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# Comparative study of testis histology and haematology of *Clarias gariepinus* exposed to phytochemicals of *Moringa oleifera* and *Carica papaya* leaf powder

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**Abstract**
This work was carried out to know the responses of *Clarias gariepinus* broodfish testis and blood when exposed to varied levels (0.50, 1.00, 1.50 and 2.00g/l) of *Moringa oleifera* and *Carica papaya* leaves powder for 8 weeks in a renewable bioassay. Results showed that temperature, nitrite and pH were within the same range with no significant difference ($P>0.05$). There was significance difference ($P<0.05$) between some blood parameters; RBC, platelets, Hb, while data from the rest showed no significant difference ($P>0.05$). Moringa had the highest value in RBC and Hb, while *C. papaya* had the highest value in WBC. Analysis on the Gonadosomatic index showed significance difference ($P<0.05$) with Moringa having the highest value (0.19±0.008) and while *C. papaya* and control showed no significance difference ($P>0.05$). There was no significance difference ($P>0.05$) in the condition factor but the values indicate a favourable condition for the fish as it was greater than 2. Histological analysis had no difference in testicular structure between the treatments as compared with the control.

**Keywords:** Testis histology and haematology, *Clarias gariepinus*, phytochemicals, *Moringa oleifera*

## Introduction
Aquaculture is a fast-growing sector in Nigeria, contributing less than 5% of the total fish supply but at a growth rate of about 2% per year. Among the culturable fishes in Nigeria, *C. gariepinus* is a major tropical aquaculture species and the most popular among fish farmers and consumers. Fish farming has contributed greatly to the availability of food in Nigeria and the world over with products from fish farming widely exported and traded to earn income (Adeparusi *et al*., 2010) [1]. Fisheries sector employs over 44.5 million people and a lot of them are from developing countries. Also, industries engaged in the marketing, supply and distribution of fish product create job opportunities for over 150 million individuals (Adeparusi *et al*., 2010) [1].

The African catfish (*Clarias gariepinus*) belongs to the family Clariidae and is the most cultivated fish in Nigeria, and highly demanded freshwater fish all over the world due to its resistant to stress, ability to tolerate a wide range of environmental conditions, high stocking density under culture conditions and relatively fast growth rate. This fish is found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as man-made habitats, such as ponds or even urban sewage systems. Due to the high demand of quality fish and fish dietary proteins, there have been increases in various researches in different ways to improve fish fertility to meet the demand and target productivity in aquaculture, with a dramatic movement from synthetic drugs to medicinal plants of natural importance.

*Carica papaya* is a soft-wooded perennial plant that has a life span of 5–10 years although commercial plantations are usually replanted. It normally grows a single – stemmed tree with a crown of large leaves emerging from the apex of the trunk but plant stands may become multi-stemmed when damaged (Adebiyi *et al.*, 2002) [2]. The fruit, seeds, leaves contained novel biologically active compounds which are potent as therapeutics. C. *papaya* leaves have been reported to contain high amounts of vitamin A, D, E, K, B, C and minerals like magnesium, sodium and iron.

Moringa leaf (*Moringa oleifera*) is the most widely cultivated species in the genus moringa, the only genus in the plant family moringaceae.

Common names include moringa, drumstick tree etc. In developing countries, moringa has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care (Makkar and Becker, 1997; Gidamis *et al*., 2003) [30, 26]. Earlier studies have shown that *M. oleifera* is a promising protein source for use in diet of Tilapia (Richter *et al*., 2003) [14]. Moringa leaves are readily eaten by cattle, sheep, goats, pigs, chickens and rabbits. It can also be used as food for herbivorous fish species. Several studies demonstrate that significant proportions of traditional fodder can be replaced with moringa leaf. Aregheore, (2002) [6] reported that a significant weight was gained over traditional fodder when 50% of fodder contained Moringa. Research on moringa in the aspect of aquaculture has mostly been directed towards nutrition because of their well-known source for proteins and vitamins Cho *et al.*, 1976 [27]; Francis *et al.*, 2001 [28]; Alegbeleye *et al*., 2001 [3]; Nwanna *et al*., 2008 [17]. Little has been done towards its effect in breeding and this research would add to knowledge bank concerning its use in breeding. Therefore, this study was aimed at investigating the effect of pawpaw (*Carica papaya*) and moringa leaves powder on the testis of *Clarias gareipinus* when exposed to varied concentrations.

