ELECTROLYTES AND TRACE METALS IN MALE ALBINO RATS studied in male albino rats (100-150g body wt). The pair-fed controls received basal feed diet daily for six weeks. Results showed a significant (P<0.05) dose dependent elevation of serum CI,

Department of Pharmacology, Faculty of Basic Medical Science, College of Medical Sciences, University of Calabar, P.M. B. 1115, Calabar, Nigeria. E-mail: victorbraide@yahoo.com. Tel: +2348034048101

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EFFECT OF DIETARY GARCINIA KOLA SEED ON SELECTED SERUM ELECTROLYTES AND TRACE METALS IN MALE ALBINO RATS

P. O. AGADA AND V. B. BRAIDE*

Summary: The effects of various doses of dietary intake of Garcinia kola seed powder [incorporated in animal feed at levels of 5% w/w, 10% w/w and 20% w/w and fed daily for six weeks] on serum levels of selected electrolytes (K+, Na+, Cl, HCO3-, Mg2+ and Ca2+) and trace metals (Cu2+, Zn2+ and Mn2+) were studied in male albino rats (100-150g body wt). The pair –fed controls received basal feed diet daily for six weeks. Results showed a significant (P< 0.05) dose dependent elevation of serum Cl–, HCO3–, Mg2+, Cu2+, Zn2+ and Mn2+. These findings point to a possible relationship with already documented histopathology of various organs (such as the gonads) induced by Garcinia kola seed. It is also conjectured that the documented antibacterial, anti-inflammatory, anti-spasmodic, antidiabetic and antifertility effects of Garcinia kola seed may have underlying involvement of alterations in body levels of trace metals and electrolytes.

Key Words: Garcinia kola seeds; electrolytes; trace metals

Introduction

Garcinia kola Heckel (Guttiferae) is a large fruit tree that abounds in the rain forest belt of Southern Nigeria. The seed (“bitter kola”) is used in Nigerian herbal medicine to treat diarrhoea, hepatitis, asthma, dysmenorrhea or menstrual cramps (Dalziel, 1937). The alkaloid and biflavonoid extracts of G. kola seed exhibited the following effects: dose–dependent spasmylytic effects on uterine and gastrointestinal smooth muscle (Braide, 1989); deterioration of reproductive function (Braide et al, 2003); anti-inflammatory and antipyretic effects (Braide, 1993); antihepatotoxic effect (Akintonwa and Essien, 1990; Braide, 1991; Iwu et al, 1990); anti-inflammatory and antidiabetic activity (Iwu et al, 1990). Chronic ingestion of G. kola seed was observed to induce histopathological changes in liver parenchymal cells, renal tubular epithelium and duodenal villous epithelium (Braide, 1990; Braide and Grill, 1990). The ingestion of G. kola seed also caused mild bronchodilation in man (Orie and Ekon, 1993). Other effects of G. kola seed extracts include protection against carbon tetrachloride induced erythrocyte damage (Adaramoye and Akinloye, 2000) and inhibitory effect on lipid peroxidation (Adegoke et al, 1998; Farombi et al, 2000; Farombi, 2002; Farombi et al, 2002, 2004). These spectra of effects, directly or indirectly, involve at the biochemical level electrolytes and trace metals. For example, the spasmylytic effect of G. kola seed on smooth muscle could be due to inhibition of transmembrane influx of calcium ions into the cytosol.

Hisataka et al, (2004) studied the effects of various concentrations of electrolytes (Na+, K+, Ca2+, Mg2+) on the developmental competence of bovine oocytes and demonstrated that magnesium concentration increased blastulation rate, follicle size and maturation, as well as development of the oocyte. It is noteworthy that electrolytes and trace metals gain entry into the body mainly from dietary sources via gastrointestinal ingestion (Linder 1991); and trace metals (eg. Zinc) are accumulated in organs such as kidney, liver, pancreas and gonads (Mc Cormick and Cunningham, 2005). Testicular atrophy has been associated with depletion of zinc in the testis; while increased zinc in the prostate and decreased zinc in the testis are correlated with age and decline in testicular activity (Oldereid et al, 2005).

