Diet Fortification with *Curcuma longa* and *Allium cepa* Ameliorates 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Induced Dyslipidemia and Oxidative Stress in Wistar Rats


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ABSTRACT

Industrial and environmental activities continuously release chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) that are hazardous to human health. This research seeks to assess the potentials of *Allium cepa* and *Curcuma longa* in suppressing 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity. Forty (40) male Wistar rats weighing 121.77±16.23 g were divided into eight groups of five (5) rats per group. Positive control was fed standard diet, and given 2 µg/kg/week of TCDD in 0.5ml DMSO while normal control was fed standard diet and given 0.5ml dimethyl sulfoxide (DMSO)/kg body weight/week instead of TCDD. Other groups received 2µg/kg/week of TCDD in 0.5ml DMSO and were fed varied inclusion levels of powdered *Allium cepa* and *Curcuma longa*. On the 35th day of the experimental period, the rats were sacrificed and serum prepared from collected blood for antioxidant enzymes, malondialdehyde (MDA) and lipid profile determinations. All treated groups had significantly (p<0.05) higher activities of antioxidant enzymes when compared with the positive control. Malondialdehyde concentration was significantly (p<0.05) lower in the treated groups than the positive control (187.87±5.65 nmoll). Lipid profiles were significantly improved upon by the inclusion of *Allium cepa* and *Curcuma longa*. Therefore, we conclude that dyslipidemia and oxidative stress due to exposure to dioxin in rats could be suppressed by consumption of 2.5% to 5% inclusion levels of powdered onion and/or turmeric in the diet.

Keywords: 2,3,7,8-tetrachlorodibenzo-p-dioxin; glutathione peroxidase; glutathione reductase; malondialdehyde; *Allium cepa*; *Curcuma longa*.

INTRODUCTION

Dioxins are generic term for polychlorinated dibenzo-dioxins and polychlorinated dibenzofurans. They are among the persistent organic pollutants as their chemical stability and absorbability combine to make them last long in the body (WHO, 2016). Dioxins do bioaccumulate particularly in adipocytes. They can also bio-magnify and move long distances in the food chains (Letcher et al., 2009; Shaw et al., 2014). They are very toxic, the most toxic of which is 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) (Ciftci et al., 2018; Natalija et al., 2010). They exert their toxicity by activating the Ah-receptor in the cytoplasm of vertebrates. The activation could cause compromised liver function, poor sensory abilities and distorted cognitive development (Chen et al., 2006). Although known for their carcinogenicity, dioxins also mediate lipid peroxidation, cell membrane disruption, DNA and protein damage, atherosclerosis, hypertension and other related disorders in humans via oxidative stress (Natalija et al., 2010; Sally and Linda, 2009). According to WHO (2016), the lifetime tolerable amount of dioxins is 70 pg/kg body weight/per month while acceptable dietary intake according to Van den Berg et al. (1998) is 4 pg TEO/kg body weight/day. Since dioxins exert their lethal effects through oxidative stress (Hassoun et al., 2006), antioxidant-enriched diets could be helpful in attenuating the effects. Turmeric has been widely studied for its function as antioxidant, which is attributed to curcumin, its major bio-active component (Priyanka et al., 2017). Onions have been reported to possess antioxidant activities due to their flavonoids, anthocyanins and flavonols contents (Ana et al., 2021). To the best of our knowledge, there is little information with regards to fortification of diets with turmeric and/or onion as a safe and natural means of suppressing dioxin-induced toxicity. The present study investigated the anti-dyslipidemic and oxidative balancing properties of diets fortified with powdered turmeric and/or onion in Wistar rats induced with dioxin toxicity.

MATERIALS AND METHODS

Feed Ingredients

Soy bean meal (SBM), corn oil, palm oil, bone meal, cellulose, salt mix, methionine and pre mix were purchased from Dutsin-Ma central market. Corn starch was prepared from corn after soaking in water for 48 hours.