Haematological features are vital parameters that can be used as an effective way of monitoring physiological and pathological changes in fishes. It is also used to evaluate physiological status of fish. Changes in haematological parameters depend on fish species, age, the cycle of the sexual maturity of spawners and diseases. Normal ranges of various blood parameters in fish have been established by different researchers. The analysis of blood indices has shown to be a necessary approach for analyzing the health status of farmed fish as these indices provides consistent information on disorders pertaining to metabolism, stress and deficiencies before they evident in a clinical platform. Blood chemistry can be used to evaluate the health of fish. Exogenous factors such as management, diseases, stress and water quality, always exert major changes in blood makeup.

Histology is an important tool to assess reproductive fish health and to show the initial signs of lesions or alterations not easily identifiable during the macroscopic examination of fish tissue. Results from a histological assessment can provide better insight into the environment (Yonkos *et al*., 2000) [24]. However, it is important for the normal state of any animal to be well known in order to provide a template for comparison with suspected abnormal state. Yonkos *et al* (2000) [24], stated that identification of tissue lesions requires a baseline appreciation of normal tissue conditions, as histological alterations can occur in target organs that are not necessarily the result of toxicant exposure. Dada, 2009 [9] reported that the use of medicinal plants as a fertility enhancer in aquaculture has now received much attention, with the shift from synthetic drugs However, some plants have been shown to have deleterious impact on aquatic organisms (Adeparusi *et al*., 2010) [1].

## Materials and Methods

**Experimental layout:** African mud fish, *C. gariepinus* adults (9 in number) were obtained from a standard fish farm in Port Harcourt, Rivers State and transported in aerated containers to the University of Port Harcourt Demonstration fish farm. Fish were acclimated for 14 days in 9 plastic tanks measuring 40cm×30cm×26cm (length, width and depth) each. Feeding was done with commercial feed (Coppens) twice daily (morning and evening) at 2% body weight throughout the period of the experiment which lasted for 8 weeks.

**Treatments:** Four treatments levels of 0.50, 1.00, 1.50 and 2.00g/l exclusive of control were used for each of the plants (*Moringa oleifera* and *Carica papaya*).

Moringa and pawpaw leaves used were washed to remove dirt particles, sun dried properly and ground into fine powder. These leaves were added to water (20L) in aquaria with fish at 0.50, 1.00, 1.50 and 2.00g/l. concentrations levels except in control tank.

**Collection of samples:** After 8 weeks, fish were weighed and blood obtained using a 5ml syringe and dispensed into tubes containing Lithium heparin anticoagulant. The samples were labeled properly and taken to the laboratory for haematological analysis. The gonads of fish were obtained by laceration of the fish abdomen. Upon removal, the milt sac was weighed with a sensitive weighing scale then kept in a petri dish before being taken to the laboratory for histopathological examination. Fulton's condition factor (K) and gonadosomatic index were both calculated using standard methods. Physico-chemical parameters such as Ammonia, alkalinity, temperature, pH, conductivity, turbidity and dissolved oxygen were adequately monitored. Ammonia - Nitrogen (NH3-N) with the phenate method of ammonia determination (APHA, 1998) [5], temperature measurements was determined with a mercury-in glass thermometer, pH with291 Mk 2 pH meter, conductivity with Horiba water checker, turbidity with a probe was inserted in water and the turbidity values obtained were read using the Horiba water checker measured by standard methods according to APHA, 1998.Dissolved Oxygen (DO) with Winkler's method (APHA, 1998).

**Experimental design:** Completely randomized design (CRD)

**Data analysis:** Analysis of variance (ANOVA) was used to analyze the results between the treatment groups. Test of significance was at 95% probability.

