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Methanolic extract of *Garcinia kola* elicits diuretic activity with alteration in circulating electrolyte concentration in male Wistar rats

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ABSTRACT: **Objective**: *Garcinia kola* is a kolaviron-containing nut native to tropical African and cultivated extensively in the new world tropics. Several studies have reported its effects on body weight and reduction in body fat without undesirable side effects as well as on gastric secretion. This study was designed to investigate the diuretic activity of methanolic extract of *Garcinia kola* (MEGCO) in male Wistar rats. **Design and Method**: Adult male Wistar rats were randomly allotted into control (distilled water, *po*), Furosemide (*po*), MEGCOA (200 mg/ kg), MEGCOB (400 mg/ kg), MEGCOC (600 mg/ kg), groups with 5 rats/group. The extract was prepared as previously described and the treatment lasted for 4 weeks. Urine electrolytes, serum electrolytes, serum uric acid, urea and creatinine as well as liver ALT and AST were assayed using standard colorimetric method. Urine volume and diuretic indices were also monitored. **Results**: Treatment of all rats with different doses of *Garcinia kola* did not significantly alter body weight but increased kidney weight (400 mg and 600 mg/kg doses) and increased urine volume, urine Na+, K^+ and Cl^- concentration, did not alter serum Na^+, but 600 mg/kg of MEGCO increased serum K^+ and Cl- concentration. In addition, 600 mg/k of MEGCO increased hepatic ALT but not AST activity, increased serum creatinine, urea and uric acid concentration when compared with control and furosemide-treated groups. **Conclusion:** The present study demonstrates that methanolic extract of *Garcinia Kola*, particularly 600 mg/kg dose causes diuresis, natriuresis and kaliuresis, but put the animals at risk of renal toxicity and electrolyte imbalance.

Keywords: Diuresis; *Garcinia kola*, potassium; renal toxicity, sodium.

Introduction

It is noteworthy from several studies that modern medicine cannot be regarded as a realistic treatment option for a significant proportion of the world population, particularly developing and underdeveloped countries (Anna *et al*. 2019). In the 21st century, the influence of plant-based pharmaceuticals is evident and the use of herbs as therapy for various diseases is widespread. The efficacy of some of these herbs in disease treatment has been confirmed by several studies. About 80% of the world population relies on herbs and plant-based medication for primary care (Buba *et al*. 2016). *Garcinia Kola* is a potent medicinal plant found in West and Central Africa. This plant has been dubbed "Wonder Plant" due to the medicinal importance of every part of the plant (Ekene *et al*. 2014). The *Garcinia Kola* can be described as a medium-large tree, which grows up to 15-17 meters in height (Iwu, 1993). The fruit is smooth and it weighs about 30 – 50 grams; it is 5-10cm in diameter and a single fruit could have 1-4 seeds (Juliana *et al*. 2006).

Garcinia Kola is of cultural significance among all Nigerian tribes particularly the Yoruba and Igbo tribes of Nigeria (Atilade, 2004). To natives of the Yoruba land it is known as "Orogbo" and the Igbos call it "Aku ilu" (Dalziel, 1937). It is commonly served to visitors at naming ceremonies, weddings, and other social events; it is a sign of peace and acceptance of guests (Ekene *et al*. 2014). The bitter chew-sticks common to West Africans are products of the roots of the *Garcinia Kola* plant (Otor *et al*.2001). Furthermore, the increase in the commercial value of *Garcinia Kola* has made natives of communities where it is endemic keen-on its cultivation. Its commercialization has been known to have a significant impact on the standard of living of people in rural communities. The production and sale of *Garcinia Kola* is usually a family business in rural communities and the proceeds are earmarked towards domestic expenditure like feeding, school fees, and family ceremonies (Atilade, 2004). *Garcinia Kola* is believed to cleanse the digestive system and excessive consumption does not cause abdominal problems (Buba *et al*. 2016). A mixture of ground *Garcinia Kola* seeds and honey is used to make a traditional cough medication (Buba *et al*. 2016). *Garcinia Kola* has been employed as an antidote for snake bites, therapy for cough, and vomiting (Buba *et al*. 2016). It enjoys notoriety in Africa as a poison antidote and has a plethora of traditional medical applications (Konziase, 2015; Buba *et al*. 2016).

