Combined Antioxidant Recovery Properties of Fruit Juice Extracts of Cucumber and Water Melon on Lipid Profile and Gonadal Steroid Levels of Cadmium Induced Testicular Damage on Male Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

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ABSTRACT

The antioxidant protective effects of fruit juice of cucumber and watermelon on lipid profile of cadmium induced toxicity on male albino rats was investigated. Forty male rats were divided into eight groups. Group NC served as normal control group while group PC was positive control that was not treated but induced with cadmium. Groups I to VI received high dose and low dose of juice of Cucumber and Watermelon respectively. Excluding the normal control group, other groups were fed with lard 14 days before treatment commenced. Doses of 0.8 mg/kg-high dose and 0.4 mg/kg-low dose for cucumber and watermelon respectively. At the 4th and 6th week, biochemical parameters were assayed. Results revealed that the levels of total cholesterol, LDL, VLDL and...
triglyceride significantly (P<0.05) were decreased compared to positive control but HDL was increased in treatment groups compared to positive control. Pretreatment with cucumber and watermelon juice indicated that total cholesterol, LDL, VLDL and triglyceride significantly (P<0.05) were decreased compared to positive control but HDL was increased in treatment groups compared to positive control. The result also revealed an increase in testosterone levels in treated groups after 4 weeks of administration of whole extract of cucumber and watermelon when compared to their week 2 values. Testosterone level in positive control was also reduced significantly from 1.5±0.14 ng/ml to 0.46±0.31 ng/ml. Histological evaluation of the testes of normal control group revealed that the interstitium was intact with leydig cells present and maturing germ cells embedded in normal seminiferous tubules while the other groups that were induced with cadmium only showed morphology of testes with empty seminiferous tubules and consolidated interstitial spaces.

Keywords: Cadmium; water melon; cucumber; lipid; toxicity.

1. INTRODUCTION

It is recognized in recent years that environmental problems have exponentially increased, due to the growing needs of human and population [1]. Humans are exposed to harmful environmental contaminants at different stages of life especially in reproductive stage. A number of these contaminants are heavy metals and toxic. Cadmium (Cd), one of the foremost toxic metals widely dispersed in environmental and occupational settings has been found to reduce male fertility [2].

Cadmium enters the atmosphere via several ways [3,4]. Through erosion, volcanic activity, river transport and weathering [5]. As an alloy, in electroplating of other metals and as a pigment which contaminate air, water and land. The extensive use in the manufacture of alkaline batteries and plastics, and the major source of cadmium available in the rural regions is because of human activities like phosphate fertilizing, fuel combustion and waste burning [5].

Bioaccumulation is the process which cadmium enters the food chain in different animals and human tissues [6]. The usual means for cadmium exposure are smoking, breathing of contaminated air and eating contaminated seafood and water [7]. Chronic cadmium toxicity can lead to renal failure among others [8]. The influence of cadmium is induced in organs and cells by altering antioxidant defense system and increasing the production of reactive oxygen species (ROS) [9,10]. Several reports have referred to contribution of cholesterol and other lipids in the maturation and functionality of sperm and the acrosome response procedure [11]. Impaired sperm capacity for capacitation and acrosome reaction has been linked to hypercholesterolemia in quantitative and qualitative alterations in sperm membrane lipids [12].

It is proven that tissue levels of lipid peroxide is an indicator in oxidative stress [13]. Furthermore, investigations have recorded acute cadmium exposure is linked to elevated lipid peroxidation in sex organs in males and other organs [14,15]. Obesity can be induced by HFD intake like lard. It can also induce inflammation and oxidative stress. Several data indicate that elevated levels of ROS and inflammation and in the brain can be linked to over nutrition [16]. Free radicals inadequately neutralized by antioxidants causes’ cumulative body harm and oxidative stress [17].

When lipids are oxidatively degraded and free radical collects electrons from lipid in cell membranes resulting in cell damage is known as lipid peroxidation. This process occurs through mechanism of chain reaction of free radicals. Polyunsaturated fatty acids are mostly influenced this procedure because of their chemical configuration that contains reactive hydrogen atom. Like other radical reactions, lipid peroxidation comprises of three basic stages: initiation or instigation, propagation and termination [18].

