

## Penile Erectile Properties And Elemental Analysis Of The Methanolic Seed Extract Of *Garcinia kola* (Heckel) In Some Experimental Animals

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**ABSTRACT:** The search for pharmacological agents that increase male virility which can address issues of erectile dysfunction remains novel because of their significance in healthy life span of the males. Plant-based pharmaceuticals are targets because of their availability and cost-effectiveness.

The effects of the methanolic seed extract of *Garcinia kola* were investigated at different oral doses (125, 250 & 500 mg/kg body weight) and duration (20 & 60 days) in male wistar rats. The extract was also tested *in vitro* on phenylephrine pre-contracted corpus cavernosum smooth muscles of the rabbit. Elemental analysis of the extract was also carried out.

Results of the findings showed that the extract at the dose of 250 mg/kg b. wt significantly increased the frequency of mount, intromission and ejaculation in both duration of administration compared to control (P<0.05). These effects were independently affected by dose and duration of treatments. Similarly, the mean number of litters per female rats paired with male rats treated with the extract was highest with that which received 250 mg/kg for both durations and lowest with 500 mg/kg. The extract caused a concentration-dependent relaxation of an isolated corpus cavernosum while the elemental analysis revealed the presence of Cu, Fe, Zn, Mg, K and Na. The results in general support the traditional claim of the usefulness of *Garcinia kola* seeds in the management of erectile dysfunction. However, we suggest the moderation of such use against the findings that usage for longer durations and in high doses will not confer any advantage but deleterious effects.

**Key Words:** *Erectile Dysfunction, Garcinia kola, Infertility, Reproductive Performance, Methanolic Extract.*

### I. INTRODUCTION

The inherent desire of mankind to tackle health problems dates back to prehistoric periods. One of such problems is that involving reproductive failure, most often manifesting as infertility in both males and females [1, 2]. Curiously, there appears to be aggressive pursuits for medicinal products that can increase sexual performance of the males. This will continue to drive research interests in the areas of male sexual enhancing drugs including those of plant-based. Problems of male reproductive health are often grossly underestimated but true situations have shown that male factors contribute in equal proportion to infertility outcomes as those of the females [3]. On the other hand, sexual violence sequel to abnormal urge sometimes due to uncontrolled use of chemical agents appears to be on the increase. The indiscriminate use of herbal preparations from traditional medicine practitioners (TMP) to solve problems of sexual dysfunction or even increase virility of the normal male folks could be contributing to such social vices.

Erectile dysfunction (ED), commonly defined 'as the persistent inability to achieve or maintain penile erection sufficient for satisfactory sexual performance for at least 3 months' [4, 5], is a common sexual disorder among males of active sexual age group. ED affects the quality of life of affected individuals and their spouses. Increased in life expectancy and prevalence of diseases such as diabetes and cardiovascular disorders have increased the need to pay more attention to issues of erectile disorders as these have been shown to be on the increase worldwide [6, 7]. Many other risk factors such as those of psychological imbalances, neurologic disorders, smoking, aging and some medications have been identified as causes of ED [8, 9]. Current pharmacological agents for non-surgical interventions in ED are unaffordable by many in developing countries [10-13]. Research interest is therefore being shifted to plant-based sources for cheaper alternative pharmacological interventions in the management of ED, given the fact that its etiology is multi-factorial and remains unclear [14, 15]). *Garcinia kola*, popularly known as 'bitter kola' is a plant that grows naturally in Nigeria and other West African sub-region [16]. It is commonly used and eaten during social occasions and in normal daily life due to its traditionally claimed aphrodisiac properties. The aim of this study was to investigate part of this claim and whether this varies with the duration of treatment, given the facts that evidence supporting such claims are most often and anecdotal.