The phytochemical constituents of *Garcinia Kola* seeds include; flavonoids, saponins, tannins, phenols, glycosides, and alkaloids (Adesuyi, 2012). Although the seeds have been deemed safe for consumption, anti-nutrients such as oxalate and phytate were observed (Konziase, 2015). Flavonoids are the most abundant phytochemicals in *Garcinia Kola* seeds; they are antioxidants with low molecular weight which scavenge free radicals and convert them to harmless molecules and also influence several aspects of immune cell activation (Nworu *et al*. 2008). They also provide protection for the Central Nervous System against oxidative and excitotoxic stress (Ijomone *et al*. 2012). Kolaviron is a major active component of Garcinia Cola seeds; it is riched in bioflavonoids that consists of GB-1, GB-2, and Kola flavanone (Tchimene *et al*., 2015). Kolaviron is known to possess sedative and anti-inflammatory properties; it acts both centrally and at the periphery and this legitimizes its traditional use as a pain reliever and anti-inflammatory (Anna *et al*. 2019). In a recent study, kolaviron was administered to a fruit fly (Drosophila melanogaster) and it was observed that the fly had a protracted life-span which was due to its antioxidative and anti-inflammatory properties (Farombi *et al*. 2018). Many of the properties of kolaviron have been elucidated in animal models, including antioxidant, hepatoprotective, anti-inflammatory, anti-malarial, anti-microbial, anti-diabetic, anti-ulcer, anti-cancer, anti-asthma, anti-arthritis and anti-hypertensive (Buba *et al*. 2016; Quadri *et al*. 2019).

Diuretics have been known to be effective in treating hypertensive patients, it has been observed that it reduces systolic and diastolic blood pressures in hypertensive patients. Administration of diuretics in combination with other agents forms the basis of therapy for many hypertensive patients (Shah *et al*. 2004). The natriuretic property of diuretics is what causes the decrease in total body sodium; potent diuretics act at a site where a large quantity of sodium is normally reabsorbed. Consequently, the amount of sodium excreted in the urine and accompanying fluid loss can be enhanced immensely with these agents by

increasing the dose (Shah *et al*. 2004). Sodium excretion and fluid loss play an important role in the management of edema and hypertension (Koushik *et al*. 2014). Herbal medicine is an essential source of diuretics, mono and poly-herbal preparations have been employed as diuretics. According to an extrapolation, more than 650 mono and poly-herbal preparations in the form of decoction, tincture, tablets, and capsules from more than 75 plants are in clinical use (Chopra *et al*. 1986). Herbs were popularly used as traditional therapy for some renal diseases and a lot of plants have been reported to show potent diuretic activity (Koushik *et al*. 2014).

There have been several studies that investigate the efficacy of herbal medicine as diuretics. *Garcinia Kola* is also suggested to act as an effective loop diuretic (Quadri *et al*. 2019). However, studies investigating the diuretic potentials of *Garcinia Kola* and in particular its effect on electrolyte balance are limited. Therefore, the present study was designed to investigate the diuretic activity of methanolic extract of *Garcinia Kola* (MEGCO) and its effects on the circulating electrolytes in male Wistar rats.

Materials and Methods

Plant collection and authentication

Garcinia Kola seed were sourced locally in Ado-Ekiti, Nigeria, and authenticated by a botanist in the Department of Agricultural science, Afe Babalola University where a voucher specimen was documented.

Drug and chemicals

Furosemide 40 mg manufactured by (Mancare Pharmaceuticals Pvt. Ltd) marketed as a diuretic drug was used as the standard drug. Assay kits for Biochemical parameters were purchased from Randox by the use of appropriate kits agent; Teco Laboratories at Ado-Ekiti.

Plant extraction

The methanolic extraction of *Garcinia cola* seed was prepared as previously described by Tendel *et al*. (2011). Fresh *Garcinia kola* seed were collected and washed with clean water, cut into small pieces and air dried under shade. 2.55 kg of dried *Garcinia cola* seeds were pulverized with an Electric pulverizer into fine powdered seed (1.95kg) with 3000 cm3 of methanol, macerated in a polytron Homogenizer for 48 hours and the solution was filtered under vacuum using Buchner funnel and Whatman No.1 filter paper (Whatman International Ltd, Maidstone, UK). The filtrate was evaporated under reduced pressure using a rotary evaporator and later freeze dried in a lyophilizer (Ilshin Lab. Co.Ltd, Seoul, Republic of Korea). The extract obtained was kept in a desiccator prior to use.

Animal care and management

Twenty-five (25) adult male Wistar rats weighing 160-200g were used for this study. They were purchased from the Animal House of the College of Health Science, Afe Babalola University, Ado-Ekiti where the study was carried out. The rats were housed in plastic cages under natural light/dark cycle in the laboratory and allowed to have access to standard rat chow (ABUAD Farm, Nigeria) and water ad libitum. They were allowed to acclimatize in the laboratory for two weeks before the commencement of the study. The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the protocol was approved by the Institutional Ethical Review Board of Afe Babalola University, and every effort was made to minimize both the number of animals used and their suffering**.**

Grouping and administration

The animals were allotted into five (5) groups of n=5 namely: control, Furosemide, MEGCOA, MEGCOB and MEGCOC groups. Control group received vehicle (distilled water), Furosemide group received 20 mg/kg of Furosemide, MEGCOA group received 200 mg/kg of MEGCO, MEGCOB group

received 400 mg/kg of MEGCO and MEGCOC group received 600 mg/kg of MEGCO. The administration was carried out by oral gavage and it lasted for 4 weeks.

