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## Original Research Article

## Coenzyme Q10 and N-acetyl cysteine modulates the haematological parameters, markers of oxidative stress and membrane bound phosphatase in spleen toxicity induced by aniline hydrochloride

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## ABSTRACT

Aniline is widely used chemical for industrial purpose. Exposure to aniline can lead to various health issues in human being. The most common toxic effect of aniline is splenotoxicity which is produced via the generation of oxidative stress. The present study examines the protective effect of coenzyme Q10 and N-acetyl cysteine on spleen weight, body weight, endogenous antioxidants and membrane bound ATPases in aniline hydrochloride (AH) induced spleen toxicity in rats. Adult male albino rats were divided into five groups, each group consists of six rats. Toxicity was induced by administration of AH (100 ppm, p. o) in drinking water for 30 days. Body weight, markers of oxidative stress and haematological parameters were assessed at the end of treatment period. Treatment rats received Coenzyme Q10 (CoQ10, 10 mg/kg/day/p.o) and N- acetyl cysteine (NAC, 300mg/kg/ day p.o) alone and in combination for 30. AH toxic rats showed a significant elevation of spleen weight, WBC count, iron content, LPO level, NO level and Ca<sup>++</sup> ATPase whereas a significant decrease in body weight, haemoglobin, RBCs level, Protein content, GSH and Na<sup>+</sup>/K<sup>+</sup> and Mg<sup>++</sup> ATPase were observed. Administration of Coenzyme Q10 (CoQ10, 10 mg/kg/day/p.o) and N- acetyl cysteine (NAC, 300mg/kg/ day p.o) together for 30 consecutive days shows a significant decrease in spleen weight, WBC level, iron content, LPO level, NO level whereas significant increase in body weight, hemoglobin level, RBCs level Protein content, GSH level, Na<sup>+</sup>/K<sup>+</sup> ATPase, Ca<sup>++</sup> and Mg<sup>++</sup> ATPase when compared with aniline treated group and CoQ10 or NAC alone treated groups. These findings indicate the synergistic & protective effect of CoQ10 and NAC in aniline induced spleen toxicity in rats compared to alone antioxidants.

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## 1. Introduction

Aniline, a toxic aromatic amine, is widely used in various chemical industries. The clinical symptoms of aniline exposure include cyanosis, weakness, dizziness, headache, stupor, loss of coordination, and coma.<sup>1</sup> Exposure to aniline is reported to produce spleen toxicity in rats. Various mechanisms by which aniline induces toxicity

are iron overload, protein oxidation, oxidative stress and methaemoglobin formation in the spleen.<sup>2</sup> Oxidative stress plays an important role in spleen toxicity and the treatment with good antioxidants may help to prevent the early symptoms of spleen toxicity.<sup>3,4</sup> CoQ10 is a powerful antioxidant with the potential to scavenge free radicals thereby protecting the cells from oxidative stress. It has been reported to prevent many disease conditions.<sup>5,6</sup> NAC is a thiol mucolytic chemical that functions as a powerful antioxidant. NAC is employed as a therapeutic agent in

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conditions defined by the development of oxidative stress, such as cardiovascular illnesses, inflammatory reactions, and malignancies.<sup>7,8</sup> Considering the involvement of oxidative stress in spleen toxicity and the beneficial role of antioxidants, the present study was designed to assess the effects of CoQ10 and NAC in aniline hydrochloride induced spleen toxicity in rats.

## 2. Materials and Methods

### 2.1. Drugs and chemicals

Aniline HCL was procured from Loba Chemical, India. CoQ 10 was procured from Zydus Cadila, Ahmedabad. NAC was procured from Loba Chemie Pvt. Ltd. Mumbai. 5,5 dithiobis (2 -nitrobenzoic acid) [DTNB] was procured from SD Fine Chem. Limited, Mumbai. N-(1- naphthyl) ethylenediamine dihydrochloride were purchased from Hamedia lab Pvt. Ltd., Mumbai. All the other chemicals used in the study were of analytical grade and procured from standard supplier.

### 2.2. Experimental animals

Male wistar rats (200-250g) were used in the study. The rats were maintained under standard laboratory conditions at temperature  $23 \pm 1^\circ\text{C}$ , relative humidity 45– 55, and 12 h light and 12 h dark cycles throughout the experiments as per CPCSEA guidelines. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of SSDJ College of Pharmacy, Neminagar, Chandwad.

### 2.3. Experimental protocol

The rats were divided into five groups of six rats each. Group I: served as control and received distilled water. Group II: Rats received AH (100ppm in drinking water) for 30 days. Group III: Rats received AH (100ppm in drinking water) and CoQ10 (10mg/kg/p.o) for 30 days. Group IV: Rats received AH (100ppm in drinking water) and NAC (300mg/kg/p.o) for 30 days. Group V: Rats received AH (100ppm in drinking water) and CoQ10 (10mg/kg/p.o) and NAC (300mg/kg/p.o) for 30 days. The dose of CoQ10 and NAC was selected based on previous study from our laboratory.

