Original Article

Hepatoprotective Effects of N-Acetyl Cysteine against Ammonium Nitrate-Induced Oxidative Stress in Male Wistar

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Abstract

Background: Ammonium nitrate (NH_4NO_3) is an important component of fertilizers; excessive buildup of NH_4NO_3 from industrial and occupational exposure can alter the antioxidant system and induce oxidative stress, which can impair liver functions. N-acetyl cysteine (NAC) is an antioxidant and anti-inflammatory nutritional supplement. This study evaluated hepatoprotective activities of N-acetyl cysteine in male Wistar rats exposed to ammonium nitrate.

Materials and Methods: Twenty male Wistar rats were grouped into four consisting of five rats in each group. The groups are control, NH_4NO_3 (300mg/kg), NAC (50mg/kg), and NH_4NO_3 + NAC. The administrations of NH_4NO_3 and NAC were via oral gavage for 36 days. The liver functions: alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), liver antioxidant and oxidant markers were evaluated spectrophotometrically. The liver histology was done.

Results: The study showed an observable increase in serum ALP, ACP, LDH, ALT, AST activities, and liver oxidative markers (malondialdehyde, nitric oxide, and reactive nitrogen species) in the NH_4NO_3 group. There is a depletion of antioxidant markers (superoxide dismutase, catalase, glutathione, thiol, and total antioxidant capacity) in the NH_4NO_3-treated group. Furthermore, the NH_4NO_3 group liver histology shows scarring around the central vein with infiltration of inflammatory cells, with evidence of perisinusoidal fibrosis and periportal fibrosis. Treatment with NAC effectively prevents NH_4NO_3 from elevating liver function enzyme activities, oxidative markers, and modulating antioxidant markers. Also, NH_4NO_3 + NAC group shows few scarring around the central vein, the morphology of the hepatocytes and sinusoids appear normal, with a few indications of perisinusoidal fibrosis.

Conclusion/Recommendations: The findings suggest that N-acetyl cysteine stabilizes liver free radicals, reduces liver function enzyme activities, enhances endogenous antioxidant activities, and prevents liver damage in Wistar rats exposed to ammonium nitrate.

Keywords: N-acetyl cysteine, ammonium nitrate, oxidative stress, liver antioxidants

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Introduction

The importance of liver in the metabolism of substances especially the detoxifying and removal of wastes and toxic is crucial for life. Hepatocyte are susceptible to toxic chemicals such as vinyl chloride, polychlorinated biphenyls, pesticides, lead acetate, paints, and ammonium nitrate that is a major component of fertilizer. The oral exposure of liver to several chemicals have been reported to cause liver damage. Industrial exposure chemicals have been reported to associated with portal hypertension, liver fibrosis, angiosarcoma of the liver, hepatotoxicity via

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inflammation, mitochondrial dysfunction, oxidative stress and depletion of antioxidant enzymes.[1]

Ammonium nitrate (NH_4NO_3) is one of the major environmental chemicals that containing the ammonium and nitrate ions. It is a major component of nitrogenous fertilizers. Excessive use can accumulate in plants and water, which may have adverse effects on plants and human health.[2] Human exposure to nitrate is from foodstuffs, drinking water, and occupations such as fertilizer manufacturing workers.[3] Nitrates/nitrites have been reported to induce oxidative stress through free radical generation.[3] High levels of nitrate exposure can cause several health.[4] Ammonium nitrate has been reported to damage the liver *via* increased reactive oxygen species (ROS), lipid peroxidation, and reduced antioxidant enzymes.[5,6] Reactive oxygen species cause oxidative stress, inducing pathology by damaging lipids, proteins, and DNA. Oxidative damage is the first mechanism that is often tested for toxicants such as ammonium nitrate.[7] A study reported that NH_4NO_3 causes liver damage via oxidative stress, increases liver enzymes, and degeneration of hepatocytes.[5]

N-acetylcysteine (NAC) as a nutritional supplement is an antioxidant and anti-inflammatory against established mucolytic and paracetamol poisoning.[8] It has been demonstrated to have strong antioxidant effects in animal models and thus an effective candidate for prevention of the diseases associated with reactive oxygen species (ROS). Traditionally, it is supposed to function as a disulphide bond reductant, reactive oxygen species scavenger, and glutathione biosynthetic precursor.[9] The antioxidant properties of NAC have been demonstrated to promote glucose metabolism in the lead acetate exposed male Wistar rats.[10]

Thus, this study aimed to examine the hepatoprotective effects of N- N-acetyl cysteine in male Wistar rats subjected to ammonium nitrate through the examination of the hepatic functions, liver endogenous antioxidant activity, oxidative stress markers, and liver histology.

