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EFFECTS OF METHANOL EXTRACT OF *Carpolobia lutea* ROOT ON THE REPRODUCTIVE FUNCTION OF MALE RABBITS (*Oryctolagus cunnucilus*).

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ABSTRACT

Carpolobia lutea (cattle stick) is a shrub of 15ft height which is patronized traditionally for the management of reproductive dysfunction. However, its effect on reproductive system has not been scientifically reported. The aim of this study was to investigate the effects of *Capolobia lutea* root extract on male reproductive parameters in rabbits. Fifteen male rabbits (1.5 – 1.8kg) were randomly assigned into 3 groups: group 1: (control) received 1% tween 20, group 2 and group 3: received 40mg/kg and 80mg/kg of *Carpolobia lutea* root extract dissolved in 1% tween 20 respectively for 28 days. Blood samples were obtained pre and post-administration of the extract for the analysis of Serum Testosterone, Luteinizing Hormone and Follicle Stimulating Hormone. The animals were weighed, sacrificed and dissected, while relative testicular weight and histology of the testes was done and caudal epididymis was used for spermiogram. Relative testicular weight showed dose dependent decrease and significant only at 80mg/kg body weight when compared with control. Sperm count increased significantly at 40mg/kg but not at 80mg/kg body weight. Sperm motility showed dose dependent increase and significant only at 80mg/kg body weight. Sperm viability had no significant differences. Sperm morphology showed that mid-piece had a dose dependent increase in abnormalities and significant only at 80mg/kg body weight, tail abnormalities showed significant decrease at 80mg/kg body weight when compared with control animals. Serum Testosterone, LH and FSH had no significant changes. Tissue histology of the testes expressed dose dependent damage. This study have shown evidence that, *C. lutea* root extract may not have a positive effect on the reproductive parameters. Thus more studies are needed to elucidate its suspected role in the enhancement of male reproductive function.

Key words: Reproduction, Infertility, Remedy, Herbs, *Carpolobia lutea*

INTRODUCTION

Infertility and the inability to attain sexual satisfaction is a major cause of marital disharmony, it is a global phenomenon with some portion of every human population affected (American Society of Reproductive Medicine, 1997). However, it is usually seen as a private matter to be resolved by the affected couples or the individual (Fidler et al. 1999). A couple is said to be infertile when pregnancy has not occurred after at least twelve months of regular sexual activity without the use of any contraceptives (Idrisa. 2005).

Sexual dysfunction is found in men of all ages, ethnicities, and cultural backgrounds, it arises from a variety of problems, most notably are alterations in sperm quality; hormonal level and erectile and ejaculatory dysfunction. However, the above pathophysiological conditions may arise from the following factors: lifestyle, advancing in age, environmental and occupational hazards; obesity, weight gain and loss, eating disorders, under nutrition, malnutrition, excessive exercise (Rakesh et al. 2013). For there to be a normal sexual intercourse and sexual fulfillment in male, the male sexual organs and hormonal factors related to erection must function optimally. Inability to perform this function effectively is referred to as sexual dysfunction which could cause divorce, loss of self-confidence, anxiety, depression, lowered life satisfaction, frustration, grief, fear, guilt, helplessness, reduced job performance, loss of social status, social stigma, social isolation and alienation, community ostracism, physical violence, to affected individuals(Van Balen et al. 2002).

Male sexual and reproductive dysfunctions can be assessed by seminal fluid analysis, androgenic hormone assay, follicular stimulating hormone, and luteinizing hormone (Jungwirth et al. 2012). Scrotal ultrasonography can be used to evaluate the scrotal volume. Testicular biopsy has a minimal role in the evaluation of male

factor infertility (Jungwirth et al. 2012). Treatment options include orthodox and traditional remedy. The uses of herbal products have remained a source of hope for many in the developing countries. Some of those plants that have been used include: Telferia occidentalis (Akang et al. 2010), Chlorophytum borivilianum (Thakur et al. 2009), *Carpolobia lutea* (Mitaine-Offer et al. 2002) etc.

