A gradual increase with time was observed in superoxide radicals in the kidneys of animals with BDL [13]. Tokac et al. [14] showed that BDL was able to induce DNA damage in liver and kidneys apart from oxidative stress.

Many experimental studies have reported beneficial effects of antioxidants in cholestasis [15, 16]. N-acetyl-L-cysteine (NAC) is a potent antioxidant that exerts its antioxidant effect by reacting directly with electrophiles or by facilitating generation of non protein thiols (GSH) after uptake by the cells [17]. NAC has shown to exert protective effect during acute kidney injury following prolonged pneumoperitoneum in rats [18] and kidney dysfunction (glomerular filtration) after the induction of chronic renal failure [19]. Previous work from our laboratory has also shown the protective effect of NAC in hepatic encephalopathy following BDL [4]. Therefore, this study aims to assess the protective efficacy of NAC on oxidative damage, activity of antioxidant enzymes in kidney tissue following experimental BDL.

Materials and Methods

Chemicals

The chemicals used in the study were of analytical grade, purchased from Sigma Chemical Co. (St. Louis, MO, USA), Merck (Mumbai, India), and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). NAC was purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Kit

for urea estimation was purchased from Reckon Diagnostics Pvt. Ltd. (Vadodara, India).

Animals and Treatment Schedule

Male Wistar rats weighing between 220 and 250 g were procured from the Central Animal House of the University. The animals had free access to pellet diet and water. The protocols followed were approved by the Institutional Animal Ethics Committee of the University. The rats were randomly segregated into four groups with each group having 5 animals each.

Sham control (SC) Animals underwent identical laparotomy without BDL.

SC + NAC Animals underwent sham surgery and after one week were orally gavaged with NAC (dissolved in water) at a dosage of 100 mg/kg for a period of two weeks.

BDL Animals underwent BDL surgery.

BDL + NAC Animals underwent BDL surgery and after one week were orally gavaged with NAC (dissolved in water) at a dosage of 100 mg/kg for a period of two weeks.

BDL Surgery

The BDL surgery was performed as described by Kountouras et al. [20]. Rats were anaesthetized using ketamine-xylazine (45 mg/kg ketamine and 5 mg/kg xylazine). The abdomen was shaved and disinfected with 10% (w/v) povidine iodine and a midline laparotomy was performed. The bile duct was carefully isolated, doubly ligated and cut between the two ligatures. Thereafter, the muscular and skin incisions were closed with absorbable sutures. During surgical procedure the body temperature of animals were maintained at 37 ± 1 °C and after the surgical procedure rats were allowed to recover.

Collection of Serum and Tissue Samples

Three weeks after the surgery blood was drawn from the orbital sinus of animals under mild ether anesthesia. Approximately 2 ml of blood was collected in test tube and left undisturbed at room temperature for 30 min. Serum was collected after centrifugation at 2000 g for 10 min. The animals were sacrificed by cervical dislocation immediately after collection of blood. Liver and kidneys were dissected, rinsed in ice cold isotonic saline and stored at -80 °C for further analysis.

Preparation of Homogenates

Potter-Elvehjem-type glass homogenizer was used to prepare 10% (w/v) homogenate in ice-cold 50 mM phosphate buffer saline (PBS), pH 7.4. The homogenate was

centrifuged at 1000g for 15 min at 4 °C to remove nuclei and unbroken cells. The pellet was discarded and supernatant was again centrifuged at 12000g for 20 min to obtain post-mitochondrial supernatant and crude mitochondrial pellet. Various biochemical assays were performed in different fractions of liver and kidney samples.

Kidney Function Tests

Urea Estimation

Assay for urea was performed in serum using commercially available kit. Results were expressed as mmoles/L.

Creatinine Estimation

Creatinine levels were estimated in the serum according to the method of Jaffe's [21] with slight modifications. Appropriate amount of sample was taken and N/12 sulphuric acid was added followed by the addition of sodium tungstate. After centrifugation supernatant was taken and then alkaline picrate was added. The absorbance of chromophore was measured at 520 nm using spectrophotometer and the results were expressed as nmoles/L.

