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PHYTOCHEMICAL ANALYSIS AND EFFECTS OF CHRONIC AND SUB-CHRONIC ADMINISTRATIONS OF METHANOLIC SEED EXTRACT OF *GARCINIA KOLA* (HECKEL) ON SOME PHYSIOLOGICAL PARAMETERS IN MALE RODENTS: RELEVANCE ON TRADITIONAL CLAIM OF ITS APHRODISIAC PROPERTIES

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ABSTRACT

The plant, *Garcinia kola*, is indigenous to most tropical rain forest of West Africa. Its consumers have for long traditionally claimed the seeds and other parts possess aphrodisiac properties. The animals were treated with the extract by oral administration in doses of 125 mg/kg, 250 mg/kg and 500-mg/kg-body weight in their respective groups for either 20 days or 60 days. Control group received normal saline 1 ml/100g of body weight accordingly for the same period. The results from this investigation showed that the methanolic seed extract produced a concentration-dependent decrease on blood pressure of an anesthetized male cat, similar with that of acetylcholine, and was blocked by atropine in a competitive manner. The extract cause significant increase on onset of sleep in male rats ($P<0.05$) and also decreases the duration of sleep ($P<0.05$). The haematological analysis showed that the extract has anti-thrombotic property through significant increase of bleeding and clotting time ($P<0.05$) and decrease of platelet counts ($P<0.05$). Both the duration of treatment with the extract and variation of the doses had significant influence on the outcome of the measured parameters.The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, glycosides, steroids, flavonoids and carbohydrates. Following abundant andrelevant literatures which indicate the correlation between the results of this study and aphrodisiac properties, it is suggested that the results can serve as scientific evidence that supports the traditional claim that *Garcinia kola* possesses aphrodisiac property that may justify its long-term consumption.

Key words: Aphrodisia, Methanolic Extract, Phytochemical Analysis, Blood Pressure, Garcinia kola, Anti-thrombosis.

INTRODUCTION

Garcinia kola (Hussein RA *et al.,* 1982), commonly called "bitter kola" is indigenous to most rain forests of tropical Africa (Dalziel JM, 1937). In most communities of these climatic regions, it is a common traditional claim and belief among the folks that consumption of *Garcinia kola* seeds or other parts can improve "alertness" and male fertility or sexual behaviors (Atilade AA, 2004).

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Because of this traditional claim or belief, the action of *Garcinia kola* has been a subject of intense scientific investigation on different biological and physiological systems in attempts to verify its pharmacological relevance on such systems. An active constituent called kolavirone, perhaps among others, has been identified and reported as having some pharmacological properties (Adaramola DA and Akinloye O, 2000; Adaramoye AD, 2010; Braide VP, 1990).

The seeds of *Garcinia kola* have also been reported to have ulcerogenic activity (Oluwole FS and Obatomi AB, 1990) anti-hepatotoxic effects (Adaramoye

AD *et al*., 2005; Bartlik BD *et al*., 1995) anti-oxidant properties, erythropoietic effects (Esomonu UG *et al*., 2005), appetite suppressant property (Uko OJ *et al.*, 2001), anti-atherogenic effect (Ajani EO *et al.,* 2008), anti-trypanosomial property (Ogbadeyi EO *et al.,* 2011) and bronchodilator effect (Orie NN and Ekon EU, 1993).

This study intends to investigate the anti-thrombotic and anti-hypnotic effects of the methanolic seed extract as well as its effect on blood pressure. Discussion of the results, though useful on their own rights, will however be tailored on their significance on the traditional claim that the seeds possess aphrodisiac properties. Nonetheless, it is worthy to note that crude plant extracts are known to contain multiple active principles that may act either synergistically or antagonistically. The present study therefore attempts to find out if there is any relationship between the effects of the extract on the physiological parameters measured and the traditional claim of its aphrodisiac properties.

MATERIALS AND METHODS

Experimental Animals

10-16 weeks old male wistar rats weighing between 210-300g were obtained from the Animal House Unit, University of Jos. They were housed in stainless steel cages and handled under ethical conventional conditions and guidelines for the use and care of laboratory animals. They were fed freely with standard solid nutritional pellets and water *ad libitum* until the commencement of experiments. Male cats were purchased from a market in Jos metropolis. They were kept at the Animal House Unit to acclimatized until the commencement of the experiment. They were similarly fed as the rats.

