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**Original Research Article**

# Effects of Garcinia Kola Seed on Some Trace Elements in Albino Rats

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## Abstract

The effect of varied doses of Garcinia kola seed on serum levels of trace elements; Copper, Zinc, and Manganese were studied. A total of 30 Albino rats weighing 100-150g, divided into five groups of six rats per group were used. Group 1 served as control and received distilled water only. Group 2, 3, 4, and 5 received 9g, 18g, 36g, and 72g of Garcinia kola seed powder respectively. The trace elements were analysed by colorimetric and atomic absorption spectrophotometric method. The statistical analysis was analysed using statistical packages for social sciences (SPSS) version 20.0 and the probability value of $P<0.05$ was considered significant. Result obtained on weight showed that there was progressive decrease ($P>0.05$) which are statistically insignificant. The result obtained on trace elements showed that there was a progressive statistically increase ($P<0.05$) in mean value of Copper in group 2,3,4, and 5 ( 114.23 ± 17.01µ g/dl, 123.35 ± 10.01µg/dl, 151.08 ± 4.82µg/dl and 159.55 ± 7.63µg/dl), Zinc (89.38 ± 18.96µg/dl, 105.05 ± 8.76µg/dl, 131.00 ± 2.45µ g/dl, and 133.47 ± 3.07µg/dl), and Manganese (15.65 ± 2.30nmol/L, 15.70 ± 2.57nmol/L, 30.77 ± 4.95nmol/L and 34.28 ± 5.63nmol/L) when compared with the control group; Copper (103.52 ± 14.21µg/dl), Zinc (88.08 ± 15.54µg/dl) and Manganese (15.17 ± 2.17nmol/L). The changes in the levels of some trace elements is due to flavonoids, saponins, tannins and glycosides content of Garcinia kola seed which contains polyphenolic compound which at moderate dose is beneficial but toxic at high dose. This study concluded that Garcinia kola seed interferes with trace elements (Copper, Zinc and Manganese) and result in copper, zinc and manganese toxicity. They possess good antioxidant properties and may also affect the function of vital organs such as liver, kidney and brain and result in liver cirrhosis, kidney necrosis and hypothension.

**Keywords:** Trauma Etiology Maxillofacial Fractures Epidemiology Injury pattern Incidence.

# INTRODUCTION

Garcinia kola Heckel (Guttiferae) is a large fruit tree that abounds in the rain forest belt of Southern Nigeria. Garcinia kola seed referred to as bitter kola in African country is usually known as “Namiji goro” in Hausa and “Akilu” in ethnic group [1]. The seed (“bitter kola”) is used in Nigerian flavouring medication to treat movableness of the bowels, hepatitis, asthma, hurting or discharge cramps [2] Phytochemical studies have shown that the seed contains a variety of phytochemicals, including flavonoids, saponins tannins and cardiac glycosides [3]. The alkaloid and biflavonoid extracts of G. kola seed exhibited the following effects: dose–dependent spasmolytic effects on uterine and gastrointestinal smooth muscle [4]; deterioration of reproductive function [5]; anti-inflammatory and anti-pyretic effects [6]; antihepatotoxic effect [7]; and antidiabetic activity [8].

Chronic ingestion of Garcinia kola seed was observed to induce histopathological changes in liver parenchymal cells, renal tubular epthelium and duodenal villous epithelium [9, 10]. The ingestion of G. kola seed additionally caused delicate bronchodilation in man [11]. The seeds area unit edible associated area unit consumed as an adjuvant to truth kola (Cola nitida) and for medicative functions [12]. In ethnomedicine, genus Garcinia kola has been used as a purgative, antiparasitic and antimicrobial agent, streptococcus tonsilitis, diarrhea, bronchitis, associated as an aphrodisiac [2]. The good toxicological profile and the longtime usage of Garcinia kola have indicated its suitability for clinical trials.