Diuretic study

Diuretic potential of methanolic extract of *Garcinia Kola* was determined following the method of Lipchitz *et al*., 1943. The rats were allowed to acclimatized in metabolic cages fabricated by (Central Technological Laboratory and Workshops (CTLW), Afe Babalola University, Ado-Ekiti, according to Ohasu R Model; Ohasu, Pine Brook, New Jersey, USA) for one week before the commencement of the study. For urine collection, the rats were fasted overnight with free access to water after which their bladders were emptied by gentle of the pelvic area and by pulling the base of the tails.

Prior to the day of sacrifice urine samples were collected 2 hours intervals for 6 hours. Urine volume and electrolyte concentrations were determined using appropriate biochemical kits. The urine volume was expressed in ml.

Evaluation of diuretic activity (Lipschiz Test)

Urine volume (m1) and Na+, K+ and Cl- concentration (mEq/L) in the urine were determine and various indices for diuretic action were calculated (Lipschitz*et al*., 1943). The ratio of urine volume of the experimental group to that of the control group was taken as a measure of the diuretic index for any given dose of the extract.

Diuretic Index = Urine Volume of Test group (Ve)/Urine volume of Control Group (Vc)

Indices of 1.0 and more are regarded as a positive effect or potent diuretics. The diuretic activity is considered to be positive if the diuretic index values are greater than 1.50, moderate if the values are between 1.00 and 1.50, mild if the values lie in between 0.27 and 1.00 and there is no diuretic activity if the values is <0.72 (Abdala *et al*. 2008). Since diuretic index is prone to variability, a parameter knows as Lipschiz value was calculated. To obtain the Lipschiz value, urine volume of the extract treated rats was compared to that of the group that receive the standard drug (Furosemide) (Mukherjee, 2002).

$$\text{Lipchitz value (or diuretic activity)} = \frac{\text{Urine Volume of Test Group (Ve)}}{\text{Urine Volume of standard Group (vr)}}$$

Sodium Assay

Sodium was assayed in accordance with the method described by (Trinder, 1951). To 1.0ml of uranyl acetate 2.1mM and magnesium acetate 20 mM in ethyl alcohol, 50 u of the sample (urine) was added. The test tubes were shaken vigorously for proper mixing for 3 minutes and then centrifuge at 1500 revolution/minutes for 10 minutes to obtain a clear supermarket and 50 potassium ferrocyanide, non-reactive stabilizers, and fillers were added. Absorbance was recorded at 550nm against blank containing distilled water. Concentration (cone) of sodium was calculated using this formula:
Calculation:

$$\frac{\text{Abs. of unknown}}{\text{Abs. of STD}} X\ Conc.of\ STD\ \left(\frac{mEq}{L}\right) = \ Chlorine\ Conc.\left(\frac{mEq}{L}\right)$$

Abs. = Absorbance, S = Sample, STD = Standard (150 mE/L)

Potassium Assay

Potassium was assayed according to the method of (Terri and Sesin, 1958). To 1.0ml of sodium tetraphenylboron 2.1mM, 0.01ml (10µ) of samples (urine) was added. The resulting solution was mixed and incubated at room temperature for 3 minutes. Absorbance was then recorded at 500 mm against blank containing distilled water. Concentration of potassium was calculated using this formula:

Calculation:

$$\frac{\text{Abs. of unknown}}{\text{Abs. of STD}} X\ Conc.of\ STD\ (mEq/L) = \ Chlorine\ Conc.(mEq/L)$$

Abs. = Absorbance, S = Sample,
STD = Standard (150mE/L)

Chloride assay

Chloride was assayed according to the previous method of (Skeggs and Hochstrasser, 1964). To 1.5ml of mercuric nitrate o.o58mM, mercuric thiocyanate 1.75mM, mercuric chloride 0.74mM and ferric nitrate 22.3m M, 0.01(10µ) of sample (urine) was added. Absorbed was the recoded at 480 nm against blank containing distilled water. Concentration of chloride was calculated using this formula:

Calculation;

$$\frac{\text{Abs. of unknown}}{\text{Abs. of STD}} X\ Conc.of\ STD\ \left(\frac{mEq}{L}\right) = \ Chlorine\ Conc.\left(\frac{mEq}{L}\right)$$

Where, Abs. = Absorbance, S = Sample, STD = standard (100mEq/L)

At the end of two weeks of the study period, the rats were sacrificed using sodium pentobarbitone (50 mg/kg *ip*) and blood sample was collected through cardiac puncture into heparinized bottle, centrifuged and plasma was collected for biochemical analysis.