Preparation of the Extract

Garcinia kola seeds were purchased from reputable dealers in a market in Jos metropolis, plateau state, Nigeria. They were authenticated at the School of Forestry, Jos, where a voucher specimen numbered GCL0153/04 was prepared and kept. The seeds were washed, de-husked and cut in small pieces. They were then dried under the shade in an open space in the laboratory. Thereafter, they were manually grounded to powder with mortar and pestle. The extract was obtained using a modified method (Adegboye MF *et al*., 2008).

150g of the powdered seeds were extracted with methanol in a soxhlet extractor. The extract was evaporated to dryness in a vacuum evaporator at 40^0C repeatedly until a constant yield of 57.9 g (38.6%). The extract was reconstituted with distilled water for the purpose of the experiment.

Phytochemical Analyses

Test for Alkaloids

The method described by Trease and Evans (Trease G and Evans WC, 1983).

was used. 0.5 g of the extract was weighed and mixed with 3 ml of 1 % aqueous hydrochloric acid (HCL) on a stream bath and stirred very well and the solution thereafter filtered with Whatman's filter paper to get a clean filtrate. Few drops of Wagner's reagent, Dragendoff's reagent and picric acid solution were separately treated with 1 ml each of the filtrate. They were then repeatedly observed for precipitation and confirmation of the presence of any alkaloids.

Test for Saponins

This test was carried out using the method of Wall (Wall ME *et al.,* 1952). 0.5 g of the extract was weighed and dissolved in 5 ml of distilled water in a test tube. This was then shaken very well and then warmed for 5 minutes while observing for the formation of froths as evidence for the presence of saponins

Test for Tannins

0.5 g of the extract was dissolved in 1 ml of distilled water. It was carefully shaken, filtered and mixed with 5 % ferric chloride. The mixture was observed for blue-green precipitate as evidence for tannins (Trease G and Evans WC, 1983).

Test for Anthraquinones

0.5 g of the extract was weighed in a test tube. 5 ml of chloroform was added into it and shaken for 5 minutes. The solution was then filtered and the filtrate mixed with equal volume of 10 % ammonium solution. Thereafter, the mixture was observed for red coloration in the ammonical layer as evidence for the presence of anthraquinones (Wall ME *et al.,* 1952).

Test for Glycosides

100 mg of the extract was weighed in a test tube and 2.5 ml of a diluted sulphoric acid was added and immersed for 15 minutes in boilingwater contained in a bath. It was then allowed to cool and thereafter neutralized with 2 ml of 20 % potassium hydroxide. 5 ml of Fehling's solutions A and B was added and boiled for 3 minutes while observing for a brick-red precipitate as evidence of the presence of glycosides.

Test for Steroids

100 mg of the extract was dissolved in 2 ml of chloroform and 1 ml of concentrated sulphoric acid was added. The mixture was then observed for a brown coloration as evidence of the presence of steroids as described by Sofowora (Sofowora A, 1982).

Test for Flavonoids

2 g of the extract was dissolved in 10 ml acetone in a conical flask and then immersed for 3 minutes in warm water contained in a bath. The mixture was then filtered immediately and 5 ml of the filtrate was mixed with lead

subacetate solution and observed for yellow precipitates as evidence for the presence of flavonoids.

Test for Carbohydrates

100 mg was dissolved in 3 ml of distilled water in an inclined test tube and then mixed with 3 drops of Molisch reagent. Thereafter, 1 ml of concentrated sulphoric acid was carefully added down the side of the test tube such that the acid forms a layer beneath the aqueous solution without mixing or shaking. It was then observed for reddish ringed precipitate at the junction of the liquid as evidence for the presence of carbohydrates (Trease G and Evans WC, 1983).