Carpolobia lutea (Polygalaceae) is a shrub or small tree up to 15ft high (Hutchinson and Dalziel,1954), and is widely distributed in West and Central areas of Tropical Africa (Mitaine - Offer etal. 2002). It is known by many common names such as cattle stick (English), Abekpok Ibuhu (Eket), Ikpafum, Ndiyan, Nyayanga (Ibibio), Agba or Angalagala (Igbo) and Egbo oshunshun (Yoruba) (Etukudo, 2003; Muanya, 2008).*Carpolobia lutea* have been tested for its gastrointestinal effect, anti-diabetic effect and anti-malaria. The leaf is used to cure rheumatism, fever, pains, insanity, dermal infection, venereal diseases, sterility and to promote child birth. In addition, it is used as vermifuge and stomach medicine (Burkil, 1984; Muanya and Odukoya, 2008). The leaf is equally reported to have anti-inflammatory and anti-arthritis properties (Iwu and Ayanwu. 1982). Fever with diarrhea, headache, leprosy, snakebite, venereal disease and wounds are reported to be cured by the leaf extract as well (Ajibesin et al. 2005). The anti-diarrhea and anti-ulcerogenic potential of crude ethanol extract of *Carpolobia lutea* leaf have been established experimentally in rodents (Nwafor and Barsey, 2007) while the root is used to facilitate childbirth, treat sterility, headache, worm infestation and also has aphrodisiac and stimulant properties (Mitaine–Offer et al. 2002). Phytochemical screening of this plant revealed the presence of chemical such as flavonoids, simple sugar, alkaloids, tannins, saponins, phlobatannins, cardiac glycosides, and anthraquinones. Flavonoid is an antioxidant that could be beneficial in cases of reproductive dysfunction caused by oxidative stress.

MATERIALS AND METHOD

Plant Material:

Carpolobia lutea root was harvested from Alure forest, Oyo. Different parts of the plant were collected and taken to Forestry and Research Institute of Nigeria, (FRIN) Ibadan, for identification on 17th of October 2013 and herbarium number (109755) was allocated to this herb.The Carpolobia lutea root was washed, chopped into pieces with cutlass and oven dried at 40^0C until the weight of this herb became relatively constant at human laboratory. The Carpolobia lutea root was grinded with a milling machine to finer particles.

The extraction was done at the Department of Pharmacognosy, University of Ibadan. 4kg of grinded plant was soaked in 15litres of absolute methanol in a glass bowl at room temperature for 72hours. The solvent was then filtered. The filtrate was concentrated in a rotary evaporator while the residue was discarded. The yield (a brown, oily substance) was stored in a refrigerator at - 4^0C.Determination of LD50 of Carpolobia lutea root:

The LD50 of *Carpolobia lutea* root extract was done according to Organization for Economic Community Development (OECD) guideline for testing of chemicals 423 (2001). Six female Wistar rats weighing 90grams were obtained from central animal house, University of Ibadan and acclimatized for five days. The animals were randomly selected and marked to permit individual identification. The animals were fasted prior to administration of *Carpolobia lutea* root extract but water was supplied. Three animals were used for each step. The dose level used as the starting dose is 300mg/kg, after 24 hours, another 300mg/kg was repeated for the same animal, this was followed by 2000mg/kg with another 3 sets of animal on the 3rd day and the 4th day.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days.

Experimental Design:

Fifteen male rabbits were grouped into three (1, 2, and 3), each group has five rabbits.
The animals were acclimatized to laboratory condition for a period of 2 weeks before the commencement of the study.
Group 1 is control group and received oral administration of 1% between 20 solution.

Group 2 received oral administration of 40mg of the C. Lutea extract /kg body weight.
Group 3 received oral administration of 80mg of the C. Lutea extract /kg body weight.

Sample Collection and processing:

Pre and Post-administration bleeding was done by collecting blood via the retro-orbital sinus with 70µL heparinised capillary tube into plain bottles (Ezzai, 1995). Samples were centrifuged at 3000rpm for 15 minutes. The sera were separated into sterile plain bottles and stored at -20°c before analysis. ELISA kits were used to determine the serum level of Testosterone, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) (Dialab, Austria) and testosterone (Rapid lab, UK).