### 2.4. Assessment of spleen toxicity

At the end of treatment period, body weight, spleen weight, water intake, and feed consumption were noted. Blood was withdrawn from retroorbital plexus using glass capillary and serum was separated using high speed centrifuge. Blood was used for the estimation of haemoglobin contents (Sahli's haemometer method), red blood cell (RBC) count, and white blood cell (WBC) count using haemocytometer. Serum was used for the estimation of total iron contents<sup>9</sup> and protein contents on biochemistry analyser using Span

diagnostic kit. The animals were sacrificed by euthanasia. Spleen were immediately transferred to ice-cold water, it was homogenised, centrifuged and the supernatant was used for the estimation of endogenous antioxidants such as lipid peroxidation (LPO),<sup>10</sup> reduced glutathione (GSH),<sup>11</sup> tissue nitrite level.<sup>12</sup> The spleen sediment was used for the estimation of membrane bound ATPases such as  $\text{Na}^+/\text{K}^+$ ,<sup>13</sup>  $\text{Ca}^{++}$ ,<sup>14</sup> and  $\text{Mg}^{++}$ <sup>15</sup> ATPase.

### 2.5. Statistical analysis

Values are expressed as mean  $\pm$  SEM, (n=6). One way ANOVA followed by Dunnett's test. Level of significance is considered as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to treatment group.

## 3. Results

### 3.1. Effect of CoQ10, NAC Alone and their Combination on Body weight, Water Intake, and Feed Consumption

Body weight, spleen weight, water intake, and feed consumption were monitored at the end of treatment period. Rats administered with AH showed a significant reduction in water intake, feed consumption and water intake whereas spleen weight was found to be significantly increased as compared to normal control rats. Chronic treatment with CoQ10, NAC, and CoQ10 + NAC shown a significant recovery in alteration of water intake, feed consumption, spleen weight, body weight as compared to AH-treated rats. Combination of CoQ10+ NAC shown restoration in all above parameters as compared to CoQ10 and NAC alone (Table 1).

### 3.2. Effect of CoQ10, NAC alone and their combinations on RBC, WBC, and Haemoglobin Level.

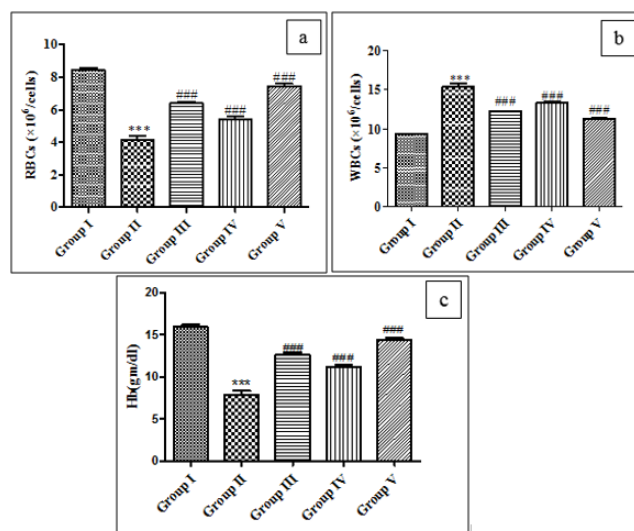
RBCs count and % Hb were significantly decreased whereas WBC count was significantly increased in AH-treated rats as compared to normal control rats. Treatment with CoQ10 and NAC showed a significant increase in RBC count and % Hb as compared to AH treated rats. The WBC count was significantly reduced in AH treated as compared to normal control rats. Alone and combination of antioxidants treatments significantly improved the WBC count in rats as compared to AH-treated group. Combination was found to be more effective as compared to CQ10 alone and NAC alone (Figure 1 a,b and c).

Values are expressed as mean  $\pm$  SEM, (n=6). One way ANOVA followed by Dunnett's test. Level of significance is considered as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to treatment group.

**Table 1:** Effect CoQ10, NAC alone and their combination on body weight, water intake, feed consumption

| Groups | Body weight (g) | Spleen weight (g) | Feed intake (g) | Water Intake (ml) |
|--------|-----------------|-------------------|-----------------|-------------------|
| I      | 287.2 ± 5.32    | 0.589 ± 0.028     | 21.1 ± 0.96     | 40.4 ± 1.54       |
| II     | 212.6 ± 3.36*** | 1.284 ± 0.014***  | 11.8 ± 0.68***  | 20.8 ± 1.07***    |
| III    | 229.6±3.42###   | 0.847 ± 0.146###  | 14.2±0.37##     | 25.4±0.55##       |
| IV     | 233.3±3.03###   | 0.989 ± 0.026#    | 14.6±0.40##     | 25.1±0.49##       |
| V      | 247.2±1.91###   | 0.789 ± 0.014###  | 17.5±0.91###    | 30.5±1.26###      |