## II. MATERIALS AND METHODS

### 2.1 Experimental Animals.

10-14 weeks old Wistar rats of both sexes weighing between 200 and 260 g were obtained from the Animal House Unit, University of Jos. They were housed separately according to sexes in stainless steel cages and handled under ethical conventional conditions and guidelines for the use and care of laboratory animals [17]. They were fed freely with standard solid nutritional pellets and water *ad libitum* until the commencement of experiment. Male rabbits were purchased from a reputable dealer in Jos and housed in the Animal House to acclimatized and were handled in same condition.

### 2.2 Preparation of the Extract

*Garcinia kola* seeds were purchased from reputable dealers from a market in Jos metropolis, North Central Nigeria. They were authenticated at the School of Forestry, Jos, where a voucher specimen GCL0153/04 is kept. The seeds were washed, de-husked and cut in small pieces. They were then dried under the shade in the laboratory. Thereafter, they were grounded to powder with mortar and pestle. The extraction was obtained using a modified method [18].

150g of the powdered seed was extracted with methanol in a soxhlet extractor. The extract was evaporated to dryness in a vacuum evaporator at 40°C until a constant yield of 57.9g (38.6%) following repeated weighing was obtained. The extract was reconstituted in distilled water for the purpose of the experiment.

### 2.3 Acute Toxicity Test (LD<sub>50</sub> Determination).

The acute toxicity test was carried out using the Lorke method [19] to determine the LD<sub>50</sub> of the crude extraction.

### 2.4 Sexual Behaviours and Reproductive Performance

20 male rats were divided into 4 groups of 5 rats each. Group 1 (control) was administered normal saline 1 ml/100g body weight while groups 2, 3 and 4 were administered the extract in daily oral doses of 125, 250 and 500 mg/kg body weight respectively for 20 days consecutively. Concentrations were prepared such that final volumes used were not more than 1ml each. After the last dose, the animals were allowed to stay for 24 hours and thereafter each male rat in each group was matched with 2 untreated virgin female rats in a stainless steel cage in accordance with a natural mating method [20]. This was done with a slight modification by allowing the male rats to stay longer while pregnant females were allowed to litter. While together, the frequency of mount, intromission and ejaculation were observed and recorded for the first and last 30 minutes within 48 hours of matching. The males were then allowed to continue staying with the females for the next 48 hours after which they were separated. The females were monitored daily for development of pregnancy and eventual littering. The first female to litter was removed with its litters into a separate cage. At the end the number of litters for each female was recorded and the mean for both calculated for each group.

The same procedure was repeated in a second set of 20 male rats divided into 4 groups of 5 rats each but treated with normal saline (control) and the extract with same doses but for duration of 60 days consecutively.

### 2.5 Effects of the Extract on Corpus Cavernosum Smooth Muscles

Three matured male rabbits weighing between 3.5-4.8 kg were purchased from a reputable dealer in Jos metropolis. In accordance with some described methods [21, 22], the animals were anaesthetized with sodium pentobarbital (40 mg/kg) and exsanguinated through the carotid artery. The penises were removed and the corpus cavernosa carefully dissected. A section of the corpus cavernosum measuring 3 mm × 3 mm × 8 mm was made and mounted in a 50 ml organ chamber containing tyrode solution (PH 7.2) maintained at 37°C and aerated with gas containing 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The tissue was allowed to equilibrate for 90 minutes. During this interval it was washed with Tyrode solution at intervals of 15 minutes. The resting tension was set at 1g. Measured contractile responses to Phenylephrine, PHE (4 × 10<sup>-6</sup> g/ml) were obtained followed by increasing concentrations of the extract from 0.4-2.0 mg/ml and the corresponding decrease in contraction recorded on a tracing paper connected and driven by a kymograph. Relaxation was calculated using the expression:

$$\% \text{ Response} = \frac{\text{Sub-maximal contraction with PHE alone} - \text{Contraction in the presence of extract X}}{\text{Sub-maximal contraction with PHE alone}} \times 100$$

### 2.6 Elemental Analysis of the Extract

The elemental analysis was done by the atomic absorption spectroscopy (AAS) using an atomic absorption spectrometer [23].