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Haematological Analysis

Treatment of Animals with the Extract

20 Male rats were randomly divided into four groups of five rats each. Animals in the first group were considered as the control group and were orally administered normal saline 1 ml/100g daily for 20 days. Animals in groups 2, 3 and 4 were administered the extract at a daily doses of 125, 250 and 500 mg/kg respectively also by oral route for 20 days such that none received a volume more than 1 ml of the reconstituted solution of the extract. After the last dose, the animals were allowed to stay for the next 24 hours before the haematological analysis.

A similar procedure with another set of 20 male rats also divided into four groups of five rats each was carried out except that treatment was for 60 days instead of 20.

Determination of Bleeding Time

The rats were placed in a restrainer and their tails passed out from one of the openings. They were anaesthetized with 3 % sodium pentobarbital (0.5 ml/100 g i.p). The tail was then disinfected with methylated spirit and then pricked with a lancet about 4 mm deep and the timing of blood flow was started immediately. The blood was cleaned with filter paper at 15-second intervals until it stopped. The procedure was repeated four times on each rat to get the average bleeding time in a minute.

Determination of Clotting Time

The rats were placed in a restrainer and the tails disinfected with methylated spirit. They were anaesthetized with 3 % sodium pentobarbital (0.5 ml/100g) and drops of blood were collected from the tip of the tail by puncture on clean slides. A clean pin was used to gradually lift up the blood on the slide at 15-second interval until coagulation was observed. This procedure was repeated four times.

Determination of Platelet Counts

The rats were restrained and their tails disinfected with methylated spirit. 0.5 ml blood sample was collected into a white blood cell pipette and diluted with 0.5 ml of Boar's solution containing EDTA. 9 ml of normal saline was added and mixed for three minutes. 2 drops of the mixture was withdrawn and put into a Nabeaur counting chamber. It was allowed to settle for 20 minutes. It was then placed under a light microscope and viewed at a magnification of 40 to enable counting of the platelets.

Effect of the Extract on Blood Pressure

The male cat was anaesthetized with urethane (1.8 mg/kg) by intraperitoneal route. The femoral vein was cannulated to allow for intravenous injection of the extract and reference drugs. The carotid artery was also cannulated and connected to a pressure transducer and physiograph for recording of the blood pressure. Heparin (1000 IU/kg) was injected through the femoral vein to prevent clot formation. The trachea was also cannulated to allow for respiration. The cat was allowed to equilibrate for about thirty minutes and thereafter the baseline blood pressure was recorded, followed by intravenous administration of normal saline (1 ml/100 kg), acetylcholine ($5 \times 10^{-5} - 5 \times 10^{-2}$ mg/ml), extract (5-30 mg/kg) alone and in the presence of atropine ($2 \times 10^{-5} - 2 \times 10^{-3}$ mg/ml). A time cycle of two minutes was maintained.

Effect of Extract on Chemical-Induced Sleep

Following daily oral administration of the extract or normal saline as described in 2.4.1 and after 24 hours of the last dose for each group, the animals were administered phenbarbitone, 40 mg/kg, by intraperitoneal route. The onset and duration of sleep were observed and recorded for each animal and the mean calculated for each group.

Statistical Analysis

Results were expressed as the mean ± s.e.m. The mean values were statistically analyzed using Student's t-test and the two-way analysis of variance (ANOVA). The validity of differences in means was considered at 5 % significant level and values with $P<0.05$ were considered statistically significant.

RESULTS

Phytochemical Analysis of Methanolic Seed Extract of *Garcinia kola*

The results of the phytochemical analysis of the methanolic seed extract of *Garcinia kola* revealed the presence of some constituents as presented on table 1.

Effect of The Extract On Blood Pressure Of A Male Cat

The effect of the methanolic seed extract of *Garcinia kola* on blood pressure of a male cat as shown on figure 1 revealed that the extract produced a concentration-dependent decrease on blood pressure. This effect was similar with that produced by acetylcholine and similarly blocked by atropine in a competitive manner.

Effect on Chemical-Induced Sleep in Male Rats

The result showed that the extract caused significant increase of the onset of sleep time (P<0.05) for the duration of 60 days treatment especially at doses of 125 and 500 mg/kg compared to control (Table 1 & 2). This suggests that the duration of the treatment ($F_{1, 32}$ = 10.2157, P<0.05), rather than variation of the dose ($F_{3, 32}$ = 1.2923, P>0.05) had significant effect on the increase of the onset of sleep caused by the extract compared to control.

