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ORIGINAL ARTICLE

Reproductive Potential of Male Catfish Treated with Gel Extract of Aloe Vera Plant

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SUMMARY

The reproductive potential of male catfish treated with gel extract of Aloe vera plant was studied using twelve male fish weighing 500-560g.The fish were divided into 3 groups; A, B and C with four fish in each group. Group A was treated with 2% Aloe vera gel while group B was treated with 3% and Group C the control was treated with distil water. Blood and semen samples were collected for analysis and histology of the testis was done. Spermiogram revealed that as the concentration of the gel increased, the motility and sperm count decreased. Although the decrease in sperm motility was significant ($p<0.05$), the decrease in the sperm count was not significant ($p>0.05$) across the groups. Percentage liveability of sperm cells was significantly lower ($p<0.05$) in treated groups than control. The control group had higher semen volume, this volume was however not significantly different ($p>0.05$) from other treatments. Morphological studies showed that group B (3% group) had a significantly higher value ($p<0.05$) of total sperm abnormalities than the other groups. The best histological integrity of the testes was observed in group C (control).This study concludes that exposure of male C. gariepinus to all treatment levels of Aloe vera gel is detrimental to the reproductive potential of the catfish and could be a cause of infertility in the catfish.

Key words: *Clarias gariepinus, Aloe vera,* spermiogram and histology.

INTRODUCTION

Clarias is a genus of catfishes (order Siluriformes) of the family Clariidae, the air-breathing catfishes. They are of a wide variety of body shapes but all of them possess well-developed barbells, the whiskers, which gives them the common name catfish (Huisman and Richter, 1987).

They live in freshwater environments and are bottom dwellers and do most of their feeding there. They are also obligate air breathers, which mean they do spend some time on the surface. This species can live in very poorly oxygenated waters and is one of the last species to live in such an uninhabitable place (Bart and Dunham, 1990). They are also able to secrete mucus to prevent drying and are able to burrow in the muddy substrate of a drying body of water with pH range from 6.5 to 8.0 (Skelton, 1993 and Teugels, 1986).

The anatomical organization of the testis in catfish is variable among the families of

catfish, but the majority of them present fringed testis. In the testes of some species of Siluriformes, organs and structures such as a spermatogenic cranial region and a secretory caudal region are observed, in addition to the presence of seminal vesicles in the caudal region. The total number of fringes and their length are different in the caudal and cranial portions between species. Fringes of the caudal region may present tubules, in which the lumen is filled by secretion and spermatozoa. Spermatocysts are formed from cytoplasmic extensions of Sertoli cells; the release of spermatozoa is allowed by breaking of the cyst walls (Urbanyi *et al*., 1999).

Seminal vesicles are typically paired, multi-chambered, and connected with the sperm duct, and have been reported to play a glandular and a storage function. Seminal vesicle secretion may include steroids and steroid glucuronides, with hormonal and pheromonal functions, but it appears to be primarily constituted of mucoproteins, acid mucopolysaccharides, and phospholipids (Satish *et al*., 1999; Adeyemo *et al*., 2007 and Mazzoldi *et al*., 2007).

Aloe vera (also known as Barbados Aloe, Common Aloe, Yellow Aloe, and Medicinal Aloe) is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are lanceolate, thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower leaf surfaces (Davis *et al*., 1987). The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long (Gong *et al*., 2002). The aloe plant, being a cactus plant, is between 99 and 99.5 per cent water, with an average pH of 4.5. The remaining solid material contains over 75 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Obata, 1993 and Shelton, 1991)

Aloe vera is alleged to be effective in treatment of wounds (Vogler and Ernst, 1999). Some studies, for example, show that *A. vera* promotes the rates of healing (Davis *et al*.,1987 and Heggers,1996), while in contrast, other studies show that the healing time of wounds to which *Aloe vera* gel was applied were significantly slower (Kaufmann *et al*.,1988 and Schmidt and Greenspoon,1991). In addition to topical use in wound or burn healing, internal intake of *A. vera* has been linked with improved blood glucose levels in diabetics (Bunyapraphatsara *et al*., 1996 and Yongchaihudha *et al*., 1996), and with lower blood lipids in hyperlipidaemic patients. Compounds extracted from *A. vera* have been used as an immunostimulant that aids in fighting cancers in cats and dogs (King *et al*., 1995). *A. vera* extracts have antibacterial, antifungal activities and growth of fungi (Sumbul *et al*., 2004). The reported effects of the *Aloe vera* plant on reproduction in animals have been conflicting. Kushwaha (2013) described the latex of the plant as having inhibitory effects on reproduction. The aqueous extract of *A. vera* (*Aloe barbadensi*) was also reported by Oyewopo et al. (2011) to have antifertility effects on male rats while Modarasi and Khodadadi (2014) observed positive effects on the testosterone and histology of testes of mice treated with hydroalchoholic extract of the plant. Rodriguez et al. (1988) used the extracts of *A. vera* for dilution of semen for the artificial fertilization of sheep.

