EFFECTS OF BITTER COLA (GARCINIA COLA) EXTRACT ON STEROID HORMONES AND SELECTED ELECTROLYTES IN WEST AFRICAN DWARF BUCKS

Department of Animal Physiology,
Federal University of Agriculture, Abeokuta, Nigeria

ABSTRACT
Twelve mature West African Dwarf bucks were allotted to four treatment groups of three animals per group to determine various doses of Garcinia cola seed extract (GCSE) on testosterone, cortisol, potassium (K+), sodium (Na+) and calcium (Ca2+) in WAD bucks. Treatment A (0 mg GCSE) received saline solution; Treatment B (50 mg GCSE); Treatment C (100 mg GCSE) and Treatment D (150 mg GCSE). The animals were observed for 20 minutes after GCSE injection for behavioural response. Jugular blood samples were collected from the WAD bucks at 10 min intervals for 3 hours before and after GCSE injection. Plasma testosterone and cortisol concentrations were determined by radioimmunoassay method. Serum electrolytes were determined by atomic absorption spectrophotometry. Injection of GCSE had no effect on mean plasma testosterone concentration while cortisol significantly increased (P< 0.001) in the three treatments as compared with the control (12.21 ng/mL). The injection of GCSE induced behavioural changes in the animals resulting in depression, inability to stand, suppression of feed intake and lethargy for 15 mins after injection. Also, intravenous injection of GCSE significantly increased serum potassium (P<0.001) and sodium (P<0.05) but not calcium in the three treatments as compared in the control. These results indicate that GCSE may be involved in the regulation of cortisol, potassium and sodium secretion in bucks. Despite the reported potential benefits of Garcinia cola, its use should be with caution because GCSE has a depressant property.

Key words: Behaviour, Electrolytes, Garcinia cola, Steroid hormone.

INTRODUCTION
Garcinia cola, a largely cultivated forest tree indigenous to West and Central Africa has been referred to as a ‘wonder plant’ because almost every part of it has been found to be of medicinal importance (Hutchinson and Dalziel, 1954). It grows to a height of about 12 – 14m and produces reddish, yellowish or orange coloured fruit (Okwu, 2005; Adesanya et al., 2007). Each fruit contains 2 to 4 yellow seeds and a sour tasting pulp. Its seed is known as bitter cola and is smooth, elliptically shaped, with yellow pulp and brown seed coat. The seed is eaten in a masticatory fashion and is used to prevent and relieve colic, chest colds, cough and headache (Ayensu, 1978).

The seed which contains carbohydrate, crude fat, protein, sodium, etc. as primary compounds also contains biflavonoids (ametoflavone), xanthones, benzophenones as secondary components (Farombi et al., 2000; Pietta, 2000; Iwu, 2003; Olatunde et al., 2004; Agyili et al., 2006; Iwu et al., 1990).

Several works done on the seed extract of G. cola have confirmed its erythropoietic effects (Esonmu et al., 2005; Unigwe and Nwakpu, 2009), hypolipidermic (Koshy and Vijayalakshmi, 2001; Oluyemi et al., 2007), antihistaminic (Nakatani et al., 2002), anti-atherogenic (Adaramoye et al., 2005), antimicrobial (Iwu et al., 1999), antiviral

*Corresponding author’s e-mail: smithof@live.com
1Department of Biological Sciences, St. Cloud State University, Minnesota, USA
2Department Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta
The seed has been shown to improve growth performance in poultry (Adediji et al., 2006). Other effects of *G. cola* seed extracts include protection against carbon tetrachloride induced erythrocyte damage (Adaramoye and Akinloye, 2000), anti-diabetic and anti-hepatoxic effects (Iwu, 1993).

As a result of the multi-purpose medicinal uses of *G. cola* and its wide spread consumption by human during festivities, some studies have been carried out on the extract of various parts of the plant, especially as they affect reproduction (Farombi et al., 2004; Ibekwe et al., 2007; Ibekwe et al., 2009; Chilaka et al., 2009). Animal and human studies revealed that the extracts of *G. cola* exhibit aphrodisiac effects in male subjects (Iwu, 1993; Orie and Okon, 1993) and suppress ovulation and delay fertility in female subjects (Iwu and Igboke, 1982). The alkaloid (Fisentin and Kolaviron) and biflavonoid extract of *G. cola* seeds exhibited spasmolytic effects on uterine and gastrointestinal smooth muscle (Braide, 1989). This was attributed to possible reduction of calcium ion entry into the cytosol of smooth muscle cells and sustenance of smooth muscle contraction (Braide, 1989; Udia, 1998; Braide et al., 2003). Ibekwe et al. (2007) reported a significant reduction in serum testosterone levels of male rats following administration of crude flavonoid extract of *G. cola* seeds; thus *G. cola* seed was proposed as a means for male contraceptives (Akpantah et al., 2003). Udoh (1998) reported effects of methanolic extract of *Garcinia cola* seed leaves on reproductive organs; the author reported testicular atrophy and spermatogenesis arrest.

