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Original Article

Curcumin Ameliorates The Hepatoxic Effects of Nitrites: A biochemical Experimental Study

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ABSTRACT

Background and aim: Sodium Nitrate is widely used all over the world and subjects are at risk of their toxic effects. Its use is inevitable and thus proper prophylaxis against its toxic effects is crucial. Curcumin is a medicinal plant with different uses, and it is suggested to produce prophylactic actions against nitrate toxicity. Here we tested the effects of acute oral exposure of NaNO2 and potential ameliorative effects of pre-treatment by curcumin.

Methodology: An experimental study was designed by four groups of animals (each 10 rats). The first was the control group (animals received orally, 2ml/kg, body weight of olive oil (vehicle) daily); the second is the NaNO2 where animals received 2ml/kg, body weight of olive oil and a single dose of NaNO3 (60mg/kg, body weight). The third was the curcumin group (animals received a daily dose of 20mg/kg body weight of curcumin dissolved in 2ml/kg body weight of olive oil). The fourth group was the combination group, where animals received oral doses of curcumin as in the third group, followed by a single oral dose of NaNO2 after 4 weeks of treatment. 48 hours after oral NaNO3, animals were sacrificed, and samples were collected for serum and tissue markers of hepatic injury, oxidative stress, and lipid profile.

Results: The use of a single oral dose of NaNO2 was associated with significant increase of hepatic injury indicators (e.g., ALT, AST, and ALP) when compared to control group. In addition, this group had dyslipidemia and a significant increase of oxidative stress indicators than the control group. The use of curcumin prior to administration of NaNO2 ameliorate these toxic effects but it did not abolish it, as there was a significant difference in combined than control and NaNO2 groups.

Conclusion: A single oral dose of NaNO2 had a hepatotoxic effect. Curcumin had a prophylactic effect when administered prior to exposure to NaNO2. These actions are produced via modulation of antioxidant status.

Keywords: Hepatotoxicity; Turmeric; Nitrites; Liver Enzymes: Oxidative Stress; Lipid Peroxidation.

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INTRODUCTION

Nitrates (specifically Sodium nitrite (NaNO2)) are widely used in industry (e.g., color fixation, food preservation, flavor enhancer, antimicrobials, and rancidity prevention). It is also used as a broncho- and vasodilator at lower physiological doses \(^{(1-4)}\). However, acute exposure to higher disease or chronic exposure to low doses of nitrates and nitrites is associated with toxic effects on several organs. For example, single exposure to a single dose of (20-75 mg/kg body weight) could lead to organ damage in experimental animals. The adverse health effects include but are not limited to respiratory diseases, congenital birth defects, neurological insults, mutagenicity, and carcinogenicity. \(^{2,5}\).

The liver is susceptible to exogenous and endogenous toxic substances. Hepatotoxicity was reported after exposure to NaNO2. These toxic effects are expected to be exerted by mitochondrial injury and oxidative stress \(^{(9)}\). Thus, it is expected that any substance with phytochemical antioxidant properties could prevent NaNO2-induced hepatotoxicity \(^{(12)}\). Turmeric (with active constituent curcumin) is a phytocompound with diverse use for its medicinal actions (For example, it had antioxidant, anti-inflammatory, antiangiogenetic and anticancer actions) \(^{(13-16)}\).

At the same time, turmeric had no toxic effects, and it alleviates several toxicity-induced organ damages (e.g., cypermethrin, lead acetate, mercury, lindane, cadmium) \(^{(19-24)}\). However, data regarding its protective effects against NaNO2-induced hepatotoxicity is scarce. Thus, the current work was designed to investigate if curcumin had a protective potential against hepatotoxicity induced by acute exposure to NaNO2.

MATERIALS AND METHODS

**Chemicals:** All chemicals were obtained from Sigma Aldrich Chemical Co. Inc. (St Louis, MO, USA), and prepared by pharmacist not included in the study.

**Animals:** Forty male Wistar rats (180-220 g) were obtained from the Cairo Faculty of veterinary medicine animal house. They were kept in plastic cages, with free access to chow and water ad libitum for one week (at ambient temperature (25°C) and relative humidity of ~ 50%), and 12 hours dark and light cycles, to permit acclimatization for climate. The handling of animals throughout the study was performed according to the United States National Research Council guidelines (8th edition) for the care and use of laboratory animals \(^{[26]}\). In addition, the study protocol was approved by local research and ethics committee of the DFM, Al-Azhar University, Egypt.

