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# Effect of Formalin on Spawning Success and Organ Histology in *Clarias gariepinus*

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## ABSTRACT

In Nigeria, the analytical grade formalin is indiscriminately used in the treatment of parasitic infestation in all fish species whereas paracide F (formalin plus methanol) has been approved for use in the USA, only on the eggs of salmonids and esocids. This study was carried out to determine the effect of therapeutic dose of formalin on spawning success and the histologcal changes induced in the organs of *Clarias gariepinus*. Two broodstock each of both sexes were used with each sex represented in both control and formalin treated fish. Treatment involved a bath method of exposure of treated fish to 2 mL of 37% formalin in 20 L of water on consecutive days for a maximum of three treatments with aeration at all times. Control fish were exposed to culture water. Weight, length and water quality parameters were determined and spawining was artificially induced with ovupin®. Fish were stripped and eggs collected and examined microscopically. Following fertilization; hatching and quality of hatchlings was determined. Histological sections of skin, liver, spleen and testes of control and formalin treated broodstock were made and examined microscopically. The eggs from formalin-treated broodstock had clumped edges and irregular shape compared to the normal well differentiated edges with round ovoid shape seen in eggs from the control. Eggs of formalin treated fish did not hatch 24 h after fertilization, while control did. Histological lesions observed in organ samples from formalin treated fish include generalized massive vacoulations of the skin, multifocal necrosis of hepatocytes, massive lymphoid depleted spleen and seminiferous tubules. Organs of the control fish revealed normal histological architecture. Utmost caution and minimal dosage is hereby recommended if formalin is required as a therapeutic agent in aquaculture.

**Key words:** Endocrine disruptors, *Clarias gariepinus,* formalin, reprotoxic effects, therapeutic agent

## INTRODUCTION

The African catfish (*Clarias gariepinus*) is highly appreciated as good aquaculture specie because of its resistance to disease, ability to tolerate a wide range of environmental parameters and relative fast growth rate (Goos and Richter, 1996). It is among the most widespread freshwater fishes in Africa (Nguyen and Jensen, 2002). Its culture in Nigeria is limited by problems of high mortality in fingerlings and the resulting seed scarcity. One of the prerequisite for domestication and establishment of a sustainable aquaculture industry is the seed for grow out of the marketable product (Mylonas *et al.*, 2010). Despite the high fecundity of *Clarias gariepinus*, the hatching rates of eggs in many hatcheries in Africa are erratic; ranging from 8-70% depending on the degree of sophistication of management in the hatcheries (Macharia *et al.*, 2005). One probable cause of erratic hatching is the parasitization of catfish eggs. The common practice is to routinely control them by using antiparasitic agents (Barnes and Gaikowski, 2004). However, ectoparasidal drugs can induce pathological lesions in tissues and organs depending on its dose and dosage (Everaats *et al.*, 1993). In Nigeria's aquaculture industry, chemicals used as fungicides include malachite green, formalin and sodium chloride. They can be used together or separately as anti-parasite treatments against ectoparasites such as *Gyrodactylus*, *Dactylogyrus*, *Ichthyobodo*, *Trichodina*, *Chilodonella* and *Ichthyophthirus* (Adeyemo *et al.*, 2011).

Paracide F, a preparation containing formaldehyde (37%) and methanol (6-13%) has been used as an effective fungicide in the USA; however, it is approved only for use on the eggs of salmonids and esocids (Piper, 1982). In Nigeria, the analytical grade of formalin is the type used to treat fish and disinfect eggs of all cultured fish species. There is therefore the need to assess the effect of prophylactic treatment with therapeutic dose of formalin on spawning success and sublethal histological alterations induced in the organs of *Clarias gariepinus*.

## MATERIALS AND METHODS

**Exposure of broodstock to formalin:** Two each, male and female broodstocks weighing 1.1±0.14 kg and with a total length of 19.5±0.58 cm, were purchased from a private fish farm in Ibadan, Nigeria. Fish were acclimatized for two weeks and fed commercially prepared pellets at 3% body weight. One male and female broodstock were each exposed to formalin. The other male and female broodstocks were not exposed to any chemical and were regarded as the control for the experiment. Formalin (37% concentration based on the active ingredient), was obtained from an agro-allied store in Ibadan, Oyo state. Formalin is usually used therapeutically by fish farmers as a bath at 0.15-0.25 ml $L^{-1}$ of culture water for up to 60 min on consecutive days for a maximum of three treatments. Hence, for the purpose of this experiment, experimental broodstock was exposed to 2 mL of 37% formalin in 20 L of culture water for 30 min on consecutive days for three treatments. Fresh preparation of formalin was made at each treatment and fish were returned to clean culture water after each exposure, while control broodstock were exposed to culture water only.