There are different views based on research results (Farombi et al., 2004; Ibekwe et al., 2007; Ibekwe et al., 2009; Chilaka et al., 2009) of the effect of *G. cola* extract on reproduction. Therefore, this study was undertaken to investigate the effect of *Garcinia cola* seed extract on plasma hormones and selected electrolytes in West African Dwarf bucks.

### MATERIALS AND METHODS

All procedures used in this animal experimentation were conducted within standard ethical norms. The experiment was carried out at the Teaching and Research Farm, University of Agriculture, Abeokuta, Ogun State, Nigeria, which is located within the rainforest zone of South Western Nigeria (where there are large cultivated forest trees of *G. cola* and its production) at the latitude 7º 13’ , 49º 46’ N, longitude 3º 26’, 11º 98’ E and altitude 76 m above sea level (Amujoyegbe et al., 2008). The climate is humid with a mean annual rainfall of 1037 mm. The annual mean temperature and humidity was 34°C and 82% respectively.

Fresh *Garcinia cola* seeds were purchased, sliced into pieces and air-dried at ambient temperature for three consecutive days and grounded into fine powder. The *G. cola* was extracted in 70% ethanol for 3 days, filtered through a Whatman No.1 filter paper and allowed to evaporate to a semi-solid residue (a brownish solid paste was obtained) using water bath at 50°C. The semi-solid residue was poured into a sample bottle and left opened for 2 days to allow the evaporation of residual ethanol. The semi-solid residue (0.5 g) was constituted into 500 mg/ml in saline solution which served as the stock solution and used for the experiment.

Twelve (12) mature, two year old intact goat bucks (West African Dwarf) were used for the experiment. The animals were housed in individual pens and were supplied with water *ad libitum*. They were allowed to graze during the day for a month and given concentrate in the evening. Upon their arrival at the farm, they were treated against ectoparasite by dipping in Asuntol solution (3g/L of water) and also antibiotic (oxytetracycline). The bucks were randomly alloted into groups, consisting of three animals per group and the treatments were: Treatment A (control, 0 mg *Garcinia cola* seed extract, GCSE/Kg BW; received saline solution), Treatment B (50 mg GCSE/Kg BW), Treatment C (100 mg GCSE /Kg BW) and Treatment D (150 mg GCSE/Kg BW).

All animals were fitted with an-indwelling jugular catheter 24 hours before the start of the experiment. Animals were stanchioned and 2ml of blood was obtained from each animal at 10-minute intervals for 3 hours before the intravenous injection
of either saline solution or GCSE (pre-treatment period or period 1) and also at 10-minute interval for another 3 hours after saline solution or GCSE injection (post-treatment period or period 2). Observations were carried out on the behavioural changes before and after the injection of GCSE. After each blood collection, heparinized-saline solution (10 µL heparin/mL) was used as flushing media. Each blood sample collected was shared into ethylene diamine tetraacetate-heparin (EDTA-heparin) tubes (for plasma collection) and plain blood sample tubes (for serum collection). Plasma testosterone and cortisol concentrations were measured by radioimmunoassay kits (Gazal et al., 1998). Serum calcium, potassium and sodium concentrations were estimated by atomic absorption spectrophotometer (Perkin-Elmer Model 4000 atomic absorption spectrophotometer). The sensitivity of the assay was 2.0 ng/mL and the intra-assay coefficient of variation was 2.6 per cent while for testosterone, the sensitivity of the assay was 0.20 ng/mL. All the statistics were carried out using General Linear Models (GLM) procedures of SAS (1999).

RESULTS AND DISCUSSION
Pre-treatment mean for plasma testosterone concentration was not significantly different (P>0.05) and also the injection of GCSE had no significant effect (P>0.05) on mean plasma testosterone concentration (Fig. 1).

GCSE injection in WAD bucks significantly (P<0.001) increased plasma cortisol concentration (Fig. 2). However, there was no effect of period on plasma cortisol concentration in WAD bucks.

The injection of GCSE also had significant effect (P<0.01) on the concentration of the electrolyte as presented in Fig. 3. There was also a significant (P<0.05) effect of period on sodium concentration in the bucks. In serum potassium concentration, the pre-treatment mean showed a significant result (P<0.001). Also, there was a significant (P<0.05) effect of period on the electrolyte (Fig. 4). Pre-treatment mean for calcium was not statistically different (P>0.05) and the injection of GCSE did not significantly affect (P>0.05) the mean serum calcium concentration (Fig. 5).

