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Effect of Aqueous Extract of *Carpolobia Lutea* Stem on Some Reproductive Parameters in Adult Male Wistar Rats

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Abstract

Background: The use of medicinal plants as fertility enhancer in human is now on the increase because of the shifting of attention from synthetic drug to natural plants. *Carpolobia lutea* (CL) is a popular aphrodisiac herbal medicine, known for curing male sterility; increasing libido; induction of penile erection; enhancement of aphrodisiac prowess; enhancement of virility and male fertility; and augmentation of male sexual functions. The present study was therefore designed to investigate the fertility enhancement ability and possible side effect(s) of CL in male rats and also to provide insight and added to the existing knowledge available in the society on the usage of CL.

Methods: A total of 20 Wistar rats with average weight of 162g were randomly grouped into 4 groups (A, B, C and D) of 5 rats per group, with group A served as the control and was administered normal saline. The remaining 3 groups (B-D) were orally administered 47mg/kgbw, 94mg/kgbw and 141mg/kgbw of *Carpolobia lutea* (CL) respectively for 60 days before sacrifice by cervical dislocation method.

Results: The results obtained showed that the blood testosterone of the treated rats reduced significantly. The morphometric result was good with significant increase in cross sectional area and luminal diameters in rats exposed to 141mg/kgbw and germinal epithelial diameter in rats exposed to 47mg/kgbw and 141mg/kgbw. Semen parameters showed that the sperm count was significantly increased at the high doses of 94mg/kgbw and 141mg/kgbw. The percentage of motile sperm also increased significantly while the morphological grading showed no changes at all. The experimental rats testes showed no visible lesions or the lesion was very mild (20%), seminiferous tubules were normal and concentric with intact basal membrane and lumen containing matured sperm cells together with the interstitial spaces which are well delineated and defined.

Conclusion: It was concluded that the consumption of CL stem in any form can enhance the normal anatomy and physiology of testes at moderate dose and may account in part for the rationale behind its use in the management of sexual dysfunction.

Keywords: *Carpolobia lutea*, Rats, Testes, Spermatozoa

1.0 INTRODUCTION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times [1]. Plants synthesize hundreds of chemical compounds for various functions including defense against insects, fungi, diseases, and herbivorous mammals [17]. Numerous phytochemicals with potential or established biological activities have been identified [2]. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain [1].

Medicinal plants are of great importance to the health of individuals and societies. In Africa, particularly West Africa, new drugs are often beyond the reach of the poor. Hence, up to 80% of the population uses medicinal plants as remedy against infections and diseases, therefore countries such as Ghana, Mali, Nigeria, and Zambia practices the traditions of collecting, processing and applying plants and plants based medications as they have been handed down from age to ages [2]. Traditional medicines, with their medicinal plants as their most important components sold in market places as prescribe by traditional healer [3].

Carpolobia lutea (CL) is a popular aphrodisiac herbal medicine, and there was a report that describe the plant with the following activities: curing male sterility; increasing libido; induction of penile erection; enhancement of aphrodisiac prowess; enhancement of virility and male fertility; and augmentation of male sexual functions [4]. Common names which the plant is known for include cattle stick (English), Abekpok Ibuhu (Eket), Ikpafum, Ndiyan, Nyayanga (Ibibio), Agba or Angalagala (Igbo) and Egbo oshunshun (Yoruba) [6].

Stem and root of *Carpolobia lutea* (CL) are used as chewing stick and their decoction have been associated with aphrodisiac potentials [6, 7];. Its shrubby and smallish stems give it an ornamental use as sweeping material or broom (indiyan) in rural areas among the Ibibio tribes of Akwa Ibom State, Nigeria [6]. Concoction of the root is used in locally-made alcohol as an aphrodisiac. It also is use in the treatment of genitourinary infections, gingivitis, waist pains and internal heat [8]. The hot water extract of the root was reported to have antimicrobial, anti-inflammatory and anti-arthritic properties due to the presence of Saponins and flavonoids [9,12]. It has been found that *Carpolobia lutea* has gastro-protective effects [10], antinociceptive effects [11]; antidiarrhoeal and anti-ulcerogenic properties [13]; antimalarial activity and moderate toxicity [14]. The root is also used to facilitate childbirth, treat sterility, headache, worm infestation and as aphrodisiac and stimulant properties [5,12]. As compared with orthodox drugs, *Carpolobia lutea* is cheap, readily available, and greatly consumed by local population. It is an accepted and commonly utilized herbal booster of libido [15,16].