Watermelon (Citrullus lanatus) is a fruit with a juicy pulp that is red or pink with many seeds. Watermelon fruit contains water and sugar in 91% and 6% respectively, and is stumpy in fat. The juice comprises of vital carotenoids like lycopene, carotene and β-carotene which counteract free radicals effect in the human [19,20].

Cucumis sativus also known as Cucumber is domicile in the family Cucurbitaceae. Cucumber
is initially from Southern Asia, but now many different varieties are sold in the market. Cucumbers are usually more than 90% water [20,21].

2. MATERIALS AND METHODS

2.1 Procurement of Samples

*Cucumis sativus* (Cucumber) and *Citrullus lanatus* (Watermelon) used for this study were purchased from Choba market, in Obio akpor LGA, Rivers State and were identified and confirmed botanically by the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State, Nigeria.

2.2 Experimental Animals

Forty Wistar male albino rats of body weight 150-250g were acquired from the Animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria. They were housed separately in cages and grouped into eight groups (I-VIII). The rats were fed with grower’s mash (Top feeds) and water ad libitum for a duration of 2 weeks before the commencement of the study.

2.3 Preparation and Extraction of the Samples

The fruits were washed and the bark removed, the pulp and seeds were blended without adding water, the juice was sieved and put in a water bottle and stored in a fridge for two days. Fresh fruit juice was blended every two days.

2.4 Experimental Design

The experiment lasted for 42 days, the rats were grouped into eight groups. The choice of dose of administration of the two samples Water melon and Cucumber was adopted from the method of [22]. All the rats in the test groups were initially fed with diets comprising 600 g of grower’s mash (Top feeds) mixed with 60 g of lard.

- **NC**: Rats served as normal control.
- **PC**: Were fed with Lard (high fat diet) in order to induce hyperlipidemia and served as positive control
- **Group I**: Treated with cucumber extracts (0.8 ml – High dose/kg body weight)
- **Group II**: Treated with water melon extracts (0.8 ml-High dose/kg body weight)

Cadmium (3 mg/kg) was induced 24hours prior to sacrifice of the animals in PC group and groups I-VI while group NC was not induced with cadmium.

2.5 Sacrificing of Animals

The animals were sacrificed and blood samples were collected in anti-coagulant bottles and centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum obtained was stored using for further analyses.

2.6 Lipid Profile Test

At week two and four, blood samples were collected from the rats, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride were determined using the spectrophotometry method (i.e. using their respective reagents kits from Randox Laboratory Limited, U.K.).

2.7 Serum Testosterone Assay

Serum collected at termination was used for assaying for total testosterone. Testosterone was measured using a commercial ELISA kit (IBL) which is based on competitive binding of testosterone on immobilised antibody. Horse radish peroxidase was used for colour development and absorbance was measured at 420 nm on a plate reader (Multiskan EX). Values are reported as ng/ml of serum [23].

2.8 Histological Analysis

The organs were harvested from the treated and control rats and were placed in 10% formaldehyde. Dehydration was done with Isopropyl alcohol and these tissues were subjected to a series of increasing concentrations of Isopropyl alcohol (60%) for two hours, 80% alcohol for two hours, 95% alcohol (overnight) and absolute alcohol (100%) for two hours, in which the water is replaced by Isopropyl alcohol. These tissues were infiltrated with
paraffin and were left to equilibrate using an incubator for one hour at 60°C. These tissues were mounted on the microtome for sectioning after the decantation, solidification of paraffin around these tissues; the paraffin was thereafter trimmed out. The sections were attached to microscope slides and these slides were labeled, properly washed and allowed to dry and the slides were dipped in an adhesive solution and allowed to dry overnight. The slides were then stained with hematoxylin and the sections were examined under a microscope and interpreted.

2.9 Statistical Analysis

One way analysis of variance was performed using SPSS 21 version. The values were presented as Mean ± SD.

3. RESULTS

The results are presented in the tables and plates below.