Intravenous injection of GCSE induced behavioural changes like depressing their ability to stand and lethargy for 15 minutes after the injection. The effect of GCSE and period did not influence the testosterone concentration in the WAD bucks and corroborates earlier report in rats (Ibekwe et al., 2009). However, Braide et al. (2003) reported a significant reduction in serum testosterone and a concomitant elevation of serum FSH and LH in young adult virgin male albino Wistar rats administered with crude alkaloid extract of Garcinia cola seed orally and G. cola seed powder in diets. Ibekwe et al. (2007) also observed a decrease in serum testosterone levels of male Albino rats. Eyong and Braide (2009) reported effects of crude alkaloid extract from G. cola. The result obtained from their study showed a reduced concentration of testosterone levels but increased LH secretion in the male rats. In another study, Chilaka et al. (2009) also reported the effect of G. cola seeds that decreased serum concentration of testosterone of adult Albino mice. But Akpantah et al. (2003) and Oluyemi et al. (2007) reported the effect of oral administration of crude ethanolic extract of G. cola seed. Their result showed an increase in the peripheral testosterone production in male Sprague-Dawley rats and Wistar rats, respectively. In this experiment, a trend in the concentration of testosterone was noticed which declined immediately after the GCSE injection. Also, in the present study, it was observed that the injection of GCSE increased plasma cortisol concentrations in WAD bucks. Increased Cortisol concentration may be attributed to physiological stress imposed by the dosage of GCSE. This could probably be used as an index of the effects of ACTH secretion (Larzul et al., 2010). The high concentration of cortisol in this study may also be attributed to caffeine present in G. cola which stimulated blood levels of cortisol corroborated by Odeburmi et al. (2009) and Beaven et al. (2008). There was increase in serum potassium and sodium, although the GCSE had no effect on the calcium. Agada and Braide (2009) reported that male Albino rats fed (10% w/w and 20% w/w) with diets of Garcinia cola powder for 6 weeks had significant (P<0.05) increase in serum concentrations of calcium. The authors also observed that there were no marked changes in the serum concentrations of sodium and potassium. However, Udia et al. (2009) reported that increase concentration of methanolic extract of G. cola in Albino rats' smooth muscle preparations in vitro
produced a decrease in heights of smooth muscle contraction. This could be as a result of the reduction of calcium ion entry into the cytosol of smooth muscle cells. This then resulted into ridding the contractile apparatus in cytosol of Ca\(^{2+}\) essential for generation and sustenance of smooth muscle contraction. The high concentration of potassium in this study may be attributed to xanthone in \textit{G. cola} by dependently inhibiting the Ca\(^{2+}\) influx induced by either norepinephrine or high K\(^+\). It suggests that xanthone might act as a blocker of both receptor–operated and voltage–dependent Ca\(^{2+}\) channels (Chen and Kang, 1997; Okojie \textit{et al.}, 2009).

Eleyinmi \textit{et al.} (2006) reported the mineral composition of bitter cola seed and hull. The authors reported 86.4, 335 and 34.1 mg/ Kg of sodium (Na\(^+\)), potassium (K\(^+\)) and calcium(Ca\(^{2+}\)) in the seed of \textit{G. cola} respectively while in the hull, it was 7.1, 10.6 and 41.5 mg/ Kg of Na\(^+\), K\(^+\) and Ca\(^{2+}\). High concentrations of Na\(^+\) and K\(^+\) in the seed of \textit{G. cola} may be responsible for the increase in concentration of Na\(^+\) and K\(^+\) in this study. Na\(^+\) and K\(^+\) are required to maintain osmotic balance of body fluid. The pH of the body, regulation of muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth (NRC, 1989).
FIG 2: Plasma cortisol concentration in West African Dwarf bucks injected Garcinia cola seed extract at (A) 0 mg/Kg BW (control), (B) 50 mg/Kg BW (low), (C) 100mg/Kg BW (mid) and (D) 150 mg/Kg BW (high).
FIG. 3: Serum sodium concentration in West African Dwarf bucks injected Garcinia cola seed extract at (A) 0mg/Kg BW (control), (B) 50 mg/Kg BW (low), (C) 100 mg/Kg BW (mid) and (D) 150mg/Kg BW (high).
FIG 4: Serum potassium concentration in West African Dwarf bucks injected Garcinia cola seed extract at (A) 0 mg/Kg BW (control), (B) 50 mg/Kg BW (low), (C) 100 mg/Kg BW (mid) and (D) 150 mg/Kg BW (high).
FIG 5: Serum calcium concentration in West African Dwarf bucks injected Garcinia cola seed extract at (A) 0 mg/Kg BW (control), (B) 50 mg/Kg BW (low), (C) 100 mg/Kg BW (mid) and (D) 150 mg/Kg BW (high)
CONCLUSION
From the study it can be concluded that: the injection of GCSE had no significant effect on plasma testosterone but significantly increase plasma cortisol level of WAD bucks. Further, GCSE injection significantly increased serum potassium and sodium without affecting calcium concentrations.

ACKNOWLEDGEMENTS
The authors express their sincere gratitude to laboratory staff of the Department of Biological Sciences, Saint Cloud State University, Minnesota, USA for the radioimmunoassay analysis. Appreciation is also extended to staff of the Central Laboratory, Federal University of Agriculture, Abeokuta for the analysis on the selected electrolytes.

REFERENCES