**Experimental design:** Animals were divided into 4 equal groups (each including 10 rats): the first group (control group), where animals received 2ml/kg, body weight of olive oil (vehicle) daily by oral gavage. The second is the study Nitrous oxide group, where animals received 2ml/kg, body weight of olive oil and a single dose of NaNO2 (60mg/kg, body weight) as described by Ansari et al. \(^{(28)}\). The third is the curcumin group, where animals received a daily dose of 20mg/kg body weight of curcumin dissolved in 2ml/kg body weight of olive oil, as described by Tajik et al. \(^{(27)}\). The fourth group is the combination group, where animals received oral doses of curcumin as in the third group, followed by a single oral dose of NaNO2 after 4 weeks of treatment. Two days after the oral dose of NaNO2, animals were sacrificed and blood samples were collected directly from the portal vein in non-heparinized, test tubes, permitted to clot in room temperature, centrifuged at 3000g/minute and serum samples were collected in Eppendorf tubes and used directly for biochemical analysis (to determine liver function biomarkers, C-reactive protein, and lipid profile). The hepatic function indicators (ALT, AST, Alkaline phosphatases (ALP) were determined after the method described by Goodla et al. \(^{(22)}\). Albumin concentrations were estimated according to principle reported by Grant \(^{(20)}\).

Determination of lipid profile (total cholesterol, Triglycerides, HDL, LDL) was achieved according to methods described by Buocolo and David \(^{(29)}\) and Grant \(^{(20)}\). In addition, the high sensitivity C-reactive protein was measured by an enzyme-linked immunosorbent assay (ELISA) (rat C-reactive protein (CRP) ELISA Kit, e-Bioscience, Inc), according to manufacturer instructions. Samples of liver tissues were collected, rinsed in ice 1.15% KCl and stored at -20°C for estimation of oxidative stress biomarkers. The liver tissues were homogenized in 50 mmol/L of Tris-HCl buffer solution followed by centrifugation at 1000g/minute for 15 minutes at 4°C. The supernatants were kept for at -20°C till analysis. The oxidative stress markers, malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD), Glutathione (GSH) and glutathione peroxidase were determined according to methods described by Ohkawa et al. \(^{(30)}\), Manobola et al. \(^{(31)}\), Misra and Fridovich \(^{(32)}\), Follow et al. \(^{(33)}\), and Rotruck et al. \(^{(34)}\).

**Statistical analysis:** The collected data were represented by the arithmetic mean and standard deviation. Groups were compared by one way analysis of variance (ANOVA) with post-hoc analysis for comparison between two groups. The differences were considered significant at p value < 0.05.

RESULTS

In the current work, liver enzymes were significantly increased with a single oral dose of NaNO2. The effect was ameliorated by the prophylactic use of curcumin. But did not reach the values as in the control group. On the other side, albumin was significantly reduced in NaNO2 than the control group. The use of curcumin prevents this effect and there were no significant differences between the curcumin and control groups. The prophylactic use of curcumin ameliorates the effect but not to the normal levels (the combined group still have lower values than the control group) \(^{(21)}\). Table (1). Table (2) presented the results of the lipid profile among the study groups. The use of NaNO2 was associated with a significant elevation of total cholesterol, triglycerides, LDL and CRP than the control group. No significant differences were recorded between curcumin and control groups. However, the use of prophylactic curcumin in combined group was associated with significant improvement of lipid profiles. However, there was a significant difference between combined and control groups.