## Results

The mean values for water quality variables such as ammonia, alkalinity, temperature, pH, conductivity, turbidity and dissolved oxygen obtained in the experimental tanks during the exposure of the different life stages of *C. gariepinus* to graded levels of the two leaves are presented in Table 1. Temperature, nitrite and pH were within the same range with no significant difference ($P>0.05$). The mean values of conductivity, Ammonia, and turbidity significantly increased ($P<0.05$) while dissolved oxygen decreased with respect to leaves when compared with control (Table 1). *Moringa oleifera* leaf powder slightly improved the wellbeing of *C. gariepinus* by 2.00% higher than that of *Carica papaya* and 2.94% higher than that of control while *Carica papaya* leaf powder was 0.94% higher than control (Table 2). The phytochemicals in *M. oleifera* and *C. papaya* leaf powder did not significantly ($P>0.05$) impact on PCV and WBC but slightly raised PCV in fish by 4.80% (*M. oleifera*) and slightly decreased it by 10.10% (*C. papaya*) while WBC was raised by 0.98% (*M. oleifera*) and 8.16% (*C. papaya*) when compared with control.

Haemoglobin (Hb) and Red blood cell (RBC) were respectively raised by 3.78% (*M. oleifera*) and 5.38% (*C.*

*papaya*) and decreased by 1.21% (*M. oleifera*)) and 1.35% (*C. papaya*) when compared with control. The leaves powder raised Platelets (PLT) by 7.18% for *M. oleifera* and 1.95% for *C. papaya* (Table 3). *M. oleifera* and *C. papaya* leaf powder decreased the PCV in fish at 0.5, 1.0, 1.5 and 2.00g/l except at 2.00g/l where it was raised by *M. oleifera* by 16.44% whereas Hb was raised at all concentration levels by the two leaves except that of *C. papaya* which dropped at 1.00 (by 0.12%) and 2.00g/l (by 1.82%) when compared with control (Table 5). At 0.5 and 1.00g/l, *M. oleifera* decreased WBC and raised it at 1.5 and 2.00g/l while *C. papaya* raised it in all the concentrations except at 0.5g/l. The two plants raised Platelets (Plt) in all the concentrations when compared with control (Table 5). There was significant difference ($P<0.05$) in GSI between *Moringa oleifera* treatment and *C. papaya* as well as control. Though there was no significant difference ($P>0.05$) between control and *C. papaya* treatment (Table 4). There was no significant histological differences in the testis of fish exposed to all the treatment levels of the two leaves when compared with control (Figure 1-7).

**Discussion**

All the water parameters considered in this work were within tolerance ranges of warm water fish species as reported by Boyd and Lick, 199 [8]; Adeniji and Ovie, 1982 [4]. It is often reported that optimal requirement for African catfishes does not vary significantly ($p<0.05$) in the respective treatment levels. This suggests that the parameters did not seem to negatively influence the test fish in this study.

Moringa contain high crude protein (251g/kg DM) in the leaves, with low content of tannins and other antinutritional compounds (Nouala *et al*., 2006) [16]. Moringa leaves are rich in crude protein content which varies from 25% (Makkar and Becker, 1996 [30]; Uedeme-Naa and George, 2019) [29]. The protein comprises high levels of sulphur containing amino acids and competes well with soybeans which is usually considered as a source of high-quality plant protein (Francis *et al*., 2001) [28]. Its crude lipid contains n-3 fatty acids in the form of linolenic acid which account for about 67% of total fatty acids (Soliva *et al*., 2005) [20]. The leaves of moringa plant are significant source of carotenoids, minerals and ascorbic acid as well as iron. Earlier studies have shown that *Moringa oleifera* is considered as a promising source of protein for inclusion in fish diets at low levels (Ayotunde *et al*., 2011) [7]. Leaves of moringa are the best source of high nutrients such as crude protein, crude fat, and gross energy etc. (Grubben Denton, 2004) [11]. Fulton's condition factor (K) is useful for estimating the wellbeing of an animal physically as well as for determining growth rate in crustaceans (Rochet, 2000) [15]. It is an important parameter used in monitoring the response of a population to environmental changes over time and to assess the overall health, productivity, lipid content and growth rate of the population (Stevenson and Woods 2006) [23]. These outstanding qualities in *Moringa oleifera* plant could be responsible for the wellbeing of *Clarias gariepinus* by 2.94% better than control and 2.00% better than that of *Carica papaya* in this work. Gonadosomatic index is an index of gonad size relative to fish size. It is a good indicator of gonadal development in fish. The percentage of the body weight of fish that is used for production of eggs is determined by the gonadosomatic index. GSI is considered as an important parameter of fish biology that could provide information of reproductive status of fish (Kiran *et al.*, 2014a) [13]. GSI indicates gonadal development and maturity of fish. In this work *Moringa oleifera* plant improved on the gonadal (milt) quality of *Clarias gariepinus* by 6% higher than control and 8% better than *Carica papaya*