Other effects of G. kola seed extracts embody protection against dissolver induced RBC injury [13] and repressing impact on lipide peroxidation [14]. These spectra of effects, directly or indirectly, involve at the organic chemistry level of electrolytes and trace metals. For example, the spasmolytic effect of G. kola seed on smooth muscle could be due to inhibition of transmembrane influx of calcium ions into the cytosol [4].

Hisataka [15] studied the effects of various concentrations of trace metals on the developmental competence of bovine oocytes and demonstrated that some concentration increased blastulation rate, follicle size and maturation, as well as development of the oocyte. It is noteworthy that trace metals gain entry into the body mainly from dietary sources via gastrointestinal ingestion [16] and trace metals (eg. Zinc) are accumulated in organs such as kidney, liver, pancreas and gonads [17].

Testicular atrophy has been associated with depletion of zinc in the testis; while increased zinc in the prostate and small metallic element within the gonad square measure related with age and decline in gonad activity [18]. Several lines of proof indicate that the event of induration of the arteries is expounded to free-radical activity, super molecule peroxidation and aerophilous modifications of low-density lipoproteins (LDL). Natural antioxidants like the catalyst copper, metallic element enzyme (Cu, zinc SOD) abound. Since zinc is an essential component of Cu, Zn SOD, any deficiency of zinc could induce an increase in tissue oxidative damage [16].

An important copper containing protein called ceruloplasmin is a blue coloured glycoprotein. It is also called serum ferroxidase which promote the oxidation of ferrous ion to ferric form, which is incorporated into transferrin [16]. This implies that increase level of copper can lead to increase amount of methaemoglobin in the body since methaemoglobin result from change in oxidation state of iron from ferrous state to ferric state. Since copper interfere with absorption of zinc, zinc is therapeutically useful to reduce copper absorption in Wilson's disease [16].

Garcinia kola seed has been shown to exert inhibitor activity [19, 14] and this observation may not be unconnected with alterations in the levels of zinc in serum, and hence the body.

The prevalence of malady within the world is changing into outrageous thanks to the negligence of some traditional seed like genus Garcinia kola that are well-tried by several analysis to possess an excellent medicative effect. It is believed to clean the digestive system without side effect such as gastrointestinal problem even if a lot of it is eaten [20]. According to Sharma et al. [21], the phytochemical elements of genus Garcinia kola seed have a really broad spectrum of pharmacological activity: protective action against chemical induced hemolysis in G6PD deficient human red blood cells. Wang [22], conjointly reportable that flavonoid supplements square measure possible to be helpful for future health since it act as a potent inhibitor. The seed has been shown to have antibiotic property by Hong-XI and Song [23], and anti-inflammatory property [6] as well as antimicrobial activity [24]. Many researches have been going on since the introduction of Garcinia kola as a medicinal plant, but few studies have been recorded on the effect of this seed on trace elements. This study is set to fill the gap of evidence of the health benefits or some adverse effect of this wonder seed.

# MATERIAL AND METHODS

### Plant material

Fresh seeds of Garcinia kola were purchased from Ekeugwu Owerri market, Owerri and was identified by a Botanist in Plant Science and Biotechnology Department of Imo state University Owerri. A voucher specimen of the seed was deposited in Imo state University, herbarium.

### Preparation of Plant seed powder

Fresh seeds of Garcinia kola, purchased in season from the local markets in Owerri Imo state, Nigeria, were peeled to remove the testa, washed and air dried for 10hrs, prior to drying in an oven at $40^0$c for 12 hours. The dried seeds were then ground to a fine powder with the aid of a grinder. Different concentration of the powder were made and fed to the experimental rats.

### Experimental Animals

Thirty male albino rats weighing about 100 – 150g body weight were purchased from the animal unit of department of agriculture, Imo state university Owerri. The rats were kept in cages to acclimatize with the conditions of the animal housing facility with ambient temperature 26-$28^0$c and adequate ventilation for two weeks. The animals were fed with a customary rat mash (Pfizer Feeds, Aba, Nigeria) and received food and $H_2O$ spontaneously. Individual identification of the animals was done by the number of strokes on their tails. The food was withheld 12hours before the administration of the powder; but has free access to water.