Body weight

The initial body weights of rats were determined and recorded before the commencement of the study, and the final weights of the rats were also monitored before sacrifice using a Camry weighing balance to assess the weight gain or loss in each group.

Urine volume

The volume of urine was measured using clean and dried measuring cylinder.

Biochemical assay

Blood sample was collected by cardiac puncture and draw into separate heparinised bottle and centrifuged at 3000 rpm for 15 minutes at 4oC, using centrifuge (Centurium Scientific, Model 8881). The plasma obtained was draw into separate plain bottle for biochemical assay. Also, the liver was isolated, weighed and sliced into sections of about 1g on dry ice and then placed in 5mL of ice-cold phosphate buffered saline. The tissue was homogenized on ice. The homogenate was then centrifuge at 1500xg for 15 minutes. The clarified supernatant was frozen stored for biochemical analysis.

Serum creatinine assay

Creatinine concentration was assayed by the method of (Bartels and Bohmer, 1972). To 1.0 ml of piric acid and sodium hydroxide, 0.1ml (10) of samples was added. Absorbance was then recorded at 492nm against blank and standard containing 0.1 ml of distilled water and standard reagent respectively. Concentration of creatinine was calculated using this for formula:

Calculation:
A2 –A1 =ΔA sample or ΔA standard
Concentration of creatinine in the tissue

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} X\ Standard\ Cone.\left(\frac{mg}{dl}\right) =$$

Where;
A2 =Absorbance reading after 30 minutes, A2 = Absorbance reading after 2 minutes.
ΔA sample =Absorbance reading of standard
Standard conc. = standard concentration in mg/dl (2.07mg/dl)

Renal urea assay

Urea concentration was assayed by the method of (Chaney and Marbach, 1962). To 100µ1 of sodium nitropusside 100µ of samples was added. The resulting solution was incubated at 37 0C for 10minutes. Then, 2.50 ml of Phenol (reagents II) and Sodium hypochlorite (reagents III) was added to the incubated mixture. This was further incubated 37oC for 15 minutes. Absorbance was then recorded at 546 mn against blank and standard containing 10µ of distilled water and standard solution respectively. Concentration of urea was calculated using this formula:

Calculation:$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} X\ Standard\ Cone.\left(\frac{mg}{dl}\right) =$

Where;
A sample = Absorbance reading of sample
A standard = Absorbance reading of standard.
Stand Conc. = standard concentration in mg/dl(80.77mg/dl)

Hepatic AST and ALT

Activities of liver ALT and AST were determined according to the method of Duncan (1994).

Serum uric acid

Serum uric acid was determined as previously described (Kageyama, 1971).

Statistical analysis

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS, version 22 of statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

Results

Effect of methanolic extract of Garcinia kola on body weight in male Wistar rats

The administration of methanolic extract of *Garcina cola* did not alter the body weight when compared with control and furosemide-treated rats as shown in the table below.

Table 1: Effect of methanolic extract of *Garcinia kola* on body weight in male Wistar rats

GROUPS	INITIAL WEIGHT (g)	FINAL WEIGHT(g)	WEIGHT GAIN(g)
CONTROL	174.0±18.5	249.1±21.0	75.2±25.2
FRD	173.8±17.1	226.6±5.0	52.8±4.5
MEGCOA	162.4±36.6	192.4±49.9	30.0±6.1
MEGCOB	162.8±11.4	159.4.±7.2	(3.5±2.5)
MEGCOC	169.4±25.3	168.2±26.3	(1.2±16.4)

Data are expressed as mean±S.E.M. n=5. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (Not significant at $p<0.05$ vs. control).

Effect of methanolic extract of Garcinia kola on kidney and liver weight in male Wistar rats

As shown in table 2, the administrations of 400 mg or 600 mg/kg body weight dose of methanolic extract of *Garcinia cola* significantly increased the kidney weight but not alter the liver weight adjusted for body weight when compared with control and furosemide-treated groups. However, the dose of 200 mg/kg body weight did not affect kidney and liver weight compared with control and furosemide-treated groups**.**

Table 2: Effect of methanolic extract of *Garcinia cola* on kidney and liver weight in male Wistar rats

GROUPS	Kidney weight (g/100g *b.w*)	Liver weight (g/100g *b.w*)
CONTROL	0.30±0.02	1.47±0.74
FRD	0.40±0.01	1.95±0.61
MEGCOA	0.45±0.03	0.99±0.76
MEGCOB	0.63±0.03*#	1.45±0.71
MEGCOC	0.86±0.03*#	1.89±0.59

Data are expressed as mean±S.E.M. n=5. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test*) (Significant at *p<0.05 vs. control, #p<0.05 vs. FRD).