Materials and Methods

Experimental animals

Twenty (20) male Wistar rats of 80-100 g were procured from the animal house of the College of Health Sciences, Osun State University, Osogbo, Nigeria, and were utilized for this experiment. The rats were housed in a favourable atmosphere in open-wired, well-ventilated polypropylene cages with 5 rats per cage. The animals were acclimatized for seven days before the commencement of the experiment. The animals were allowed free access to standard animal feed in pellet form and water. Ethical approval was obtained from the College of Health Sciences Ethics Committee, Osun State University (UNIOSUNHREC 2024/009B), and guidelines for the care and use of laboratory animals were strictly followed.

Experimental design

In this study, 20 animals were divided into 4 groups comprising 5 rats per group. Animals in group one served as the control group. Animals in group 2 were exposed to NH_4NO_3 only at 300 mg/kg p.o. Animals in group 3 received 50 mg/kg p.o of NAC only. Animals in group 4 were exposed to NH_4NO_3 and NAC. The dosages were given for thirty-six consecutive days. The dosages of ammonium nitrate and NAC are in accordance with previous studies. [6, 11]

The animals were weighed and euthanized under 50mg/kg of ketamine anaesthesia, and blood samples were obtained via cardiac puncture for biochemical assays. The livers were dissected, weighed, and homogenized for biochemical and histological assays.

Relative liver weight determination

The relative liver weight was determined by finding the percentage of the ratio of liver and body weights.

Biochemical assays

The obtained blood samples were centrifuged to obtain serum, which was used for the estimation of albumin, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activities.

Albumin evaluation

Albumin level was determined with the Fortress diagnostics kit (BXC0221). The principle of albumin assay involves bromocresol green (BCG) binding with albumin to form a green BCG complex. The amount of the BCG complex is directly proportional to the albumin concentration present in the sample. The absorbance of the samples against the reagent blank was read at 650nm.

Alkaline phosphatase activity assay

Serum alkaline phosphatase was determined by the Fortress diagnostics kit (BXC0183) based on the principle that alkaline phosphatase reacts with 2-Amino-2-Methyl-l-Propanol-buffered sodium thymolphthalein monophosphate, which develops a blue chromogen after adding alkaline reagent to stop enzyme activity which was measured at 590 nm.

Acid phosphatase assay

Acid phosphatase activity was determined using a colorimetric method. The Fortress diagnostics kit (RXC0401) was used. The acid phosphatase activity test principle is based on the reaction between the 1-naphthyl phosphate and water; the product 1-naphthol reacts with 4-chloro-2-methylphenyldiazonium salt to give an azo dye. The absorbance was read at 405nm, and the change in absorbance was calculated per minute over the course of

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the reaction.

Lactate dehydrogenase assay

Abbexa's lactate dehydrogenase assay kit (abx294035) was used, and the assay was done accordance to the procedure manual. The lactate dehydrogenase catalyses the production of pyruvic acid from lactic acid under the action of NAD^+. The absorbance was read at 450nm.

Alanine aminotransferase and aspartate aminotransferase activities assay

Alanine aminotransferase and aspartate aminotransferase activities were evaluated using a colorimetric method. The procedures for evaluating the enzyme activities were carried out accordance to the Fortress diagnostics kit manual.

Antioxidant and oxidant markers assay

A portion (2g) of the harvested liver was washed in ice-cold 1.15% KCl solution, blotted with filter paper, and homogenized in ice-cold 0.1M Tris-HCl (pH 7.4) using a Teflon homogenizer fitted into a microfuge tube chilled in ice. Each sample was centrifuged at 10,000 × g for 10 minutes with a Biofuge Fresco cold centrifuge. The supernatant was used for the estimation of catalase, glutathione, thiol, total antioxidant capacity, malondialdehyde, nitric oxide, and reactive nitrogen species.