4. DISCUSSION

From Table 1, it is observed that the total cholesterol level in positive control 1.61±0.69 mmol/L was high compared to normal control 0.98±0.07 mmol/L and treated groups after 2 weeks. Further increase in positive control value 2.43±0.08 mmol/L of total cholesterol was observed after 4 weeks (Table 2) while animals fed low combination of cucumber and watermelon 0.94±0.02 mmol/L and also those fed high combination of cucumber and watermelon 0.95±0.14 mmol/L had similar values to normal control 0.94±0.09 mmol/L after 4 weeks.

Low density lipoprotein was increased in treated groups when compared to normal control after 2 weeks. Results in treated groups showed decreased values after 4 weeks compared to week 2 with values similar to normal control group in animals fed high combination of cucumber and watermelon (0.12±0.04 mmol/L) and animals fed low combination of cucumber and watermelon (0.12±0.03 mmol/L) to normal control value (0.12±0.04 mmol/L). Low density lipoprotein of positive control animals (0.24±0.05 mmol/L) was higher than normal control (0.12±0.04 mmol/L) after 2 weeks. Low density lipoprotein of positive control increased from 0.24±0.05 mmol/L after 2 weeks to 0.41±0.11 mmol/L after 4 weeks.

Very low density lipoprotein of positive control was significantly higher in positive control (0.32±0.02 mmol/L) when compared to normal control (0.13±0.01 mmol/L) after 2 weeks. Very low density lipoprotein of all treated groups was higher than normal control at 2 weeks but

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mmol/L)</th>
<th>Low density lipoprotein (mmol/L)</th>
<th>Very low density lipoprotein (mmol/L)</th>
<th>High density lipoprotein (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.98±0.07</td>
<td>0.12±0.04</td>
<td>0.13±0.01</td>
<td>0.76±0.07</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>PC</td>
<td>1.61±0.69</td>
<td>0.24±0.05</td>
<td>0.32±0.02</td>
<td>0.67±0.04</td>
<td>0.85±0.04</td>
</tr>
<tr>
<td>GRP 1</td>
<td>0.95±0.74</td>
<td>0.18±0.25</td>
<td>0.15±0.18</td>
<td>0.65±0.30</td>
<td>0.70±0.39</td>
</tr>
<tr>
<td>GRP 2</td>
<td>1.02±0.05</td>
<td>0.14±0.01</td>
<td>0.15±0.04</td>
<td>0.61±0.99</td>
<td>0.83±0.08</td>
</tr>
<tr>
<td>GRP 3</td>
<td>0.97±0.04</td>
<td>0.13±0.02</td>
<td>0.15±0.04</td>
<td>0.63±0.01</td>
<td>0.74±0.09</td>
</tr>
<tr>
<td>GRP 4</td>
<td>1.02±0.01</td>
<td>0.12±0.01</td>
<td>0.18±0.01</td>
<td>0.69±0.00</td>
<td>0.86±0.02</td>
</tr>
<tr>
<td>GRP 5</td>
<td>1.04±0.04</td>
<td>0.15±0.02</td>
<td>0.14±0.01</td>
<td>0.63±0.01</td>
<td>0.53±0.04</td>
</tr>
<tr>
<td>GRP 6</td>
<td>1.03±0.14</td>
<td>0.14±0.02</td>
<td>0.15±0.13</td>
<td>0.63±0.01</td>
<td>0.98±0.28</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD, n=5; "X" shows significant difference between week 2 and week 4.
"a" shows significant difference when compared to normal control (NC); "b" shows significant difference when compared to positive control (PC); NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2=High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4=Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6=Low concentration of whole extract of Watermelon

