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# INVESTIGATING THE TOXICITY AND ANTIMICROBIAL ACTIVITY OF *GARCINIA KOLA* EXTRACTS.

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## ABSTRACT

The fruit of *Garcinia kola* extracts were evaluated for antimicrobial and subchronic toxicity effects on liver enzymes in mice. The antimicrobial activity of the extracts was determined against some pathogenic organisms at 2mg/ml using agar dilution method. The aqueous fruit extract did not inhibit the growth of any of the test organisms. *Staphylococcus aureus, Klebsiella pneumonia, Candida albicans, Eschercichia coli, Bacillus subtilis, Pseudomonas aeruginosa.; Klebsiella pneumoniae* and *S. aureus* were sensitive to the ethanolic extract. The minimum inhibitory concentration (MIC) for *S. aureus* and *K. pneumonia* was 0.5mg/ml and 0.25mg/ml respectively. The minimum bacteriocidal concentration (MBC) for *S. aureus* and *K. pneumonia* was 1.00mg/ml and 0.50mg/ml respectively. The effects of subchronic treatment with graded doses (100mg/kgbw, 200 mg/kgbw, 300 mg/kgbw, 400 mg/kgbw, and 500 mg/kgbw) of *Garcinia kola* extracts were investigated on the liver enzymes and body weight of mice. The $LD_{50}$ of the extracts were above 500mg/kgbw. The results showed that the ethanolic and aqueous extracts of the medicinal plant did not have any significant ($P>0.05$) toxic effects on glutamate pyruvic transaminase (GPT) and total protein when compared with the control. However the aqueous extract significantly increased the glutamate oxaloacetate transaminase (GOT) levels of the treated animals when compared with the control. The aqueous extract exerted a dual effect on the body weight of the mice while 100mg/kgbw and 400mg/kgbw of the extract significantly ($p<0.05$) decreased the body weight of the mice 300mg/kgbw elevated the weight of the mice. The body weight of the

animals was not adversely affected by the administration of 200mg/kgbw and 500mg/kgbw of the aqueous extract when compared with the control. The results of this study suggest that the fruit may have beneficial effect on some liver enzymes although it may adversely affect the GOT level of mice.

**KEYWORDS:** Antimicrobial, Dose, Enzymes, Extracts, Inhibit, Toxicity.

## INTRODUCTION

Herbal remedies are commonly used in developing nations for the treatment of various ailments. This practice is an alternative to compensate some perceived deficiencies in orthodox medicine. Many medicinal plants have not been scientifically validated for their therapeutic efficiency and safety uses. For example, many herbal medicinal plants have been found to induce fatal hepatic effects, and severe acute liver failure with marked haemetological and biochemical alterations[1, 2] after prolonged administration. *Garcinia kola* fruit is one of such plants traditionally used for treatment of laryngitis, liver disease and cough.[3] The fruit also has anti-inflammatory, antimicrobial, antidiabetic and antiviral properties.[4] The fruit was widely speculated though without scientific evidence to cure Ebola virus in a recent outbreak. The continued utilization of this plant is based on long term clinical experience. Therefore, the present study was undertaken to evaluate the antimicrobial effect and medicinal significance of the subchronic treatment with *Garcinia kola* on liver enzymes and body weight of mice.

## MATERIALS AND METHODS

### *Collection and identification of plant materials.*

The seeds of *Garcinia kola* was obtained from River Basin, Minna, Niger State, Nigeria. Fresh and healthy, fruits were identified and authenticated by a taxonomist with the Herbarium Department, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria.

### Extraction of Plant Materials

#### *The aqueous extraction*

The bitter kola was washed and air dried at room temperature ($28 \pm 2^0$C). The dried kola were chopped and pulverized with a blender. Seventy-five (75g) grams of the powdered kola was extracted in 1000ml of sterile distilled water by soaking for 3 days. The extract was filtered

using a Whatman filter paper (No. 1). The filtrate was evaporated to dryness with rotary evaporator. The aqueous extract was stored at – $4^0$C until required for used.[5]

***Extraction in methanol***

A measured quantity of fifty grams (50g) of *Garcinia kola* was extracted in 500ml of 95% ethanol by refluxing for 6 hours. The resulting mixture was filtered with Whatman filter paper (No. 1) and evaporated to dryness with rotary evaporator. The resulting extract was stored at – $4^o$C until it was required for use.

