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AMELIORATIVE EFFECT OF N-ACETYL CYSTEINE ON SODIUM ARSENITE INDUCED TOXICITY AND OXIDATIVE STRESS IN MICE

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ABSTRACT

The present study was conducted to investigate the antioxidative effect of N-acetyl cysteine (NAC) against sodium arsenite-induced hepatotoxicity and nephrotoxicity in mice. Animals were divided into four groups; the first group was used as control. As group: mice treated with arsenic (As) as sodium arsenite (5 mg/kg bw/day), NAC group: mice treated with N-Acetyl cysteine (NAC) (200mg/kg bw/day) and As + NAC Group: animals treated with sodium arsenite (5 mg/kg bw /day) plus N-Acetyl cysteine (200 mg/kg bw /day). Mice were orally administered their respective doses every day for 30 days. Results showed that NAC treatment reduced the elevation of liver enzymes Alanine amino transferase (ALT), Aspartate amino transferase (AST) and alkaline phosphatase (ALP) as well as the proinflammatory cytokine TNF-α and caspase-3 activity. On the other hand, liver and renal glutathione peroxidase (GPX), superoxide dismutase (SOD) enzymes and glutathione (GSH) content significantly increased compared to arsenic group and control group. Whereas, the Malondialdehyde (MDA) content of both tissues significantly decreased in comparison with arsenic group. NAC treatment alone also improved the health status of the animal to maintain the normal level of the enzymatic antioxidant and non enzymatic antioxidant activities in liver and kidney tissues as well as caspase-3 activity and the proinflammatory cytokine TNF-α level. It can be concluded that NAC possess high antioxidant activity and more effective in preventing the injury of oxidative damage in both liver and kidney tissues during the arsenic administration.

Keywords: Arsenic, N-acetyl cysteine, Hepatotoxicity, Nephrotoxicity, Apoptosis.

INTRODUCTION

Arsenic, an extremely toxic heavy metal is a common environmental pollutant. Several studies showed that sodium arsenite (NaSO2) to be the most toxic of all arsenic compounds [1] Arsenic is immunotoxic in nature [2] and induces the generation of reactive oxygen species [3]. It has been observed that arsenic modulates lymphocyte co-receptor expression and release of cytokines in mammals [4] resulting in immunosuppression and increased susceptibility to infection [5]. The main source of environmental arsenic exposure in most populations is the drinking water in which inorganic form of arsenic predominate [6]. There is evidence suggesting that arsenic toxicity involves oxidative damage [7], mainly by the interaction of arsenic with protein thiols that are central components of redox-sensitive proteins in redox signaling and control pathways [8].Several studies have demonstrated that liver is the primary arsenic metabolizing organ [9]. N-acetyl cysteine, an aminothiol is a sulphydryl group donor and precursor of glutathione (GSH) [10]. NAC has a protective effect against liver injury in rats [11]**.**

In addition to its hepatoprotective action, NAC is also widely used as an antiangiogenic [12]**,** antifibrotic [13]**,** neuroprotective [14], renoprotective [15] and as a chelating agent in the treatment of heavy metal poisoning [16]. N-acetylcysteine is effectiveness is primarily attributed to its ability to reduce extracellular cystine to cysteine, and as a source of sulfhydryl groups [17]. Administration of NAC in intoxicated animal mainly stimulates glutathione synthesis, enhances glutathione-S-transferase activity, promotes detoxification by inhibiting xenobiotic biotransformation and it is also act as a powerful nucleophile capable of scavenging free radicals [18].

MATERIALS AND METHODS

Experimental animals

Male Swiss albino mice, weighing 25–30 g were obtained from Theodor Bilharz Inistitute, Cairo, Egypt. They were kept in plastic cages at room temperature (25 ± 2 ◦C) and humidity (55%) under a 12 h dark-light cycle. They were supplied with standard laboratory Diet and water *ad libitum*, and left to acclimatize for 1 week before the experiments. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Drugs

Sodium arsenite (soluble in dist water) purchased from Sigma Chemical Co., USA. N-Acetyl cysteine (NAC) (soluble in dist water) was purchased from Sigma Chemical Co. (USA 314-771-5750) Batch#:077k0748 with molecular weight 163.2 and chemical formula: C5H9NO3S.