Biochemical Analysis for Oxidative Stress and Antioxidant Enzymes

Lipid Peroxidation

Malondialdehyde (MDA), a measure of lipid peroxidation was quantified in the homogenates of liver and kidney according to the method of Wills [22]. Degradation products of peroxidized lipids form MDA that is quantified by reaction with thiobarbituric acid at 532 nm. The levels were calculated using molar extinction coefficient of chromophore ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) and expressed as nmoles MDA/mg protein. Lipid peroxidation was also determined by measuring the absorption at 233 nm of conjugated dienes in lipid extracts (chloroform–methanol 2:1, v/v) of liver and kidney homogenate using 50 mM sodium maleate buffer as described by Bhaskar and Subramanian [23].

Nitrite Estimation

Nitrite levels were estimated by the method of Green et al. [24]. Liver and kidney sample were mixed with equal volume of Griess's reagent (0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDA) and 1% (w/v) sulfanilamide in 5% (w/v) phosphoric acid) and incubated for 10 min at room temperature. The absorbance was read

at 546 nm and the results were expressed as nmoles of nitrite/mg protein using sodium nitrite as standard.

MTT Reduction

The reduction of MTT to blue formazan was performed to assess the activity of the mitochondrial dehydrogenases in both liver and kidney tissue as described by Sandhir et al. [25]. The results were expressed as μg of formazan formed/min/mg protein.

Thiols

Total thiols (T-SH) were quantified in the liver and kidney homogenate samples according to the method of Sedlak and Hanus [26]. To test samples, Tris–HCl buffer (0.2 M, pH 8.2 containing 0.02 M EDTA) was added followed by the addition of 0.1 M 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) in stoppered tubes. Thereafter, absolute methanol was added and samples were kept at room temperature for 15 min. The samples were then centrifuged at 3000g for 10 min and the absorbance was measured at 412 nm. The T-SH levels were calculated using molar extinction coefficient of chromophore ($13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) and results were expressed as nmoles/mg protein. GSH was measured based on the method of Roberts and Francetic [27]. Liver and kidney samples were mixed with 4% (w/v) sulphosalicylic acid, centrifuged at 1200g for 5 min to obtain supernatant which was then treated with Ellman's reagent (0.1 mM DTNB in 0.1 M sodium phosphate buffer, pH 8.0) to form 5-mercapto-2-nitrobenzoate which was read at 412 nm. The values were calculated using molar extinction coefficient of chromophore ($13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) and results were expressed as nmoles/mg protein. Protein thiols (P-SH) were calculated from the difference between the values of T-SH and GSH and results expressed as nmoles/mg protein.

Redox Ratio

GSH and glutathione disulfide (GSSG) levels were determined with fluorescence detection after reaction of the supernatant containing $\text{H}_3\text{PO}_4/\text{NaH}_2\text{PO}_4$ –EDTA or $\text{H}_3\text{PO}_4/\text{NaOH}$, respectively, of the deproteinized homogenate solution with ophthalaldehyde, pH 8.0, according to Hissin and Hilf [28]. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette and the fluorescence was measured at 420 and 350 nm emission and excitation wavelengths, respectively. The GSH and GSSG levels were determined from GSH or GSSG standards, respectively.

Superoxide Dismutase (SOD)

SOD activity was assayed according to the method of Kono [29] wherein the inhibition of nitro blue tetrazolium reduction was measured at 560 nm. Briefly, the reaction was initiated by the addition of hydroxylamine hydrochloride (20 mM, pH 5.0) to the reaction mixture containing nitro blue tetrazolium (96 μ M) and the test sample. The results were expressed as units/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50%.

Catalase (CAT)

The activity of CAT was measured in the post nuclear supernatant as described by Luck [30]. Hydrogen peroxide (12.5 mM H_2O_2 in 0.067 M sodium phosphate buffer, pH 7.0) decomposition by CAT was monitored by following the decrease in absorbance at 240 nm. The activity was estimated using molar extinction coefficient of H_2O_2 ($71 \text{ M}^{-1} \text{ cm}^{-1}$) and results were expressed as nmoles H_2O_2 decomposed/min/mg protein.