Superoxide dismutase, catalase, glutathione, thiol, and total antioxidant capacity assays

The activity of superoxide dismutase (SOD) was determined by the principle of autoxidation of pyrogallol in the presence of EDTA at pH 8.2.[12] The evaluation of serum catalase (CAT) activity was by the reaction of hydrogen peroxide with ammonium molybdate to generate a yellowish complex read at an absorbance of 405 nm.[12] The glutathione (GSH) activity was determined by SH groups reduced activity on 5,5'-dithiobis- (2,-nitrobenzoic acid) to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH.[12] Thiol and total antioxidant capacity were measured using Elabscience and Abcam colorimetric assay kits with catalog no E-BCK265-M and ab6529, respectively. The manufacturers' procedure manuals were followed stringently.

Serum malondialdehyde, nitric oxide, and reactive nitrogen species evaluation

The serum level of MDA was determined by the reaction of 2-thiobarbituric acid with MDA.[13] The nitric oxide level was estimated using Griess reagents, and the absorbance of the colour change was measured at 540 nm.[14] The liver level of reactive nitrogen species was evaluated with the previously reported method.[15]

Statistical analysis

Data collected were analyzed using Graph pad prism 8.0.1 (244). The control and test groups data were subjected to normality test using Shapiro-Wilk test and the data were normally distributed ($p>0.05$). The data were analyzed using one-way ANOVA followed by Tukey-Kramer test for multiple comparisons. Results are expressed as mean ± SEM (standard error of mean) in graphs and the level of significance was less than 0.05.

Results

Liver function

Table 1 shows the results of N-acetyl cysteine on liver functions in male Wistar rats exposed to ammonium nitrate. The relative liver weight and serum albumin level are relatively similar in all the treated groups when compared with the control group.

Alkaline phosphatase, acid phosphatase, lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activities are significantly higher in the NH_4NO_3 group compared with the control. Also, the lactate dehydrogenase activity increased ($p<0.05$) in the NH_4NO_3 + NAC group. The administration of NAC reduced ($p<0.05$) alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities.

The co-administration of NH_4NO_3 and NAC reduced ($P<0.05$) alkaline phosphatase, acid phosphatase, lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activities when compared with the NH_4NO_3 group (Table 1).

Antioxidant markers

Table 2 shows the results of liver antioxidant activity/level in male Wistar rats treated with N-acetyl cysteine and ammonium nitrate. The liver superoxide dismutase, catalase activities, glutathione, thiol, and total antioxidant capacity levels were reduced significantly in the NH_4NO_3 group relative to the control group. Also, in the NH_4NO_3 + NAC group, the level of glutathione, thiol, and total antioxidant capacity reduced ($p<0.05$) when compared with the control group. Administration of N-acetyl cysteine alone significantly improved liver superoxide dismutase, catalase activities, glutathione, and thiol levels when compared with the control group. The co-administration of NH_4NO_3 + NAC significantly increased liver catalase activity, glutathione, thiol, and total antioxidant capacity levels relative to the NH_4NO_3 group (Table 2).

Oxidative markers

Table 2 shows the results of liver oxidative markers levels in male Wistar rats treated with N-acetyl cysteine and ammonium nitrate. The liver malondialdehyde, nitric oxide, and reactive nitrogen species levels increased ($p<0.05$) in the NH_4NO_3 group when compared with the control group. The liver reactive nitrogen species level also increased ($p<0.05$) in the NH_4NO_3 + NAC group, while NAC reduced ($p<0.05$) the liver level of reactive nitrogen species when compared with the control group. In

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the NH_4NO_3 + NAC group, the level of malondialdehyde, nitric oxide, and reactive nitrogen species was reduced significantly relative to the NH_4NO_3 group (Table 2).

Liver histology

Figure 1 shows the liver histology of male Wistar rats treated with ammonium nitrate and N-acetyl cysteine. The control (A) and NAC (C) groups show normal central vein (CV) without scar, the morphology of the hepatocytes appears normal (HC), the sinusoids (S) appear normal, and there is no evidence of fibrosis in the periportal and perisinusoidal.