Effect of methanolic extract of Garcinia kola on urine volume in male Wistar rats

Administration of methanolic extract of *Garcina cola* significantly increased the urine volume after 4 hrs in furosemide and MEGCOC-treated group compared with control. The urine volume for MEGCOC-treated group was significantly higher than furosemide treated group. The urine volume of furosemide and all the extract-treated groups were significantly higher compared with control group after 6 hrs of urine collection.

Table 3: Effect of methanolic extract of *Garcinia kola* on urine volume in male Wistar rats

GROUPS	URINE VOL (ml) (2 HOURS)	URINE VOL (ml) (4 HOURS)	URINE VOL (ml) (6 HOURS)
CONTROL	0.2 ± 0.1	0.08 ± 0.1	0.05±0.1
FRD	0.25 ± 0.025	0.2 ± 0.025*	0.9±0.025*
MEGCOA	0.20 ± 0.04	0.1 ± 0.04	0.3 ±0.04$^{*\#}$
MEGCOB	0.24 ± 0.1	0.1 ± 0.1	0.4 ±0.1$^{*\#}$
MEGCOC	0.30 ± 0.01	0.3 ± 0.01$^{*\#}$	0.8 ± 0.01$^{*\#}$

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. ($^{*}p<0.05$ vs. Control; $^{\#}p<0.05$ vs. FRD).

Diuretic indices for administration of methanolic extract of Garcina kola in male Wistar rats

According to Lipchitz's test, Indices of 1.0 and more are regarded as a positive effect or potent diuretics. The diuretic activity is considered to be positive if the diuretic index values are greater than 1.50, moderate if the values are between 1.00 and 1.50, mild if the values lie in between 0.27 and 1.00 and there is no diuretic activity if the values is <0.72 (Abdala *et al*., 2008). Since diuretic index is prone to variability, a parameter knows as Lipchitz's value was calculated by adjusting for the standard (furosemide). As shown in Table 4 comparing the value obtained with Lipchitz's value, all the extract-treated groups (200 mg, 400 mg and 600 mg) show diuretic activity. However, only 600 mg/kg body weight shows diuretic activity when adjusted for furosemide (standard).

Table 4: Diuretic indices for administration of methanolic extract of *Garcina kola* in male Wistar rats

Adjusted for control			**Adjusted for furosemide**		
MEGCA	MEGCB	MEGCC	MEGCA	MEGCB	MEGCC
1.82	2.24	4.24	0.44	0.55	1.04

Effect of administration of methanolic extract of Garcina kola on urine Na+ concentration in male Wistar rats

There was a significant decrease in urine sodium (Na) concentration of MEGCOA (400 mg) –treated group after 2 hours of urine collection when compared with control. However, administration of methanolic extract of *G. cola* also led to significant increase in urine sodium concentration when compared with furosemide-treated groups. There was significant increase in furosemide-treated groups and extract-treated groups after 4 hours of urine collection compared with control. Sodium urine concentration significantly increases in extract-treated groups compared with furosemide groups after 4 hours of urine collection. After 6 hours of urine collection, the urine Na concentration significantly increases in furosemide and extract-treated groups. However, a decreased was observed in urine sodium concentration of 200mg and 400mg compared with furosemide-treated groups**.**

Table 5: Effect of administration of methanolic extract of *Garcina kola* on urine Na+ concentration in male Wistar rats

Urine Na^+ Conc. (mEq/L) (2 HOURS)	Urine Na^+ Conc. (mEq/L) (4HOURS)	Urine Na^+ Conc. (mEq/L) (6 HOURS)
70.05 ± 2.5	45 ± 2.5	25.57±2.5
56.19 ± 3.1*	55.01 ± 3.1*	64.25±3.1*
66.2 ± 1.5#	68.76 ± 1.5*#	57.91 ±1.5
79.94 ± 3.2*#	61.99 ± 3.2*#	36.85±3.2*#
69.6 ± 4.0*	62.96 ± 4.0*#	61.13 ± 4.0*

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (*p<0.05 vs. Control; #p<0.05 vs. FRD).

