INTRODUCTION

Male sexual dysfunction, which mostly includes erectile dysfunction (ED) and premature ejaculation, is the most common problem that contributes to infertility, distress, relationship problems and low quality of life [1]. While sexual dysfunction rarely threatens physical health, it can bring a heavy psychological toll; depression, anxiety, and debilitating feelings of inadequacy. ED accounts for 45% of male sexual dysfunction in Nigeria [2]. ED is experienced some of the time by most men who have reached 45 years of age, and it is projected to affect 322 million men worldwide by 2025 [3]. ED is usually underestimated in many developing countries including Nigeria [4,5] probably because it is not a life-threatening condition and due to the associated stigma. Herbal preparations have enjoyed the patronage of most people in rural and urban areas of Nigeria [6]. In Africa, several plants have been used for many years to improve sexual stimulation and performance. Ang et al. [7] reported the use of Aristolochia indica, Crocus sativus, Alpinia galanga, and Allium cepa to improve sexual activities with varying degrees of success. Carpolobia lutea, (polygalaceae) is a small plant that often grows to 15 ft in height. Its juicy fruits are consumed by people of Southern Nigeria [8]. The plant is well distributed in West and also Central Africa [9]. It is popularly known in Southwest and South Eastern Nigeria particularly among the Eket tribe as a potent aphrodisiac. Carpolobia lutea is widely used by traditional herbal practitioners to treat male erectile disorders and facilitate delivery [9]. The root decoction is reportedly used as malarial remedy [10,11], anti-inflammatory/anti-arthritis [12,13], anthelmintic and anti-sterility agent [14,9].

Though ethno-botanical survey has revealed that the root decoction of C. lutea is used to enhance sexual activity [15],

ABSTRACT

Aims: In spite of the folkloric use of the root of Carpolobia lutea as a sexual stimulant in man, there has been limited scientific proof of its efficacy. This study compares the efficacy of methanol extract of C. lutea root (MECLR) and sildenafil on the sexual activity of male rabbits. Methods: 20 adult male rabbits were grouped into four of five rabbits each. Groups 1-4 were treated orally for 28 days with 2 ml/kg 1% Tween-20 (vehicle), 40 mg/kg MECLR, 80 mg/kg MECLR, and 0.5 mg/kg sildenafil citrate (SC), respectively. Sexual activities of males from each group were assessed by cohabiting them with sexually receptive female at estrus on days 0, 1, 3, and 5 using digital camera mounted on mating arena. Serum testosterone and nitric oxide concentration of the corpora cavernosa homogenates were also determined. Results: MECLR caused a dose-dependent significant increase in mount frequency, intromission frequency and ejaculatory latency (EL) while it reduced mount latency, intromission latency and post EL (similar to SC) when compared with the control. MECLR also caused significant increase in nitric oxide concentration in corpora cavernosa but no change in serum testosterone concentration. Conclusions: Results suggest that MECLR enhances male sexual activity possibly by augmenting nitric oxide concentration. This study thus provides a novel scientific rationale for the use of C. lutea in the management of penile erectile dysfunction and impaired libido.

KEY WORDS: Carpolobia lutea, aphrodisiac, efficacy, male, rabbits

Comparative evaluation of the aphrodisiac efficacy of sildenafil and Carpolobia lutea root extract in male rabbits

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very few experimental studies have been performed to ascertain these claims of efficacy. Orthodox treatments that could serve as options for ED are also expensive, not readily available and present unpleasant side-effects. As compared with orthodox drugs, *C. lutea* is cheap, readily available, and greatly consumed by local population. This study therefore investigated and compared the aphrodisiac efficacy of methanol extract of *C. lutea* root (MECLR) with sildenafil citrate (SC) in adult male rabbits.

**METHODS**

**Plant Material**

*C. lutea* plant was collected from Alade village in Akinyele Local government, Oyo State, Nigeria, in December 2013. The plant was identified, authenticated and assigned voucher number FHI 109755 at the Forestry Research Institute of Nigeria, Ibadan, Nigeria.