The NH_4NO_3 (B) group shows scarring around the central vein (CV) with infiltration of inflammatory cells, the morphology of the hepatocytes (HC) appears normal, some sinusoids (S) appear normal, there is evidence of perisinusoidal fibrosis (PSF) and periportal fibrosis (FS). The NH_4NO_3 + NAC (D) group shows few scarring around the central vein (CV) with infiltration of inflammatory cells, the morphology of the hepatocytes (HC) appears normal, the sinusoids (S) appear normal, and there are a few indications of perisinusoidal fibrosis (PSF).

Table 1: Effect of N-acetyl cysteine on liver functions in male Wistar rats exposed to ammonium nitrate

	Control (n=5)	NH_4NO_3 (n=5)	NAC (n=5)	NH_4NO_3 + NAC (n=5)
Relative liver weight (%)	3.0 ± 0.26	3.4 ± 0.13	3.7 ± 0.62	2.9 ± 0.22
Albumin (g/dL)	3.4 ± 0.17	3.4 ± 0.23	3.6 ± 0.07	3.3 ± 0.11
Alkaline phosphatase (U/L)	52.4 ± 1.97	87.4 ± 2.84*	41.4 ± 2.14*	49.6 ± 1.57^{+}
Acid phosphatase (U/L)	4.8 ± 0.36	7.9 ± 0.37*	3.7 ± 0.13	5.3 ± 0.25^{+}
Lactate dehydrogenase (U/L)	101.8 ± 3.17	200.2 ± 8.70*	90.4 ± 3.23	132.0 ± 6.18$^{*,+}$
Alanine aminotransferase (U/L)	37.8 ± 1.39	58.2 ± 1.43*	30.4 ± 0.81*	39.8 ± 1.39^{+}
Aspartate aminotransferase (U/L)	28.8 ± 1.02	36.8 ± 1.59*	21.8 ± 1.39*	27.0 ± 1.41^{+}

Values present in mean ± S.E.M, n=5; *P<0.05 indicates a significant difference when compared to control. $^{+}$P<0.05 indicates a significant difference when compared to the NH_4NO_3

Table 2: Effect of N-acetyl cysteine on liver antioxidant activity and oxidative stress in male Wistar rats exposed to ammonium nitrate

	Control (n=5)	NH_4NO_3 (n=5)	NAC (n=5)	NH_4NO_3 + NAC (n=5)
Superoxide dismutase (U/mL)	0.51 ± 0.020	0.37 ± 0.011*	1.12 ± 0.055*	0.48 ± 0.018
Catalase activity (U/mg protein)	15.8 ± 0.86	11.8 ± 1.02*	19.2 ± 0.86*	15.2 ± 0.82^{+}
Glutathione (mM)	0.46 ± 0.018	0.23 ± 0.017*	0.57 ± 0.028*	0.32 ± 0.008$^{*,+}$
Thiol (mM)	1.24 ± 0.110	0.44 ± 0.014*	1.72 ± 0.041*	0.85 ± 0.023$^{*,+}$
Total antioxidant capacity (mM)	50.0 ± 0.71	37.6 ± 1.08*	47.8 ± 1.56	42.0 ± 0.71$^{*,+}$
Malondialdehyde (µM)	1.5 ± 0.15	2.3 ± 0.09*	1.4 ± 0.11	1.7 ± 0.08^{+}
Nitric oxide (µM)	4.62 ± 0.15	7.1 ± 0.29*	3.9 ± 0.81	5.1 ± 0.21^{+}
Reactive nitrogen species (nM)	793.0 ± 9.79	1197.0 ± 34.71*	546.2 ± 21.20*	892.8 ± 11.32$^{*,+}$

Values present in mean ± S.E.M, n=5; *P<0.05 indicates a significant difference when compared to control. $^{+}$P<0.05 indicates a significant difference when compared to the NH_4NO_3.

Discussion

This current study investigated the hepatoprotective properties of NAC in male Wistar rats' exposure to NH_4NO_3 by evaluating liver functions, lipid profile, and antioxidant/oxidative markers. In this study, the relative liver weight of all the treated groups is similar to the control animals. The relative liver weight was evaluated to assist in identifying the potential risk of NH_4NO_3 on the liver. The exposure to some pollutants/chemicals increased relative liver weight, suggesting liver hypertrophy and inflammation.[16] No changes in relative liver weight were observed in this study may be related to the sub-acute administration of NH_4NO_3.