5 g of the extract was dried in an oven and sample of it weighed. The sample were then placed in a hot furnace and ashed at 600°C for 3 hours. The furnace was cooled to 120°C and the sample were then removed and placed in a dessicator for 1 hour to cool further. They were weighed repeatedly until a constant weight was obtained.

0.5g of the ashed sample were weighed and transferred into digestive tubes. 5ml each of distilled water, concentrated trioxonitrate (IV) acid and perchloric acid were added and the content mixed properly. The tubes were placed into digestive blocks inside a fumed cupboard and the temperature set at 150°C and digested for 90 minutes. The temperature was then increased to 230°C and digested for another 30 minutes and thereafter the temperature was reduced to 150°C. 1ml of hydrochloric acid was then added to the tube within 5 minutes and the digest was not allowed to cool to room temperature to prevent formation of insoluble precipitates such as potassium percholates. More water was added to the tubes to give a convenient volume and the content was mixed and filtered and the resulting solution was used for the elemental analysis at appropriate wave lengths, temperature and current for each element.

### **2.7 Data Analysis**

Data were analyzed statistically by Student's t-test and two-way analysis of variance at  $P = 0.05$ , significance level.

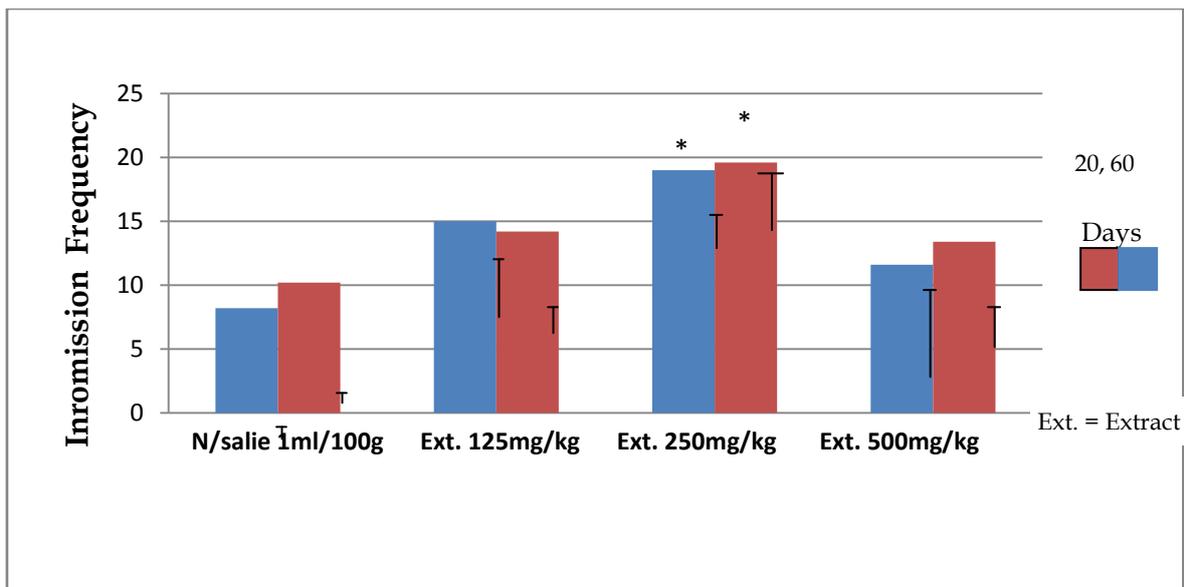
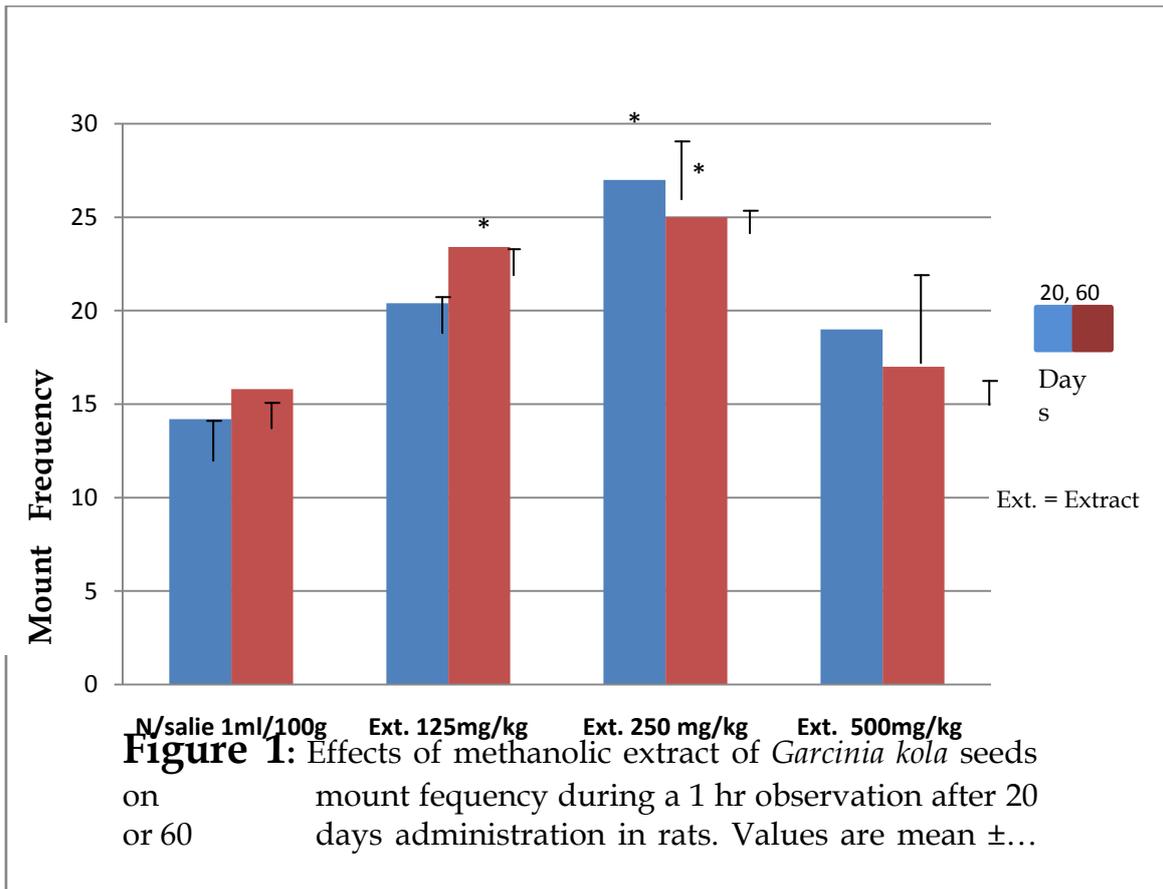
## **III. RESULTS**