Several lines of evidence indicate that the development of atherosclerosis is related to free-radical activity, lipid peroxidation and oxidative modifications of low-density lipoproteins (LDL). Natural antioxidants such as the enzyme copper, zinc superoxide dismutase (Cu, Zn SOD) abound. Since zinc is an essential component of Cu, Zn SOD, any deficiency of zinc could induce an increase in tissue oxidative damage (Disilvestro and Bloteinfujii, 1997). Garcinia kola seed has been shown to exert antioxidant activity (Farombi et al, 2002; Farombi et al, 2004) and this observation may not be unconnected with alterations in the levels of zinc in serum, and hence the body. The objective of this study is to determine if oral doses of whole seed
formulations of *G. kola* have any effect on serum concentrations of electrolytes (Na\(^+\), K\(^+\), Cl\(^-\), HCO\(_3\)\(^-\), Mg\(^{2+}\), Ca\(^{2+}\)) and trace metals (Zn\(^{2+}\), Cu\(^{2+}\), Mn\(^{2+}\)) in experimental animals.

**Materials and methods**

**Animals**

The animals used in the study were young adult, virgin male albino rats 100 – 150g body wt. obtained from the animal house of the Department of Pharmacology, University of Calabar, Nigeria. The rats were randomly selected into groups, consisting of five rats per group, housed in standard rat cages (one rat per cage) and maintained for two weeks prior to the study, to allow for acclimatization and uniform husbandry conditions of light (14 h light and 10h darkness) and ambient room temperature (28 – 30°C). The animals were fed with a standard rat mash (Pfizer Feeds, Aba, Nigeria) and received food and tap water *ad libitum*.

**Preparation of Plant Material and Administration**

Fresh seeds of *Garcinia kola*, purchased in season from the local markets in Calabar, Nigeria, were peeled to remove the testa, washed and air dried for 10hrs, prior to drying in an oven at 40°C for 12 hours. The dried seeds were then ground to a fine powder with the aid of mortar and pestle.

Four batches of feed mixture were made available for use in the study: control feed sample containing no *Garcinia kola* seed powder; feed samples that contained *Garcinia kola* seed powder at levels of 5% (w/w), 10% (w/w) and 20% (w/w) respectively. The control feed and the *Garcinia kola* feed mixtures were fed to the rats for 6 weeks; on pair-fed basis.

**Collection and Handling of Blood Serum**

The animals were anaesthetized in a chloroform chamber at the end of feeding period of 6 weeks, and blood was obtained through cardiac puncture. Blood samples from each animal were put in well-labeled nonheparinized sample tubes which were then allowed to stand for 3h in iced water and later centrifuged at 7,000g for 10 minutes. The serum was then collected and stored at – 15°C until ready for use.

**Analytical Methods**

Serum zinc, copper and manganese concentrations were determined as follows: Aliquots of serum were predigested with concentrated nitric acid (HNO\(_3\)) followed by further digestion with a mixture (10:3) of concentrated \(\text{HNO}_3\) and concentrated perchloric acid (HClO\(_4\)). The acid samples were heated in a 50ml pyrex beaker, covered with a watch glass of suitable size, on an electric hotplate. The heating was continued till all traces of HClO\(_4\) were eliminated, as was indicated by a cessation of white fume production in the beaker. The final liquid volume in the digestion beaker (about 2ml) was made up to 25ml with deionized water and assayed for Zn, Mn and Cu with an atomic absorption spectrophotometer (Jarrell –Ash Model 82–362) (Braide, 1973). Serum levels of cation electrolytes were estimated by flame photometry while the serum levels of anion electrolytes were estimated by titration procedures for chloride and bicarbonate.

**Statistical Analysis**

All data for control and experimental animals were subjected to statistical evaluation, using the Student’s t-test for significance of differences between control and experimental groups, at values of P< 0.05.