On the other hand, there was significant decrease of the duration of sleep (P<0.O5) for the duration of 60 days treatment with all the doses of the extract used as compared to control. This indicates that both the duration of treatment ($F_{1, 32}$ = 67.3343, P< 0.05) and the dose variations ($F_{3, 32}$ = 9.1015, P<0.05) are perhaps mutually exclusive on their role on the observed increase of the sleep duration.

However, it was found that there was no significant relationship between the doses used and the duration of treatment for both the onset of sleep ($F_{3, 32}$ = 2.6396, P>0.05) and the duration of sleep ($F_{3, 32}$ = 2.0517, P>0.05) .

Effect of Methanolic Seed Extract of *Garcinia kola* on Some Haematological Parameters in Male Rats

From the results, the methanolic seed extract of *Garcinia kola* induced significant increase on bleeding and clotting timesin all doses of the extract following 20 days treatment (P<0.05), but not so with 60 days treatment (P>0.05) compared to control (Table 3&4). On the other hand the extract cause significant but inconsistent decrease on platelet counts at all the doses used for both the duration of 20 and 60 days treatments (P<0.O5) compared to control (Table 5)

The duration of treatment had significant effect on bleeding time ($F_{1, 32}$ = 12.5491, P<0.05) and platelet counts ($F_{1, 32}$ = 16.5231, P<0.05) with little or no such effect on clotting time ($F_{1, 32}$ = 2.1373, P>0.05). The dose variation on the other hand had no significant effect on bleeding time ($F_{3, 32}$ = 0.3926, P>0.05) and clotting time ($F_{3, 32}$ = 0.0518, P>0.05) but significant on platelet counts ($F_{3, 32}$ =15.5621, P<0.05). Interestingly, it was found that there is no significant interaction between the doses and the duration of treatment with the extract on all the measured parameters (P>0.05). These observations therefore suggestthat both chronic and sub-chronic consumption of the seeds of *Garcinia kola* would likely cause decrease platelet counts and hence increase bleeding time.

Table 1. Phytochemical Composition of Methanolic Seed Extract of *Garcinia kola*

Constituent	Remark
Alkaloids	+
Saponins	++
Tannins	++
Anthraquinones	-
Glycosides	+
Steroids	+
Flavonoids	+
Carbohydrates	+

++ = Present in large quantity + = Present - = Absent

Table 2. Effect of Methanolic Seed Extract of *Garcinia kola* on Onset of Sleep in Male Rats. Values are (mean ± s.e.m) in Minutes

Treatment/Days	20	60
Control, N/S 1ml/100g	32.00 ± 3.85	31.90 ± 2.00
G. kola, 125 mg/kg	23.00 ± 0.65	36.70 ± 1.60*
G.kola, 250 mg/kg	26.20 ± 0.80	33.10 ± 2.70
G. kola, 500 mg/ kg	32.60 ± 2.70	35.50 ± 1.90*

n=5 * = P<0.05

Table 3. Effect of Methanolic Seed Extract of *Garcinia kola* on Sleep Duration in Male Rats. Values are (mean ± s.e.m) in Minutes

Treatment/Days	20	60
Control, N/S 1ml/100g	177.80 ± 11.50	158.70 ± 3.22
G. kola, 125 mg/kg	170.80 ± 5.40	143.20 ± 3.50*
G. kola, 250 mg/kg	161.60 ± 11.60	124.20 ± 8.10*
G. kola, 500 mg/kg	160.40 ± 10.90	137.10 ± 4.60*

n=5 * = P<0.05

Table 4. Effect of Methanolic Seed Extract of *Garcinia kola* on Bleeding Time of Male Rats. Values are (mean ± s.e.m) in Minutes