Identification of spices

Turmeric and onion were collected from gardens located in Ajawa, Rimi local government area of Katsina State in Nigeria and identified as *Curcuma longa* and *Allium cepa* respectively at Department of Biological Sciences, Federal University Dutsin-Ma Katsina State. FUDMA/PSB/00143 and FUDMA/PSB/0089 voucher numbers were respectively assigned to the plants.
Dioxin of the type 2,3,7,8-tetrachlorodibenzo-p-dioxin was obtained from LOBA CHEMIE PVT Ltd while assay kits were purchased from reputable vendor and were products of Randox Ltd.

Preparation of Spices and Feed Formulation
Onion and turmeric were dried in an envelope exposed to sunlight, pulverized and sieved using 0.05 mm sieve. The sieved samples were then mixed thoroughly in equal ratio to prepare mixed spices. Standard rodent diet was prepared by appropriately mixing corn starch, SBM, cellulose, salt, vitamin mix and mineral mix in accordance with the method of Idoko et al. (2022) as shown in Table 1.

Table 1: Standard rat chow

<table>
<thead>
<tr>
<th>FEED COMPONENT</th>
<th>CONTROL DIET (g/kg)</th>
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<tbody>
<tr>
<td>n starch</td>
<td>554.5</td>
</tr>
<tr>
<td>SBM</td>
<td>320</td>
</tr>
<tr>
<td>Cellulose</td>
<td>45</td>
</tr>
<tr>
<td>Bone Meal</td>
<td>12.5</td>
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<tr>
<td>Palm oil</td>
<td>60</td>
</tr>
<tr>
<td>Salt mix</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin/mineral mix</td>
<td>2.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Mineral mix (g/kg): CaCO₃ (15.258), CoCl₂.6H₂O (0.001), ZnCl₂ (0.001), CuSO₄.5H₂O (0.019), FeSO₄.7H₂O (1.078), MgSO₄ (2.929), MnSO₄.2H₂O (0.178), KI (0.032), KH₂PO₄ (15.559) and NaCl (5.573). Vitamin mix (g/kg diets): thiamine (0.02), riboflavin (0.03), pyridoxine (0.01), P-aminobenzoic acid (0.20), myo-inositol (2.00), biotin (0.001), menadione (0.01), ergocalciferol (0.4), choline-HCl (2.0), and cellulose (3.31), α-tocopherol acetate (50), retinal palmitate (0.4), calcium pantothenate (0.0016) and folic acid (0.0002).

Supplemented Diet Formulation
The supplemented diets were formulated by mixing the formulated rat chow with each of the prepared spices in the ratio 95:5 and 97.5 to get 5% and 2.5% inclusion levels respectively. For 2.5% inclusion levels of the spices, the standard diet was mixed with each of the spices in the ratio of 97.5:2.5.

Preparation of Dioxin Solution
In 0.5 ml dimethyl sulfoxide, 2 μg of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) dioxin was added and allowed to dissolve completely by stirring gently. The solution was administered orally to the rats using the following relationship: VT = BW x 0.5.

Where VT = volume of dioxin solution received per kg body weight; BW = body weight of individual rats.

Experimental Design
Forty (40) male Wistar rats weighing 121.77±16.23g were divided into eight (8) groups of five (5) rats per group as follow:

Group 1: Normal control was fed standard diet and given 0.5ml DMSO/week.

Group 2: Positive control was fed standard diet and given 2µg/kg/week of TCDD in 0.5ml DMSO.

Group 3: Rats on 2.5% onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO.

Group 4: Rats on 5% onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO.

Group 5: Rats on 2.5% turmeric supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO.

Group 6: Rats on 5% turmeric supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO.

Group 7: Rats on 2.5% mixture of turmeric and onion-supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO.

Group 8: Rats on 5% mixture of turmeric and onion-supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO.

All administrations of the dioxin solution were by gavage. The rats were maintained on their respective diets ad libitum for a period of five (5) weeks.

Blood Sample Collection
At the end of the 5-week treatment period, the rats were weighed then anaesthetized followed by cutting of the jugular vein. Serum was obtained from collected blood samples by centrifugation at 1500 g for 15 minutes and stored at –20 °C until required (Suragani et al., 2014).