**Weighing of Experimental Animal**

The rats were placed in a circular pan placed on a weighing balance. Their weight was quickly taken before and after experimental period.

**Experimental Design**

The thirty male unusual person rats were divided into 5 teams of six rats per cluster. Animals all told teams received, by alimentation the following.

**Group 1:** (Control) received orally in addition to normal diet and water, 2ml of distilled water on alternate days for 14days.
**Group 2:** Received normal diet with water, in addition to normal diet and water, oral dose of 9g Garcinia kola powder on alternate days for 14 days.
**Group 3:** Received normal diet with water, in addition to normal diet and water, oral dose of 18g Garcinia kola powder on alternate days for 14 days.
**Group 4:** Received normal diet with water, in addition to normal diet and water, oral dose of 36g Garcinia kola powder on alternate days for 14 days.
**Group 5:** Received normal diet with water, in addition to normal diet and water, oral dose of 72g Garcinia kola powder on alternate days for 14 days.

**Sample Collection**

The food was withheld 12hours once the experiment and there was free access to water. The rats were sacrificed and were collected by cardiac puncture with sterile syringe. Five millilitre (5ml) of blood was aspirated aseptically from the heart of each animal and was dispensed into a well labelled plain tubes. The sample was centrifuged at 3000 revolution per minute (rpm) for 5 minutes. The serum then collected and stored at $-28^0$c until ready for use.

**Biochemical assay:**

**A. Determination of Copper was done by Akita and Yiamashita** [25]

**B. Determination of Zinc**: By colorimetric method as described by Tetsuo [26], as modified by Tulip Diagnostics Private Limited, India. Catalog number: 2387.

**C. Determination of Manganese:**

By atomic absorption spectrophotometric method as described by Braide [5].

**Statistical Analysis**

The data was analysed using statistical package for social science (SPSS) version 20.0. All values were expressed as mean ± SD. Test of significance and difference between groups was carried out using student's t-test and one way analysis (ANOVA). Test with a probability value of $P < 0.05$ was considered statistically significant.

# RESULT AND ANALYSIS

**Table-4.1: The Mean Value of Body Weight of Albino Rats Treated with Different Doses of Garcinia kola Seed Powder.**

| Groups (Treatment given) | Initial weight (g) | Final weight (g) | Weight change (g) | P-value |
|---|---|---|---|---|
| Group1 (Distilled water) | 112.50±10.15 | 113.50±7.29 | 2.67±2.07 | 0.84 |
| Group 2 | 113.83±12.30 | 110.67±12.39 | 3.50±1.76 | 0.67 |

| | | | | |
|---|---|---|---|---|
| (9g) | | | | |
| Group 3 (18g) | 119.67±13.16 | 109.67±12.75 | 10.00±1.21 | 0.31 |
| Group 4 (36g) | 121.00±20.08 | 107.33±20.53 | 13.67±1.18 | 0.27 |
| Group 5 (72g) | 127.00±21.36 | 106.00±21.79 | 21.00±1.09 | 0.09 |

Table-4.1 shows the mean values of body weight of control and experimental Albino rats before and after treatment with different doses of Garcinia kola seed powder. There was decrease in body weight of group 2 (3.50±1.76), group 3 (10.00±1.21), group 4 (13.67±1.18) and group 5 (21.00±1.09) and was not statistically significant ($p>0.05$) when compared with the control group (2.67±2.07).

**Table-4.2**: **The Mean Values of Some Trace Elements of Control and Experimental Albino Rats Treated with Different Doses of Garcinia kola Seed Powder.**

| | **Group1 Distilled Water** | **Group2 9g** | **Group3 18g** | **Group4 36g** | **Group5 72g** | **P-value** |
|---|---|---|---|---|---|---|
| Copper | 103.52±14.21 | 114.23±17.01 | 123.35±10.01 | 151.08±4.82 | 159.55±7.63 | 0.001 |
| Zinc | 88.08±15.54 | 89.38±18.96 | 105.05±8.76 | 131.00±2.45 | 133.47±3.07 | 0.001 |
| Manganese | 15.17±2.17 | 15.65±2.30 | 15.70±2.57 | 30.77±4.95 | 34.28±5.63 | 0.001 |

**ANALYSIS**

Table-4.2 shows the mean value of Copper, Zinc, and Manganese of control and experimental Albino rat treated with distilled water, 9g, 18g, 36g, and 72g prepared powder of Garcinia kola seed.