Serum albumin, synthesized exclusively by the liver and serves a crucial role in maintaining oncotic pressure and antioxidant defence due to its thiol groups, especially Cys34.[17] In this study, the NAC-only group apparently, but not significantly increased serum albumin. This indicates that NAC might partially enhance hepatic protein synthesis and reflects its antioxidative and anti-inflammatory actions—likely through glutathione replenishment and preservation of albumin gene expression.[18] The serum albumin level in the NH_4NO_3 group exhibited similarity with the control and NH_4NO_3 +

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NAC groups. This suggests that administration of NH_4NO_3 might not affect hepatic protein synthesis despite the previous report that NH_4NO_3 induced oxidative or inflammatory damage, which could affect hepatic protein synthesis.

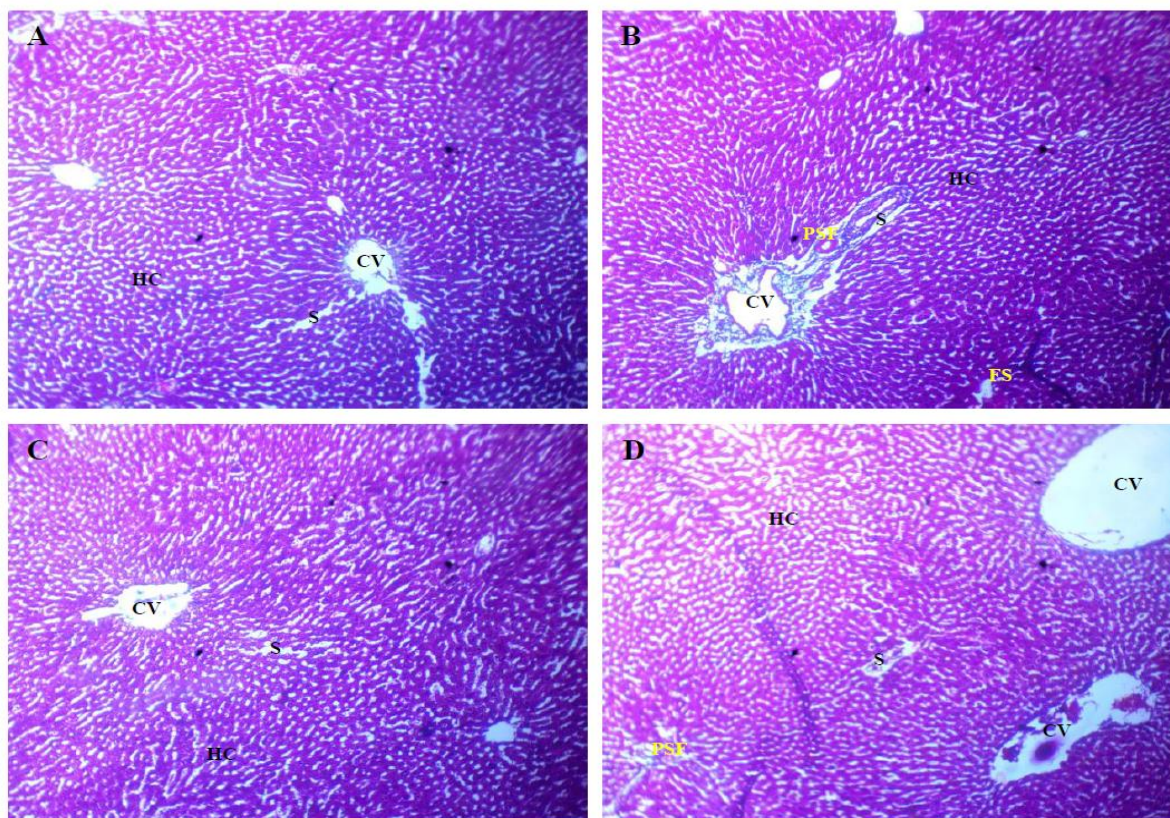

Figure 1: Effect of NAC on liver histology of Wistar rats treated with ammonium nitrate. Masson's trichrome (MT) stain, magnification ×100.