***Source of microorganisms***

The microorganisms used for this study were *Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Klebisella pneumoniae*. These organisms were obtained from NIPRD, Idu, Abuja, Nigeria.

***Standardization of Microorganisms***

Sterile nutrient broth (5ml) was inoculated with a loopful of test organism and incubated for 24 hours. An aliquot of 0.2ml from the overnight culture of the organisms were dispensed into 20ml of sterile nutrient broth and incubated for 3-5 hours to standardize the culture to $10^6$ cfu/ml. A loopful of the standard culture was used for antimicrobial assay.[5]

***Screening of extracts for antimicrobial activity.***

To test for the antimicrobial activity of the fruit extract, agar dilution method of Collins[6] as adopted by Babayi[7] was employed wherein 2mg/ml of the crude extracts were used. The controls were also included: organisms viability control, extract sterility control and media sterility control (OVC, ESC and MSC). The extracts efficacy was compared with ampiclox (Beecham) which has often been used as broad spectrum antibiotic. The experiment was done in triplicate.

***Determination of MIC***

The MIC of the crude ethanolic extract was determined by Collins[6] method.

***Determination of MBC***

The method of Okoli and Iroegbu[8] was used in determining the MBC of the crude ethanolic extract. A loopful of culture from MIC tubes showing no apparent growth was subcultured into fresh sterile broth and incubated at 37$^o$C for 24 hours. The least concentration showing no visible growth on subculturing was taken as MBC.

***Experimental Animals***

Mice of average weight 20-28g were used for this study. The animals were obtained from Institute of Medical Science Vom, Jos, Plateau State, Nigeria. They were fed with standard NIPRD formulated feed and allowed free access to water. They were maintained under standard condition of humidity (40-60%) Temperature (25 $\pm$ $2^0$C), 12 hours light and dark cycle. The mice were first acclimated to housing conditions before the commencement of any experiment. The animals were handled in accordance with NIH guide[9] for the care and use of laboratory animals.

***Determination of acute toxicity( $LD_{50}$) of the extract of Garcinia kola.***

Effects of acute administration of the extracts on mice was investigated by the method of Lorke.[10]

***Effects of extracts of G. kola on liver enzymes and body weight of mice***

Effects of subchronic administration of aqueous and ethanolic extracts of *Garcinia kola* was investigated for 28 days in mice. The method of Aniagu[11] was employed. Mice were selected by stratified randomization. They were divided into 6 groups of 11 mice each. The first group served as control and were administered equivalent volume of water only while five (5) groups were administered 100mg/kgbw, 200 mg/kgbw, 300 mg/kgbw, 400 mg/kgbw, and 500 mg/kgbw of the aqueous extracts. Five groups of 11 mice each were also administered 100mg/kgbw, 200 mg/kgbw, 300 mg/kgbw, 400 mg/kgbw and 500 mg/kgbw of ethanolic extract.

The body weight of the animals were determined weekly. The physical appearance of the mice was observed daily. The first day of administration was taken as $D_0$ while the day of sacrifice was designated as $D_{28}$. At the end of the 28 days treatment, the animals were weighed and exsanguinated under chloroform anesthesia. Blood samples were drawn from the heart of the sacrificed mice. For liver enzymes study, blood sample of the animals in each group were obtained. The samples were collected in plastic test tubes and allowed to stand for 3 hours to ensure complete clotting. The clotted samples were centrifuged at 3500rpm for 10minutes and clear serum samples were aspirated off and stored at -$4^0$C. The biochemical parameters: glutamate oxaloacetate transminase (GOT) or aspartate transaminase (AST) and glutamate pyruvate transminase (GPT) or alanine transaminase (ALT) and total protein were determined colorimetrically by standard ready to use kit obtained from Randox laboratory UK.