Determination of Indices of Dyslipidaemia
The serum concentrations of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) concentrations were estimated using commercial Randox assay kits according to manufacturers instruction. Low Density Lipoprotein (LDL) was computed as described by Friedewald et al. (1972): LDL=total cholesterol – (Triglycerides/2.2) – HDL, while atherogenic index (AI) and coronary risk index (CRI) were determined following the method of Kazemi et al. (2018).

Atherogenic Index (AI) = LDL-C/HDL-C; Coronary risk index (CRI)= (TC/HDL-C).

Determination of Oxidative Status
The serum concentration of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase and catalase were determined using commercial Randox assay kits, while MDA concentrations were estimated by measuring TBARS.

Statistical Analysis
Results are presented as mean ± standard error of mean (SEM) of 3 determinations. Analysis of variance (ANOVA) and Duncan’s new multiple range test were performed using SPSS version 16 (IBM Corp.) software package at 95% confidence interval.
RESULTS
The results of the lipid profile and atherogenic indices (Table 2) shows that the group exposed to TDDC but fed 5% mixed spices supplemented diet (group 8) had significantly (p < 0.05) lowest concentration of cholesterol. With the exception of group exposed to TDDC and maintained on 2.5% mixed spices supplemented diet (Group 7) which had significantly (p<0.05) lower triglyceride in comparison with the normal control (Group 1), all other treated groups had significantly (p<0.05) higher triglyceride when compared with the normal control but significantly (p < 0.05) lower if compared with the positive control (Group 2). All the treated groups had significantly (p < 0.05) higher HDL but lower LDL, AI and CRI as compared with the positive control (Group 2).

DISCUSSION
Increasing atherogenic index and coronary risk index as seen in the group of rats exposed to dioxin and fed standard diet correlates with increasing atherosclerosis and myocardial infarction (Donat-Vargas et al., 2020; Kazemi et al., 2018; Vennila and Pugalendi, 2012) and is consistent with the reports of Natalija et al. (2010) and Sally and Linda (2008) suggesting that exposure to dioxin is a high-risk factor for atherosclerosis. Similarly, Ida et al. (2019) reported that dyslipidemia is a major risk factor in the development of cardiovascular disease. This creates a vicious circle as damage to the heart exacerbates dyslipidemia (Upaganlawar and Balaraman, 2012).

Diet supplementation with onion and/or turmeric moved towards normalising lipid metabolism and ameliorating dyslipidemia, and may have prevented atherosclerosis in rats exposed to dioxin toxicity as seen in the significantly lower total cholesterol, triglyceride, LDL-C, atherogenic index, coronary risk index, and higher HDL in the groups exposed to dioxin toxicity but fed the supplemented diets relative to the group exposed to dioxin toxicity but given standard diet. Curcuminoids are the major bioactive compounds in turmeric and had significantly (p < 0.05) higher activities in the treated groups relative to the group exposed to dioxin toxicity but fed the standard diet. This study indicates that supplementation of spices diet with turmeric and onion has the potential to lower the concentration of cholesterol, triglyceride, LDL-C and AI, and to increase HDL-C and CRI.