**Preparation of Plant Extract**

*C. lutea* root was washed, cut into pieces and oven dried at 40°C to a relatively constant dry weight. 4 kg of the oven dried, pulverized sample was soaked in 15 l of absolute methanol in a glass bowl at room temperature for 72 h. It was then filtered with Wattmann filter paper. The filtrate was concentrated in a rotary evaporator at 40°C [16] to yield the methanol extract (brown oily substance) which was stored in a refrigerator at −4°C.

**Determination of LD$_{50}$**

Nine male Wistar rats were equally grouped into 3.50 mg/kg of MECLR was administered to the first group and subsequently observed for signs of toxicity or mortality over a 24-h period. The dose was then repeated on the absence of mortality. Same procedures were then followed for the two remaining groups at fixed dose of 300 and 2000 mg/kg body weight (BW) respectively. LD$_{50}$ was subsequently determined according to OECD 423 guideline [17].

**Experimental Animals**

20 adult male and female rabbits (6 months) weighing 1.5-2.0 kg sourced from the animal house of the Department of Veterinary Physiology University of Ibadan were used. Animal was allowed to acclimatize for 3 weeks before commencement of study. They were fed standard pelletized rodent feed and water ad libitum. Female rabbits used for the evaluation of sexual behavior were from the same strain with male and were prepared according to the method of Anders [18]. All the experimental procedures were done following guidelines of the University of Ibadan Animal Ethics Committee.

**Experimental Protocol**

20 adult male rabbits (1.5-2.0 kg) were divided into four equal groups and treated daily (orally) for 28 days with 2 ml/kg 1% Tween-20 (control), 40 mg/kg MECLR, 80 mg/kg MECLR and 0.5 mg/kg SC. The male rabbits were cohabited with sexually receptive female at estrus, and sexual activities of the male were observed and recorded on days 0, 1, 3 and 5 using a digital camera mounted on the mating arena [19]. Animals were treated 1 h prior to mating on days 1, 3, and 5 [20].

**Mating Behavior Test**

Mating behavioral tests were carried out according to the methods of Anders [18] and as modified by Gauthaman et al., [21] and Guohua et al., [22]. Healthy males showing brisk sexual activity were paired with receptive females in estrus at ratio 1:1 in the mating arena. Estrus was artificially induced by sequential administration of estradiol benzoate (10 μg/100 g) orally and progesterone (0.5 mg/100 g BW) subcutaneously, 48 h and 4 h respectively prior to pairing [23]. Estrus was confirmed as described by the methods of Marcondes et al., [24]. Pairing was also conducted 16.00 h each day in the same arena with same light intensity for 50 min. Recorded event frequencies and phases were later transcribed from the mounted digital camera. Mount frequency, mount latency, intromission frequency (IF), intromission latency (IL), ejaculatory latency (EL), and post EL (PEL) as indicators of male sexual behavior were analyzed following the adaptations of Gauthaman et al., [21] and Guohua et al., [22].

**Blood Collection and Hormone Assay**

Blood samples were collected on day 0 (basal), 5 and 28 from each animal through retro-orbital sinus with a 70 μl heparinized capillary tube into a plain serum bottle. Samples were centrifuged at 3000 revolutions per minute for 15 min to obtain serum. Serum testosterone concentration was thereafter determined using a double antibody enzyme-linked immunosorbent assay kit (Rapidlab Testosterone kit, Italy).

**Determination of Corpus Cavernosum Nitric Oxide**

Male rabbits were sacrificed by cervical dislocation on day 28. Corpus cavernosum were then excised, weighed, and immediately homogenized in phosphate buffer (pH 7.4) and centrifuged. Nitric oxide concentration in each tissue homogenate was determined using Griess reaction method [25,26]. Samples were diluted fourfold with distilled water and deproteinized by adding 1/20th volume of zinc sulfate (300 g/L) to give a final concentration of 15 g/L. After centrifugation at 1000 g for 15 min (room temperature), 100 μL of supernatant was applied to a microtiter plate well, followed by 100 μL of Griess reagent (1 g/L sulfanilamide, 25 g/L phosphoric acid, and 0.1 g/L N-1-naphthylethylenediamine). After 10 min of color development at room temperature, the absorbances were measured on a microplate reader (Titertek Multiskan MCC/340; Flow Lab, McLean, VA) at a wavelength of 540 nm. Each sample was assayed in duplicate wells. Background values were obtained by treating samples as described but using 25 g/L phosphoric acid instead of complete Griess reagent.
Statistical Analysis

Data were analyzed using prism Graph pad version 5.0 and presented as mean ± standard error of the mean. Comparison between means was done using analysis of variance. Values were considered statistically significant at \( P \leq 0.05 \).