Treatment/Days	20	60
Control, N/S 1 ml/100 g	3.50 ± 0.41	3.60 ± 0.47
G. kola, 125 mg/kg	6.20 ± 0.57*	2.63 ± 0.33
G. kola, 250 mg/kg	5.50 ± 0.35*	2.38 ± 1.45
G. kola, 500mg/kg	4.80 ± o.80*	2.75 ± 1.37

n=5 *= P<0.05

Table 5. Effect of Methanolic Seed Extract of *Garcinia kola* on Clotting Time of Male Rats. Values are (mean ± s.e.m) in Minutes

Treatment/Days	20	60
Control, N/S 1 ml/100 g	2.63 ± 0.48	2.68 ± 0.40
G. kola, 125 mg/kg	5.00 ± 0.79*	1.48 ± 0.25
G. kola, 250 mg/kg	4.50 ± 0.50*	0.94 ± 0.24
G. kola, 500 mg/kg	3.50 ± 0.77*	1.63 ± 0.32

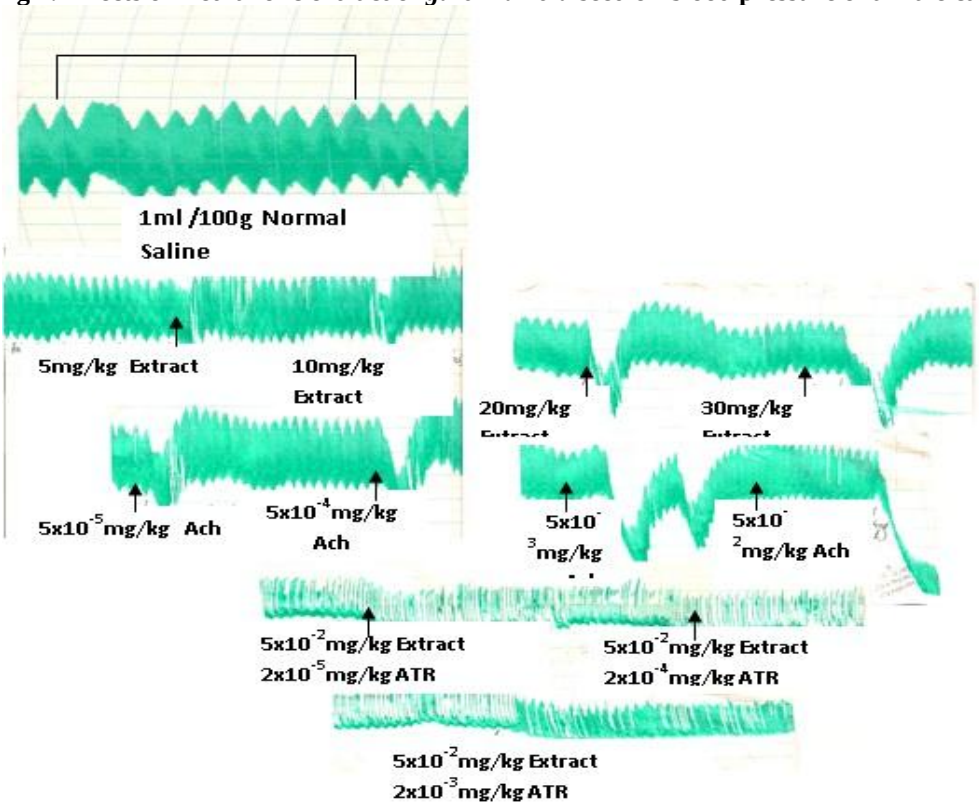
n=5 *=P<0.05

Table 6. Effect of Methanolic Seed Extract of *Garcinia kola* on Platelet Counts of Male Rats. Values are (mean ± s.e.m) x 10^3

Treatment/Days	20	60
Control, N/S 1ml/100g	60.50 ± 3.07	60.28 ± 4. 91
G. kola, 125 mg/kg	38.40 ± 2.99*	25.50 ± 0.14*
G. kola, 250 mg/kg	43.80 ± 3.76*	24.25 ± 3.21*
G. kola, 500 mg/kg	50.40 ± 3.17*	28.75 ± 3.40*

n=5 *=P<0.05

Fig 1. Effects of methanolic extract of *garcinia kola* seed on blood pressure of a male cat.