Experimental design

The animals were randomly divided into four equal groups (6 mice for each). First group: was used as Normal control group. As group: mice treated with arsenic (As) as sodium arsenite (5 mg/kg bw/day) [19], NAC group: mice treated with N-Acetyl cysteine (NAC) (200mg/kg bW/day) [20] and As + NAC Group: animals treated with sodium arsenite (5 mg/kg bw/day) plus N-Acetyl cysteine (200 mg/kg bw/day). Mice were orally administered their respective doses every day for 30 days. At the end of the experiment, Animals were sacrificed and liver and kidney were immediately removed and washed with cold saline, dried and weighed. Then the tissues were homogenized in the appropriate buffer (10%w/v), in ice-cold medium. Then, the homogenates were centrifuged at 3000 rpm for 10 min at 4 ◦C, and the resultant supernatant was used for the determination of the biochemical parameters.

Biochemical assays

TNF-α was determined by ELISA technique using a corresponding enzyme-linked immunosorbent assay kit purchased from Bender Med-Systems Company (Europe). Activity of Caspase-3 was assayed by the method of Kamada *et al*. [21] using the Caspase-3 Assay Kit, colorimetry purchased from Sigma-Aldrich Co. (USA). Total protein in tissue was determined by the method of Young [22]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined by a colorimetric method of Reitman and Frankel [23]. Alkaline phosphatase was determined colorimetrically according to the method described by manufacturer's instruction by using kit purchased from Diamond Co. Malondialdehyde (MDA) was investigated by the thiobarbituric acid test according to the method of Ohkawa et al [24]**.** The activity of superoxide dismutase (SOD) was carried out according to the method of Minami and Yoshikawa [25]. Activity of glutathione peroxidase (GPX) was determined according to the method of Gross et al [26]. Glutathione reduced (GSH) was determined according to the method of Beutler et al [27].

Statistical analysis

All results were expressed as mean ± S.E. of the mean. Statistical Package for the Social Sciences (SPSS) program, version 11.0 was used to compare significance between each two groups. Difference was considered significant when $P \leq 0.05$.

RESULTS

The results in Table 1 showed the effect of N-acetyl cysteine treatment on hepatic and renal inflammatory and apoptic marker of mice treated with arsenic. In comparison with the normal control group, there was a significant increase in the level TNF-α and apoptic marker caspase-3 activity in the liver and kidney of arsenic group (86.56%, 105.67%) and (1521.62%, 1397.89%), respectively. on the other hand, the treatment of arsenic group with NAC produced a significant depletion in the levels of TNF-α and caspase-3 activity in the liver (-36.05%,34.46%) and kidney(-73.41%,-79.55%), respectively as compared to arsenic group and (19.30%,34.79%) and (331.08%,206.31%) as compared to control group. Treatment with NAC alone maintain the normal levels of these markers in liver and kidney ($P < 0.05$) when compared to control mice and arsenic mice.

Table (1) Effect of N-acetyl cysteine treatment on hepatic and renal inflammatory and apoptic markers of mice treated with arsenic.

Parameters / Groups	Liver		Kidney	
	TNF-α (ng/g.wt.tissue)	Caspase-3 (µ.mole.PNA/min/mg protein)	TNF-α (ng/g.wt.tissue)	Caspase-3 (µ.mole.PNA/min/mg protein)
Normal control group	2010.00±88.10	0.74 ±0.05	2120.02±65.28	0.95±0.03
As group	3750.00±218.60[a] C (86.56%)	12.00±1.02[a] C (1521.62%)	4360.38±82.82[a] C (105.67%)	14.23±0.92[a] C (1397.89%)
NAC group	2006.00±102.61[ab] C (-0.19%)	0.70±0.38[ab] C (-5.40%)	2110.87±56.34[ab] C (-0.43%)	0.86±0.05[ab] C (-9.47%)
As + NAC group	2398.00±215.06[b] C (19.30%) D (-36.05%)	3.19±0.57[b] C (331.08%) D (-73.41%)	2857.63±29.34[b] C (34.79%) D (34.46%)	2.91±0.31[b] C (206.31%) D (-79.55%)

The mean difference is significant at the $P \leq 0.05$. C%=Percentage of change from normal control group. D%=Percentage of change from arsenic group. a =compared to the normal control group. b =compared to the As group.