Considering these dissenting views on its effects on reproduction in general and inadequate documentation of the *Aloe vera* gel on the reproductive potential of *Clarias gariepinus* especially the male African catfish, this study seeks to determine the effects of *Aloe vera* gel extracts on the reproductive potential of male *C.*

gariepinus.

MATERIALS AND METHODS

Study Location: This experiment was carried out at the Experimental Animal Unit (EAU) of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State.

Experimental Fish: Twelve male *Clarias gariepinus* weighing between 500g-560g were procured from a commercial fish farm in Ibadan.

Experimental Plant: *Aloe vera*. The Leaves were harvested from the premises of the Experimental Animal Unit (EAU) in the University of Ibadan, Ibadan, Oyo State. The gel was extracted by washing and sterilising the freshly harvested *A. vera* plants with clean water and alcohol respectively. The serrated edges of the plants were cut and their green barks were stripped off with the use of a sharp knife. The white coloured gel was carefully skimmed out with the knife into a plastic bowl (which had earlier been sterilised with alcohol). The 2% and 3% concentrations (El Dakar *et al.,*2008, Oyeyemi *et al*., 2011) were obtained by adding 0.2ml of gel to 10mls of distilled water and 0.3ml of gel to 10mls of distilled water respectively. Each of the mixture was thoroughly homogenised with a spatula.

Experimental Design: Twelve *C. gariepinus* fish were grouped into three of four fish per group. The fish were reared semi-intensively in plastic containers. The three treatments; (A) 2%, (B) 3% and (C) 0% were randomly assigned to the fish. Groups A and B were orally dosed,with 2% and 3% concentration of *Aloe vera* gel extract while the group C served as control and were orally dosed with distil water. The three groups were fed the same amounts of feed daily for 5 days.

Experimental Conditions: The experiment was conducted under conditions of 12hour photoperiod and 12hour darkness. Well water was used.

Experimental Procedure: The fish were acclimatized for 24hours after they were introduced into the plastic containers. The fish were fed pelletized feed at 3% body weight twice daily. A dose volume of 1ml of the desired concentration was administered to each of the fish orally, using a 2ml syringe. After the experimental treatment period of 5days, the whole fish were taken to the laboratory where blood and milt were collected and analysed.

Laboratory Procedures:

Blood collection: 2mls of blood was collected from the caudal vein and dispensed into heparinised tubes for complete blood count. Blood analysis for packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), platelets, lymphocytes and neutrophils was carried out according to the methods described by MAF, (1984). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were extrapolated from PCV and Hb values.

Milt collection: Milt was collected by sacrificing the male fish by spinal transection, after which the belly was dissected and the testes removed. Sodium citrate solution (2.9%) was used to rinse away blood clots and other tissues. The testes were placed in the buffer solution pending the time of semen release. Semen was collected by gently crushing the testes and aspirating the released milt into a 5ml syringe. Collected milt samples were examined and evaluated as described by Zemjanis (1970).

TABLE I: Spermiogram of *Clarias gariepinus* in the three treatment groups

Table S/N	Motility	% liveability	Concentration(10^6cells/ml)	Volume(ml)	Weight(mg)
A	50.00±5.12[c]	45.00±7.07[a]	249.00±12.73[a]	5.65±(0.07)[a]	0.80±((0.26)[a]
B	30.00± 0.20[a]	83.75±17.50[a]	204.00±7.94[a]	5.73±(0.12)[a]	0.49±(0.08)[a]
C	85.00±7.07[b]	98.00±0.50[b]	278.00±4.24[a]	5.75±(0.07)[a]	0.70±(0.03)[a]

The letters a and b within a column represent degree of significance. Means with the same letter are not significantly different (p>0.05) while means with different letters are significantly different (p<0.05).
A-2% group B-3% group C- control(0%) group

Statistical Analysis

Data was subjected to statistical analysis and results were presented as mean and standard deviation. Findings were adjudged to be significant at p<0.05 (Adeyemo *et al.*, 2009).