Results of the current work showed a significant increase of MDA in NaNO2 than control group in values of MDA. However, values of GSH, SOD, glutathione peroxidases and catalase were significantly reduced in NaNO2 than the control groups. The combined use of curcumin followed by NaNO2 was associated with significant improvement in these values. However, there is still a significant difference between combined and control groups (Table 3).
Table (1): Liver function indicators among study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaNO2</th>
<th>Curcumin</th>
<th>Combined</th>
<th>F</th>
<th>P</th>
<th>Post Hoc, LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>Means±SD 46.20±8.13</td>
<td>86.10±10.99</td>
<td>39.40±5.20</td>
<td>58.10±6.36</td>
<td>66.05</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>30-62</td>
<td>70-105</td>
<td>32-52</td>
<td>49-71</td>
<td></td>
<td></td>
<td>P2&lt;0.07</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>Means±SD 132.50±21.34</td>
<td>191.00±16.70</td>
<td>130.50±20.38</td>
<td>151.00±13.22</td>
<td>23.62</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>102-160</td>
<td>160-220</td>
<td>100-158</td>
<td>129-167</td>
<td></td>
<td></td>
<td>P2&lt;0.80</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>Means±SD 22.90±3.25</td>
<td>80.70±7.54</td>
<td>21.70±2.79</td>
<td>61.80±6.55</td>
<td>290.2</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>19-30</td>
<td>73-96</td>
<td>18-28</td>
<td>51-72</td>
<td></td>
<td></td>
<td>P2&lt;0.62</td>
</tr>
<tr>
<td>Albumin (g/d)</td>
<td>Means±SD 4.25±0.22</td>
<td>3.92±0.19</td>
<td>4.35±0.22</td>
<td>4.04±0.18</td>
<td>9.48</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>3.90-4.60</td>
<td>3.50-4.10</td>
<td>4.0-4.70</td>
<td>3.70-4.30</td>
<td></td>
<td></td>
<td>P2&lt;0.273</td>
</tr>
</tbody>
</table>

P1: NaNO2 vs Control; P2: Curcumin vs Control; P3: Combined versus control; P4: Curcumin vs NaNO2; P5: combined vs NaNO2 and P6 combined vs Curcumin.

Table (2): Lipid profile and CRP among study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaNO2</th>
<th>Curcumin</th>
<th>Combined</th>
<th>F</th>
<th>P</th>
<th>Post Hoc, LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>Means±SD 131.10±9.55</td>
<td>144.10±4.33</td>
<td>130.30±6.78</td>
<td>139.20±3.29</td>
<td>10.56</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>115-142</td>
<td>138-151</td>
<td>121-140</td>
<td>133-144</td>
<td></td>
<td></td>
<td>P2&lt;0.78</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>Means±SD 57.30±11.35</td>
<td>76.40±12.99</td>
<td>56.20±11.79</td>
<td>65.60±14.09</td>
<td>5.50</td>
<td>0.003*</td>
<td>P1&lt;0.002*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>39-79</td>
<td>59-99</td>
<td>40-80</td>
<td>45-91</td>
<td></td>
<td></td>
<td>P2&lt;0.85</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>Means±SD 44.90±4.17</td>
<td>25.10±3.54</td>
<td>32.70±5.12</td>
<td>50.77</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
<td>P3&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>39-52</td>
<td>19-31</td>
<td>25-41</td>
<td>45-25</td>
<td></td>
<td></td>
<td>P4&lt;0.001*</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>Means±SD 57.00±5.56</td>
<td>86.70±11.51</td>
<td>51.70±5.52</td>
<td>73.70±10.57</td>
<td>28.52</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>49-68</td>
<td>72-108</td>
<td>48-67</td>
<td>59-89</td>
<td></td>
<td></td>
<td>P2&lt;0.74</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>Means±SD 12.80±2.85</td>
<td>33.70±6.39</td>
<td>12.40±2.41</td>
<td>32.60±6.71</td>
<td>56.36</td>
<td>&lt;0.001*</td>
<td>P1&lt;0.001*</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>9-19</td>
<td>27-48</td>
<td>10-17</td>
<td>25-48</td>
<td></td>
<td></td>
<td>P2&lt;0.85</td>
</tr>
</tbody>
</table>

P1: NaNO2 vs Control; P2: Curcumin vs Control; P3: Combined versus control; P4: Curcumin vs NaNO2; P5: combined vs NaNO2 and P6 combined vs Curcumin.