The use of haematological technique in fish culture for toxicological research, environmental monitoring and fish health conditions have grown rapidly in recent times (Gabriel *et al*., 2007; Akinrotimi, 2008; Akinrotimi *et al.*, 2011) [25, 32, 31]. Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al*., 2013) [12]. Haematological components, which consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals (Oyawoye and Ogunkunle, 2004) [19]. The haematological parameters of fish are important for evaluating the physiological conditions, disease as well as determining the effect of diet and other environmental factors in cultured fish (De Pedro *et al*., 2005) [10]. Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood. According to Isaac *et al.* (2013) [12] Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume shows a better transportation and thus results in an increased primary and secondary polycythemia. Packed cell volume (PCV) was raised, 4.80% above control by *Moringa oleifera* plant and was decreased, 10.10% by *Carica papaya* when compared with control. *Moringa oleifera* plant raised the volume of haemoglobin flow in this work by 3.78% to the health benefit of *C. gariepinus* which agrees with the work of Ugwuene, 2011 [33] ; Omiyale, Yisa, and Ali-Dunkrah, 2012 [18] ; Soetan *et al*., 2013 [22] ; Isaac *et al*., 2013 [12] who reported that haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals. Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury. Red blood cells (RBC), Platelets (Plts) and White blood cells (WBC) were respectively raised to 5.38%, 7.18% and 0.98% above control by *Moringa oleifera* while *Carica papaya* raised platelets to 1.95% and WBC to 8.16% above control. This implies that the combination of *Moringa oleifera* and *carica papaya* leaves could be suitably used to improve on the health status of *C. gariepinus* and other aquatic lives. Histology is the study of the microanatomy of cells, tissues, organs as seen and observed through the microscope. Its primary center is the examination of the correlation between structure and function. It is an important tool to assess reproductive fish health and to show the initial signs of lesions or alterations not easily identifiable during the macroscopic examination of fish tissue. There were no histological differences in the testicular structure of both the *Moringa oleifera* and *C. papaya* leaves treatments which corroborated with the report of Soetan and Olaaiya (2013) [21]. This also underscores that at the various treatment levels, there may not been any disparity in testicular structure of *C. gariepinus* broodfish.

## Conclusion

This work has shown the haematology and also the comparative analysis of the testes of *C. gariepinus* broodfish when exposed to varied concentrations of *M. oleifera* and *C. papaya* leaves powder. The results from the work shows that these phytochemicals have a profound effect on the haematology parameters of the fish though there was no disparity in the testicular structure of the specimens. By this work, Moringa leaf has a very positive impact on the Packed cell volume(PCV); Haemoglobin(Hb); Red Blood Cell (RBC) and Platelet (Plt) of fish hence farmers should incorporate *M. oleifera* leaves in either the feed or water to promote good health which is vital in the growth process of fish especially brood stock. Also *C. papaya* leaf is recommended in the treatment of fish disease due to its boost in White Blood Cell (WBC) production.

**Table 1:** showing water quality parameters taken over the experimental period

| Treatments | Ammonia (mg/l) | Temperature (ºC) | pH | Dissolved oxygen(g/ml) | Nitrites |
|---|---|---|---|---|---|
| Moringa | $0.36^{a}$ | $28.8^{a}$ | $6.25^{b}$ | $3.10^{b}$ | 0.001 |
| Pawpaw | $0.42^{a}$ | $27.9^{b}$ | $6.47^{a}$ | $3.18^{b}$ | 0.001 |
| Control | $0.18^{b}$ | $29.3^{a}$ | $6.30^{c}$ | $4.67^{a}$ | 0.001 |

**Table 2:** Condition factor of *C. gariepinus* across the treatments and control

| Treatments | Control | Moringa | Pawpaw |
|---|---|---|---|
| Condition factor | $2.64\pm0.372^{a}$ | $2.80\pm0.28^{a}$ | $2.69\pm0.41^{a}$ |