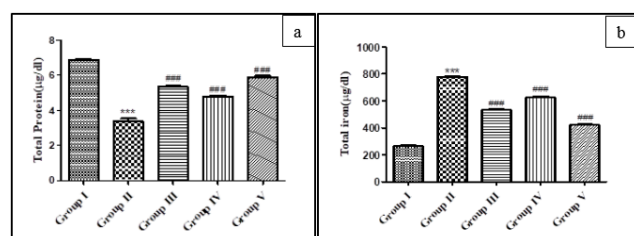
Values are expressed as mean ± SEM, (n=6). One way ANOVA followed by Dunnett's test. Level of significance is considered as \*p < 0.05. \*\*p < 0.01, \*\*\*p < 0.001 compared to control group. #p < 0.05, ##p < 0.01, ###p < 0.001 compared to treatment group.



**Fig. 1:** Effect of CoQ10, NAC alone and their combination on RBC (1a), WBC (1b) and haemoglobin (1c) level in AH induced spleen toxicity

### 3.3. Effect of CoQ10, NAC alone and their combination on Serum total protein and total iron contents

A significant decrease in the level of serum protein and increase in serum iron content were observed in AH intoxicated group. Treatment with CoQ10 in combination with NAC showed a significant increase in total protein and significant decrease in total iron content as compared to AH intoxicated rats. The combination shown additive effects in maintaining protein and iron level towards normal compared to alone antioxidants (Figure 2 a and b).

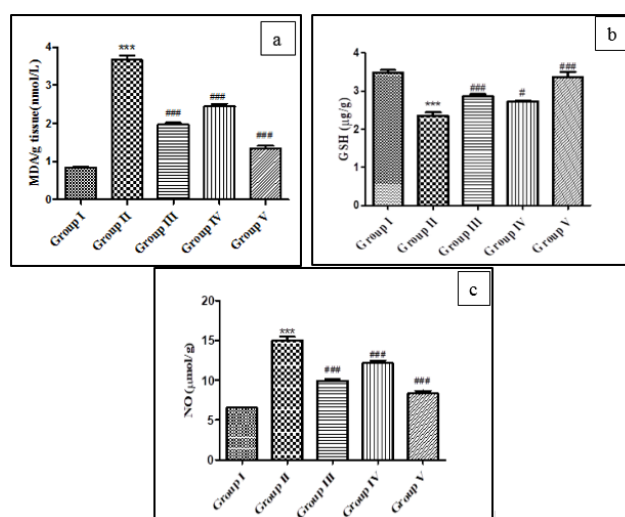


**Fig. 2:** Effect of CoQ10, NAC alone and their combination on total protein (a) and Iron content (b)

Values are expressed as mean ± SEM, (n=6). One way ANOVA followed by Dunnett's test. Level of significance is considered as \*p < 0.05. \*\*p < 0.01, \*\*\*p < 0.001 compared to control group. #p < 0.05, ##p < 0.01, ###p < 0.001 compared to treatment group.

### 3.4. Effect of CoQ 10, NAC alone and their combination on LPO (MDA), GSH and tissue nitrite levels

The lipid peroxidation marker, MDA, endogenous antioxidant GSH, and tissue nitrite (NO) level were measured in spleen tissue homogenate. MDA and NO levels were found significantly ( $p < 0.001$ ) increased and on the other hand tissue GSH was significantly decreased in AH-intoxicated rats as compared to control group. Chronic treatment with CoQ10 in combination with NAC shown a significant ( $p < 0.001$ ) decrease in MDA and NO level (Figure 3 a and c) and a significant ( $p < 0.001$ ) increase in GSH level as compared to AH- group (Figure 3b). The combination was found to be more effective in reducing lipid peroxidation and restoration antioxidant defense compared to CoQ10 and NAC alone treated groups.



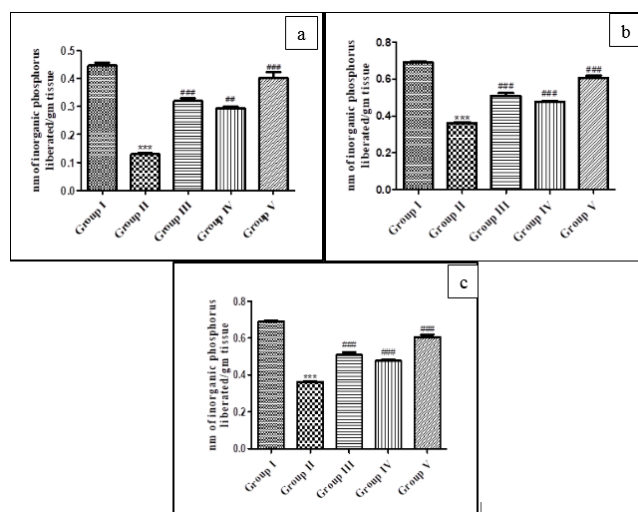
**Fig. 3:** Effect of CoQ10, NAC alone and their combination on LPO (MDA) (a), GSH (b) and NO Level (c).