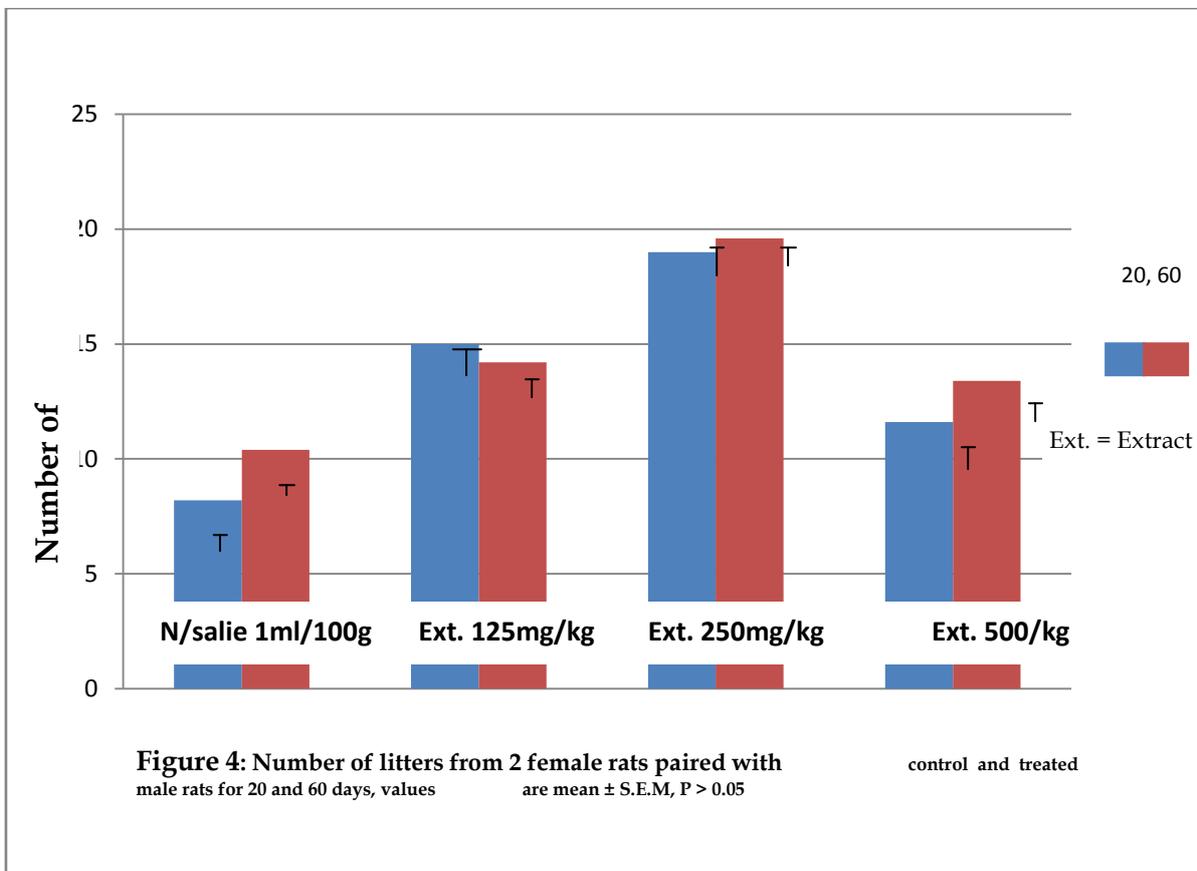
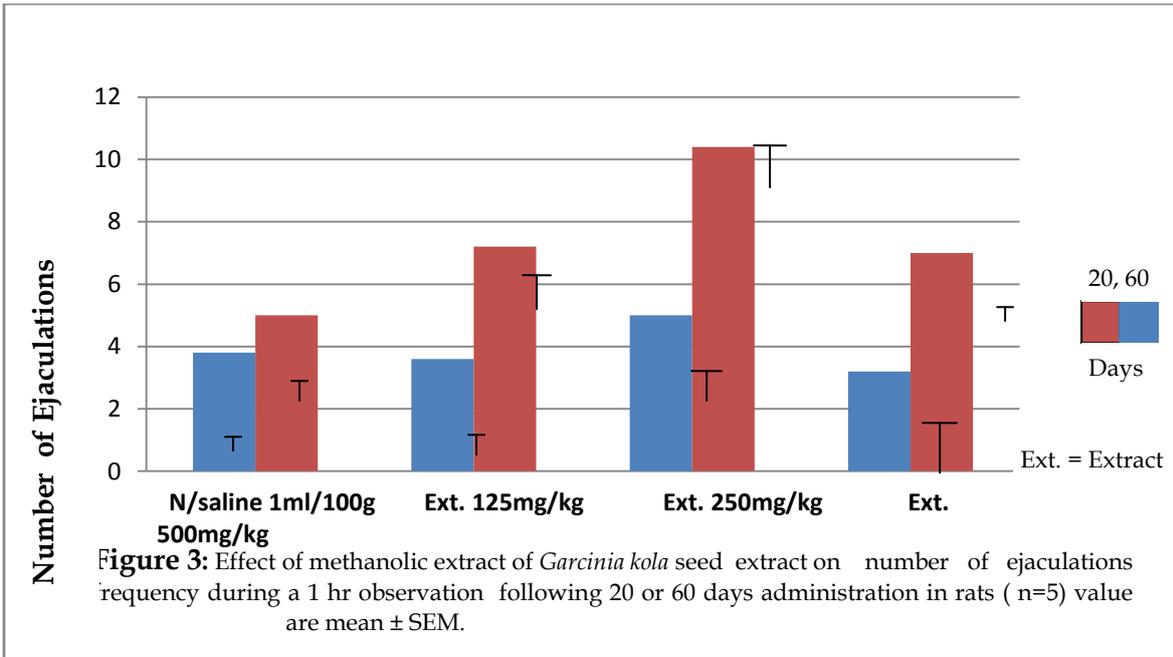
### **3.1 Acute Toxicity Test**