Effect of administration of methanolic extract of Garcina kola on urine Cl concentration in male Wistar rats

There was a significant decrease in urine chloride concentration in the furosemide/extract-treated groups (200 mg and 400 mg) after 2 hours of urine collection but no significant change in MEGCOC (600 mg) compared with control. However, MEGCOB (400 mg) and MEGCC (600 mg) were significantly higher than furosemide-treated groups. No significant change in urine chloride concentration. After 6 hours of urine collection there was significant decrease in urine chloride concentration compared with control group. However, no significant alteration in urine chloride concentration in all the extract-treated groups when compared with furosemide-treated group as shown in Table 6.

Table 6: Effect of administration of methanolic extract of *Garcina kola* on urine Cl concentration in male Wistar rats

GROUPS	Urine Cl^- Conc. (mEq/L) (2 HOURS)	Urine Cl^- Conc. (mEq/L) (4HOURS)	Urine Cl^- Conc. (mEq/L) (6 HOURS)
CONTROL	122.31 ± 5.2	173.72 ± 5.2	159.6 ±5.2
FRD	72.03 ± 8.5*	150.16 ± 8.5	123.16 ±8.5*
MEGCOA	68± 6.0*	152.49 ± 6.0	155.93 ±6.0
MEGCOB	98.32±10.0*#	144.35 ± 10.0	138.19±10.0
MEGCOC	121.75± 2.0	161.86 ± 2.0	137 ± 2.0*

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (*p<0.05 vs. Control; #p<0.05 vs. FRD).

Effect of administration of methanolic extract of Garcina kola on urine K^+ concentration in male Wistar rats

The potassium urine concentration significantly increased in all the extract-treated groups compared with control and furosemide-treated groups after 2 hours of urine collection. The urine potassium concentration also significantly increased in all the extract-treated groups compared with control and furosemide groups after 4 hours of urine collection. Furosemide-treated group and extract treated groups significantly increased when compared with control groups. However, the urine potassium was significantly higher in MEGCOC (600 mg)-treated group compared with furosemide group as shown in Table 7.

Table 7: Effect of administration of methanolic extract of *Garcina kola* on urine K^+ concentration in male Wistar rats

GROUPS	Urine k^+ Conc. (mEq/L) (2 HOURS)	Urine k^+ Conc. (mEq/L) (4HOURS)	Urine k^+ Conc. (mEq/L) (6 HOURS)
CONTROL	11.16 ± 1.2	10.79 ± 1.2	11.27 ±1.2
FRD	11.33 ± 1.9*	11.39 ± 1.9	16.98 ±1.9*
MEGCOA	16.2± 1.5*#	17.61 ± 1.5*#	17.61 ±1.5*
MEGCOB	17.61±0.9*#	15.61 ± 0.9*#	18.61±0.9*
MEGCOC	17.61± 2.0*#	18.61 ± 2.0*#	26.13 ± 2.0*#

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (*p<0.05 vs. Control; #p<0.05 vs FRD).

Effect of administration of methanolic extract of Garcina kola on serum K^+, Na^+ and Cl^- in male Wistar rats

Administration of methanolic extract of *Garcinia Kola* did not significantly alter the serum Na^+ concentration compared with furosemide and control groups. In addition, the administration of MEGCO did not alter serum potassium concentration except for MEGCOC-treated group when compared with control and furosemide treated groups. Similarly, administration of MEGC at 400 mg and 600 mg/kg body weight led to significant increase in serum Cl^- level compared with control and furosemide-treated groups, whereas 200mg/kg body weight did not alter the serum Cl^- level when compared with control but significantly increased when compared with furosemide-treated groups.

Table 8: Effect of administration of methanolic extract of *Garcina kola* on serum K^+, Na^+ and Cl^- in male Wistar rats

GROUPS	Serum K^+ Conc. (mmol/l)	Serum Na^+ Conc. (mmol/l)	Serum Cl^- Conc. (mmol/l)
CONTROL	4.24± 0.12	84.79± 4.2	69.23± 1.5
FRD	3.03 ± 0.5	76.89 ± 8.5	54.82 ± 3.5*
MEGCOA	3.63± 0.7	80.25± 6.0	71.68± 2.0*#
MEGCOB	4.49± 0.2	79.66± 10.0	93.50± 1.2*#
MEGCOC	4.70± 0.1*#	81.15± 2.0	83.89± 2.0*#

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (*p<0.05 vs. Control; #p<0.05 vs. FRD).

Effect of administration of methanolic extract of Garcina kola on serum uric acid, urea and creatinine concentration in male Wistar rats

Serum uric acid has been documented as a pro-inflammatory biomarker. The administration of MEGCO (600 mg/kg) did not alter the serum uric acid concentration except in MEGCOC-treated group where a significant increase was observed with control and furosemide groups. In addition, administration of MEGCO significantly increased serum urea concentration in all the extract-treated and furosemide groups compared with control. However, serum urea level of MEGCOC (600 mg)-treated group is significantly higher compared with furosemide-treated groups. Likewise, administration of MEGCO did

not significantly altered the serum creatinine level except at 600 mg-treated groups that significantly increased when compared with furosemide and control groups as shown in table 9.