Keys
A: Control group; B: NH_4NO_3 group; C: NAC group; D: NH_4NO_3 + NAC group.
CV: Central vein;
HC: Hepatocytes;
FS: Periportal fibrosis;
PSF: Perisinusoidal fibrosis.

Alkaline phosphatase is an enzyme that present in the liver especially in the canalicular and sinuoidal membranes. It is an indicator of bile duct integrity and hepatobiliary damage.[19] The rise in ALP in the NH_4NO_3 group in this study points to the hepatocellular or biliary injury possibly as a result of oxidative stress, which could affect hepatocyte membrane and lead to the release of ALP into the blood. In this study, there was a substantial reduction of ALP in the NAC and with NH_4NO_3 co-administration. This finding indicates that NAC was able to reverse the NH_4NO_3-induced hepatotoxicity and, perhaps, inhibit the basal ALP activity by its antioxidant activity. The NAC also has the potential to stabilize the action on liver cells membranes, by restoring glutathione and neutralizing reactive oxygen species (ROS) that protect the liver against oxidative damage,[9] thereby restoring the ALP levels.

The serum concentration of ACP activity in this study was greatly high in NH_4NO_3 group. The observed elevation in ACP after the NH_4NO_3 could be a reflection of liver lysosomal membrane damage that cause the liberation of ACP into the liver lysosome.[20] The high concentration of ACP in the NH_4NO_3 group was minimized as a result of NAC co-treatment. The capacity of NAC to lower the ACP in NH_4NO_3 group implies that its antioxidant activity could reverse the lysosomal demise brought by NH_4NO_3. Such a reduction is probably because NAC can stabilize lysosomal membranes and lower the oxidative stress baseline, and this decrease could be associated with its glutathione-replenishing and free radical-scavenging activity.[9]

Lactate dehydrogenase is an important hepatic enzyme that is required for converting lactate to pyruvate to regenerate glucose in the Cori cycle. Serum LDH activity

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was greatly increased in the rats administered with NH_4NO_3 showing possibility of hepatocyte damage. This aligns with the observation that inflammatory cytokines causes the generation of reactive nitrogen and oxygen species, which destabilize hepatic cell membrane and induce LDH leakage.[21] The serum LDH activity is slightly high despite NAC co-treatment with NH_4NO_3, as compared to the control. Although the NAC inhibits the increase in serum LDH activity in co-treatment with NH_4NO_3. This implies that although NAC helps in sustaining antioxidant activity, it may inhibit the damage of hepatocyte membrane caused by NH_4NO_3 through the increase of LDH activity.[22]

The present research found that serum ALT and AST activities were increased in NH_4NO_3-treated group which showed that the NH_4NO_3 may causes liver damage. It aligns with the research indicating that nitrates (such as ammonium salts) lead to increased ALT and AST because of oxidative damage of the liver.[23] In addition, the NH_4NO_3 is reported to cause the oxidative stress and disruption of the membrane in the hepatocytes, resulting in the increase of ALT and AST. [5, 24] The increase in ALT and AST shows the evidence of hepatocellular and lysosomal membrane damage that is likely caused by oxidative stress induced by NH_4NO_3. A similar observation reported that reactive oxygen species cause liver lysosome membrane instability and enzyme leakage into the bloodstream.[25] The NAC-only and co-administration with NH_4NO_3 exhibited a considerable decrease in the activities of ALT and AST, which demonstrates the strong hepatoprotective property of NAC. Through the restoration of glutathione reserves, elimination of reactive oxygen species, and maintenance of cell membrane integrity.[9]