Calculation of the acute toxicity test revealed that the  $LD_{50}$  is about  $3,215 \pm 52.68$ mg/kg.

### **3.2 Effects of the Extract on Mount, Intromission, Ejaculation, and Reproductive Performance.**

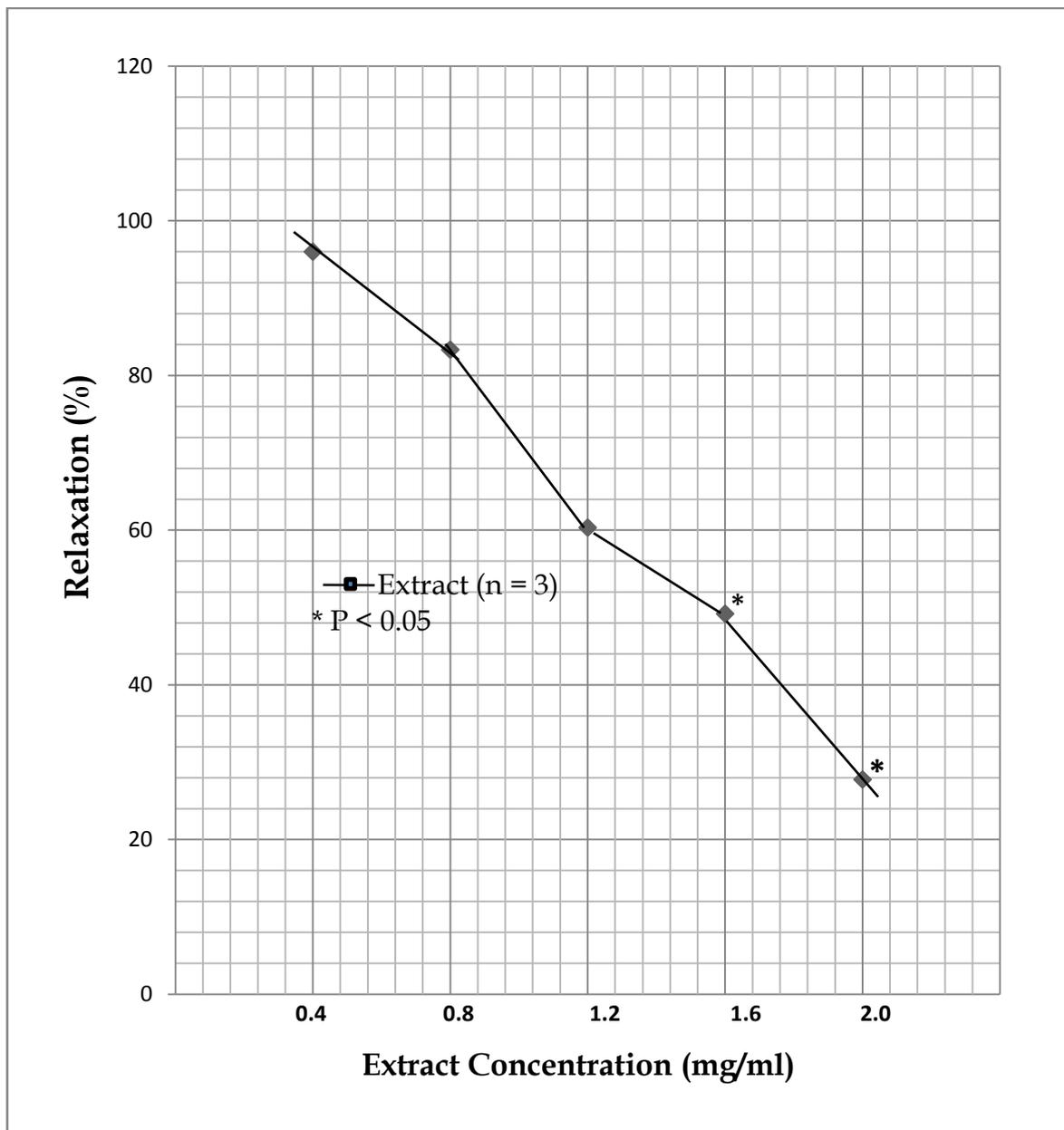
The results showed that there was no significant difference in mount frequency ( $F_{1, 32}=0.0615$ ,  $P>0.05$ ) and intromission frequency ( $F_{1, 32}=0.4942$ ,  $P>0.05$ ) for both the 20 and 60 days administrations of the extract, suggesting that duration of treatment has little or no effect on these parameters while there was significant difference in the number of ejaculations for the same periods ( $F_{1, 32}=14.8665$ ,  $P<0.05$ ), suggesting dependence of this parameter on duration of treatment. On the other hand, there was significant difference in mount frequency ( $F_{3, 32}=62.3666$ ,  $P<0.05$ ) and intromission frequency ( $F_{3, 32}=9.6321$ ,  $P<0.05$ ) at the doses of extract used suggesting that dose variations rather than duration of treatment affected these parameters, but there was no significant difference in the number of ejaculations ( $F_{3, 32}=2.4792$ ,  $P>0.05$ ), suggesting that the durations rather than the doses affected it. The effect of the interactions between the doses and durations of treatment was significant on mount frequency ( $F_{3, 32}=7.7197$ ,  $P<0.05$ ) while on the other hand the effect of interaction between the doses and durations of treatment was not significant on intromission ( $F_{3, 32}=0.2496$ ,  $P>0.05$ ) and ejaculation ( $F_{3, 32}=0.9101$ ,  $P>0.05$ ), suggesting that there was an effect of interactions between the doses and durations of treatment on intromission and ejaculation. Generally, the extract significantly ( $P<0.05$ ) increased mount and intromission frequencies in rats treated with 250 mg/kg body weight for both 20 and 60 days compared with control (Figure 1 & 2) but not so ( $P>0.05$ ) on ejaculation (Figure 3). The number of litters per female rats paired with treated male rats was highest ( $P>0.05$ ) with the group treated with 250 mg/kg for both 20 and 60 days (Figure 4).





### 3.3 Effects of the Extract on Phenylephrine Pre-contracted Corpus Cavernosum Smooth Muscles.

The extract caused concentration-dependent relaxations of the corpus cavernosum smooth muscles in corpus cavernosum pre-contracted by Phenylephrine (0.4mg/ml, 4.00±0.31%; 0.8mg/ml, 16.67±0.58%; 1.2mg/ml, 39.67±0.93%; 1.6mg/ml, 50.81±0.80%; 2.0mg, 27.76±0.57%). The relaxation was significant (P<0.05) at concentrations between 1.6 and 2.0 mg/ml of the extract compared with the maximum contraction induced by phenylephrine. (Figure 5).