Animal Sacrifice and Organ Collection:

At the end of 28th day of the study, the animals were sacrificed by cervical dislocation which involved snapping of the spine of the rabbits by applying pressure on the upper third of the neck.

The animals were dissected and the testis, epididymis, liver and kidney were collected. The testes were cleared of adherent tissues, weighed with electronic weighing balance (Hobby King Technology Ltd) and fixed in 10% formalin

RESULTS

The results revealed that LD_{50} = 2500 mg/kg body weight. Relative testicular weight showed dose dependent decrease and significant only at 80mg/kg body weight when compared with control (Fig. 3).

Sperm count increased significantly at 40mg/kg (Fig. 4). Sperm motility showed dose dependent increase and significant only at 80mg/kg (Fig. 5). Sperm viability had no significant differences (Fig. 5). Sperm morphology showed that mid-piece had a dose dependent increase in abnormalities and significant only at 80mg/kg body weight, tail abnormalities showed significant decrease at 80mg/kg body weight when compared with control animals. Serum Testosterone (Table 1), LH (Fig. 1) and FSH (Fig. 2) had no significant changes. Tissue histology of the testes expressed dose dependent degree of damage (Fig 6).

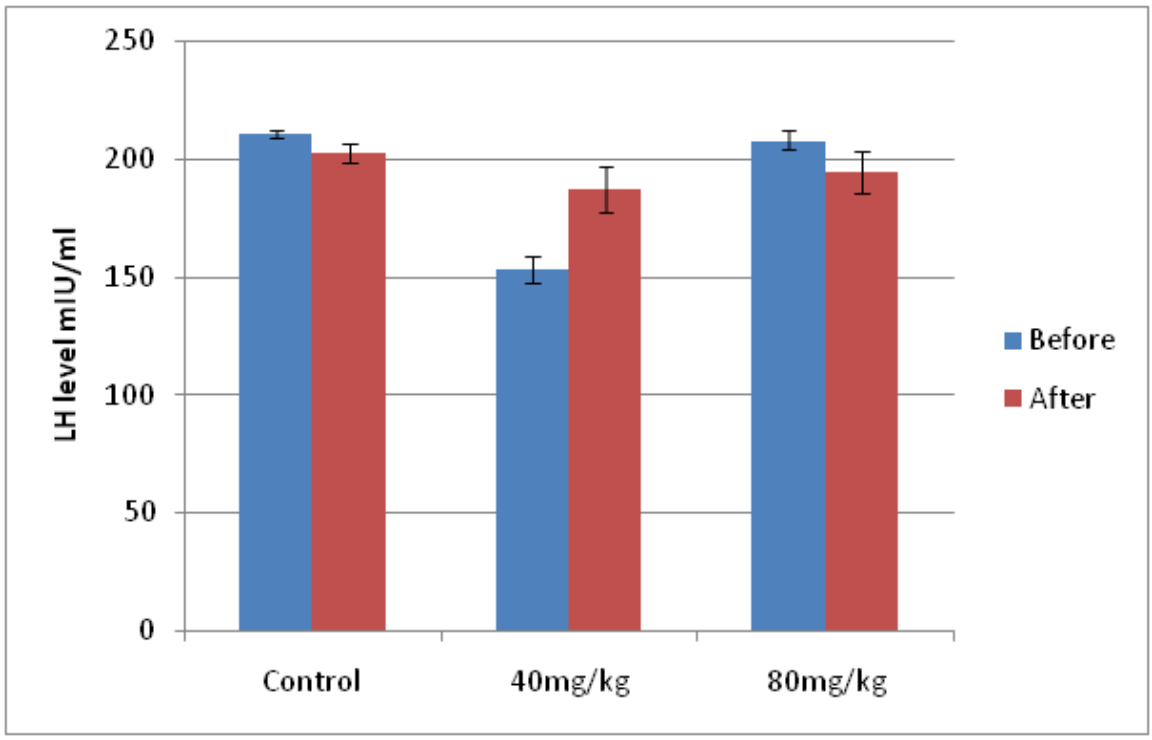


Fig. 1: Effect of *Carpolobia lutea* Root Extract on Serum Levels of Luteinizing Hormone (Before And After Treatment) Values in Mean ± SEM, n=5