Table 2: Lipid panels and atherogenic indices in dioxin toxicity-induced rats treated with Curcuma longa and/or Allium cepa.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tot. Chol. (mg/dl)</th>
<th>Chol. (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>AI</th>
<th>CRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>55.00±0.58**</td>
<td>87.67±1.45*</td>
<td>10.00±0.58*</td>
<td>27.47±0.79*</td>
<td>2.77±0.19*</td>
<td>5.53±0.29*</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>94.67±2.4†</td>
<td>198.33±2.19*</td>
<td>5.00±0.58*</td>
<td>41.00±1.5*</td>
<td>10.33±1.57†</td>
<td>19.5±2.57†</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>65.33±0.88°</td>
<td>110.1±1.152*</td>
<td>11.0±1.10°</td>
<td>32.33±0.71†</td>
<td>3.00±0.36*</td>
<td>6.04±0.54*</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>64.33±1.67**</td>
<td>139.6±0.88*</td>
<td>16.0±1.00°</td>
<td>20.4±1.39*</td>
<td>1.28±0.12°</td>
<td>4.04±0.21°</td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td>60.67±1.33**</td>
<td>131.33±2.40*</td>
<td>14.00±1.00°</td>
<td>20.40±2.16*</td>
<td>1.50±0.27*</td>
<td>4.39±0.4*</td>
<td></td>
</tr>
<tr>
<td>G6</td>
<td>60.00±1.15°</td>
<td>133.11±0.33*</td>
<td>10.67±0.88*</td>
<td>20.1±1.24*</td>
<td>3.57±0.39†</td>
<td>5.70±0.48*</td>
<td></td>
</tr>
<tr>
<td>G7</td>
<td>52.00±1.15°</td>
<td>58.00±1.53°</td>
<td>9.00±1.00°</td>
<td>31.4±0.40°</td>
<td>3.57±0.38*</td>
<td>5.89±0.49*</td>
<td></td>
</tr>
<tr>
<td>G8</td>
<td>50.33±0.33°</td>
<td>100.00±1.15°</td>
<td>9.67±0.67°</td>
<td>20.67±0.98*</td>
<td>2.17±0.23*</td>
<td>5.26±0.36*</td>
<td></td>
</tr>
</tbody>
</table>

The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different at p>0.05. G1 = Normal control was fed standard diet and given 0.5ml DMSO/week; G2 = Positive control was fed standard diet and given 2µg/kg/week of TCDD in 0.5ml DMSO; G3 = Rats on 2.5% onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G4 = Rats on 5% onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G5 = Rats on 2.5% turmeric supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO. G6 = Rats on 5% turmeric supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G7 = MIX-Rats on 2.5% mixture of turmeric and onion-supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G8 = MIX-Rats on 5% mixture of turmeric and onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; TC = total cholesterol; TG = triglyceride; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; AI = atherogenic index; CRI = coronary risk index.
supplemented diet which could also improve lipid metabolism and prevent dyslipidaemia.

Table 3: Serum activities of antioxidant enzymes and serum concentration of MDA in dioxin toxicity-induced rats treated with Curcuma longa and/or Allium cepa.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT (u/l)</th>
<th>SOD (u/l)</th>
<th>GPx (u/l)</th>
<th>GR (nmol/l)</th>
<th>MDA (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>4.97±0.09a</td>
<td>9.57±0.07b</td>
<td>10.00±0.15d</td>
<td>4.83±0.12b</td>
<td>98.53±1.34d</td>
</tr>
<tr>
<td>G2</td>
<td>2.37±0.12a</td>
<td>7.33±0.23a</td>
<td>5.67±0.18a</td>
<td>3.23±0.15a</td>
<td>187.87±5.65a</td>
</tr>
<tr>
<td>G3</td>
<td>4.57±0.09a</td>
<td>18.67±0.34d</td>
<td>7.67±0.33c</td>
<td>6.13±0.13d</td>
<td>75.40±0.31c</td>
</tr>
<tr>
<td>G4</td>
<td>4.83±0.12g</td>
<td>14.40±0.32c</td>
<td>9.40±0.21a</td>
<td>4.47±0.29a</td>
<td>62.00±4.16b</td>
</tr>
<tr>
<td>G5</td>
<td>4.10±0.06d</td>
<td>18.57±0.22d</td>
<td>7.43±0.33a</td>
<td>6.80±0.06a</td>
<td>76.77±0.39c</td>
</tr>
<tr>
<td>G6</td>
<td>4.90±0.06i</td>
<td>24.13±0.45a</td>
<td>7.97±0.88c</td>
<td>8.03±0.09a</td>
<td>26.53±1.25a</td>
</tr>
<tr>
<td>G7</td>
<td>3.67±0.03c</td>
<td>15.17±0.46c</td>
<td>7.47±0.15c</td>
<td>5.63±0.07c</td>
<td>53.87±3.06b</td>
</tr>
<tr>
<td>G8</td>
<td>3.20±0.12a</td>
<td>8.93±0.18b</td>
<td>8.73±0.27d</td>
<td>6.27±0.12d</td>
<td>74.47±2.19</td>
</tr>
</tbody>
</table>