The data in Table 2 showed the effect of N-acetyl cysteine treatment on liver enzymes AST, ALT and ALP activities of mice treated with arsenic. It was revealed that, daily

administration of As in male mice resulted in significant elevation in the levels of AST (45.71%), ALT (16.07%) and ALP(136.67%) as compared to the normal control group. In contrast, the treatment of As group with NAC produced significant reduction in the levels of AST, ALT and ALP (-24.42%, -6.00% and -44.91%), respectively as compared to the As group and (10.12%, 9.10% and 30.36%) as compared to control group. In addition, Treatment with NAC alone caused significant ($P < 0.05$) decrease in the level of these enzymes compared to control and arsenic group.

Table (2) Effect of N-acetyl cysteine treatment on hepatic AST, ALT and ALP of mice treated with arsenic

Parameters / Groups	AST (mg/g tissue)	ALT (mg/g tissue)	ALP (U/L)
Normal control group	1171.90±16.43	1110.65±8.50	65.20±6.70
As group	1707.60±34.77[a] C (45.71%)	1289.20±15.12[a] C (16.07%)	154.31±6.30[a] C (136.67%)
NAC group	1167.32±18.07[ab] C (-0.39%)	1104.15±14.62[ab] C (-0.58%)	60.16±4.20[ab] C (-7.73%)
As+ NAC group	1290.50±80.17[b] C (10.12%) D (-24.42%)	1211.74±22.99[b] C (9.10%) D (-6.00%)	85.00±5.50[b] C (30.36%) D (-44.91%)

The mean difference is significant at the $P \leq 0.05$. C%=Percentage of change from normal control group. D%=Percentage of change from arsenic group. a =compared to the normal control group. b =compared to the arsenic group.

Table3: show the levels of enzymatic and non-enzymatic antioxidants in liver and kidney of different groups. As treatment caused a significant decrease in the activities of the glutathione metabolizing enzymes SOD and GPX as well as GSH ($P < 0.05$) and increase in the levels of MDA content ($P < 0.05$) in liver and kidney tissues when compared to control animals. However, As + NAC treated group showed a significant increase in the activities of SOD, GPX and GSH content ($P < 0.05$) in both liver and kidney as compared to As-treated animals and control animals. Whereas, the level of MDA content recorded significantly decreased ($P < 0.05$) in As + NAC group in comparison to arsenic and control group. NAC treatment alone showed significant increase in the activities of both the antioxidant enzymes and decrease in MDA content ($P < 0.05$) when compared to control animals and As animals.

Table (3) Effect of N-acetyl cysteine treatment on MDA, SOD, GPx and GSH levels in the liver and kidney tissue of mice treated with arsenic

Groups / Parameters		Normal control group	As group	NAC group	As + NAC group
Liver	**MDA** (n.mole/gm)	31.15±1.00	65.12±2.52[a] C (109.05%)	34.74±2.10[ab] C (11.52%)	46.64±3.00[b] C (49.72%) D (-28.37%)
	SOD (μ g/gm)	410.81±7.61	275.64±8.32[a] C (-32.90%)	397.73±3.28[ab] C (-3.18%)	383.42±3.12b C (-6.66%) D (39.10%)
	GPx (mg/gm)	465.30±19.00	350.73±13.00[a] C (24.62%)	471.73±13.00[ab] C (1.38%)	410.06±31.63[b] C (-11.87%) D (16.91%)
	GSH (mg/g tissue)	28.62±1.92	12.92±4.23[a] C (-54.85%)	30.12±2.34[ab] C (5.24%)	22.42±1.39[b] C (-21.66%) D (73.52%)
Kidney	**MDA** (n.mole/gm)	35.29±3.12	105.34±4.32[a] C (198.49%)	30.45±2.14[b] C (13.71%)	45.34±3.62[b] C (28.47%) D (-56.95%)
	SOD (μ g/gm)	400.92±6.25	121.54±3.24[a] C (-69.68%)	410.34±8.34[ab] C (2.35%)	370.24±6.23[b] C (-7.65%) D (204.62%)
	GPx (mg/gm)	570.34±15.28	320.49±10.92[a] C (- 43.80%)	581.21±11.23[ab] C (1.90%)	480.42±121.62[b] C (-15.76%) D (49.90%)
	GSH (mg/g tissue)	25.73±2.35	11.81±2.34[a] C (-54.10%)	27.62±1.92[ab] C (7.34%)	20.81±1.62[b] C (-19.12%) D (76.20%)