***Statistical analysis and data evaluation***

Various statistical tests such as one way ANOVA, T-test and confidence limit were employed in the analysis of the data generated. Differences between means was considered significant when $P \leq 0.05$.

## RESULTS

***Antimicrobial activity of crude plant extracts***

The results of the antimicrobial activity of the crude fruit extracts of *Garcinia kola* on test organisms are shown in Table 1. The results revealed that the aqueous extract did not inhibit the growth of any of the test organisms. The ethanolic extract however inhibited the growth of *S. aureus* and *K. pneumoniae.* The MIC (Table 2) of ethanolic extract for *S. aureus* and *K. pneumonia* was 0.5mg/ml and 0.25mg/ml respectively. The minimum bactericidal concentration (MBC) for *S. aureus* and *K. pneumonia* was 1.00mg/ml and 0.50mg/ml respectively.

**Table 1: Antimicrobial activity of aqueous extracts of *Garcinia Kola* extracts against selected organisms**

| | **Extracts** | |
|---|---|---|
| Test organism | Aqueous | Ethanolic |
| *Bacillus subtilis* | - | - |
| *Candida albicans* | - | - |
| *Escherichia coli* | - | - |
| *Pseudomona aeruginosa* | - | - |
| *Klesiella pneumonia* | - | + |
| *Staphylocuccus aureus* | - | + |
| -: No activity | | |

+: Activity

**Table 2: Minimum inhibitory concentration of ethanolic extract of *Garcinia kola* against selected organisms.**

| | **Test organisms** | |
|---|---|---|
| Concentration of extract (mg/ml) | *Klebsiella pneumonia* | *Staphylococcus aureus* |
| 2.00 | + | + |
| 1.00 | + | +* |
| 0.50 | +* | + |
| 0.25 | $+^0$ | - |
| 0.175 | - | - |

- : No Activity
+: Activity
*: Bactericidal Concentration
0: Bacteriostatic concentration

***Acute toxic effects of Garcinia kola extracts***

The results revealed that all doses (10mg/kgbw – 500mg/kg) of the aqueous and ethanolic extracts had no any demonstrable acute toxic effects in all the groups of the mice.

***Effects of extracts of Garcinia kola on liver enzymes***

The effects of aquoues and ethanolic extracts of *Garcinia kola* on liver enzymes are shown in Tables 3 and 4. The results revealed that ethanolic extract did not significantly (P>0.05) affect glutamate oxaloacetate transminase (GOT), glutamate pyruvic transaminase (GPT) and total protein of the mice at the doses (100mg/kgbw – 500mg/kgbw) tested. The aqueous extract significantly increased the GOT level of the treated mice when compared with the control. The GPT and total protein levels were not affected by the aqueous extract when compared with that of the control.

**Table 3: Effects of aqueous extract of *Garcinia kola* on liver enzymes after 28 days of treatment**

| | Treatment (mg/kgbw) | | | | | |
|---|---|---|---|---|---|---|
| Liver enzymes | Control | 100 | 200 | 300 | 400 | 500 |
| GOT (UI/mg) | $16.53\pm1.94^{a}$ | $20.98\pm4.8^{ab}$ | $24.30\pm4.83^{ab}$ | $24.82\pm3.50^{ab}$ | $26.56\pm2.91^{ab}$ | $26\pm5.95^{ab}$ |
| GPT (UI/mg) | $63.23\pm4.44^{a}$ | $74.67\pm1.22^{a}$ | $85.33\pm1.46^{a}$ | $79.33\pm3.60^{a}$ | $81.60\pm4.02^{a}$ | $84.80\pm4.5^{a}$ |
| Total protein (µmol/L) | $6.78\pm0.14^{a}$ | $7.21\pm0.37^{a}$ | $7.27\pm0.38^{a}$ | $7.68\pm0.06^{a}$ | $7.12\pm0.22^{a}$ | $7.56\pm0.95^{a}$ |