Values are expressed as mean ± SEM, (n=6). One way ANOVA and Dunnett's test. Level of significance is

considered as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to AH treated group.

### 3.5 Effect of CoQ10, NAC Alone and their Combination on Membrane Bound Phosphatases ( $\text{Na}^+/\text{K}^+$ , $\text{Ca}^{++}$ , and $\text{Mg}^{++}$ ATPase)

The activities of membrane bound phosphatase such as  $\text{Na}^+/\text{K}^+$  ATPase,  $\text{Ca}^{++}$  ATPase, and  $\text{Mg}^{++}$  ATPase in the spleen were estimated. The level of  $\text{Na}^+/\text{K}^+$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$ ATPase was significantly ( $p < 0.001$ ) decreased in AH treated rats compared to control group. Treatment with CoQ10, NAC for 30 days shown significant ( $p < 0.001$ ) increase in the level of  $\text{Na}^+/\text{K}^+$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$  ATPase as compared to AH-treated group. (Figure 4a, b and c).



**Fig. 4:** Effect of CoQ10, NAC alone and their combination on  $\text{Na}^+/\text{K}^+$  ATPase (a),  $\text{Ca}^{++}$  ATPase (b), and  $\text{Mg}^{++}$  ATPase (c).

Values are expressed as mean  $\pm$  SEM, (n=6). One way ANOVA and Dunnett's test. Level of significance is considered as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to AH treated group.

## 4. Discussion and Conclusion

The principal and significant sign of aniline toxicity is the formation of methaemoglobin (MetHb). During hepatic clearance of aniline, it produces its secondary metabolites participating in the formation of MetHb. The formation of MetHb interferes badly with the  $\text{O}_2$  carrying capacity of the blood. MetHb and phenyl hydroxyl amine (PHA), are the two metabolites of aniline contributing to the toxicity of aniline especially the splenic toxicity.<sup>16</sup> At the time of erythrocyte scavenging mechanism, the phagocytes, mainly, the macrophages, themselves get activated, and thus lead to an increased production of highly reactive species, such as hydroxyl radical and ferryl cation, which is results in the observed injury.<sup>17</sup> In the present study, altered body weight,

feed and water intake along with the spleen hypertrophy indicated toxicity caused by AH.

In the present study AH exposed rats showed significant reduction in the level of haemoglobin, decreased RBC, and raised WBC count as compared to control rats. Treatment with CoQ10 and NAC showed significant improvement in the level of haemoglobin and RBC and WBC count, which might be due to the strong antioxidant/free radical scavenging activity of CoQ10 and NAC.

AH administered rats showed a significant increase in iron load and decrease in protein contents. Iron plays a significant role as a mediator of aniline-induced splenotoxicity. AH toxicity causes accumulation of iron which may catalyze excessive formation of reactive oxygen species and damage proteins, nucleic acids, and lipids.<sup>17</sup>

The aniline exposure can lead to induction of lipid peroxidation in the spleen, which are accompanied by morphological changes as vascular congestion, increased red pulp cellularity due to increase sinusoidal cells and fibroblast, capsular thickening and formation of fibrous tissue in the capsule and throughout the parenchyma.<sup>18</sup> AH treatment resulted in greater formation of MDA-protein adducts in the spleen, suggesting that MDA generated as a consequence of lipid peroxidation produces structural modification of native protein, which can alter their functional properties and thus, contribute to splenic toxicity induced by aniline. Free radical generated by aniline can also alters ATPases level.<sup>19</sup>

CoQ10, is a fat-soluble vitamin-like molecule that functions as a natural antioxidant. It is a key component of the mitochondrial electron transport chain (ETC), which is responsible for ATP generation<sup>20</sup> and protects protects cells from oxidative stress.<sup>21</sup> N-acetylcysteine (NAC) is a thiol mucolytic chemical that works as a powerful antioxidant<sup>22</sup> by reacting with a few oxidants, such as nitric oxide and hypochlorous acid.<sup>23</sup> The combination of CoQ10 and NAC might be potentiating the antioxidant effects of each other and thereby producing significant effects compared to diseased rats. In conclusion, present study reveals that the combination of CoQ10 and NAC shown better protection as compared to antioxidant used alone by preventing the oxidative and nitrosative stress in AH-treated spleen toxicity in rats.

## 5. Conflict of Interest

The authors declare no relevant conflicts of interest.

## 6. Source of Funding

None.

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