Table 2. Effects of different concentrations of cucumber and watermelon on lipid profile of cadmium induced testicular damage in Wistar albino rats after four weeks of treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mmol/L)</th>
<th>Low density lipoprotein (mmol/L)</th>
<th>Very low density lipoprotein (mmol/L)</th>
<th>High density lipoprotein (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.94±0.09</td>
<td>0.12±0.04</td>
<td>0.13±0.01</td>
<td>0.79±0.07</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>PC</td>
<td>2.43±0.08&lt;sup&gt;a,x&lt;/sup&gt;</td>
<td>0.41±0.11&lt;sup&gt;a,x&lt;/sup&gt;</td>
<td>0.47±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.706±0.02&lt;sup&gt;a,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 1</td>
<td>0.80±1.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18±0.04</td>
<td>0.13±0.01</td>
<td>0.67±0.11</td>
<td>0.37±0.02&lt;sup&gt;b,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 2</td>
<td>0.89±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13±0.05</td>
<td>0.13±0.01</td>
<td>0.61±0.02</td>
<td>0.43±0.01&lt;sup&gt;b,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 3</td>
<td>0.94±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12±0.04</td>
<td>0.13±0.05</td>
<td>0.69±0.07</td>
<td>0.35±0.11&lt;sup&gt;b,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 4</td>
<td>0.95±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12±0.03</td>
<td>0.14±0.02</td>
<td>0.76±0.05</td>
<td>0.31±0.03&lt;sup&gt;b,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 5</td>
<td>1.00±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13±0.07</td>
<td>0.09±0.06</td>
<td>0.68±0.15</td>
<td>0.20±0.12&lt;sup&gt;b,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 6</td>
<td>0.98±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13±0.04</td>
<td>0.14±0.02</td>
<td>0.63±0.18</td>
<td>0.3±0.03&lt;sup&gt;b,x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD, n=5; "X" shows significant difference between week 2 and week 4; "a" shows significant difference when compared to normal control (NC); "b" shows significant difference when compared to positive control (PC); NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2=High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4=Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6=Low concentration of whole extract of Watermelon

Table 3. Effects of different concentrations of cucumber and watermelon on gonadal steroid (testosterone) of cadmium induced testicular damage in Wistar albino rats after two weeks and four weeks of treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>WK 2</th>
<th>WK4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.60±0.28</td>
<td>1.50±0.14</td>
</tr>
<tr>
<td>PC</td>
<td>1.50±0.14</td>
<td>0.46±0.31</td>
</tr>
<tr>
<td>GRP 1</td>
<td>1.01±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85±0.07&lt;sup&gt;a,x&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 2</td>
<td>1.01±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 3</td>
<td>1.15±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16±0.28</td>
</tr>
<tr>
<td>GRP 4</td>
<td>0.95±0.07&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 5</td>
<td>2.14±0.205&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.46±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GRP 6</td>
<td>1.01±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD, n=5; "X" shows significant difference between week 2 and week 4; "a" shows significant difference when normal control (NC) is compared with other groups; "b" shows significant difference when positive control (PC) is compared with other groups; NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2=High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4=Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6=Low concentration of whole extract of Watermelon
concentration of whole extract of Cucumber; GRP 2= High concentration of whole extract of Watermelon; GRP 3= High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4= Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5= Low concentration of whole extract of Cucumber; GRP 6= Low concentration of whole extract of Watermelon

reductions were observed after 4 weeks in very low density lipoprotein levels of treated groups. Group 5 animals fed low concentration of whole extract of cucumber and watermelon showed the most reduced level 0.09±0.06 mmol/L when compared to normal control 0.13±0.01 mmol/L of very low density lipoprotein after 4 weeks. Very low density lipoprotein of positive control animals increased from 0.32±0.02 mmol/L to 0.47±0.02 mmol/L after 4 weeks.

Low density lipoprotein and very low density lipoprotein consistently increased in positive control after weeks 2 and week 4 of study compared to normal control and treated groups. High Density Lipoprotein values after 2 weeks was lower in positive control and all treated groups compared to normal control. After 4 weeks, high density lipoprotein level of treated groups increased when compared to their levels in week 2. High density lipoprotein of positive control animals reduced from 0.67±0.04 mmol/L to 0.53±0.03 mmol/L after 4 weeks which is low compared to normal control (0.79 ± 0.07 mmol/L).