**Table 3:** Haematology of *C. gariepinus* exposed to *C. papaya* leaf and *M. oleifera* (Mean±S.D)

| Parameters | Control | % control | *M. oleifera* | *% control* | *C. papaya* | *% control* |
|---|---|---|---|---|---|---|
| PCV | $34.75\pm0.5^{a}$ | 35.10 | $39.5\pm29.10^{a}$ | 39.90 | $24.75\pm2.75^{a}$ | 25.00 |
| Hb | $8.85\pm0.12^{ab}$ | 32.48 | $9.88\pm0.77^{a}$ | 36.26 | $8.52\pm0.84^{b}$ | 31.27 |
| RBC | $4.28\pm0.22^{c}$ | 31.99 | $5.00\pm0.21^{a}$ | 37.37 | $4.10\pm0.39^{bc}$ | 30.64 |
| WBC | $8.65\pm0.25^{a}$ | 30.29 | $8.93\pm1.82^{a}$ | 31.27 | $10.98\pm1.37^{a}$ | 38.45 |
| PLT | $155.00\pm7.07^{c}$ | 30.29 | $191.75\pm22.70^{a}$ | 37.47 | $165.00\pm6.78^{ab}$ | 32.24 |

Key: PCV = Packed cell volume; Hb = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; PLT = Platelet.

**Table 4:** Gonadosomatic index (GSI) of *C. gariepinus* across treatments and control (Mean±S.D).

| Treatment | Control | Moringa | Pawpaw |
|---|---|---|---|
| GSI | $0.16\pm0.02^{bc}$ | $0.19\pm0.008^{a}$ | $0.15\pm0.02^{bc}$ |

**Table 5:** Haematology of *C. gariepinus* adult exposed to *Moringa oleifera and pawpaw* leaf powder

| Parameters | Control (0.00g/l) | | T1 (0.5g/l) | | | | T2 (1.0g/l) | | | | T3 (1.5g/l) | | | | T4 (2.0g/l) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *M. oleifera* | | *C. Papaya* | | *M. oleifera* | | *C. Papaya* | | *M. oleifera* | | *C. Papaya* | | *M. oleifera* | | *C. Papaya* | |
| PCV | 35 | 11.99% | 28 | 9.59% | 26 | 8.91% | 25 | 8.56% | 23 | 7.88% | 22 | 7.53% | 28 | 9.59% | 83 | 28.43% | 22 | 7.35% |
| Hb | 8.8 | 10.68% | 9.3 | 11.29% | 9.2 | 11.17% | 9.5 | 11.53% | 8.7 | 10.56% | 9.7 | 11.77% | 8.9 | 10.80% | 11 | 13.35% | 7.3 | 8.86% |
| RBC | 4 | 9.90% | 4.8 | 11.88% | 4.5 | 11.14% | 4.9 | 12.13% | 4.3 | 10.64% | 5 | 12.38% | 4 | 9.90% | 5.3 | 13.12% | 3.6 | 8.91% |
| WBC | 8.7 | 9.85% | 7.3 | 8.27% | 9.7 | 10.99% | 7.5 | 8.49% | 10.2 | 11.55% | 9.9 | 11.21% | 11.2 | 12.68% | 11 | 12.46% | 12.8 | 14.50% |
| PLT | 150 | 9.51% | 175 | 11.10% | 170 | 10.78% | 180 | 11.41% | 167 | 10.59% | 187 | 11.86% | 168 | 10.65% | 225 | 14.27% | 155 | 9.83% |

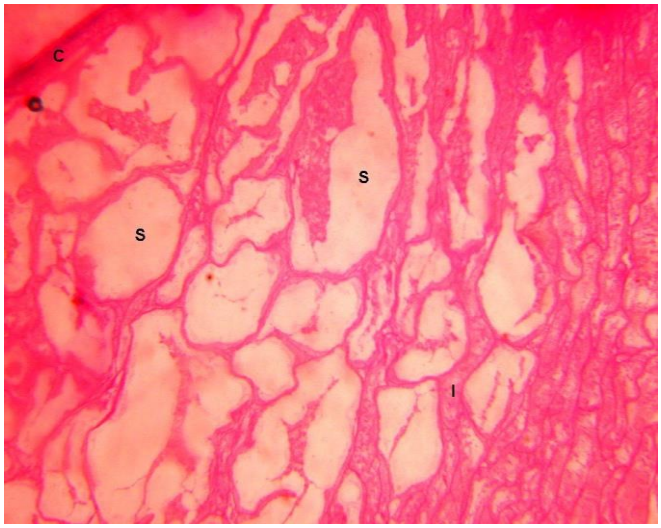


**Fig 1:** Testis of *C. gariepinus* exposed to *C. papaya* leaf powder at 0.00g/l. Note the seminiferous tubules (S) with thin layer of germinal cells (double arrow). Magnification: x100.