The result showed there was progressive increase in the mean value of Copper in group 2 (114.23±17.01), group 3 (123.35 ± 10.01), group 4 (151.08 ± 4.82), and group 5 (159.55 ± 7.63) as the doses increased. This increase was statistically significant (P= 0.001) when compared with the control group (103.52±14.21).

The result showed there was progressive increase in the mean value of Zinc in group 2 (89.38 ±18.96), group 3 (105.05±8.76), group 4 (131.00±2.45), and group 5 (133.47±3.07) as the doses increased. This increase was statistically significant (P= 0.001) when compared with the control group (88.08±15.54).

The result showed there was progressive increase in the mean value of Manganese in group 2 (15.65±2.30), group 3 (15.70±2.57), group 4 (30.77±4.95), and group 5 (34.28±5.63) as the doses increased. This increase was statistically significant (P= 0.001) when compared with the control group (15.17±2.17).

# DISCUSSION

Garcinia kola is one of the important medicinal plant of economic important. The consumption of genus Garcinia kola seed to an exact concentration have an impact within the body. In this present study, there was a dose dependent increase

in the serum concentration of copper. This findings correlates with the study of Eleyinmi [27] which shown that Garcinia kola seed have a significant increase in the level of Copper. The observed changes in concentration of this trace element Could have a considerable implications for therapeutic and toxicological response, following the use of Garcinia kola seed in traditional medicine. This observed increases in Copper levels in serum, following consumption of Garcinia kola seed, lends credence to the conclusion drawn from studies [28] in which the natural antioxidant, copper-zinc superoxide dismutase, were implicated in protection against oxidative damage to tissues, free radical activity and liquid peroxidation of cell membranes.

This present study has shown a dose dependent increase in the serum concentration of Zinc. This findings correlates with the study of Agada and Braide [6] which shown that there was dose- dependent increased level of Zinc in albino rats fed with Garcinia kola seed powder. According to Dilina do [29] metallic element induces the synthesis of metallothioneins, which are proteins effective in reducing chemical group radicals and sequestering reactive element species made in trying situations.

In this present study, there was a dose dependent increase in the serum concentration of Manganese. This findings correlates with the study of Agada and Braide [6], which shown that there was dose dependent increased level of manganese in albino rats fed with Garcinia kola seed powder. The increased serum levels of manganese, as observed in the present study could conceivably explain the effect of Garcinia kola seed on smooth muscle activity [4]. This observed increases in manganese levels in serum, following consumption of Garcinia kola seed, lends credence to the conclusion drawn from studies [28] in which the natural antioxidant, manganese superoxide dismutase, were implicated in protection against oxidative damage to tissues, free radical activity and liquid peroxidation of cell membranes.

However, changes in the serum levels of these trace elements can be used as a biomarker of cellular immunity.

# CONCLUSION

In conclusion, high doses of Garcinia kola seed cause copper, zinc and manganese toxicity. Copper toxicity may result in cirrhosis of the liver, necrosis in kidneys and the brain, gastrointestinal distress, lesion, low blood pressure and fetal mortality [30]. Zinc toxicity may result to iron deficiency anaemia, decrease level of high density lipoprotein and increase level of low density lipoprotein [31]. Manganese toxicity (manganism) may result to neuron death [32].