Superoxide dismutase activity in this research also had a significant reduction in the NH_4NO_3 group, a fact which may suggest that NH_4NO_3 causes oxidative stress in liver. The reduction of SOD is consistent with previous study in rats that treated with ammonium salts where liver and brain SOD activity declined dramatically upon exposure to nitrate.[5] Conversely, NAC administration had a great impact on SOD activity, as it may increase the endogenous antioxidant defense in the liver. NAC also restores intracellular glutathione that indirectly enhances the enzymatic ability of SOD. In addition, NAC was also found to increase SOD expression and activity in different tissue types.[26] Ammonium nitrate and NAC co-treatment depicted the apparent increase in the liver SOD activity. This finding indicates that although NAC has some protection effect; this was not sufficient to completely replenish the level of SOD in the presence of NH_4NO_3.
It was also found in the result of this study that the catalase activity in the liver was significantly reduced after the administration of NH_4NO_3. This implies the presence of oxidative stress caused by NH_4NO_3 could overstretch the endogenous liver antioxidant enzymes.[5] The NAC-only group had a considerable increase in catalase activity as compared to the control and NH_4NO_3 group. This indicates that NAC can help to increase the effects of endogenous antioxidant systems in the liver.[27] In contrast to liver SOD activity in the NH_4NO_3 and NAC co-treatment group, the catalase activity was enhanced indicating that the antioxidant effect of NAC was adequate to improve the catalase activity during persistent oxidative injury of the NH_4NO_3.

Glutathione is an essential intracellular antioxidant, which directly interacts with reactive species and contributes to enzymatic detoxification. The NH_4NO_3-only and the NH_4NO_3 + NAC groups demonstrated in this study a significant decrease in the hepatic glutathione level as compared to the control. This reduction suggests that there could be oxidative stress due to the presence of NH_4NO_3 that could drain GSH because it tries to counteract higher concentration of reactive oxygen and nitrogen species. A previous result that matched the report on GSH loss in rats intoxicated with NH_4NO_3.[5] On the contrary, the GSH level in the NAC-only group increased significantly as compared to the control and NH_4NO_3 groups. This highlights the long-standing abilities of NAC as a precursor of glutathione, a significant replenishment of intracellular GSH stock and strengthening of antioxidant defense.[9] Besides, the liver GSH level of the group treated with NH_4NO_3 and NAC was greater than the NH_4NO_3 group, indicating that NAC may prevent the NH_4NO_3 oxidative damage in the liver. This is consistent with the researchs showing that NAC is able to salvage the GSH levels in liver tissues despite continuous exposure to toxicity. [28, 29]

Thiol groups are important components of cellular responses to oxidative stress since they have a non-enzymatic antioxidant effect that helps to maintain the redox balance.[30] Thiols showed a strong reduction in the NH_4NO_3-treatment group and this implies the formation of free radicals, loss of antioxidant defences and this could results in hepatic oxidative injuries as seen in this study. The hepatic effects of NH_4NO_3 may be the negative influence on the thiol level in the liver through formation of reactive nitrogen species including NO. Conversely, the thiol levels of the NAC-only group increased significantly because it acts as a precursor of glutathione synthesis.[30] Besides, the rise in thiol concentration in the NH_4NO_3 + NAC group compared to the NH_4NO_3 group supports the hepatoprotective and antioxidant effect of NAC.
Total antioxidant capacity is a complex value that shows the sum of all antioxidants in plasma and tissues, both enzymatic and non-enzymatic.[31] The NH_4NO_3 group in this study noted a reduction in the total antioxidant capacity, which is consistent with previous studies showing that NH_4NO_3 exposure is associated with oxidative and nitrosative stress.[32] On the other hand, the

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improvement in total antioxidant capacity of both the NAC-only and in the presence of NH_4NO_3 indicate that NAC has the ability to facilitate the antioxidant defence, which facilitates the rise of catalase activity, glutathione and thiol levels recorded in the present research.