The mean difference is significant at the P ≤ 0.05. C%=Percentage of change from normal control group. D%=Percentage of change from arsenic group. a =change at P<0.05 in comparison with negative control group. b =Significant change at P <0.05 in comparison with arsenic group.

DISCUSSION

The present study demonstrated an increase in the levels of hepatic and renal proinflammatory cytokines TNF-α in As group compared to normal control group. These observations supported by Das et al [28]. and Tseng [29] they found that arsenic administration caused NF-κB activation with subsequent cascade of events responsible for tissue injury. Elevated TNF-α is known as an important step for activation of the NF-κB signaling pathway [30]. The increased proinflammatory cytokines could come from the inflammatory cells located in liver and kidney which could play a role in As-induced

hepatotoxicity and nephrotoxicity [31]. Also, the present study demonstrated that arsenic administration resulted in elevation of caspase-3 activity leads to apoptosis.

This result agreement with Majumdar et al [32] and Singh and Rana [33] who demonstrated that activation of caspase family of proteases and hepatocellular apoptosis as a result of arsenic administration. They also found that increased generation of reactive oxygen species and activation of NF-κB was to be correlated with arsenic-induced cellular apoptosis. Generation of reactive oxygen species, alterations in the signal cascade and an imbalance in antioxidant levels, in turn triggers cellular apoptosis in cells. The action of arsenic-induced apoptosis is complex. H_2O_2 is apparently involved in the induction of apoptosis by arsenite [34].

H_2O_2 may play a role as a mediator to induce apoptosis through release of cytochrome c to cytosol, activation of caspase 9 and caspase 3 activities and apoptotic cell death [35]. Recently, many researchers have reported that arsenic trioxide (As2O3) induced apoptotic cell death occurs probably because As_2O_3 acts directly on the mitochondria or binds to adenine nucleotide translocator (ANT) in the mitochondria, inducing the opening of mitochondrial permeability transition pore (MPTP) and release of Cyt c [36],which facilitates the formation of the apoptosome, containing the adaptor apoptotic protease-activating factor (Apaf-1) and the initiator caspase-9 complex, by dATP and finally induces apoptosis in cells [37].

The resulting oxidative stress may also affect the levels and functions of redox-sensitive signaling molecules, such as AP-1, NF-κB, and p53, derange the cell signaling and gene expression systems, and/or induce apoptosis [38]. Previous studies showed that agents which inhibit TNF-α production and NF-κB activation effectively ameliorated arsenic-induced tissue injury [39].

Moreover, the present study revealed that NAC treatment significantly inhibited the expression of caspase-3, an executioner of cell apoptosis, in liver and kidney tissue of mice treated with arsenic. The antiapoptotic activity observed with NAC treatment can be attributed to its antioxidant and anti-inflammatory actions that reduced TNF- α production, and inhibition of apoptosis. In agreement with the present results, previous studies showed that N-acetylcysteine (NAC), an antioxidant, prevents the activation of NF-κB by cytokines and lipopolysaccharide (LPS) in certain cell lines [40, 41]. NAC can also prevent apoptosis

and promote cell survival by activating mitogen-activated protein kinases (MAPK) pathways [42].