**Result of the Histological Assessment of the Testes**

![Plate 1](image1.jpg) Photomicrograph of testes of normal control rat showing seminiferous tubules and interstitial spaces  
H & E X 400

![Plate 2](image2.jpg) Photomicrograph of rat testes fed lard but untreated showing empty seminiferous tubules and consolidated interstitial spaces  
H & E X 400
Plate 3. Photomicrograph of rat testes treated with high concentration of cucumber showing interstitium intact with leydig cells present and maturing germ cells embedded in seminiferous tubules. H & E X 400

Plate 4. Photomicrograph of rat testes treated with high concentration of watermelon showing normal seminiferous tubules intact with matured germs cells

H & E X 400

Plate 5. Photomicrograph of rat testes treated with high concentration of cucumber and watermelon showing abundance of germ cells embedded in seminiferous tubules. H & E X 400

Plate 6. Photomicrograph of rat testes treated with low concentration of cucumber and watermelon showing abundance of matured germ cells embedded in normal seminiferous tubules. H & E X 400

Plate 7. Photomicrograph of rat testes treated with low concentration of cucumber showing scanty germ cells embedded in normal seminiferous tubules. H & E X 400

Plate 8. Photomicrograph of rat testes treated with low concentration of watermelon showing average quantity of germ cells embedded in normal seminiferous tubules. H & E X 400

Triglyceride level was increased in positive control animals 0.85±0.04 mmol/L after 2 weeks when compared to normal control 0.28±0.01 mmol/L. Also, triglyceride level of treated groups was significantly reduced from week 2 values with
marked reduction in group 5 animals. Triglyceride of positive control animals further increased to 1.706±0.02 mmol/L when compared to normal control 0.27±0.01 mmol/L.

Although the HFD provoked an elevation in lipid profile of the rats, administration of cucumber and watermelon juice was able to attenuate the damage caused by high fat diet in some animal groups due to the lipid lowering properties of cucumber and watermelon juice because of their antioxidant potential [24]. Lipid profile markers of treated groups were statistically higher after 2 weeks when compared to normal control but was reduced to level of normal control after 4 weeks. Positive control animals' lipid profile indices were observed to be significantly increased compared to normal control. High density lipoprotein of positive control was observed to reduce significantly compared to normal control. More so, triglyceride for treated group 2 was significantly increased when compared to normal control. This results may also be attributed to components of the plant juice especially watermelon which is rich in citrulline. Citrulline is responsible for release of nitric oxide, enhancing nitric oxide release reduces aortic blood pressure and decreases lipid peroxidation in the liver [25].

Table 3 shows increase in testosterone levels in treated groups after 4 weeks of administration of whole extract of cucumber and watermelon when compared to their week 2 values. Testosterone level in positive control was also reduced significantly from 1.5±0.14 ng/ml to 0.46±0.31 ng/ml.

Treated groups had higher testosterone values after week 4 when compared to week 2. This findings is harmony with those obtained by Nawal et al. 2015 on protective effects of zinc on reproductive profile of male rats exposed to cadmium, zinc influenced increase in testosterone [26].

Excluding group 5 animals, all treated groups showed significant (P>0.05) increase when compared to normal control after 2 weeks of administration of whole extract of cucumber and watermelon. After 4 weeks of pretreatment with whole extract of cucumber and watermelon, treated groups showed increase which was statistically not significant from normal control. There was reduction in testosterone of positive control animals after 4 weeks when compared to the level after 2 weeks.

5. CONCLUSION

Histological evaluation of the testes of normal control animal revealed that the interstitium was intact with leydig cells present and maturing germ cells embedded in normal seminiferous tubules. Animals injected cadmium only showed morphology of testes with empty seminiferous tubules and consolidated interstitial spaces. Morphological alterations caused by different dose of pretreatment was observed, this may be because of the direct impact of cadmium on rat testes. There were reductions in germ cell concentration in wistar rats pretreated with low dose of individual whole extracts compared with normal control group while other groups exhibited morphology similar to that of control animals owing to the potential of whole extract of cucumber and watermelon in alleviating the effect of cadmium chloride on the testes. This result is harmony with those obtained by Obianime et al. 2009 when antioxidants caused a dose dependent effect on testes induced toxicity of male wistar rats and also the work of Adaikpoh et al, 2009 where pretreatment with vitamin E attenuated the effect of cadmium chloride on rat testes [27,28].

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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