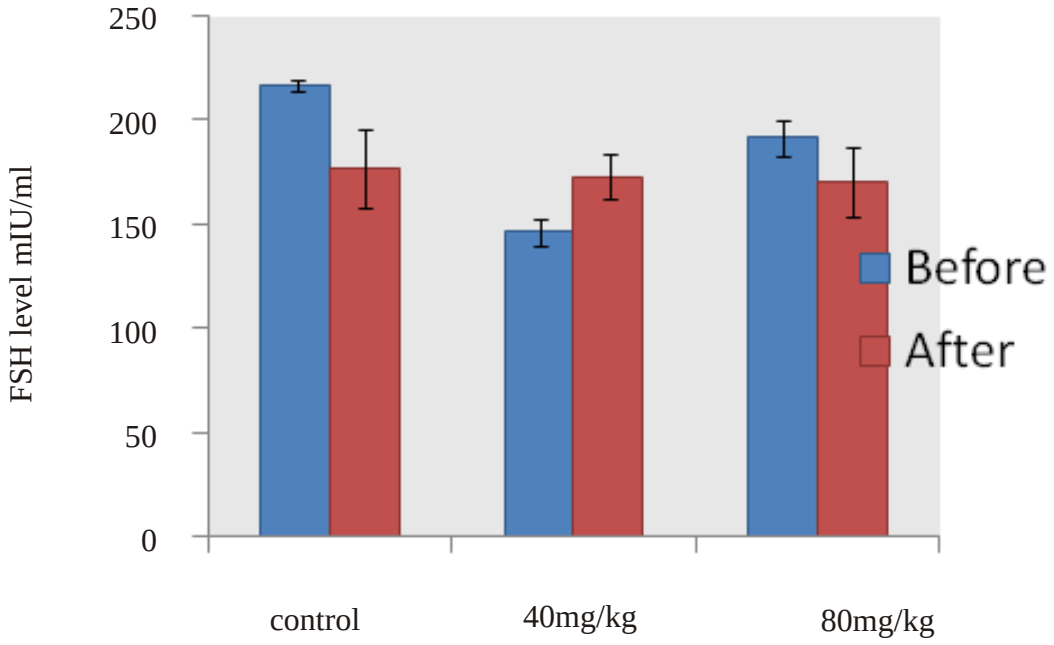


Fig. 2: Effect of *Carpolobia lutea* Root Extract On Serum Level Of Follicle Stimulating Hormone (Fsh) (Before And After Treatment) Values in Mean ±SEM, n=5