Table 9: Effect of administration of methanolic extract of *Garcina kola* on serum uric acid, urea and creatinine concentration in male Wistar rats

GROUPS	Serum Uric acid Conc. (mg/dl)	Serum Urea Conc. (mmol/l)	Serum Creatinine Conc. (µmol/l)
CONTROL	6.47± 0.3	1.33± 0.16	35.75± 5.0
FRD	4.56 ± 0.8	3.63 ± 0.3*	36.33 ± 3.0
MEGCOA	4.70± 0.7	4.09 ± 0.56*	46.63± 4.0
MEGCOB	5.40± 0.2	4.70± 0.2*	46.82± 3.2
MEGCOC	7.28± 0.1$^{*\#}$	4.79± 0.1$^{*\#}$	50.69± 0.9$^{*\#}$

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. ($^{*}p<0.05$ vs. Control; $^{\#}p<0.05$ vs. FRD).

Effect of administration of methanolic extract of Garcina kola on hepatic AST and ALT activities in male Wistar rats

Administration of MEGCO did not significantly altered hepatic AST activity when compared with control and furosemide treated groups. In addition, methanolic extract of *Garcina cola* significantly increased hepatic ALT activity at 400 mg and 600 mg/kg when compared with furosemide-treated group. However, the extract-treated groups were not significantly different from control except 600 mg-treated group that was significantly higher than control group**.**

Table 10: Effect of administration of methanolic extract of *Garcina kola* on hepatic AST and ALT activities in male Wistar rats

GROUPS	AST (u/g prot.)	ALT (u/g prot.)
CONTROL	9.58 ± 0.90	4.30 ± 0.50
FRD	7.52 ± 1.20	3.20 ± 0.40
MEGCOA	7.26 ± 0.70	3.04 ± 0.7
MEGCOB	9.11 ± 0.50	5.22 ± 0.20$^{\#}$
MEGCOC	9.5 ± 0.80	9.50 ± 0.95$^{*\#}$

Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. ($^{*}p<0.05$ vs. Control; $^{\#}p<0.05$ vs. FRD).

Discussion

This study examined the potential diuretic activity of *Garcinia Kola* using a Wistar rat model. This assessment involved the comparison of the volume of urine excreted and circulating electrolytes of animal that were administered with a methanolic extract of *Garcinia Kola* and animals that were administered with Furosemide, a standard diuretic. This study demonstrated that an effective dose of *Garcinia Kola* induced diuresis and altered electrolyte balance. Administration of 400 mg/kg and 600 mg/kg of the extract significantly increased urine volume after at 6 hours. The diuretic indices were tested by the Lipchitz's method and the tests demonstrated that all the extract-treated groups (200 mg/kg, 400 mg/kg and 600

mg/kg) showed diuretic activity. However, the diuretic index of 600 mg/kg was higher (1.04) when adjusted for furosemide (standard). The results also show that 400 mg/kg and 600mg/kg of the extract significantly increased the concentration of Na^+ and K^+ in the urine. The 600 mg/kg concentration of the extract altered the Cl^- concentration in an inverse manner, thereby significantly reducing its concentration in the urine. Furthermore, 400 mg/kg and 600 mg/kg of the extract did not alter the serum Na^+ concentration but the 600 mg/kg significantly increased the serum K^+ concentration. It was also observed that 400 mg/kg and 600 mg/kg of the extract also increased serum Cl^- concentration. In addition, administration of 600 mg/kg of the extract significantly increased serum uric acid, urea and creatinine. The 600 mg/kg concentration of the extract also increased the alanine transaminase activity but did not alter aspartate transaminase activity in the tissues.

Since the basic function of a diuretic is the removal of excess water from the body by increasing urine formation and excretion, the marked increase in urine volume of animals treated with *Garcinia Kola* relative to control underscores the efficacy of *Garcinia Kola* as a diuretic agent. The Lipchitz's test has been used over the years as a common test of the potency of diuretics (Mejo *et al*. 2020). Consequently, this study employed Lipchitz's test and the results demonstrated that all extract treated groups showed diuretic activity. However, in order to emphasize the efficacy of the extract as a diuretic the Lipchitz's value was calculated while adjusting for furosemide (Standard diuretic). It was observed that the group treated with 600 mg/kg of the extract was higher (1.04) when adjusted for furosemide (Standard diuretic). Although the presence of the bioflavonoid group in *Garcinia Kola* gives it several pharmacokinetic advantages like the survival of first pass metabolism which inactivates most monomeric flavonoids, (Olaleye *et al*. 2000), the consequent increase in bioavailability that comes from an increase in dose may be the reason for the dose dependent effect.