**Figure 5:** Relaxation Effect (%) of the Extract on Phenylephrine Pre-Contracted Corpus Cavernosum Muscles. Values are mean ± SEM (% Relaxation)

### 3.4 Elemental Analysis of the Extract.

Results of the elemental analysis revealed the presence of trace elements that include; copper (Cu), iron (Fe), zinc (Zn), magnesium (Mg), potassium (K), and sodium (Na) (Table 1).

**Table 1: Elemental Analysis of Methanolic Extract of *Garcinia kola* Seed**

Element	Concentration (mg/L)
Se	Nil
Zn	4.5801
Cu	0.6792
Fe	3.3585
Ca	3.0799
Mg	23.2992
K	16.5245
Na	96.8325

## IV. DISCUSSIONS AND CONCLUSION

Frequencies of mount, intromission and ejaculation are important parameters in libido assessment in males, but they are also believed to have psychogenic components. Male sexual behaviours consist of complex processes involving both central and peripheral mechanisms. Given the observations that increased sexual performance usually correlates with fertility [24, 25], and based on data of this study, the extract showed positive modulator effects on motivation to engage in sexual functions at a dose of 250 mg/kg. This observation corroborates the findings of a similar study which showed that an ethanolic extract of *Garcinia kola* at 200 and 400 mg/kg for 28 days enhanced libido and potency in male rats with increased testosterone secretions [26]

There are different mechanisms involved in sexual arousal which could be centrally or peripherally mediated. However, drugs that interfere with the autonomic system most often have negative effects on erection [27-29]. Information on effects of *Garcinia kola* on corpus cavernosum is scarce, but the present study showed that the extract contains active compounds that induced relaxation of the corpus cavernosum in a concentration-dependent manner. It can be recalled that multiple mechanisms are usually involved in the relaxation of the corpus cavernosum as part of the penile erectile process. Of equal note also is the fact that crude plant extracts are known to contain multiple active principles that may act either synergistically or antagonistically. Basically, pharmacological agents that induce relaxation of the corpus cavernosum are believed to cause penile erection through vasodilation that increases blood flow and pressure of the cavernosal arteries. Such agents have formed the basis for pharmacological interventions in non-surgical treatment of erectile dysfunctions [30]. The cavernosal smooth muscles are rich with adrenergic innervations that are believed to maintain the integrity of the penis [31, 32], though  $\alpha_{1a}$  seems predominant. Indeed, studies have shown that  $\alpha_1$ -adrenoceptor agonists that contract the cavernosal smooth muscles do so through the  $\alpha_{1L}$  isoform,  $\alpha_{1L}$ . This is because such agonists are said to display low affinity for  $\alpha_1$  antagonists such as prazosin and tamsulosin [33-35]. Other notable mechanisms also involved in cavernosal relaxation include blockade of Phosphodiesterase type 5 (PDE-5) enzyme,  $\alpha_2$ -adrenergic antagonists, stimulation of guanylylcyclase that liberates endothelin-derived relaxing factor (EDRF) or nitric oxide (NO) and potassium channel opening.

Trace elements have been shown to be important components of semen and may contribute to virility [36]. This study revealed the presence of some of these elements in our extract especially zinc which is a critical element in spermatogenesis [37-41]. Zinc possesses antioxidant property which could be protective on sperm cells from scavenging elements [42, 43]. It also has aphrodisiac effects that boost testosterone in zinc-deficient individuals [44-46].

From this preliminary investigation, the results showed promising penile erectile properties of the extract, though the specific mechanism remains to be determined. However, the aqueous extract had not shown such effect in an earlier study [47]. Nonetheless, high intake and long term use of *Garcinia kola* should be discouraged to avoid deleterious effects evident from a part of the results of this study.

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#### **Conflict of Interest**

There is no conflict of interest on this study.

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