The end product of lipid peroxidation is malondialdehyde (MDA), which is a good indicator of oxidative stress. The high levels of MDA in the NH_4NO_3 group, in this research, prove that NH_4NO_3 has hepatotoxic property and pro-oxidant molecule that triggers lipid peroxidation in the hepatocytes, resulting in the excess production of MDA.[5] NAC-alone group exhibited lower levels of MDA indicating that it has a possible ability of enhancing the endogenous antioxidant system and a proactive role under normal conditions in lowering basal lipid peroxidation. Moreover, co-administration of NAC with NH_4NO_3 showed significant reduction of MDA level and this is an indication that NAC protects by inhibiting lipid peroxidation through its component of thiol that could play a role in inhibiting lipid peroxidation.[33, 34]
Nitric oxide (NO) is a small, diffusible, gaseous molecule, which is a biologic messenger and is involved in vasodilation, neurotransmission, and immune defence. Nevertheless, in the high concentrations, NO causes oxidative and nitrosative stress that causes cell damage.[35] Results of this study indicated that the NO level was significantly elevated in the NH_4NO_3 group indicating that nitrosative stress was induced by ammonium nitrate exposure. This aligns with the fact that nitrogen based compounds have the potential to enhance the generation of ROS and RNS, the formation of peroxynitrite and subsequent cellular damage.[35] On the other hand, the NAC-only and the NH_4NO_3 + NAC co-treatment group showed the significant decrease in the liver NO level. It would mean NAC may have an effective effect of lowering the production of NO or detoxifying it. It might be because of the antioxidant effect of NAC that can be linked to glutathione replenishment that buffers oxidative or nitrosative damage.[34] The fact that NAC can decrease the amount of NO even below control implies that the involvement of NAC also has a stabilizing effect on redox-sensitive signaling that may suppress the excess production of NO in physiological and pathological conditions.

Reactive nitrogen species (RNS) are extremely reactive compounds that are formed out of nitric oxide (NO), as well as its reaction with superoxide and other radicals. The major ones are peroxynitrite (ONOO-), nitrogen dioxide (NO_2), and dinitrogen trioxide (N_2O_3) that may damage lipids, proteins, DNA, and mitochondrial membranes. [35, 36] This experiment revealed that the levels of liver RNS were highly increased with exposure to NH_4NO_3, which means that this results in high levels of nitrosative stress. The NH_4NO_3 + NAC co-treatment group demonstrated higher RNS than the control group but less than the NH_4NO_3 group, which indicates the hepatoprotective effect of NAC. Significant decrease in the levels of liver RNS was observed in the NAC-only group relative to control, which proved its antioxidant and anti-nitrosative effects. The mechanisms of NAC include blocking the expression of iNOS, raising the concentration of intracellular glutathione (GSH), and neutralizing NO and peroxynitrite. [34, 37] The retardation of nitrosative stress by NAC was emphasized by the fact that the levels of RNS in all groups were reduced compared to NH_4NO_3 but were still above normal, which showed that NAC reduces but does not completely reverse the accumulation of RNS due to toxic exposure.

This study has indicated that the liver histology after the NH_4NO_3 treatment has demonstrated mutilation around the central vein which could be as a result of the inflammatory cell infiltration that is evident in the liver. The scar in the central vein observed is evident by the excessive fibrous connective tissue seen in the perisusoidal and periportal areas of the liver in the current study. Although the hepatocytes' morphology and some sinusoids appeared normal after the exposure to NH_4NO_3, this may be due to the short duration of administration of the NH_4NO_3. The effects of NH_4NO_3 on mutilation around the central vein caused by the infiltration of inflammatory cells may be explained by other results obtained in this study. The administration of NH_4NO_3 caused elevation of ALP, ACP, ALT, and AST, which are markers of hepatotoxicity. Also, there is an increase in malondialdehyde, NO, reactive nitrogen species, and a reduction in the level of endogenous antioxidant markers by the NH_4NO_3, which may be responsible for the infiltration of inflammatory cells observed in the liver histology due to oxidative stress. The hepatotoxic effect of NH_4NO_3 seen in this study supported the previous report.[5] The administration of NAC reduced the observed scar around the central vein, perisinusoidal fibrosis, and infiltration of inflammatory cells caused by NH_4NO_3 in this study. The antioxidant activity of NAC, the ability to suppress hepatotoxic manifestations, and the liver oxidative stress measured in the current study may be closely connected with the ability of the substance to protect hepatocytes against damage caused by NH_4NO_3.[30] Conclusively, N-acetyl cysteine inhibits the hepatotoxicity caused by ammonium nitrate through decreasing the hepatotoxic enzyme indicators, liver oxidative stress markers, and increasing the antioxidant activities of the liver endogens. The results in this research indicate that people need to exercise caution in occupational and industrial applications of ammonium nitrate to avoid the damage of the liver.

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Conflict of interest/Declaration
All the authors declare no conflict of interest

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