In this study the change in urine volume was not associated with body weight change. However, there was a significant increase in the kidney weight in groups treated with higher concentrations of the extract (400 mg/kg, 600 mg/kg). Relative organ weight has been indicted as an index of the toxic effects of a chemical compound (Quadri *et al*. 2019). Therefore, the increase in kidney weight could imply renal hypertrophy, which is an indication of renal toxicity. This is consistent with recent study by Quadri *et al*. that suggested that higher doses of kolaviron (active substance in *Garcinia kola*) caused derangement in biomarkers of renal function urine volume, serum creatinine, urinary albumin/protein (Quadri *et al*. 2019). One of the salient attributes of diuretics is that they reduce reabsorption of sodium and chloride at different parts of the nephron, as a result increasing urinary sodium and water losses (Hunt *et al*. 2009, Dickstein *et al*. 2010). The excretion of sodium in the urine (natriuresis) which was observed in the groups treated by the extract underscores the natriuretic potential of of *Garcinia Kola*. Therefore, this study suggests that effective dose of the extract can be an adjuvant treatment for hypernatremia and hypertension and this supports previous studies by (Adaramoye, 2012, Akomolafe *et al*. 2017). Bolstering this assertion is the observation of the increase in the urine potassium concentration of groups treated with the extract compared to control. Studies have also shown that the risk of developing hypertension increases with reduce urine potassium excretion (Quadri *et al*. 2019). Therefore, this present study suggests that the methanolic extract of Garcinia Kola causes diuresis, natriuresis and kaliuresis.

Garcinia Kola did not affect the serum sodium concentration and this observation corroborates a past study by (Agada and Braide, 2009). Likewise, the treatment only affected serum potassium concentration in groups that were treated with 600mg/kg of the extract. There was a significant decrease in serum potassium concentration compared to the control and furosemide groups, which implies that administration of 600 mg/kg of the extract causes potassium-dependent electrolyte depletion. In addition, the serum chloride concentration was significantly increased in all the extract treated groups compared with control and furosemide groups, which is inconsonance with earlier studies (Agada and Braide, 2009). The administration of Garcinia Kola also altered serum uric acid concentration at 600 mg/kg. Uric acid has been earlier documented as a biomarker of proinflammatory response (Camilo *et al*. 2020). The present study indicates that in addition to diuresis, natriuresis and kaliuresis, *Garcinia Kola* also promotes proinflammatory responses at a dose 600 mg/kg. However, there was no significant change in serum uric acid concentration at 200 mg/kg and 400 mg/kg. Although serum urea was significantly increased in all the

treated groups compared to control, creatinine was only higher in the groups treated with 600 mg/kg of the extract compared to control. This suggests that the administration of Garcinia Kola could predispose the experimental animals to renal toxicity. Previous studies have used increased urea levels to indicate renal toxicity, this is because it is water soluble and an increase in the serum levels of urea indicates that the capacity of the kidneys to expel this substance has been reduced (Meotti *et al*. 2003).

ALT (Alanine Transaminase) and AST (Aspartate Transaminase) are liver function markers that indicate tissue or cellular toxicity (Meotti *et al*. 2003, Saad *et al*. 2018); Administration of Garcinia Kola increased hepatic ALT at 400 mg/kg and 600 mg/kg and as a consequence, at these doses the animals are predisposed to tissue toxicity. This could be due to the fact that ALT concentration is higher in the liver of rats while AST can be found in a myriad of tissues, although AST concentrations are usually higher in muscular tissues and the liver (Uko *et al*. 2001). Therefore, the high ALT levels could be an indication of hepatotoxicity in groups treated with higher concentration of the extract. In addition, at 200 mg/kg there was no alteration in the AST and ALT activities which imply the non-toxicity of lower doses to the liver and this is consonant with a previous study by Kalu *et al*. (Kalu *et al*. 2016). Therefore, this study suggests that the apparent toxicity of the methanolic extract of *Garcinia Kola* is dose dependent. The findings of this study suggest that *Garcinia Kola* can be employed as a potential diuretic agent and can serve as adjuvant therapy for conditions like hypernatremia, hyperkalemia, and hypertension.

Conclusion

In conclusion, the effective dose (600 mg/kg) of the methanolic extract of *Garcinia Kola* caused diuresis, natriuresis and kaliuresis but put the animals at risk of renal toxicity and electrolyte imbalance.

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