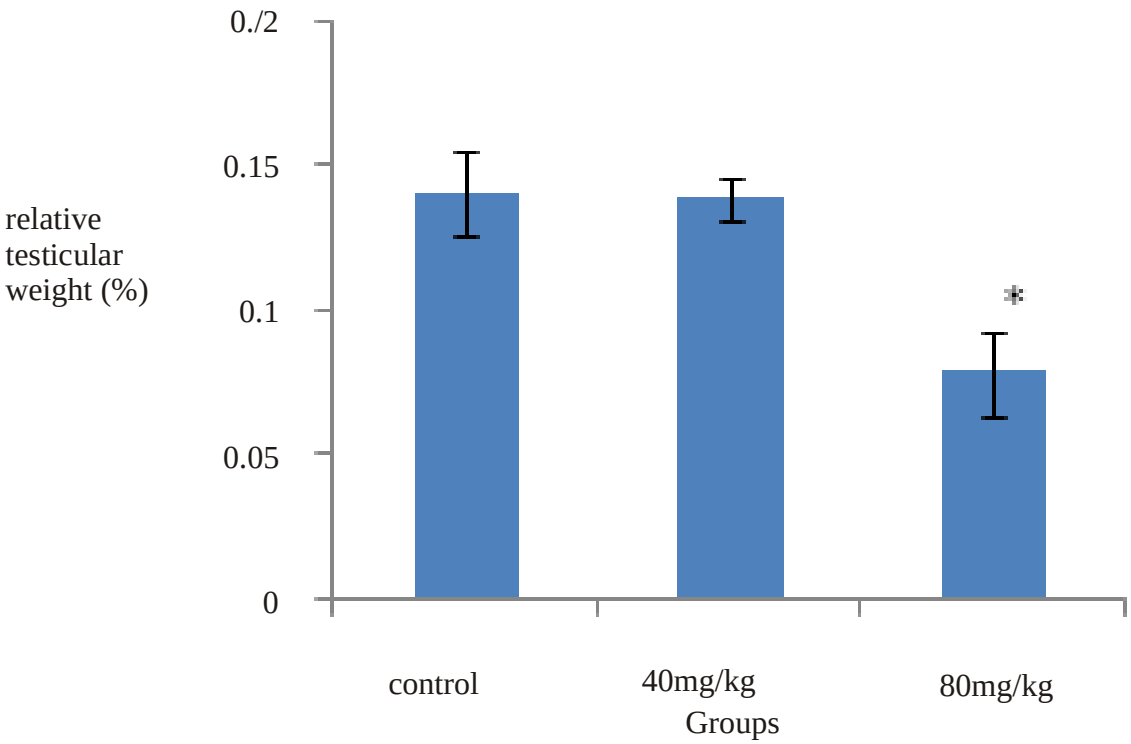


Fig. 3: Effect of *Carpolobia lutea* Root Extract On Relative Testicular Weight
Values in mean ± sem, n=5
*p < 0.05 as compared with control and 40mg/ kg groups

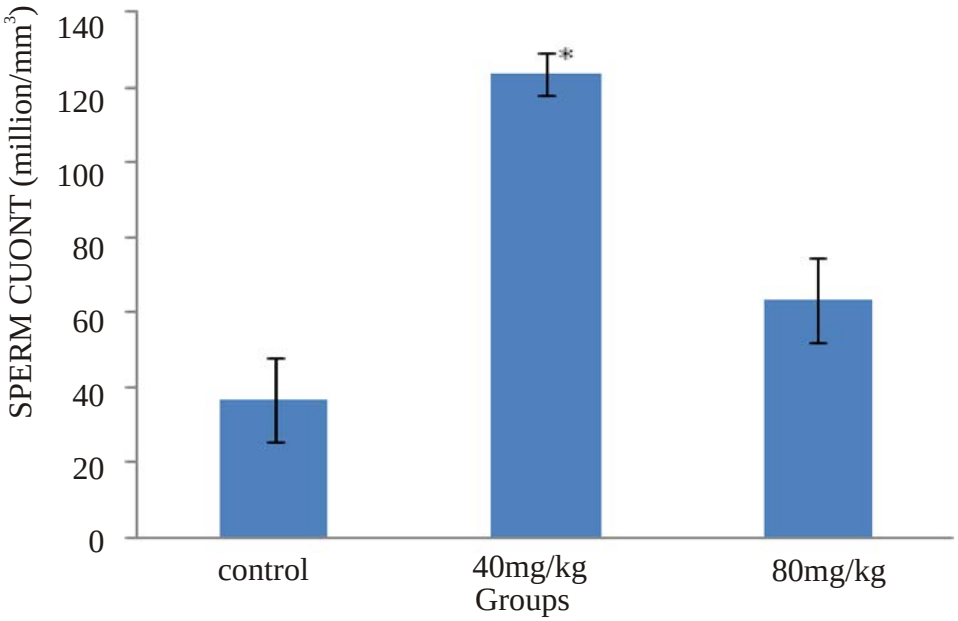


Fig. 4: Effect of *Carpolobia lutea* Root Extract on Sperm Count
(Values in Mean ± SEM, n=5)
*P ≤ 0.05 as compared with the control and 80mg/kg group

Table 1: Effect of *Carpolobia lutea* Root Extract on Serum Level of Testosterone
There is no significant difference between the pre-test and post-test
Values in Mean ± SEM

Pre-treatment (µg/mol)	Post-treatment (µg/mol)	
CONTROL	19.71 ± 6.65	6.68 ± 8.33
40mg/kg	27.56 ± 10.49	10.08 ± 8.81
80mg/kg	10.07 ± 6.08	16.54 ± 6.83