Values are expressed as mean ± S.E.M for N = 11

GOT: Glutamic oxaloacetate transaminase

GPT: Glutamic pyruvic transaminase

Row mean data carrying the same superscript do not differ significantly from each other (P>0.05)

**Table 4: Effects of aqueous extract of *Garcinia kola* on liver enzymes after 28 days of treatment**

| | Treatment (mg/kgbw) | | | | | |
|---|---|---|---|---|---|---|
| Liver enzymes | Control | 100 | 200 | 300 | 400 | 500 |
| GOT (UI/mg) | 32.43±1.94[a] | 32.77±3.34[a] | 32.20±3.02[a] | 29.42±3.90[a] | 34.35±3.06[a] | 32.90±3.12[a] |
| GPT (UI/mg) | 63.33±4.40[a] | 76.67±4.20[a] | 85.00±1.55[a] | 71.67±1.55[a] | 91.33±1.51[a] | 84.09±1.20[a] |
| Total protein (µmol/L) | 6.78±0.15[a] | 7.01±0.43[a] | 7.19±0.44[a] | 7.44±0.46[a] | 7.23±0.37[a] | 6.59±0.47[a] |

Values are expressed as mean ± S.E.M for N = 11

GOT: Glutamic oxaloacetate transaminase

GPT: Glutamic pyruvic transaminase

Row mean data carrying the same superscript do not differ significantly from each other (P>0.05)

***Effect of extracts of G. kola on body weight of mice.***

The effects of *G. kola* on body weight of mice are shown in Figures 1 and 2. The results showed that ethanolic extracts of the medicinal plant did not affect the body weight of the mice after 28 days of exposure. The aqueous extract (200mg/kgbw and 500mg/kgbw) did not significantly affect the body weight of the mice when compared with the control. However, 100mg/kgbw and 400mg/kgbw of the aqueous extract exerted a significant decrease on the body weight of the mice when compared to that of the control. There was elevation in weight of the animals treated with 300mg/kgbw of the extract.