Glutathione Peroxidase (GSH-Px)

GSH-Px catalyzes the oxidation of GSH from H_2O_2 to form GSSG which was monitored at 340 nm for 3 min [31]. GSH (30 mM) levels were maintained by the addition of glutathione reductase (160 units/mg protein) and NADPH (6 mM) which immediately converted GSSG produced to GSH. GSH-GPx activity was calculated using molar extinction coefficient of NADPH ($6.22 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$) and the results were expressed as nmoles NADPH oxidized/min/mg protein.

Glutathione Reductase (GR)

GR activity was assayed in the post mitochondrial supernatant according to the method of Carlberg and Manner-vick [32]. The reaction was initiated by addition of phosphate buffer (0.067 M, pH 6.6), EDTA (15 mM), oxidized glutathione (60 mM) and NADPH (6 mM in 1% NaHCO_3) to the test sample and absorbance read at 340 nm. Enzyme activity was calculated using molar extinction coefficient of NADPH ($6.22 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$) and the results were expressed as nmoles NADPH oxidized/min/mg protein.

Glutathione-S-Transferase (GST)

The activity of GST was quantified according to the method of Habig et al. [33]. To the kidney mitochondrial supernatant, phosphate buffer (0.3 M, pH 6.5), 1-chloro-2,

4-dinitrobenzene (CDNB) (20 mM in 95% alcohol), was added and the rate of formation of GSH and CDNB conjugate was read at 340 nm. The activity of enzyme was calculated using molar extinction coefficient of conjugate ($9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) and results were expressed as nmoles CDNB-GSH conjugate formed/min/mg protein.

Estimation of Protein

The protein content was estimated according to the method of Lowry et al. [34].

Statistical Analysis

The results were expressed as mean $\pm$ standard deviation (SD) and the differences between groups, assessed by ANOVA. Post hoc analysis was performed for inter-group comparisons using the least significance difference test. Values with $p < 0.05$ were considered to indicate a significant difference between groups.

Results

Effect of NAC on Serum Urea and Creatinine levels Following BDL

Table 1 depicts the levels of kidney function markers viz. urea and creatinine. There was no significant change in the serum urea levels following BDL; however NAC supplementation to BDL animals significantly increased the serum urea levels as compared to BDL rats. The serum creatinine levels were 2.5-fold higher in the BDL rats as compared to the SC. NAC treatment significantly ameliorated the BDL induced increase in serum creatinine levels.

Table 1 Effect of NAC administration on blood urea and creatinine following BDL

	Urea (nmoles/L)	Creatinine (nmoles/L)
SC	4.38 $\pm$ 0.71	105.38 $\pm$ 24.06
SC + NAC	4.82 $\pm$ 0.35	87.69 $\pm$ 11.85
BDL	4.7 $\pm$ 0.57	268.84 $\pm$ 18.88*
BDL + NAC	5.64 $\pm$ 0.12**	208.46 $\pm$ 7.80**

Values are expressed as Mean $\pm$ SD

n = 5

* Significantly different from control group

** Significantly different from untreated BDL group

*/** ($P < 0.05$)

Effect of NAC on Oxidative Stress and Antioxidant Enzymes in BDL Rats

Lipid Peroxidation

A significant increase in MDA levels in liver (58%) and kidney (35%) were observed in BDL rats as compared to SC. NAC administration to BDL rats resulted in significant decrease in MDA levels in liver (42%) and kidneys (30%) as compared to BDL rats (Tables 2, 3). Similarly a significant increase was observed in the conjugated dienes in liver (70%) and kidneys (41%) following BDL as compared to SC. NAC supplementation to BDL rats showed a significant decrease in the conjugated dienes in both the liver (31%) and kidneys (25%) as compared to untreated BDL rats.