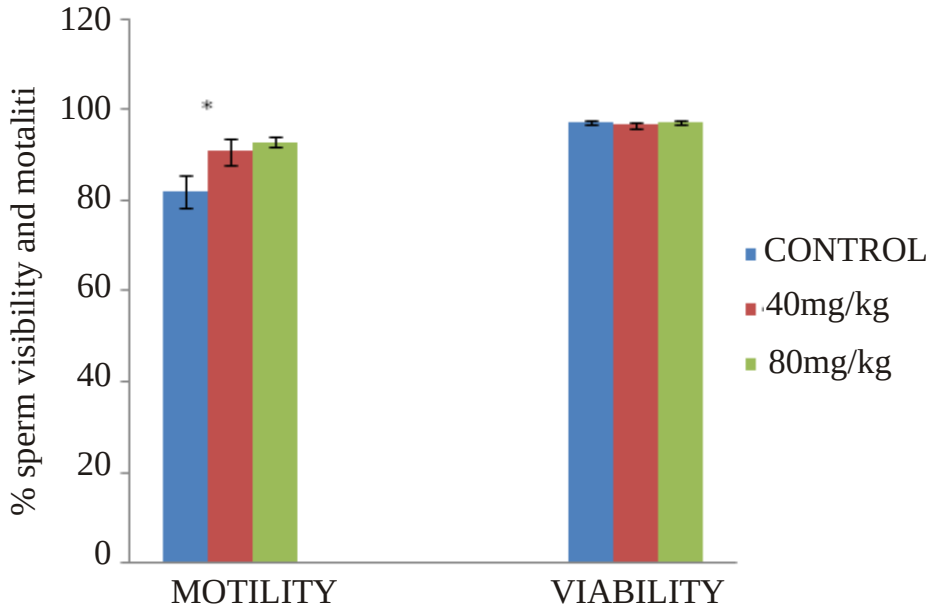
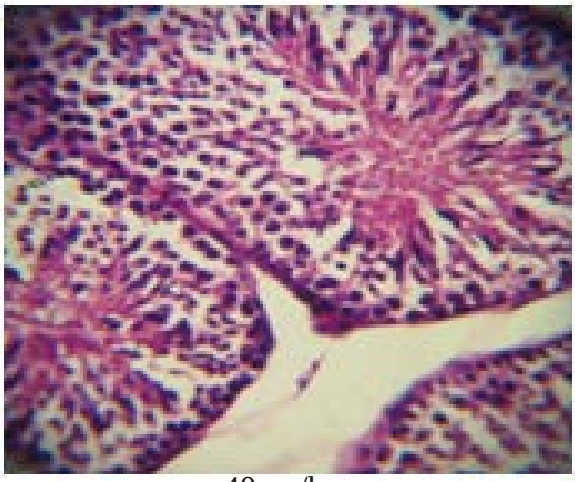
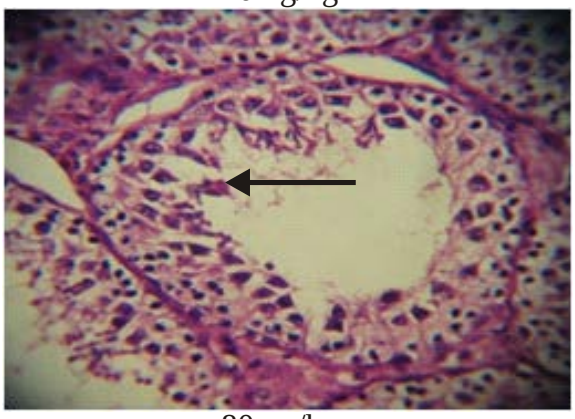


Fig. 5: Effect of *Carpolobia lutea* Root Extract on Percentage Sperm Motility and Viability
Values in mean ± SEM, n=5
*p ≤ 0.05 significant different in sperm motility at 80mg compared to control.
Sperm viability of control and treated rabbits with no difference.