**Assessment of water quality:** Water quality assessment was carried out daily for both the treatment and the control. The water quality parameters determined are: alkalinity, ammonia, carbondioxide, chloride, dissolved oxygen, nitrite, pH and hardness. Water quality parameters were determined using Hach® water quality test kits.

**Artificial spawning of broodstocks:** Spawning was induced in the females (both treatment and control) using Ovupin® according to recommended manufacturer's dosage rate of 0.5 mL $kg^{-1}$. Twenty-four hours later, fish were stripped of egg into dry sterile petri dish. Egg samples were obtained for histological assessment. The remaining eggs were mixed with the milt from corresponding male broodstock and fertilization was activated with distilled water. Fertilized eggs were spread on carcaban in two separate flow-through hatching system for the treatment and control at a constant flow-rate of 3.5 L $min^{-1}$. The set-up was allowed to run for 24 h to allow for hatching of the fertilized eggs. Newly hatched frys swam into fresh water, while the unhatched and dead eggs were siphoned out. The flow-through system was allowed to run for 4 days, while regression of yolk sac, growth rate and abnormalities in hatchlings were monitored daily using camera-mounted light microscope.

**Histological assessment:** After sacrificing the male broodstocks to obtain milt; necropsy was performed and skin, liver, spleen and testes were harvested and preserve in Bouin's fluid for 24 h, after which tissues were fixed in 10% phosphate-buffered formalin until processing. Processing involved dehydrating tissues, putting them into a xylene phase and impregnating them with paraffin wax under vacuum. Following this process, the tissues were embedded in wax and sectioned on a microtome into 5 μm sections. Selected sections were floated and stretched on a hot-water bath, mounted on clean glass slides and placed on a warming tray to dry and adhere. Following staining with haematoxylin and eosin; sections were covered with a coverslip and mounted on a light-microscope for evaluation by the pathologist (Kiernan, 1990). Abnormalities were documented using a digital camera.

## RESULTS AND DISCUSSION

The results of the water quality parameter are presented in Fig. 1. Subsequent to three consecutive days of treatment of broodstocks with formalin days at therapeutic level in formalin with adequate aeration, stripped eggs didn't flow out as easy and fast as that of the female in the control. Also, the quantity of eggs stripped was considerably low compared to that of control. Normal eggs from the control were in clusters and had well-defined edges and ovoid shape (Fig. 2), while eggs from formalin treated broodstock were in clusters, but were clumped and had irregular edges (Fig. 3). The fertilized egg of the control hatched and developed normally (Fig. 4), while that of formalin treated broodstock did not.

**Histological alterations:** In formalin treated broodstock, compared to normal testes (Fig. 5), liver (Fig. 7), spleen (Fig. 9) and skin (Fig. 11) the histological alterations observed was disrupted and