RESULTS

Effect of MECLR on Toxicity/Lethality and LD\( \text{50} \)

No mortality was recorded when the starting dose of 50 and 300 mg/kg was repeated as well as the first administration of 2000 mg/kg. Only one animal died after repeating the dosage of 2000 mg/kg [Table 1]. 2500 mg/kg BW was obtained as the LD\( \text{50} \) of MECLR in this study.

Effect of MECLR on Mount and IL

As shown in [Figures 1 and 2], there was significant, dose-dependent decrease in mount and IL in MECLR treated rabbits when compared to control. In addition, there was significant decrease when MECLR treated rabbits were compared to SC treated group.

Effect of MECLR on Mount and IF

There were significant, dose-dependent increases in mount and IF when MECLR treated rabbits were compared to control and SC groups [Figures 3 and 4].

Effect of MECLR on EL and PEL Period

As shown in [Figures 5 and 6], there were significant dose-dependent decreases in ejaculatory and PEL periods in MECLR treated groups as compared to control and SC treated groups.

Effect of MECLR on Serum Testosterone and Corpora Cavernosa Nitric Oxide Concentration

As shown in [Table 2] there were no significant differences in serum testosterone concentrations when MECLR treated groups were compared to control, and SC treated group. However, nitric oxide concentration increased significantly in MECLR (80 mg/kg), and SC treated groups as compared to control [Figure 7].

DISCUSSION

The background for classifying any medicinal plant as having the potential to stimulate and enhance sexual vigor was enunciated by Ratnasooriya and Dharmarsiri [27]. They opined that medicinal plant with a tendency to stimulate and enhance sexual behavior should produce a statistically significant increase in mount and IF and also reduce significantly mount and IL; since these indices are indicators of sexual arousability, motivation, and vigor. Results from this study indicated that MECLR at 40 and 80 mg/kg BW enhanced sexual activity comparable to SC.

Table 1: Effects of MECLR on toxicity/lethality and LD\( \text{50} \) in male rabbits

| Number of animals | Dosage (mg/kg BW) | Number of lethality |
|-------------------|-------------------|---------------------|
| 3                 | 50                | 0                   |
| 3                 | 300               | 0                   |
| 3                 | 2000              | 1                   |

MECLR: Methanol extract of Carpobobia lutea root, BW: Body weight

Table 2: Effect of MECLR on serum testosterone concentration (ng/mol)

| Group             | Day (0) (basal) (ng/mol) | Day 5 (ng/mol) | Day 28 (ng/mol) |
|-------------------|--------------------------|---------------|-----------------|
| Control           | 7.18±3.39                | 15.65±5.97    | 5.05±3.98       |
| 40 mg/kg BW       | 8.78±3.23                | 18.80±0.79    | 7.70±0.75       |
| 80 mg/kg BW       | 8.25±4.05                | 10.53±0.62    | 9.56±0.54       |
| 0.5 mg/kg BW SC   | 5.05±4.21                | 17.80±0.83    | 0.90±0.64       |

Data presented as mean±standard error of mean., \( n = 5 \), MECLR: Methanol extract of Carpobobia lutea root, BW: Body weight, SC: Sildenafil citrate

Figure 1: Effect of methanol extract of Carpobobia lutea root on mount latency. *\( P \leq 0.05 \) when compared with control group; #\( P \leq 0.05 \) when compared with sildenafil citrate group; $\leq 0.05$ when compared with day 0 (Basal)