CONTROL



40mg/kg



80mg/kg

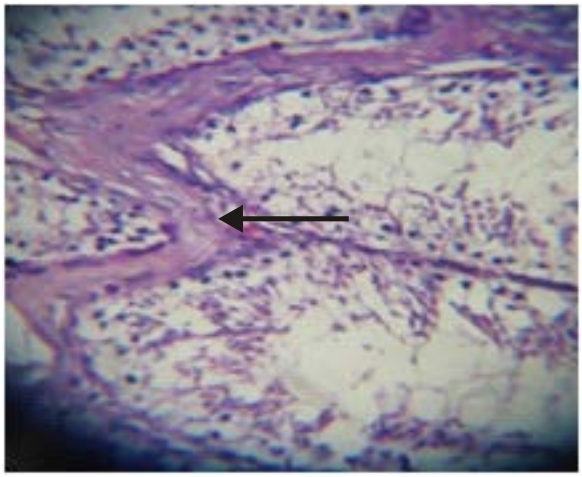


Fig. 6: Photomicrograph of Tissue Histology of the testis of control and treated rats.

Control testicular histology showing normal architecture; 40mg/kg showing seminiferous tubules with maturation arrest, with few germ cells and empty widened lumen (blue arrow); 80mg/kg showing seminiferous tubules with thick propria (white arrow) sloughing of the germ cells, and reduction of sertoli cells.

DISCUSSION

Hypothalamic pituitary gonadal axis is an axis very essential in ensuring normal steroidogenesis and spermatogenesis through release of follicle stimulating hormone (FSH) and luteinizing hormone(LH). FSH released from anterior pituitary acts on sertoli cells thereby facilitating the release of primodial germ cell, while LH also from the anterior pituitary acts on the leydig cells to cause maturation of spermatozoa.In this study, *Carpolobia lutea* administered at 40mg/kg and 80mg/kg body weight showed no significant changes in serum LH, FSH, and Testosterone levels of male rabbits when compared with control group or pretreated group. This result is in contrast with Jyoti et al. (2010) where it was reported that the leaf extract of *Ocimum sanctum* significantly reduce serum FSH and LH level while testosterone is increased. This observation indicates that hypothalamic-pituitary-gonadal axis may not have been affected by *Carpolobia lutea* root extract.

The quality of sperm is one of the important indices of a reproductive function. Alteration in sperm indices can cause a reduction in male fertility. In this present study, sperm count was significantly increased at a dose of 40mg/kg, while sperm motility was significantly increased at 80mg/kg. This result may probably suggest that *Carpolobia lutea* may have exerted its effect at the testicular level, as reproductive hormones were not significantly affected, and caused release of immature spermatozoa as maturation arrest was seen in tissue histology of testis for 40mg/kg group. It could also be that sperm count that was found to be significantly increased in 40mg/kg is sperm produced before commencement of administration as spermatogenic cycle in rabbit is 51 to 56 days and administration of *Carpolobia lutea* extract to this animals lasted only for 28days.Considering the dose dependent decrease in relative testicular weight, which was significant at 80mg/kg body weight, and dose dependent damage showed in the tissue histology of the testes, liver and kidney, this could suggest the detrimental effect of increased dose of *Carpolobia lutea*root extract on the testicular weight and its toxicity on the tissues of the testis, liver and kidney, which is in line with the toxicity findings on the leaf extract

of *Carpolobia lutea* (Nwidu et al. 2012), and also in agreement with the report of Chen et al (1998), which reportedthat treatment of humans with *Panax notoginseng* causes reduction in sperm count and motility in human. The Saponin content of the *Panax notoginseng* could account for the reduction observed(chen et al. 1998). Phytochemical screening of *Carpolobia lutea* root extract revealed that saponin is one of the major constituent, this could probably contribute to the ability of *Carpolobia lutea* to reduce sperm count and motility in this present study.

CONCLUSION.

With reference to reduction in relative testicular weight, damages seen in the tissue histology of the testes and non significance changes in reproductive hormone status. This study have shown evidence that, *C. lutea* root extract may not have a positive effect on the reproductive parameters. However, more studies are needed to elucidate its suspected role in the enhancement of male reproductive function.

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