RESULTS

Spermiogram (Table I) revealed that as the concentration of the gel increased the motility decreased. A significant difference (P<0.05) was observed in the motility (%) across the groups. Group C (85.00±7.07) had the highest motility followed by groups A (50.0±5.12) and B (30.00±0.20) respectively. The sperm count (10^6 spermatozoa/ ml) also decreased with increasing concentration of the *A. vera* gel. Group C (control) (278.00±4.24) had the highest sperm count followed by groups A (249.00±12.73) and B (204.00±7.94) respectively though the differences were not significant (P> 0.05).

Morphological studies (Table II) indicated that group C (6.50± 0.51) had the lowest number of abnormal sperm cells(%) while group B (11.03± 0.42) had the highest followed by group A (7.27 ±0.24).The values obtained for groups C and A were significantly different (P< 0.05) from group B.

Table 3 shows the result of haematology across the three groups. PCV (%) in group A (6.0± 1.41) was lower than B (9.0± 1.41) which was lower than group C (10.0± 1.41) but the differences were not significant (P> 0.05). Haemoglobin value (gm%) was observed in an ascending order with group A having 1.8± 0.14, B, 2.7± 0.28 and C, 3.1± 0.42. The RBC values (10^{12}/L) followed the same pattern. Significant differences (P< 0.05) were seen in the haemoglobin and RBC values of the three groups. WBC value (10^9/L) was lower in the group C (4.75± 0.21) than in A (5.6± 0.28) and B (5.00± 0.14) the differences between them were significant (P< 0.05). Neutrophils (%) in 2%, 3% and control groups were significantly different (p<0.05) from one another. The control had the least value (30.00±1.41) while the 2% group had the highest value (80.00±2.83). The 3% group had a value of 69.00±4.24. Lymphocyte count (%) revealed an ascending pattern of 2%, (20.00±1.41), 3%, 30.00 ±1.41, and control 40.00±2.83 with a significant difference (p<0.05) from one another. Platelet value (10^9/L) was highest in the group B (15.00±2.83), group C, 10.00±1.41 and group A had the least value of 8.00±1.41. The 2% and 3% groups are significantly different (p<0.05) from each other.

TABLE II: Morphological characteristics of spermatozoa of fish treated with varying Aloe vera gel concentrations

S/N	Tailless head	Headless tail	Rudimentary tail	Bent tail	Curved tail	Curved mid-piece	Bent mid-piece	Coiled tail	Looped tail	Total abnormal	Total normal	Total cell
A	0.82 ±0.01^{b}	0.57 ±0.13^{b}	0.74± 0.11^{ab}	1.15 ±0.02^{a}	0.74 ±0.11^{b}	1.39 ±0.14^{b}	1.39 ±0.09^{b}	0.33 ±0.24^{a}	0.17 ±0.01^{b}	7.27± 0.24^{b}	92.73 ±0.24^{a}	612.50 ±10.61^{c}
B	1.17 ±0.00^{a}	1.38 ±0.06^{a}	1.13± 0.18^{a}	1.42 ±0.23^{a}	1.05 ±0.05^{a}	2.05 ±0.17^{a}	2.18 ±0.01^{a}	0.29 ±0.06^{a}	0.38 ±0.06^{a}	11.03 ±0.42^{a}	88.97 ±0.42^{b}	1196.50±4.95^{a}
C	0.67 ±0.12^{b}	0.82 ±0.09^{b}	0.52± 0.09^{b}	1.04 ±0.02^{a}	0.82 ±0.09^{ab}	1.04 ±0.02^{b}	1.11 ±0.08^{c}	0.30 ±0.21^{a}	0.22 ±0.10^{ab}	6.50± 0.51^{b}	93.50 ±0.51^{a}	676.50 ±12.02^{b}

The letters "a", "b" and "c" within a column represent degree of significance. Means with the same letter are not significantly different ($p>0.05$) while means with different letters differed significantly ($p<0.05$)

TABLE III: Haematological result of the three groups

S/N	%PCV	gm% Hb	$X10^{12/L}$ RBC	$X10^{9/L}$ WBC	$X10^{9/L}$ Platelets	FL MCV	Pg MCH	% MCHC	% lym	% neut
A	06.00± 1.41^{a}	1.8.00.00 ±0.14^{b}	2.60±0 .28^{b}	5.60±0 .28^{a}	08.00±1 .41^{b}	23.00± 2.83^{a}	06.00± 1.41^{a}	30.00± 1.41^{a}	20.00± 1.41^{c}	80.00± 2.83^{a}
B	09.00± 1.41^{a}	2.7.00±00 .28^{ab}	3.02±0 .01^{ab}	5.00±0 .14^{ab}	15.00±2 .83^{a}	29.00± 1.41^{a}	9.50±2. 12^{a}	31.00± 1.41^{a}	30.00± 1.41^{b}	69.00± 4.24^{b}
C	10.00± 1.41^{a}	3.10±0.42^{a}	3.34±0 .00^{a}	4.75±0 .21^{b}	10.00±1 .41^{ab}	27.00± 1.41^{a}	08.00± 1.41^{a}	31.50± 0.71^{a}	40.00± 2.83^{a}	30.00± 1.41^{c}

The letters a, b and c within a column represent degree of significance. Means with the same letter are not significantly different ($p>0.05$) while means with different letters are significantly different ($p<0.05$).