Beneficially, at moderate doses Garcinia kola seed possess good antioxidant property. It is used in herbal medicine to treat diarrhea, hepatitis, asthma, dysmenorrheal, or menstrual cramp. It also possess antimicrobial, antiviral, anti-inflammatory, antidiabetic, hepatoprotective activities [6, 7, 8]

## APPENDICES

### APPENDIX I

| **COMPOSITION OF REAGENTS** | | |
|---|---|---|
| **COPPER (Cu)** | | |
| **REAGENT COMPOSITION** | | |
| **Contents** | **25ml** | **75ml** |
| **L1:** Buffer reagent(pH 8.2) | 12.5ml | 37.5ml |
| **L2:** Colour Reagent | 12.5ml | 37.5ml |
| **S:** Copper Standard (200µg/dl) | 2ml | 2ml |
| **ZINC (Zn)** | | |
| **REAGENT COMPOSITION** | | |
| **Contents** | **25ml** | **75ml** |
| **L1:** Buffer reagent(pH 8.2) | 20ml | 60ml |
| **L2:** Colour Reagent | 5ml | 15ml |

| | | |
|---|---|---|
| **S:** Copper Standard (200µg/dl) | 2ml | 2ml |
| **MANGANESE (Mn)** | | |
| **REAGENT COMPOSITION** | | |
| **Contents** | | |
| 0.5% Lanthanum solution | 13.37g/L Lanthanum chloride | |
| Stock standard of 18 nmol of Mn/L is purchased commercially. | | |

**APPENDIX II**

**STATISTICAL TERMS AND FORMULAR**

1. Mean (x) = $\frac{\sum x}{\sum n}$

2. Standard deviation (sd) = $\sqrt{\frac{\sum(x-x)^2}{\sum n}}$

3. Student's t-test (t-cal) = $\frac{xc-xt}{sp\sqrt{\frac{1}{nc}+\frac{1}{nt}}}$

$SP = \sqrt{\frac{(nc-1)sc^2+(nt-1)st^2}{nc+nt-2}}$

Where
$\sum$ = Summation
$\chi$ = Individual data (sample size)
$\bar{\chi}$ = mean
$\bar{\chi}e$ = mean of Control
Sd = standard deviation
$\bar{\chi}t$ = mean of test
Sp = pooled estimation of the common population
nc = sample size of control
nt = sample size of test
$sc^2$ = variance of control
$st^2$ =variance of test

The student's t-test was used to determine the difference between the control and the test was statistically significant by using 50% (0.05) level of significant.

**Hypothesis Testing**

Ho = Null hypothesis (xc = xt)
Hi = Alternate hypothesis (xc ≠ xt)

Ho is rejected if t-cal ≥ + t-tab or t-cal ≤ - t-tab when Ho was rejected, Hi was accepted and it was concluded that the difference between the test and the control was statistically significant.

Ho is accepted if t-cal ≤ + t-tab or t-cal ≥ - t-tab when Ho was accepted, Hi was rejected and it was concluded that the difference between the test and control was not statistically significant.

**APPENDIX III**

**WEIGHTS OF THE ALBINO RATS BEFORE THE EXPERIMENTS**

| Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|
| 102.00 | 120.00 | 140.00 | 139.00 | 138.00 |

| | | | | |
|---|---|---|---|---|
| 110.00 | 110.00 | 106.00 | 100.00 | 150.00 |
| 100.00 | 135.00 | 109.00 | 150.00 | 110.00 |
| 125.00 | 106.00 | 112.00 | 102.00 | 140.00 |
| 118.00 | 100.00 | 122.00 | 114.00 | 104.00 |
| 120.00 | 112.00 | 129.00 | 121.00 | 120.00 |
| 112.50±10.15 | 113.83±12.30 | 119.67±13.16 | 121.00±20.08 | 127.00±21.36 |

**APPENDIX IV**
**WEIGHT OF THE ALBINO RATS AFTER THE EXPERIMENTS**

| Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|
| 105.00 | 115.00 | 130.00 | 124.00 | 118.00 |
| 112.00 | 108.00 | 97.00 | 85.00 | 131.00 |
| 105.00 | 133.00 | 100.00 | 138.00 | 92.00 |
| 120.00 | 102.00 | 102.00 | 89.00 | 120.00 |
| 118.00 | 98.00 | 110.00 | 100.00 | 80.00 |
| 121.00 | 108.00 | 119.00 | 108.00 | 95.00 |
| 113.50±7.29 | 110.67±12.39 | 109.67±12.75 | 107.33±20.53 | 106.00±21.79 |