In an acute toxicity studies, the median LD_{50} was calculated to be 3340 mg/kg, 3240.4 and 1414.2mg/kg for the ethanol fraction, crude ethyl acetate and ethyl acetate fraction, respectively [15,20]. Despite these streams of fertility findings, there is need to carry out scientific investigation on the male sexual parameters of the plant at various doses of 47, 94 and 141 mg/kg (body weight) which was very much lower than the lethal dose to corroborate the ethnomedical use of the plant as an aphrosidiac and against other male reproductive dysfuntions. Therefore, this study seeks to evaluate the androgenic and testicular effects of crude extract of *C. lutea* stem on male rats.

2.0 METHODOLOGY

2.1 Plant Material

Carpolobia lutea was obtained from Jagun Herbs market, Ogbomoso, Oyo State, Nigeria. The leaf was identified and authenticated in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology Ogbomoso, Nigeria. The plant material was pulverized to form a powder. 100g *Carpolobia lutea* powder was soaked in 400ml of distilled water for 72hours in a standard room temperature. The extract was stored in airtight container until use.

2.2 Animals

Animals used were twenty (20) adult male Wistar rats with an average weight of 162g. The rats were housed in a well-ventilated covered cages maintained under standard dark-light cycle at normal room temperature. Feeds and water were given to the rats ad libitum. All rules applying to animal safety and care were observed thoroughly.

2.3 Grouping

Rats were weighed and acclimatized for two (2) weeks and randomly grouped into 4 groups (A, B, C and D) of 5 rats per group with group A served as the control administered normal saline. The remaining 3 groups were orally administered 47mg/kg, 94mg/kg and 141mg/kgbw respectively for 60 days.

2.4 Collection of Organ, Histological Processing and Histomorphometric Analysis

At the end of the administration, the rats were sacrificed by cervical dislocation method. The semen from the epididymis was collected for analysis of the sperm parameters (count, motility and morphology) [22].

Testis from each group was excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They are fixed in Bouin's fluid for 24 hrs. The tissues, after fixation, were washed in water to remove excess fixative. Washed tissues were dehydrated through a graded series of ethyl alcohol, cleared with xylene and embedded in paraffin wax. Sections were cut at 3µm with microtone blade, and mounted on clean glass slide. The sections were routinely stained with haemotoxyllin and eosin. The stained slides were observed (100X and 200 X) in research microscope and photographed.

Histomorphometric analysis was done using 'Imagej®' (an open source image processing software, designed for scientific multidimensional images): the size of the seminiferous tubules was measured in the peripheral and the central regions, from four different fields of every section of the testis. Two diameters at right angle to each other, passing through the center of the tubule were measured. One was considered the long diameter (luminal diameter) and the other was the short diameter (germinal epithelial diameter). Tubular profiles that were round or nearly round were randomly chosen and measured (cross sectional area) in each rat. The readings obtained were tabulated and statistically analyzed.

Statistical analysis of the data was carried out using GraphPad Prism 5 package to determine the results which were expressed as Mean ± SEM and the difference between the mean values was analyzed using one-way analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant results

3.0 RESULTS

3.1 Hormonal Assay

The concentration of the testosterone in the testes of the treated rats reduced significantly ($P<0.05$) when compared to the control in dose dependent manner (Figure 1).