The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly (p<0.05) different; G1 = Normal control was fed standard diet and given 0.5 ml DMSO/week; G2 = Positive control was fed standard diet and given 2 µg/kg/week of TCDD in 0.5ml DMSO; G3 = Rats on 2.5% onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G4 = Rats on 5% onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G5 = T-Rats on 2.5% turmeric supplemented diet, and given 2 µg/kg/week of TCDD in 0.5ml DMSO. G6 = Rats on 5% turmeric supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G7 = MIX-Rats on 2.5% mixture of turmeric and onion-supplemented diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO; G8= Rats on 5% mixture of turmeric and onion supplemented diet, and given 2µg/kg/week of TCDD in 0.5 ml DMSO; SOD = Superoxide dismutase; GPx = Glutathione peroxidise; GR = Glutathione reductase; CAT = Catalase; MDA = Malondialdehyde.

Our finding on dyslipidaemic effect of onion supplemented diet in rats exposed to dioxin could result from LDL being converted to HDL via activation of lecithin-cholesterol acyltransferase as reported by Ige and Akhigbe (2013) and/or by increased excretion of bile acids or inhibition of cholesterol absorption as demonstrated by Guan et al. (2010). These could be attributed to its rich fibre and flavonoids (Hamauzu et al., 2011). Our result is consistent with the finding of Huang et al. (2021) who reported that supplementation of onion has beneficial effect on dyslipidaemia. It is evident from our finding that diet supplementation with onion could prevent atherosclerosis, a common feature of dioxin toxicity since according to Mattiuzzi et al. (2020), controlling blood lipid decreases the risk of atherosclerosis and/or cardiovascular disease.

The improvement observed in the lipid and atherogenic status of the groups of dioxin-exposed rats fed diets supplemented with onion and/or turmeric could be through scavenging excess free radicals generated by dioxin as indicated by significantly higher activities of the studied antioxidant enzymes and lower MDA levels in the exposed groups fed supplemented diets. Experiments have demonstrated that dioxin particularly TCDD cause DNA damage, peroxidation of lipids and consequently tissue damage as a result excess generation of free radicals. Free reactive oxygen species attack lipids which negatively affect lipid fluidity leading to eventual cell death. Lipid peroxidation intermediates are toxic and could affect the DNA (Dianzani and Barrera, 2008). This is one of the major mechanisms of oxidative damages to tissues. Turmeric improves insulin sensitivity in metabolically disturbed rats (Imam et al., 2022) and therefore could prevent degenerations of lipid. The major biologically active components of turmeric, curcumin lowers production of free radicals, provokes the synthesis of antioxidant enzymes (Alm-Eldeen et al., 2013) and prevents formation of hydroxy radicals (Anand et al., 2008). Our finding is consistent with the reported hepatoprotective effects of turmeric against TCDD toxicity which according to Ciftci et al. (2011) was by elevating the levels of enzyme activities involved in free radical scavenging. Diet supplemented with onion also exhibited beneficial effects against oxidative stress in rats exposed to dioxin. Onion is rich in quercetin, a flavonoid seen at the centre of its biological activities. Seref et al. (2014) had reported onion attenuated oxidative stress in Cd-induced oxidative stress in rats. Earlier, Obioha et al. (2009) had postulated antioxidative properties of onion to be by sparing the consumption of SOD, GSH-Px and catalase or by forming Cd-flavonoid complex. Although the mixed spices had beneficial effects in attenuating development of dyslipidaemia and oxidative stress, it is not clear at this point if the combination is superior to the use of the individual spices in mitigating dioxin toxicity.
CONCLUSION
Exposure to TCDD causes atherosclerosis and oxidative stress in rats which could be suppressed by consumption of 2.5% to 5% inclusion levels of powdered onion and/or turmeric. However, such supplementation should be done with caution since we are not able to establish in this study if any deleterious effect is associated with such inclusion levels and therefore necessitate further investigations.

REFERENCES