In the present study, As treatment resulted in a significant increase in the level of hepatic enzymes AST, ALT and ALP when compared to control. This elevation might be due to arsenic treatment caused liver damage which resulted in enhancement of the hepatic pro-inflammatory cytokine TNFα. This result is in agreement with Tan et al [43] and Pan et al [44] who reported that chronic arsenic exposure induces hepatic oxidative stress due to increased ROS generation and/or reduced anti-oxidant capacity. On the other hand, NAC Treatment ameliorated hepatic injury in terms of the AST, ALT and ALP levels against As treated mice. The reason may be due to NAC with its anti-inflammatory and antioxidant effects could prevent As-induced oxidative damage and hepatic dysfunction. This evidence of liver damage are consistent with the previous findings by Santra et al [45] who mentioned that administration of N-acetylcysteine (NAC) stimulates the synthesis of glutathione, which exhibited therapeutic effects on As-induced liver fibrosis in mice. Flora [46] reported that arsenic-induced liver cell injury in mice is associated with induction of oxidative stress, the perturbations in the mitochondrial redox state and arsenic-induced apoptosis of hepatocytes and all these changes were reduced in intensity and/or modified by pretreatment with NAC. Hepatoprotection by NAC could be due to effective detoxification of electrophiles generated by arsenic as well as its rapid elimination/excretion from the body [47].

The present investigation revealed that arsenic treatment caused a significant increase in lipid peroxidation (MDA) content and decreased glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and reduced glutathione (GSH) content in liver and kidney tissue. The main cause of the arsenic induced liver injury and renal injury is the formation of free radicals. In support to the current results, Gupta and Flora [48] explained that inhibition of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the hepatic tissue of arsenic exposed animals suggests disturbed antioxidant ratio, resulting in oxidative stress. SOD and GPx are the most important defense enzymes against toxic effects of oxygen metabolism. SOD accelerates the dismutation of superoxide to H_2O_2. A decrease in the activity of SOD can be due to enhanced superoxide production during arsenic metabolism [49]. Increased production of free radicals and inhibition of antioxidant enzymes have been possible mechanisms to explain arsenic-induced oxidative damage [50].

GSH is an important cellular antioxidant, and depletion of GSH enhances As-induced cytotoxicity and aberrant gene expression [51]. One of the mechanisms by which arsenicals produce toxic effects in liver and kidney is through their interaction with cellular sulfhydryl groups in proteins or elsewhere [52]**.** As (III) and As (V) have been shown react with GSH to form As-GSH complexes [31]. Arsenicals and arsenothiols have been shown to produce a significant depletion in hepatic GSH up to a 35% along with liver injury [53]**.** Consistent with the literature, the present study observed up to 54% depletion in cellular GSH following repeated As administration. Also, an increased level of lipid peroxidation content was observed in the liver and kidney tissue of arsenic intoxicated mice. On this line, Nain and Smits [54] reported that lipid peroxidation is one indication of As toxicity in rats**.** The present results suggested that during the heavy metal treatment the accumulation arsenic and its toxicity effect is pronounced mainly in the kidney tissues to release more and more amount of LPO content was liberated. Because the release of Lipid peroxidation is mainly initiated by free radicals and is the oxidative deterioration of poly unsaturated fatty acids which is synthesized in intoxicated animals [46]. Bera et al [55] reported that an increased LPO level has a positive correlation with the dose of arsenic.

Various studies reported that arsenic could participate in the cellular oxidation–reduction reactions resulting with the formation of excess ROS such as superoxide anion($O^{-}2$) and hydroxyl radical (OH_) via a chain reaction causing oxidative stress [56]. In the present study, NAC treatment enhancement the levels of antioxidant enzymes SOD and GPX beside GSH content and ameliorated the levels of MDA in liver and kidney as compared to arsenic group. These results may be due to the antioxidant scavenging properties of NAC to remove the ROS like OH; O and H_2O_2 radicals which librated from arsenic treatment. This result was coincide with Gurer et al [57] who reported that, N-Acetylcysteine (NAC) is a thiol-containing antioxidant that has been used to reduce various conditions of oxidative stress. Its antioxidant action is attributed to GSH synthesis; therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS) [58]. Moreover, the treatment with NAC in arsenic intoxicated mice could deplete cellular stores of the GSH and is an effective intervention against oxidative stress developed due to arsenic exposure [45]. NAC can cross the cell membrane therefore provide intracellular effects [46]. The decreased level of lipid peroxidation suggested that respective tissues eliminated As and free radicals [59]. Thus, NAC has a strong ability to restore the impaired pro-oxidant/antioxidant balance in metal poisoning.

CONCLUSION

In conclusion, the present study shows that NAC treatment mitigates arsenic intoxication-induced hepatic and renal oxidative damage in mice, which could be due its antioxidant nature and free radical scavenging properties.

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