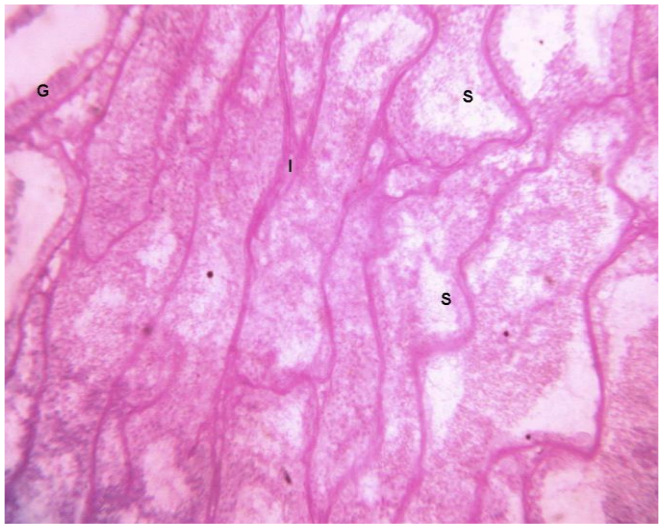


**Fig 2:** Testis of *C. gariepinus* exposed to *M. oleifera* powder at 1g/l. Note the seminiferous tubules (S) with thin layer of germinal cells (arrow). Magnification: x100.

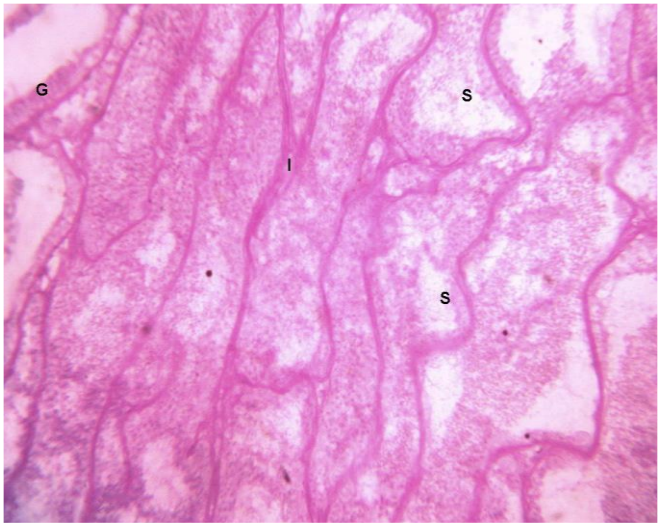


**Fig 3:** Testis of *C. gariepinus* exposed to *M. oleifera* leaf powder at 1.5g/l. Note the seminiferous tubules (S) with thin layer of germinal cells (arrow). Magnification: x100.

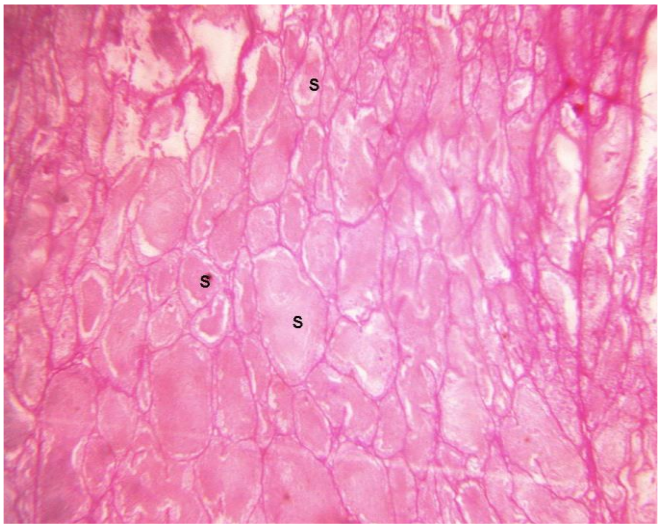


**Fig 4:** Testis of *C. gariepinus* exposed to *M. oleifera* powder at 2g/l. Note the distended seminiferous tubules (S) with thin layer of germinal cells (arrow). Magnification: x100.