**Results**

**Effect of *G. kola* seed diet on serum electrolytes**

Male rats fed for 6 weeks on diets containing various levels of *G. kola* seed powder (G KP) were studied at the end of the feeding period. The diets contained GKP at levels of 5% (w/w), 10% (w/w) and 20% (w/w) respectively. The control feed and the *Garcinia kola* feed mixtures were fed to the rats for 6 weeks; on pair-fed basis.

The animals were anaesthetized in a chloroform chamber at the end of feeding period of 6 weeks, and blood was obtained through cardiac puncture. Blood samples from each animal were put in well-labeled nonheparinized sample tubes which were then allowed to stand for 3h in iced water and later centrifuged at 7,000g for 10 minutes. The serum was then collected and stored at – 15°C until ready for use.
Table 1. Effects of Garcinia kola seed powder (GKP) diet on cation electrolyte levels in serum of male rats after feeding for 6 weeks.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Serum concentration (mM/l)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$K^+$</td>
<td>$Na^+$</td>
<td>$Ca^{2+}$</td>
<td>$Mg^{2+}$</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>2.64</td>
<td>137</td>
<td>620</td>
<td>30.44</td>
</tr>
<tr>
<td>0% w/w GKP (5)</td>
<td></td>
<td>2.74 ± 0.1</td>
<td>136 ± 2.07</td>
<td>841.5 ± 151.4</td>
<td>80.44 ± 2.7*</td>
</tr>
<tr>
<td>5% w/w GKP (5)</td>
<td></td>
<td>2.88 ± 0.22</td>
<td>138 ± 1.79</td>
<td>1,039 ± 175*</td>
<td>160.32* ± 35.2</td>
</tr>
<tr>
<td>10% w/w GKP (5)</td>
<td></td>
<td>2.96 ± 0.25</td>
<td>138 ± 0.71</td>
<td>1,515* ± 157.2</td>
<td>502.5* ± 303.8</td>
</tr>
<tr>
<td>20% w/w GKP (5)</td>
<td></td>
<td>3.04 ± 0.28</td>
<td>138 ± 0.71</td>
<td>1,644* ± 157.2</td>
<td>522.5* ± 303.8</td>
</tr>
</tbody>
</table>

* Significantly different from controls (p < 0.05). (a) Number in parentheses represents the number of animals in each diet group. (b) The values represent means ± S.D.

Table 2. Effects of Garcinia kola seed powder (GKP) diet on anion electrolyte levels in serum of male rats after feeding for 6 weeks.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Serum concentration (mM/l)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chloride</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Control 0% GKP (5)</td>
<td></td>
<td>97.6 ± 3.6</td>
<td>23.4 ± 0.5</td>
</tr>
<tr>
<td>5% w/w GKP (5)</td>
<td></td>
<td>97.0 ± 1.6</td>
<td>24.8 ± 0.8</td>
</tr>
<tr>
<td>10% w/w GKP (5)</td>
<td></td>
<td>102.4 ± 1.8*</td>
<td>24.4 ± 0.5</td>
</tr>
<tr>
<td>20% w/w GKP (5)</td>
<td></td>
<td>103.8 ± 1.3*</td>
<td>27.0 ± 0.7*</td>
</tr>
</tbody>
</table>

* Significantly different from controls (p <0.05), (a) Number in parenthesis represents the number of animals in each diet group, (b) The values represent means ± S.D.

Table 3. Effects of Garcinia kola seed powder (GKP) diet on levels of copper, zinc and manganese in serum of male rats after feeding for 6 weeks.

<table>
<thead>
<tr>
<th>Diet (5)</th>
<th>Serum concentration (mg/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cu</td>
<td>Zn</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>11.6 ± 3.53</td>
<td>2.44 ± 0.49</td>
</tr>
<tr>
<td>5% w/w GKP (5)</td>
<td></td>
<td>16.3 ± 2.40</td>
<td>3.54 ± 0.68</td>
</tr>
<tr>
<td>10% w/w GKP (5)</td>
<td></td>
<td>25.6 ± 4.48</td>
<td>3.96 ± 0.44</td>
</tr>
<tr>
<td>20% w/w GKP (5)</td>
<td></td>
<td>54.8 ± 3.91(*)</td>
<td>6.80 ± 2.18(*)</td>
</tr>
</tbody>
</table>

(*) Significantly different from controls (p<0.05), (a) Number in parenthesis represents the number of animals in each diet group, (b) The values represent means ± S.D.