Nitrite Levels

Nitrite levels were significantly enhanced in the liver (57%) and kidneys (30%) following BDL as compared to the SC rats. NAC administration to BDL rats significantly lowered nitrite levels in liver by 37% whereas nitrite levels in kidneys were not significantly affected as compared to untreated BDL animals (Tables 2, 3).

MTT Reduction

MTT reduction, a marker of mitochondrial dehydrogenases, was decreased significantly in the liver (30%) and kidneys (51%) of BDL rats as compared to the SC animals (Tables 2, 3). However, upon NAC

supplementation to BDL rats the rate of MTT reduction was increased significantly by 30% in the liver tissue, whereas no effect was observed in the kidneys as compared to the BDL rats.

Thiols

Thiol levels in liver and kidney tissue for various groups have been depicted in Tables 2 and 3. T-SH levels were significantly decreased in liver (32%) and in kidneys (28%) of BDL animals as compared to the SC. NAC supplementation to BDL rats significantly restored the T-SH levels by 29% as compared to untreated BDL rats. In BDL rats, GSH levels were significantly decreased by 24 and 25% as compared to SC rats in liver and kidney tissue respectively. NAC supplementation to BDL rats increased GSH levels by 29 and 27% in liver and kidney tissue as compared to BDL rats. P-SH levels were decreased significantly in liver (32%) and kidneys (29%) of BDL animals as compared to SC that were increased by 29% in liver and kidneys following NAC supplementation to BDL rats as compared to untreated BDL animals.

Redox Ratio

GSH/GSSG ratio a biomarker of oxidative stress was significantly decreased in the liver (28%) and kidneys (34%) of BDL rats as compared to SC (Tables 2, 3). NAC supplementation was effective in increasing the GSH/GSSG ratio in both liver (39%) and kidney (44%) tissue as compared to BDL rats.

Table 2 Effect of NAC administration on lipid peroxidation, conjugated dienes, nitrite levels, MTT reduction, thiols and redox ratio in liver following BDL

	Liver			
	SC	SC + NAC	BDL	BDL + NAC
Lipid peroxidation (nmoles of MDA/mg protein)	1.07 ± 0.16	0.99 ± 0.16	1.70 ± 0.30*	0.98 ± 0.16**
Conjugated dienes (nmoles of dienes/mg protein)	3.37 ± 0.73	3.42 ± 0.28	5.74 ± 0.92*	3.91 ± 0.84**
Nitrite levels (nmoles of nitrite/mg protein)	3.73 ± 0.95	4.04 ± 0.44	5.86 ± 1.10*	3.67 ± 0.82**
MTT reduction (μmoles of formazan formed/min/mg protein)	0.62 ± 0.05	0.59 ± 0.07	0.43 ± 0.02*	0.56 ± 0.01**
Total thiols (nmoles/mg protein)	75.62 ± 4.79	75.32 ± 6.14	50.95 ± 3.16*	65.93 ± 1.82**
Non protein thiols (nmoles/mg protein)	3.34 ± 0.16	3.29 ± 0.22	2.54 ± 0.12*	3.29 ± 0.21**
Protein thiols (nmoles/mg protein)	71.92 ± 4.85	72.02 ± 5.93	48.47 ± 3.25*	62.66 ± 1.90**
GSH/GSSG Ratio	8.16 ± 0.89	8.20 ± 0.56	5.85 ± 1.11*	8.16 ± 0.99**

Values are expressed as Mean ± SD

n = 5

* Significantly different from control group

** Significantly different from untreated BDL group

*/** ($P < 0.05$)

Table 3 Effect of NAC administration on lipid peroxidation, conjugated dienes, nitrite levels, MTT reduction, thiols and redox ratio in kidneys following BDL