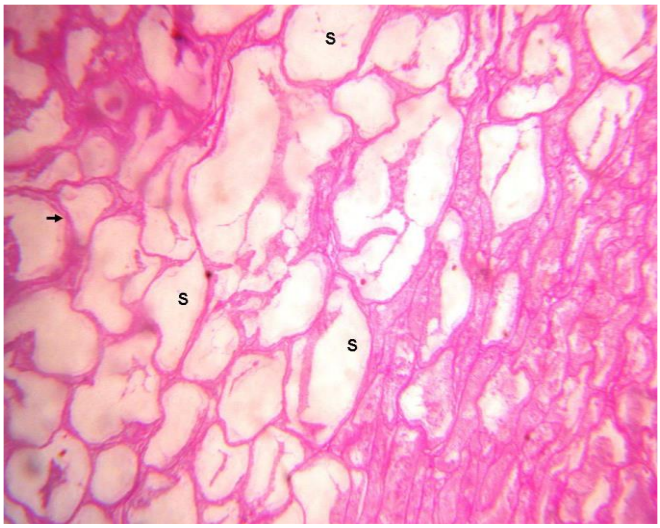


**Fig 5:** Testis of *C. gariepinus* exposed to *C. papaya* leaf powder at 1g/l. Note the seminiferous tubules (S) with thin layer of germinal cells (arrow). Magnification: x100.

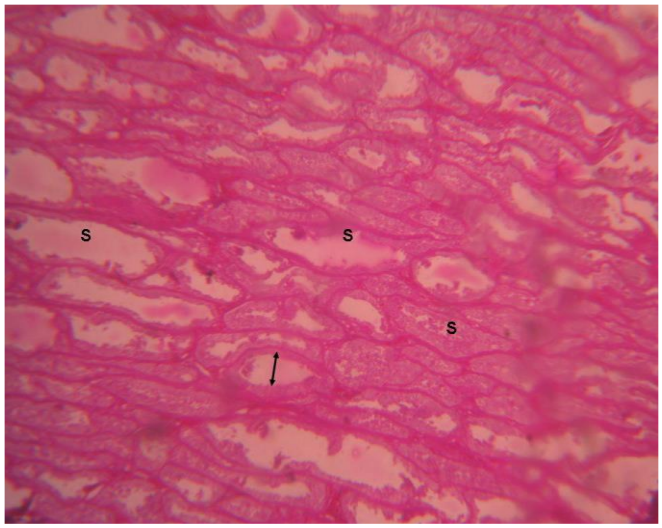


**Fig 6:** Testis of *C. gariepinus* exposed to *C. papaya* leaf powder at 1.5g/l. Note the seminiferous tubules (S) with thin layer of germinal cells (double arrow). Magnification: x100.

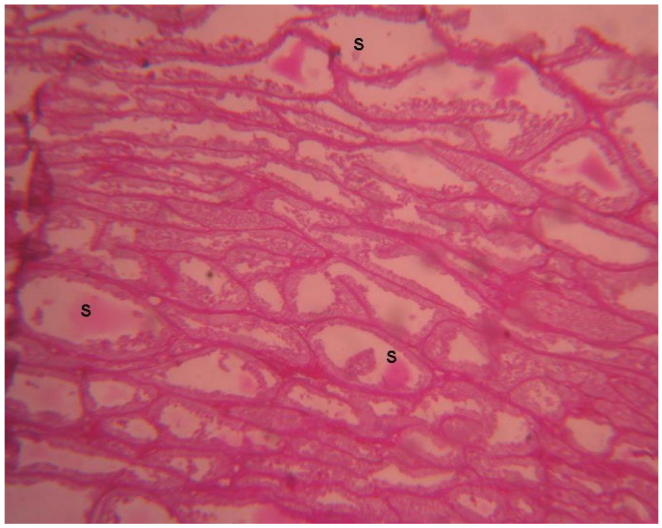


**Fig 7:** Testis of *C. gariepinus* exposed to *C. papaya* leaf powder at 2g/l. Note the seminiferous tubules (S) with thin layer of germinal cells (double arrow). Magnification: x100

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