LEGEND:

PCV-packed cell volume Hb-haemoglobin RBC- red blood cell

MCV- mean corpuscular volume MCH- mean corpuscular haemoglobin volume

MCHC- mean corpuscular haemoglobin volume Lym-lymphocyte Neut-neutrophil

HISTOPATHOLOGY

Histopathological examination of the testes of the three groups showed varying levels of disparities in the histology of the interstitia and germinal layers of the seminiferous tubules.

Fig. 1: The testes of the 2% group showed a reduction in the width of the interstitium. Mild disruption of the germinal compartments was also observed.

Fig. 2: The testes of the 3% group showed a marked degeneration and reduction of both interstitium and germinal cells (spermatozoa).

Fig. 3: The testes of the control revealed normal germinal layer and interstitium.

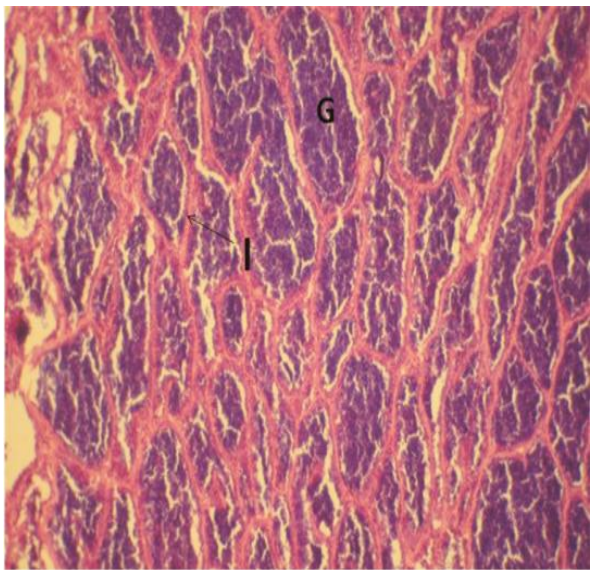

Figure 1: Cross-section of the testis of the 2% group
Testis of the 2% group showing an increase in the width of the interstitium (I) and a mild germinal layer(G) disruption. (100x)

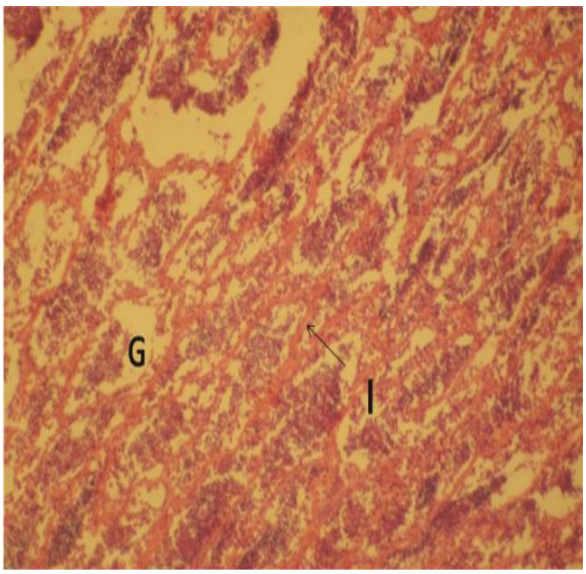

Figure 2: Cross-section of the testis of the 3% group
Testis of the 3% group showing marked degeneration of the interstitium (I) and germinal layer (G) of the seminiferous tubule (100x)