	Kidneys			
	SC	SC + NAC	BDL	BDL + NAC
Lipid peroxidation (nmoles of MDA/mg protein)	1.11 ± 0.24	1.26 ± 0.20	1.5 ± 0.11*	1.04 ± 0.13**
Conjugated dienes (nmoles of nitrite/mg protein)	4.31 ± 0.59	4.66 ± 0.73	6.11 ± 0.61*	4.58 ± 0.61**
Nitrite levels (nmoles of nitrite/mg protein)	4.03 ± 0.71	3.76 ± 0.43	5.27 ± 0.6*	4.72 ± 0.29
MTT reduction (μmoles of formazan formed/min/mg protein)	0.49 ± 0.09	0.49 ± 0.22	0.24 ± 0.07*	0.45 ± 0.02
Total thiols (nmoles/mg protein)	81.12 ± 8.66	80.52 ± 8.85	58.12 ± 7.65*	75.13 ± 8.25**
Non protein thiols (nmoles/mg protein)	11.61 ± 1.5	11.5 ± 0.59	8.7 ± 0.41*	11.1 ± 0.7**
Protein thiols (nmoles/mg protein)	69.5 ± 9.17	69.02 ± 9.23	49.41 ± 7.42*	64.02 ± 7.66**
GSH/GSSG Ratio	6.29 ± 0.59	6.32 ± 0.90	4.21 ± 0.29*	6.09 ± 0.70**

Values are expressed as Mean ± SD

n = 5

* Significantly different from control group

** Significantly different from untreated BDL group

*/** ($P < 0.05$)

Table 4 Effect of NAC administration on activity of antioxidant enzymes in kidneys following BDL

	Kidneys			
	SC	SC + NAC	BDL	BDL + NAC
Superoxide dismutase (units/mg protein)	2.17 ± 0.17	2.23 ± 0.28	2.60 ± 0.10*	1.80 ± 0.11**
Catalase (μmoles H ₂ O ₂ decomposed/min/mg protein)	48.94 ± 2.91	46.98 ± 4.25	36.62 ± 4.05*	43.66 ± 5.96**
Glutathione peroxidase (nmoles of NADPH oxidized/min/mg protein)	86.8 ± 2.4	81.2 ± 3.6	71.2 ± 5.04*	84.5 ± 3.9**
Glutathione reductase (nmoles of NADPH oxidized/min/mg protein)	72.7 ± 6.2	67.6 ± 11	52.2 ± 2.4*	73.2 ± 2.1**
Glutathione-s-transferase (nmoles of CDNB hydrolyzed/min/mg protein)	47.9 ± 5.4	44.9 ± 8.3	32.9 ± 1.6*	41.2 ± 5.3

Values are expressed as Mean ± SD

n = 5

* Significantly different from control group

** Significantly different from untreated BDL group

*/** ($P < 0.05$)

Antioxidant Enzymes

As is evident from the data in Table 4 there was a significant increase in SOD activity in kidneys (20%) following BDL as compared to SC rats. NAC supplementation to BDL animals significantly decreased (31%) the SOD activity as compared to BDL rats. BDL significantly reduced the activity of CAT by 25% as compared to sham animals (Table 4). However, NAC treatment to BDL rats significantly increased the CAT activity by 19% in the kidneys as compared to untreated BDL rats. BDL decreased the activity of GSH-Px by 18% and GST by 32% as compared to SC rats. NAC supplementation to BDL rats was able to restore the activity of GSH-Px (19%) significantly whereas no significant effect was observed in the activity of GST enzyme as compared to untreated BDL rats (Table 4). BDL rats showed a significant decrease (28%) in

GR activity as compared to SC, however NAC supplementation to BDL rats increased the GR activity by 40% as compared to BDL animals.

Discussion

Previous report from our laboratory revealed increase in serum bilirubin, ammonia levels, alkaline phosphatase, transaminases along with an increase in the liver hydroxyproline and collagen content following BDL. Animals developed liver failure, jaundice and hyperammonia indicating cirrhosis [4]. In the present study no significant difference was observed in the serum urea levels following BDL as compared to SC rats. The exact reason for this is not known but this may be due to the hepatic damage following BDL [35]. However, a significant increase was

observed in serum urea levels in BDL animals treated with NAC suggesting that NAC may influence urea levels by restoring liver functions. Serum creatinine levels are strong prognostic markers of renal function in cirrhosis [36]. Three weeks after BDL surgery a 2.5 fold increase in the creatinine levels indicated impaired renal function due to cirrhosis induced renal injury. NAC treatment was successful in ameliorating serum creatinine levels in the BDL animals. NAC has been shown to reduce creatinine levels along with exerting protective effect against renal injury as a remote organ after skeletal muscle ischemia–reperfusion in rats [37].