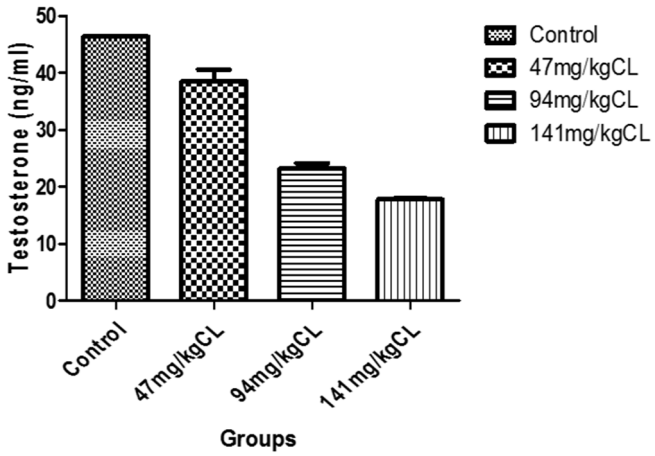

Figure 1. Concentration of testosterone in CL- treated rat groups and control.

3.2 Histomorphometric Analysis and Johnsen's Score

A significant increase ($p<0.05$) in the cross sectional area (CSA) of seminiferous tubule was observed in Group D treated with 141mg/kg body weight of CL when compared with the control group. Significant increase ($p<0.05$) in luminal diameter (LD) was also observed in Group D that received 141mg/kg body weight of CL compared to the control group, while significant increase ($p<0.05$) were observed in the germinal epithelium diameter (GED) of Group B (47mg/kg) and group D (141mg/kg) when compared with the control. In the Johnsen's score analysis, a non-significant increase ($p>0.05$) results were observed in all the treatment groups when compared to the control group (Table 1). These results showed a good morphometric grading and good Johnsen's score as a result of the effect of aqueous extract administration of CL.

3.3 Semen Analysis Results

The effect of *Carpolobia lutea* on sperm parameters is shown in Table 2. There was significant ($P<0.05$) increased number of spermatozoa in group C and group D and a non-significant ($P>0.05$) motility grading was observed in all treatment groups except group D which

Table 1. Effects of CL on histomorphometric parameters and Johnsen's score in experimental rats' testes compared to the control rats

Group	CSA (10^{10}µm)	LD (10^{6}µm)	GED (10^{4}µm)	Johnsen's Score
A	7.4 ± 0.32	0.05 ± 0.001	0.043 ± 0.002	0.19 ± 0.009
B	8.0 ±0.32	0.06 ± 0.002	0.052 ± 0.004*	0.15 ± 0.015
C	9.1 ± 1.11	0.06 ± 0.001	0.044 ± 0.003	0.14 ± 0.023
D	12.2 ±0.48*	0.08 ± 0.001*	0.049 ± 0.001*	0.212 ± 0.015

CSA: cross sectional area, LD: lumen diameter, GED: germinal epithelium diameter. Values are expressed as Mean ± SEM. *P<0.05

Table 2: Effects of CL on sperm parameters in the epidydimis of experimental rats compared to the control group

Groups	Sperm count (10^6/ml)	Sperm motility (%)	Sperm morphology (%)
A	21.03 ± 4.649	50.00 ± 10.070	40.00 ± 5.774
B	15.07 ± 2.278	33.33 ± 6.009	40.00 ± 5.774
C	48.07 ± 1.849*	70.00 ± 5.774*	36.6 ± 8.819
D	65.07 ± 14.111*	66.67 ± 8.819	40.00 ± 5.774

Values are expressed as Mean ± SEM. *P<0.05

showed a significant (P<0.05) motility grading. However, there were no significant morphological differences (P>0.05) between the treated groups and the control group.

3.4 Histological Analysis

Histological findings in the control group depicted no alteration in the histomorphological presentation as seen across the testicular profiles. A normal cytoarchitecture of the testes in rats showing a normal shape and arrangement of seminiferous tubule with intact basement membrane and Leydig cells with progressive proliferation of spermatogenic cells to produce matured spermatozoa (Figure 2A). Group B rats showed mild loss of luminal content (Figure 2B). Group C rats showed no visible lesions or the lesion was very mild (10%) (Figure 2C). Group D rats showed normal and concentric seminiferous tubules with intact basal membrane and lumen containing matured sperm cells together with the interstitial spaces which are well delineated and defined (Figure 2D).