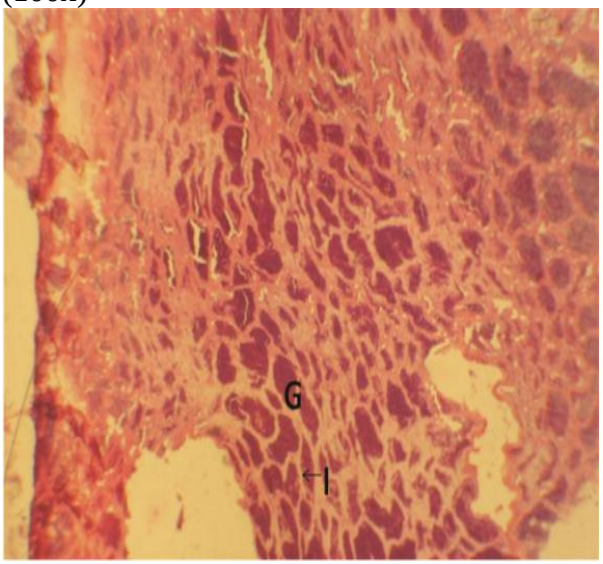

Figure 3: Cross-section of the 3 testis of the control group
Testis of the control group showing a normal interstitium (I) and germinal layer (G). (100x)

DISCUSSION

This study showed that the administration of *Aloe vera* gel extract to African catfish did not affect semen concentration and volume, the effect was however significant on liveability with the higher values recorded in the control (group C) than the treated groups. However, motility was significantly lower ($p<0.05$) in treated groups than control and sperm morphological abnormalities were significantly higher ($p<0.05$) in group B than groups A and C (Table I). This suggests that the *Aloe vera* gel possesses some anti-semen properties and exposure of animals to it especially in high concentration may adversely affect their semen characteristics (Oyeyemi *et al.*, 2011). This is similar to the report of Jorsaraei *et al*. (2008) who experimented with the extracts of ginger plants on human sperm, and found that the plant extracts can have adverse effects on sperm parameters especially when used in high concentrations. The values obtained for sperm morphological abnormalities (tailless head, headless tail, rudimentary tail, bent tail, curved tail, curved mid-piece, bent mid-piece, coiled tail, looped tail, total abnormal

sperm cells) from the treated groups were higher than those obtained from the control group. Also, these values were higher for the fish in 3% group than for those in the 2% group. This may be due to the fact that *Aloe vera* gel extract has toxic properties that are injurious to sperm cells and increases the risk of infertility and sterility (Oyewopo *et al.*, 2011). The effects of these properties of the extract on sperm morphology are more pronounced with increasing concentration of the extract. This is dissimilar to the report of Rodriguez et al. (1988) who used *Aloe vera* extract to dilute semen for artificial fertilization of ewes. The dissimilarity could be due to the differences in the nature of the two studies;this study was conducted in-vivo while Rodriguez *et al*. (1988) did theirs in-vitro.

The histological integrity of the testes was found to be compromised by the *Aloe vera* gel extract. The germinal layer and interstitium of the seminiferous tubules were normal for the control group while the germinal layer was disrupted and the width of the interstitium reduced in the treated 2% and 3% groups. This degeneration was more pronounced in the 3% group than the 2% group (Plates 2 and 1 respectively). This implies that *Aloe vera* gel extract is toxic to the male reproductive organ and the higher its concentration, the more toxic it is. This agrees with the findings of Kushwaha (2013) that *A. vera* latex caused histological damage to the gonads of *Oreochromis niloticus.* This is dissimilar to the report of Amin and Hamza (2006) who reported the effects of Roselle and ginger extracts against cisplatin-induced spermiotoxicity in rats, showing that not all plant extracts are injurious to spermatozoa.

The haematology of the catfish studied was not unaffected by the gel extract of the *Aloe vera* plant. There was no significant effect ($p>0.05$) on the PCV however there was a significant effect ($p<0.05$) on the lymphocytes and the neutrophils (Table III). The treated groups showed lesser values in packed cell volume (PCV), platelet count, haemoglobin concentration, red blood cell (RBC) count as compared to those of the control group. The reduction in platelet count may reduce the efficiency of the clotting process with the resultant increase in blood loss. This suggests that the gel extract possesses anticoagulant properties. The decreases in the PCV, haemoglobin and RBC values indicate that the gel extract may have anti- haematinic properties. The observed increase in neutrophils can be attributed to the effect of the gel on the immune system. Its bactericidal action stimulates the immune system particularly when there is an ongoing infection in the body, leading to a proliferation of neutrophils which are the main inflammatory cells seen in bacterial infections. Constituents of the *Aloe vera* gel such as anthraquinones (Sims *et al*., 1971) and salicylic acid confer upon the gel its antibacterial properties. The lymphocytosis observed is also a response to the effect of the gel extract on the immune system. *Aloe vera has* been found to cause immunomodulation (Green, 1996, and Sheets, 1991).

In conclusion, the results of this study show that *Aloe vera* gel has negative impacts on sperm motility, liveability concentration and morphology and thus detrimental to the fertility of fish. Therefore, its use in breeding fish or inclusion in fish feed or water should be done with caution.

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