Discussion

This study has shown that in male young adult rats, following ingestion of Garcinia kola seed for six weeks, there was marked does-dependent elevation in serum concentrations of chloride, bicarbonate, calcium, magnesium, copper, zinc and manganese. The observed changes in concentrations of the electrolytes (anionic and cationic) and the trace metals (Cu, Zn, Mn) could have considerable implications for therapeutic and toxicological responses, following the use of G.kola seeds in traditional medicine.

Calculations of the anion gap from results presented in Tables 1 (for $[Na^+]$ and $[K^+]$) and 2 (for $[Cl^-]$ and $[HCO_3^-]$) suggest clearly a dose-dependent decrease in the value for anion gap, as follows: Controls (18.64mmol/L); 5% w/w GKP (16.94 mmol/L); 10% w/w GKP (14.08mmol/L); and 20% w/w GKP (10.16 mmol/L). The decrease in anion gap, in response to increasing dosage of GKP treatment is due to the increased levels of chloride and bicarbonate in serum; especially when the GKP dosage was as high as 20% (w/w) in the diet. The anion gap is usually increased in most cases of metabolic acidosis. In this study, GKP could not have been said to induce metabolic acidosis, since the anion gap was markedly decreased. It is suggested that GKP may
have induced some kind of metabolic alkalosis; since the bicarbonate level in serum was markedly elevated. The anion gap represents those negative ions not normally measured routinely in clinical practice, including phosphate, sulphate, lactate, ketoacids and albumin. A subsequent study (Odubayo, 2009) showed no indication that *G. kola* seeds had any effect on serum albumin levels.

It is noteworthy that GKP also caused marked dose-dependent enhancement of the serum levels of calcium, magnesium, copper, zinc and manganese. Calcium is the main ion carrying the inward current during the rising phase of the action potential in smooth muscle; and spontaneous action potentials in guinea pig taenia coli and uterus are also dependent on the calcium concentration, whereas manganese blocks the production of action potentials in smooth muscles. Although excess calcium in extracellular fluid may increase the amplitude of action potential and enhance smooth muscle contractility, these effects are opposed by the membrane stabilization produced by calcium. Braide (1989) and Udia (1998) attributed the antispasmodic and spasmyolytic effect of *Garcinia kola* seed extracts to possible reduction of calcium ion entry into the cytosol of smooth muscle cells and/or the sequestration of calcium ions in the extracellular fluid and/or active calcium extrusion from the cytosol thereby ridding the contractile apparatus in cytosol of Ca^{2+} essential for generation and sustenance of smooth muscle contraction. The enhanced serum levels of calcium and manganese, as observed in the present study, could conceivably explain the effect of *Garcinia kola* seed on smooth muscle activity.

The serum level of copper was markedly increased by treatment with *Garcinia kola* seed. Caeruloplasmin is the alpha-2-globulin that complexes with copper in blood; and without caeruloplasmin, there is a rise of free (unbound) copper in the blood along with excessive entry of Cu^{2+} into tissues (Lee et al., 1976). It is of interest to recall the study by Odubayo (2009) which showed that the extract from *Garcinia kola* seed did reduce markedly the globulin fraction of serum proteins in rats. The observed increases in manganese, copper and zinc levels in serum, following consumption of *Garcinia kola* seed, lends credence to the conclusion drawn from studies (Disilvestro and Blotein-Fujii, 1997) in which the natural antioxidants, copper-zinc superoxide dismutase and manganese superoxide dismutase, were implicated in protection against oxidative damage to tissues, free radical activity and lipid peroxidation of cell membranes.

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**References**