DISCUSSIONS AND CONCLUSION

The phytochemical analyses revealed that the methanolic seed extract of *Garcinia kola* contains alkaloids, glycosides, saponins, tanninis, flavonoids, steroids and carbohydrates. This result is similar to that of Monago and Akhidue (Monago CC, Akhidue V, 2002). Among these constituents, alkaloids, saponins and flavonoids have been reported as possessing some critical functions in male fertility (Udoh PB *et al.*, 2002; Salvati G *et al.*, 1996; Koumanov F *et al.*, 1982) a property that is traditionally associated with the consumption of *Garcinia kola* seeds. The presence of flavonoids, specifically kolavirone, a biflavonoid, have been isolated from *Garcinia kola* and some of its pharmacological activities reported (Akatanwa A and Essien AR, 1990). Flavonoids are known for their actions as antioxidants (Pietta PG, 2000). This could be protective to sperm cells and beneficial in the treatment of male infertility, although this observation remains controversial (Jovanovic SV et al., 1991; Terao J, 2009; Agarwal A, 2004; Agarwal A and Sekhon LH, 2010) .

The results of the effects of the extract on blood pressure suggest that the extract possesses a blood pressure reducing property being mediated through a cholinergic mechanism that is sensitive to the blockade effect of atropine. The blood pressure reducing effect of *Garcinia kola* in rats has earlier been reported from a a similar study by Naiho and Ugwu (Naiho A and Ugwu AC, 2009).

Although there are five sub-types of muscarinic receptors (M_1-M_5), only M_3 is said to generally cause vascular smooth muscles relaxation through generation of nitric oxide (NO) that can manifest as reduction in blood pressure (Walch L *et al.*. 2001). Indeed, dilatation of vascular beds by exogenously administered acetylcholine is said to be specifically due to M_3 receptor sub-type (Caulfield M and Birdsall NJM, 1998). Furthermore, the role of acetylcholine in the regulation of penile erection has been inferred from some limited neuro-pharmacological studies that involve muscarinic agonists and antagonists (Anderson KE, 1998; Maeda N *et al*., 1990). These findings suggest that cholinergic mechanisms may have regulatory roles in penile erection. It is therefore conceivable that the blockade effect by atropine could partly explain the effect of *Garcinia kola*on its penile erectile properties (Anderson KE, 2001; Bukar BB *et al*., 2016).

The effect on chemical induced sleep suggests that the extract possesses a stimulatory or anti-hypnotic effect that may have implications on its traditionally claimed aphrodisiac property. Sleep disturbances such as narcolepsy are usually associated with sexual dysfunction in men (Karacan I, 1986). Loss of libido secondary to sleepiness has been reported. While acute sleep deprivation produces hyper sexuality, chronic sleep loss on the other hand correlates with loss of sexual interest.Similarly, it has been reported that psychostimulant agents such as amphetamine that reduce sleep can reverse sexual dysfunctions in men, though when used chronically such same drugs inhibit sexual functions (Braide VP, 2001; Montejo AL *et al*., 2001). These effects may offer explanations to the chronic consumption of *Garcinia kola* seeds for recreational purposes and by long distance drivers in order to prevent sleep or increase their sexual urge. This finding therefore serves as experimental evidence that the extract possesses stimulatory effect that may enhance sexual function.

Results of the haematological analysis showed that the extract possesses anti-thrombotic effect. This is in agreement with similar findings earlier reported (Sigman M, 2000; Olajide O *et al*., 2011). This property may also be relevant to the traditional claim that *Garcinia kola* possesses aphrodisiac property. This could be useful in venous thrombosis and other similar disorders associated with hyperhomocysteinemia in male patients with oligozoospermia (Rees MM and Rodgers GM, 1993; Undas A *et al.,* 2005; Varghese S and Asif S, 2013). Equally, this could also be useful in infertile males with varicocele which sometimes is due to renal vein thrombosis (Kleinclauss F *et al*., 2001).

In conclusion, results of this study revealed and served as preliminary scientific evidence that the methanolic seed extract of *Garcinia kola*significantly induced some physiological effects that could be relevant to the traditional claims that it possesses aphrodisiac property and this may justify its chronic consumption by users.

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CONFLICT OF INTEREST

There is no conflict of interest on this study

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