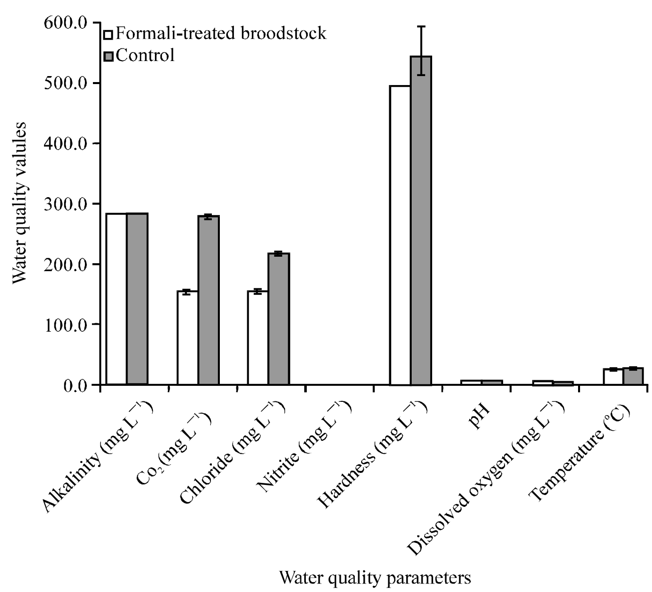


Fig. 1: Water quality parameters of formalin treated broodstock and control

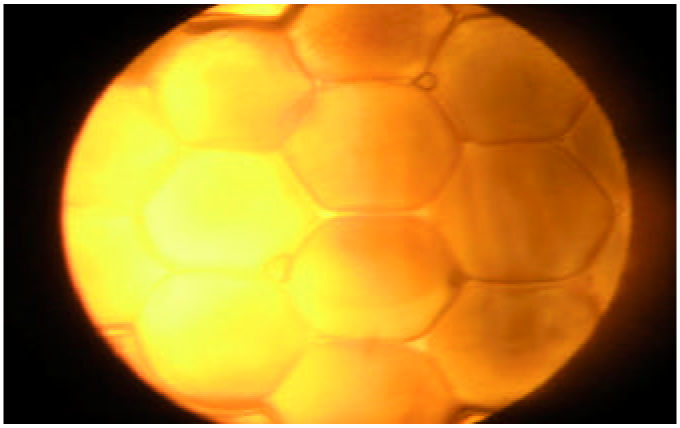

Fig. 2: Normal (control) eggs in clusters with well-defined edges and ovoid shape

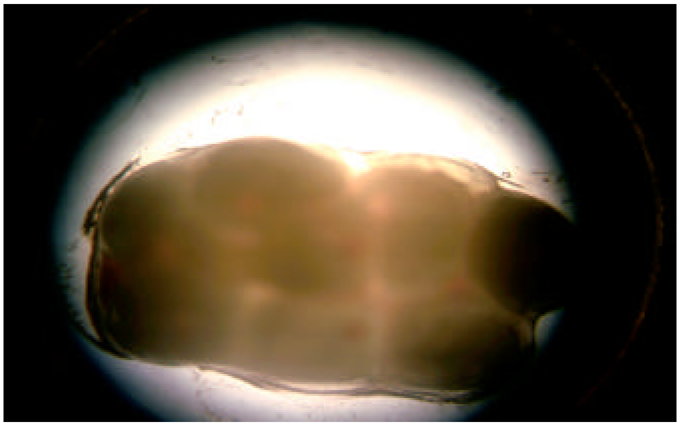

Fig. 3: Eggs from formalin treated broodstock in clusters with clumped and irregular edges

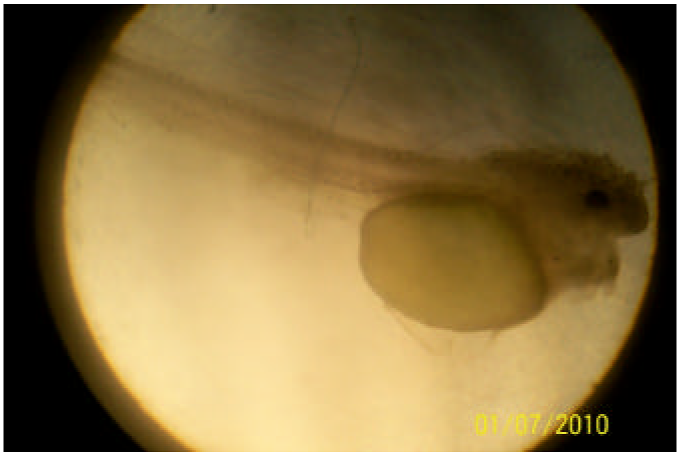


Fig. 4: Fish larvae 24 h after hatching with yolk and well differentiated eye in the control

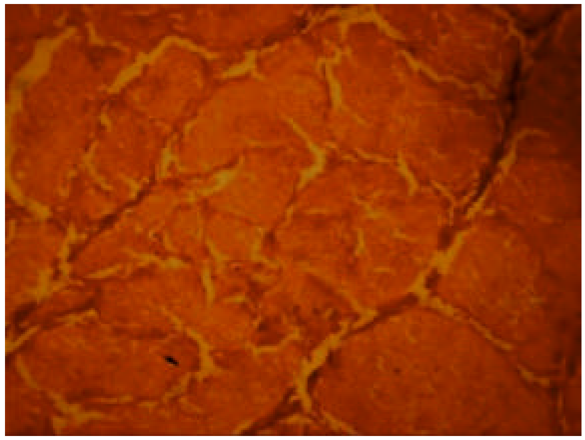

Fig. 5: Normal histology of the testes (H and E x 100)