The levels of MDA and conjugated dienes in liver and kidneys from BDL animals were observed to be significantly higher as compared to those observed in SC animals. High MDA and conjugated dienes represent the extent of membrane injury via generation of free radicals. Moreover, conjugated dienes reflect the initial phase of lipid peroxidation preceding that of aldehyde products [38]. An increase in serum bile acid levels and ammonia levels has been reported previously from our laboratory in BDL rats [4] suggesting that bile acids and ammonia act as pro-oxidants and endotoxins causing direct tissue damage mediated by ROS or indirectly through tissue resident macrophages to generate ROS [7]. NAC supplementation to BDL animals was able to significantly lower lipid peroxide levels. These findings can be attributed to the restoration of the pool of total intracellular GSH which is often depleted as a consequence of oxidative stress or inflammation or neutralization of lipid peroxides and ROS [39]. Liver and kidney nitrite levels were found to be significantly elevated in the present study following BDL. It has been shown earlier that nitric oxide (NO) formation and expression of inducible nitric oxide synthase is increased during obstructive jaundice that in turn leads to the formation of harmful peroxynitrite anion (ONOO^-) leading to cellular damage and apoptosis [40]. As suggested by our study, NAC administration was able to reduce the nitrite levels that may be mediated through elimination of harmful ONOO^- . Decreased MTT reduction observed in BDL rats suggests altered renal mitochondrial functions thereby accelerating ROS generation [41]. NAC was able to increase the liver and renal MTT reduction which might be due increased GSH levels.

A significant decrease in the T-SH, GSH and P-SH levels along with GSH/GSSG ratio was observed in liver and kidney tissue of BDL rats as compared to the SC rats. The results are in agreement with the previous studies showing a decrease in GSH levels in liver and renal tissue following BDL as GSH oxidation leads to extensive redox change [3]. NAC administration was able to restore liver and kidney thiols and GSH/GSSG ratio in BDL rats. This can be attributed to the ubiquitous distribution of acylases, that

catalyzes the deacetylation of NAC in turn improving redox status and GSH synthesis inside the cells [42]. NAC has also been shown to increase intracellular GSH and ameliorate renal ischemia-reflow injury, [43] kidney damage from *cis*-platinum [44] and other nephrotoxic insults [45].

Our results reveal significant increase in SOD activity whereas CAT activity was decreased in the kidney of BDL rats. These observations show that body's ability to scavenge or inactivate free radicals is compromised in BDL model [4]. A decrease in the activity of CAT has also been reported earlier in renal tissue following BDL [16]. Increased SOD activity could be an adaptive response to the increased superoxide radicals thereby increasing H_2O_2 levels. This combined with decrease in CAT activity might lead to accumulation of H_2O_2 in the tissues resulting in the formation of hydroxyl radicals leading to oxidative insult. However, NAC was able to restore the activities of these antioxidant enzymes in BDL rats. The activities of GPx, GR and GST in the kidney tissue of BDL rats were found to be significantly decreased. GR is responsible for the conversion of GSSG to GSH and decrease in its activity suggests altered GSH/GSSG ratio. GST is a free radical scavenging enzyme and decrease in its activity reflects enhanced generation of ROS following BDL. NAC supplementation accentuated the activity of GPx, GR and GST that might be due to improved redox status or direct renal protective mechanisms [42].

It is evident from the present study that BDL induced hepatic damage through oxidative stress and compromised antioxidant enzymes plays an important role in the progression of cirrhosis induced renal dysfunction. The findings of present study provide evidence that NAC exerts its beneficial effect through attenuation of oxidative stress and restoration of thiol levels. Based on the results NAC is suggested to be a potential therapeutic agent capable of minimizing renal dysfunction following liver cirrhosis.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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