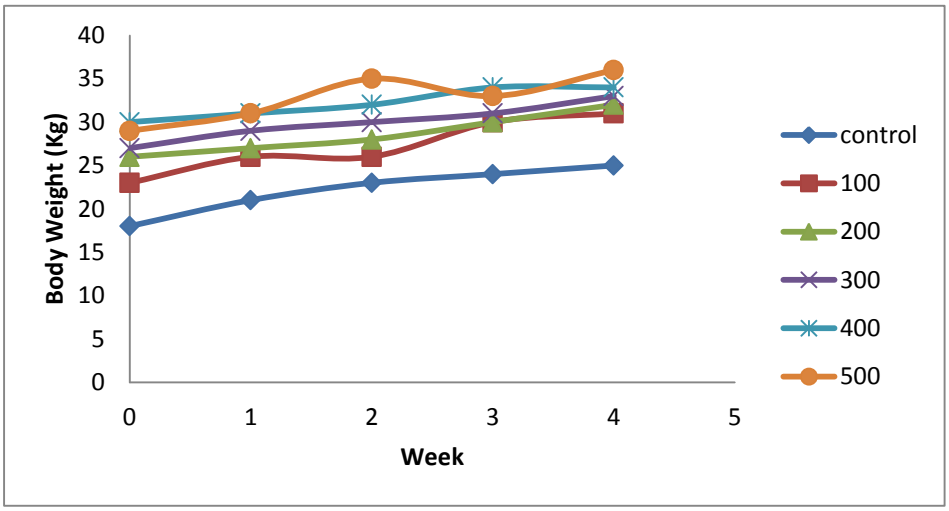


**Figure 1: Effect of aqueous extract of *Garcinia kola* on weekly body weight of mice**

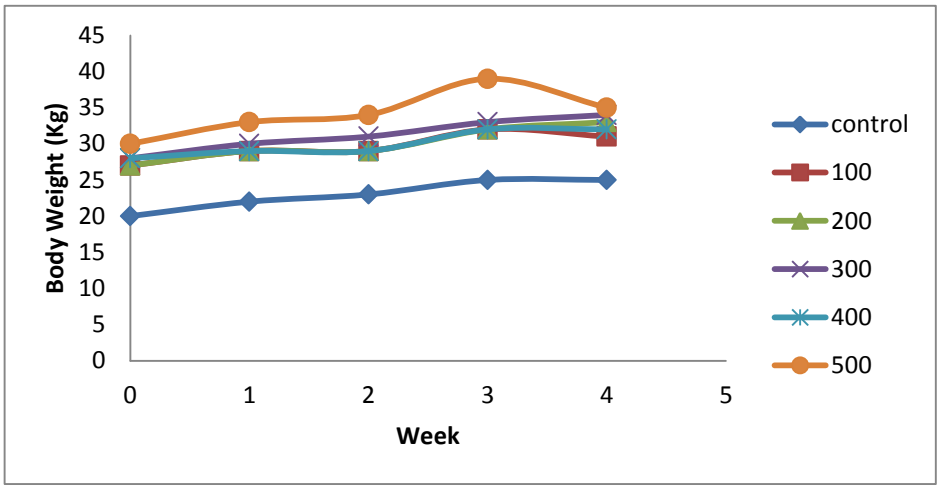


**Figure 2: Effect of Ethanolic extract of *Garcinia kola* on weekly body weight of mice**

## DISCUSSION

The extraction of bioactive components from medicinal plants facilities study leading to synthesis of more active drugs with reduced toxicity.[12] The crude ethanolic extracts of *G. kola* demonstrated inhibitory effects on some pathogenic organisms of medical importance. The inhibitory effects shown by the ethanolic extracts may be due to the presence of some phytochemical components reported by Iwu[3] and Okunji.[13] The antimicrobial properties of ethanolic extracts of *G. kola* was attributed to the presence of benzophenone. Research involving the bioassay fraction of *G. kola* seeds showed mixtures of triterpenes, phenolic compounds, benzophenones, kolanone with potent antimicrobial properties.[14]

The lack of activity demonstrated by aqueous extract of *G. kola* might not be unconnected to the fact that during extraction the components were diluted or denatured sufficiently so as to reduce the antimicrobial properties to a considerable extent or that the components were present in trace amounts.

The interesting thing about these findings is that on purification of the extract, it is highly likely that even at much lower concentration, results obtained may be similar or even better. However it is not uncommon to find that extracts are denatured after fractionation.[8] However if the extract is not denature on purification and the purified compound is obtained it could be standardised and packaged for use as phytomedicine.

The preliminary acute toxicological evaluation of the plant extracts revealed an oral $LD_{50}$ value greater than 500mg/kgbw. Therefore, the medicinal plant can be categorised as nontoxic based on the scale proposed by Lorke.[10]

Some herbal remedies have hepato and nephrotoxic effects.[15, 16] Damage to these organs often results in elevation in clinical chemical parameters such as serum enzymes; glutamate oxaloacetate aminotransminase (GOT), glutamate pyruvate transaminase (GPT), urea and total protein.[17, 18]

The present study showed that ethanolic extract of *Garcina kola* was not toxic to the liver enzymes. However, the aqueous extract at all the doses (100mg/kgbw – 500mg/kgbw) may adversely affect the GOT level of animals.

This suggests that the medicinal plant extract may adversely affect the physiological functions of the liver. Similarly the aqueous extract of the medicinal plant exerted a dual effect on the body weight of the mice. Therefore, people should exercise caution in the use of the extract. More so, in the rural population where people use this plant, facilities for monitoring the blood and biochemical parameters are absents. The results of the present study experimentally indicate the effectiveness of *Garcinia kola* in healing infections caused by *S. aureus* and *K. pneumoniae*. *Garcinia* biflavonoid (Kolaviron) isolated from *Garcinia kola* have demonstrated inhibitory effects against methicilin-resistant *Staphylococcus aureus* (MRSA)[19] and vancomycin-resistant Enteroccoci (VRE).[20]

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**Conflict of interest**

The authors have declared that there is no conflict of interest.

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