Figure 2: Effect of methanol extract of Carpobobia lutea root intromission latency. *\( P \leq 0.05 \) when compared with control group, #\( P \leq 0.05 \) when compared with sildenafil citrate group, $\leq 0.05$ when compared with day 0 (Basal) \( n = 5 \)
This is evidenced by the statistically significant reduction in mount/intromission, and PEL; and the statistically significant increase in mount and IF of both MECLR and sildenafil-treated groups as compared with control. Yakubu and Jimoh [28] have earlier reported on the capability of aqueous C. lutea root extract in enhancing mount/IF and EL after paroxetine-induced sexual impairment in male rats. The increase in sexual activity observed in the animals treated with MECLR could be due to phytochemicals present in the plant. C. lutea belongs to the polygalacaea families who are rich in saponins and flavonoids [28,29]. Medicinal plants such as Tribulus terrestris [21], Anemopaegma arvense [30], Arrabidaea chica [31] and Turnera diffusa [32] which also contain phytochemicals like saponins and flavonoids have been documented to enhance sexual activities. Flavonoids are widely distributed in flowering plants and therapeutic potential ascribed to them include antioxidant and hemodynamic activities. The antioxidant potential helps provide protection against cellular damage to erectile tissues that can cause ED as a result of stress [33] or Leydig cells damage leading to decrease in testosterone and loss of libido [34]. Flavonoids ability to enhance hemodynamic flow benefits the activity of nitric oxide synthase that stimulates the production of nitric oxide. Nitric oxide then activates guanylylcyclase to produce cyclic GMP (cGMP) a potent vasodilator. Saponins are regarded as adaptogens or anti-stress agents. Although, their mechanism of action is still unclear, they have been reported to help improve non-specific resistance of the body after exposure to various stressing factors [35]. Saponins like flavonoids can help to enhance penile erection by preventing the damaging effect of stress on erectile tissues. In addition some saponins have also been reported to inhibit phosphodiesterase 5 [22] thereby potentiating activity of the potent vasodilator cGMP.
Testosterone have always been assumed to play a major role in male erectile function as evidenced by the observation that men with marked decrease in testosterone concentration have a significant reduction in the frequency, amplitude and rigidity of erection [36,37]. However, the level of testosterone required to cause ED is debatable. Testosterone is a steroid hormone produced from cholesterol. In males, it is primarily synthesized in the Leydig cells of the testes under the influence of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH regulates the number of Leydig cells in the testes while LH controls how much testosterone the Leydig cells produce by regulating the expression of 17-β-hydroxysteroid dehydrogenase (an enzyme that mediate the rate limiting-step of testosterone synthesis). Result from this study shows that there is no significant difference in serum testosterone concentration of male rabbits treated with MECLR despite its aphrodisiac potential. Similar observation was reported by Gonzales et al., [38] when the root extract of Lepidium meyenii produces no significant effect on serum reproductive hormones (LH and Testosterone) but with aphrodisiac and fertility-enhancing property. This observation may imply that the increase sexual activity in MECLR treated rabbits may not be mediated through the hormonal (testosterone) pathway.

There was significant increase in cavernosa nitric oxide (NO) concentration in 80 mg/kg MECLR treated rabbits comparable to sildenafil-treated groups. Nitric oxide (NO) synthesis is enhanced by NO synthase [39]. The ability of MECLR to enhance cavernosa concentration of NO may help explain its aphrodisiac potential. Panax ginseng used as a sexual stimulant has been reported to enhance nitric oxide synthesis in the corpora cavernosa [32]. Production of Nitric oxide is known to activate guanayl cyclase to produce cGMP a potent vasodilator that acts by lowering intracellular calcium.

Comparatively, sildenafil exerted more potent action than MECLR in this study. This is quite understandable as MECLR used is still in its crude and unpurified form. It will be useful in future to compare the intracellular mechanisms of action of MECLR against sildenafil. It will also be appropriate to investigate the effect of MECLR on cavernosa NO concentration on injection of inhibitors of NO synthase. Furthermore, it will be interesting to know if MECLR like sildenafil can inhibit the breakdown of cGMP by serving as inhibitors of phosphodiesterase V (the prominent phosphodiesterase found in male cavernosa) without accompanying side effects associated with sildenafil.

CONCLUSION

The result from this study shows that MECLR enhances sexual activity in male rabbits by augmenting NO concentration in the corpus cavernosum. This provides a novel scientific basis for the folkloric use of this plant in stimulating and enhancing sexual activity.

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