Table (3): Indicators of oxidative stress among study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaNO2</th>
<th>Curcumin</th>
<th>Combined</th>
<th>F</th>
<th>P</th>
<th>Post Hoc, LSD</th>
</tr>
</thead>
</table>
| MDA (umol/ M
g Protein) | Means±SD 3.07±0.53        | 8.35±1.16                  | 3.05±0.45                   | 5.61±1.10                  | 83.16 | <0.001* | P1<0.001*     |
| Min.-Max.      | 2.1-4.1                      | 5.9-9.6                    | 2.20-3.80                   | 4.20-8.10                  |     |       | P2<0.95       |
| GSH (umol/ M
g Protein) | Means±SD 10.91±1.99      | 5.41±1.02                  | 10.76±1.55                  | 6.89±1.34                  | 33.22 | <0.001* | P1<0.001*     |
| Min.-Max.      | 7.9-13.5                     | 3.9-7.1                    | 8.66-13.10                  | 4.9-9.3                    |     |       | P2<0.82       |
| SOD (U/ M
g Protein) | Means±SD 48.82±4.41        | 17.70±3.11                 | 48.70±3.02                  | 28.60±3.20                 | 195.27 | <0.001* | P1<0.001*     |
| Min.-Max.      | 39-54                        | 12-22                      | 44-55                       | 24-33                      |     |       | P2<0.93       |
| GPx (U/ M
g Protein) | Means±SD 18.0±4.69       | 9.90±3.90                  | 18.60±3.10                  | 13.0±1.88                  | 13.74 | <0.001* | P1<0.001*     |
| Min.-Max.      | 7.0-25                       | 7-20                       | 11-23                       | 10-15                      |     |       | P2<0.71       |
| CAT (U/mmol H2O2
 consumed/mgProtein) | Means±SD 801.90±29.01    | 383.0±62.90                | 799.50±27.73                | 608.9±35.47                | 231.24 | <0.001* | P1<0.001*     |
| Min.-Max.      | 760-850                      | 290-520                    | 750-840                     | 570-680                    |     |       | P2<0.49       |

P1: NaNO2 vs Control; P2: Curcumin Vs control; P3: Combined versus control; P4: Curcumin vs NaNO2, P5 combined vs NaNO2 and P6 combined vs Curcumin.

DISCUSSION

Humans are widely exposed to nitrites through different environmental routes (e.g., contaminated water). In addition, canned foods are significant sources of exposure and induced toxicities. However, this exposure is unavoidable. Hence, the importance of prophylactic substances for those exposed to nitrates and nitrites. Here we tested the acute effects of a single dose of NaNO2 and possible ameliorative effects of curcumin. The results showed that the single oral dose is associated with significant changes in chemical indicators of liver injury. This could be attributed to significant changes in lipid profile and oxidative stress markers (i.e., the liver injury is mediated by toxic stress response). The use of curcumin for two weeks before acute exposure is associated with significant reduction of liver injury and oxidative stress indicators. The reported significant elevations of liver enzymes, ALT, AST, and ALP are markers of liver injury as reported in previous studies. In addition, the significant reduction of serum albumin may be related to destruction of hepatocytes and reported early in liver damage. The reduction of albumin synthesis could be explaining increased values of CRP due to switching of the direction of its synthesis.

Lipids are vital molecules for normal cellular function, structure, and homeostasis. The liver is a key player in the lipid’s metabolism. So, liver injury (acute or chronic) is expected to be reflected on the levels of lipids. This is confirmed by the results of the current work and these results agree with previous studies. The reduction in the lipids was suggested to be occurred by lipolysis, through stimulation of hormone sensitive lipase.

The use of curcumin was associated with significant amelioration of the toxic effects of NaNO2. Previous studies proved the ameliorative effects of curcumin on lipid profile.

The oxidative stress induced by NaNO2 is consistent with previous studies. NaNO2 is a highly reactive compound, that could oxidize, reduce or nitrolyze compounds. It also transformed into different compounds.
(e.g., nitrous acid, nitric oxide, and nitrates) 61; and reported oxidative stress can be explained by these compounds. The use of curcumin could neutralize or ameliorate these oxidative stress as it had anti-oxidative actions. anti-oxidative actions play a hepatoprotective action, as reported previously (45–47).

Previous reports indicated that the pre-treatment of animals with curcumin before exposure to toxic agents results in significant improvement of the oxidative stress and its biomarkers (e.g., MDA). Thus, curcumin play a role against NaNO2-induced cellular damage (46,48).

This action of curcumin had been confirmed in earlier studies and against different toxic agents (Heavy metals, lead acetate paracetamol, and lindane) on different organs. The antioxidant ability of curcumin is further evidenced by increased levels of glutathione and activities of antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalse) in the livers of the treated rats (30, 48-51).

To summarize, the current work proved the hepatic effects of a single oral dose of NaNO2 and indicated the protective (ameliorative) effects of curcumin against these hepatic effects. These actions are produced via modulation of antioxidant status.

Authors financial and conflict of interest disclosure: none to be disclosed.

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2. Ansari FA, Ali SN, Arif H, Khan AA, Mahmood R. Acute oral dose of sodium nitrite and indicated the protective (ameliorative) antioxidant enzymes (against different toxic agent improvement of the oxidative stress and its biomarkers (e.g, MDA) previously neutralize or ameliorate these oxidative stress as it had anti).


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