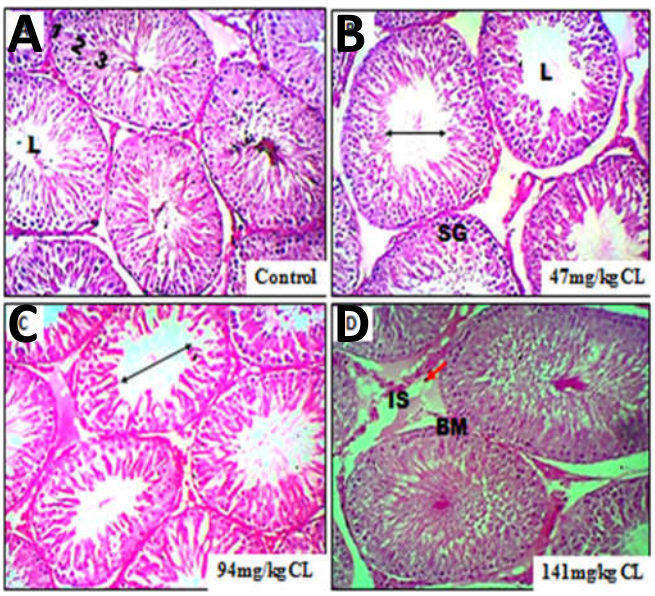

Figure 2. Photomicrographs showing rat testicular cyto-architecture of the seminiferous epithelium with basement membrane (BM), the lumen (L) and interstitial space (IS) containing interstitial cells, spermtogonium (SG) are well demonstrated across the testicular sections. H&E stain, X200

4.0 DISCUSSION

Testosterone is a vital biomarker of androgenicity and it is a major androgen secreted by the testes in nature, the major source of about 95% is known as the interstitial cells of Leydig. The level of serum testosterone as observed in this study is meaningfully reduced in the rats exposed to the extract of CL in dose dependent manner and this could be as a result of decrease in the stimulatory action by the extract on hypothalamic-pituitary axis that is responsible for the synthesis and secretion of testosterone of the male rats. This is in agreement with the reports of Yakubu and Jimoh [4] who opined that serum testosterone and antioxidant profile in testes of the animals were significantly decreased as a result of oxidative stress. On the other hand, it was reported that there was increase in serum testosterone concentration by methanol extract of *C. lutea* root at 100 and 200 mg/kg body weight as an indication that the extract has androgenic activity by presumably expediting some committed enzymatic step reactions in the testosterone generative pathway [19].

Following various doses administered, *C. lutea* notably did not altered testicular architecture when compared with the control rats, and the testicular profiles were characterized with well-defined histological structures. This is in agreement with the reports of Ejike *et al.*, [18] who reported that *C. lutea* extract plays a protective function in ameliorating testicular damage caused by cadmium in rats. This is probably due to the extract's potential in the management of testicular dysfunction and fecundity in animals. Also improved histomorphological changes of the testes were also observed when *Ozoroa paniculosa* at 50mg/kg and 250mg/kg were used to treat testicular dysfunctional in rats [23].

The significant increase in sperm count and sperm motility observed in the rats treated with higher doses of the extract could be as a result of aphrosidiac activities of *C. lutea* on the reproductive cells. Increase semen quality observed was also evident by the result from the Johnsen's score data. This finding is in support with previous work of Neeruganti, *et al.,* [21] that reported a significant increase in semen parameters in animals treated with aphrosidiac methanolic extract of *Buchanania axillaris* (linn.) leaves at a higher concentration. This is also corroborated by Nkosinathi *et al.,* [23] who reported the improved of total sperm count in the group of animals treated with the leaf extract of *M. procumbens* at 250mg/kg.

Based on this preliminary data, it can be concluded that the herb, *C. lutea* is a safe drug without any known adverse effects and can be very useful in enhancing the male sexual activity and treating various sexual disorders. It is therefore recommended that continuous usage of CL as a food ingredient should be encouraged at dosage considered in the study. However, further detailed studies are needed to confirm the usefulness of this plant extract in treating sexual disorders.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

WOA conceived and designed the study, contributed to data analysis tools, manuscript writting and supervised the study; **AA** contributed to study design, data collection, performed data analysis and monitor the bench work; **MTA** contributed to data analysis tools, analysis of data and monitoring of the rats during the acclimatization and the experimental period

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