**APPENDIX V**
**RAW DATA FOR ALBINO RATS IN CONTROL GROUP (GROUP 1)**

| S/N | Copper (µg/dl) | Zinc (µg/dl) | Manganese (nmol/L) |
|---|---|---|---|
| 1 | 101.40 | 83.40 | 17.20 |
| 2 | 130.50 | 100.30 | 18.50 |
| 3 | 95.10 | 70.50 | 15.90 |
| 4 | 88.60 | 73.20 | 12.40 |
| 5 | 100.80 | 90.70 | 14.70 |
| 6 | 105.20 | 110.40 | 17.10 |
| | 103.52 ± 14.21 | 88.08 ± 15.54 | 15.17± 2.17 |

**APPENDIX VI**
**RAW DATA FOR ALBINO RATS FED WITH 9g OF GKP (GROUP 2)**

| S/N | Copper (µg/dl) | Zinc (µg/dl) | Manganese (nmol/L) |
|---|---|---|---|
| 1 | 120.10 | 96.40 | 18.20 |
| 2 | 102.80 | 70.50 | 12.80 |
| 3 | 110.60 | 78.60 | 15.60 |
| 4 | 88.60 | 69.80 | 10.70 |
| 5 | 133.20 | 111.00 | 19.10 |

| | | | |
|---|---|---|---|
| 6 | 130.10 | 110.00 | 17.50 |
| | 114.23 ± 17.01 | 89.38 ± 18.96 | 15.17 ± 217 |

**APPENDIX VII**
**RAW DATA FOR ALBINO RAT FED WITH 18g OF GKP (GROUP 3)**

| S/N | Copper (µg/dl) | Zinc (µg/dl) | Manganese (nmol/L) |
|---|---|---|---|
| 1 | 117.50 | 91.20 | 11.50 |
| 2 | 119.60 | 100.10 | 14.50 |
| 3 | 125.30 | 112.60 | 18.80 |
| 4 | 109.60 | 102.30 | 15.90 |
| 5 | 130.10 | 110.50 | 13.30 |
| 6 | 138.00 | 113.60 | 16.60 |
| | 123.35 ± 10.01 | 105.05 ± 8.76 | 15.10 ± 2.57 |

**APPENDIX VIII**
**RAW DATA FOR ALBINO RAT FED WITH 36g OF GKP (GROUP 4)**

| S/N | Copper (µg/dl) | Zinc (µg/dl) | Manganese (nmol/L) |
|---|---|---|---|
| 1 | 150.20 | 135.30 | 37.50 |
| 2 | 146.50 | 130.20 | 29.50 |
| 3 | 160.00 | 132.20 | 36.20 |
| 4 | 152.60 | 128.50 | 29.20 |
| 5 | 148.20 | 129.30 | 26.40 |
| 6 | 149.00 | 130.50 | 25.80 |
| | 151.0 ± 84.82 | 131.00 ± 2.45 | 30.77 ± 4.95 |

**APPENDIX IX**
**RAW DATA FOR ALBINO RATS FED WITHN 72g OF GKP (GROUP 5)**

| S/N | Copper (µg/dl) | Zinc (µg/dl) | Manganese (nmol/L) |
|---|---|---|---|
| 1 | 168.30 | 136.10 | 40.00 |
| 2 | 168.60 | 131.20 | 38.60 |
| 3 | 155.20 | 132.20 | 39.50 |
| 4 | 149.90 | 138.10 | 28.20 |
| 5 | 160.20 | 133.20 | 30.40 |
| 6 | 155.10 | 130.00 | 29.00 |
| | 159.55 ± 7.63 | 133.47 ± 3.07 | 34.28 ± 5.63 |

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