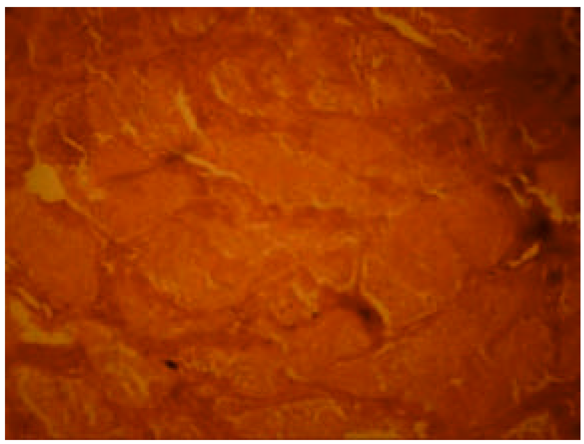

Fig. 6: Disrupted and depleted seminiferous tubules observed in formalin exposed broodstock (H and E x 100)

depleted seminiferous tubules (Fig. 6), multifocal necrosis of hepatocytes (Fig. 8), massive lymphoid depletion in the spleen (Fig. 10), necrosis and vacuolation of the skin of (Fig. 12), respectively. An important challenge currently facing the field of aquatic toxicology is to clearly identify and quantify population-level effects in fish exposed to endocrine disrupting chemicals (EDCs). Exposure of sexually differentiated fish to EDCs have been reported to result in a decrease in the bioavailability of sex hormones and gonadotropins (Bayley *et al*., 2003; Balch *et al*., 2004) which results in altered vitellogenesis in females, causing detrimental effects on oogenesis and egg quality, ultimately leading to developmental abnormalities, increased embryo and sac fry mortality and even spawning inhibition (Orlando *et al*., 2004).

The inability of the fertilized eggs from formalin exposed broodstock to hatch therefore suggests that formalin may have a disruptive effect on the reproductive process even at therapeutic dose. Several full life cycle tests with known xenoestrogens (ethinylestradiol and bisphenol A) have reported decreased hatching and swim-up success in offspring produced from adult exposed females, suggesting a possible link between exposure to xenoestrogens and decreased egg quality (Hill and Janz, 2003; Versonnen and Janssen, 2004; Tilton *et al*., 2005).

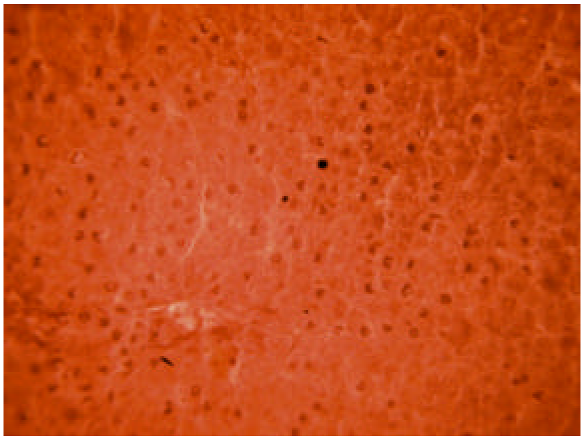

Fig. 7: Normal histology of Liver as observed in the control (H and E x 600)

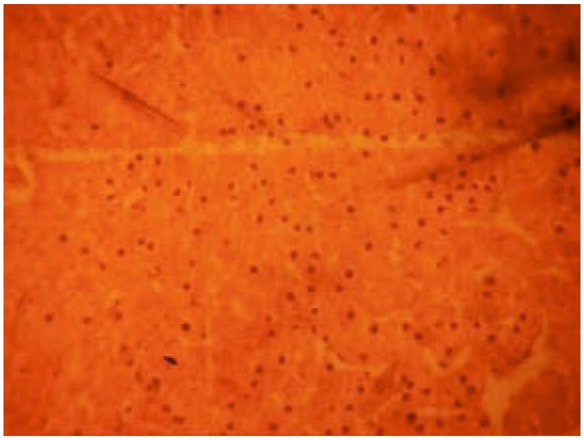

Fig. 8: Multifocal necrosis of hepatocytes in liver of formalin exposed broodstock (H and E x 400)

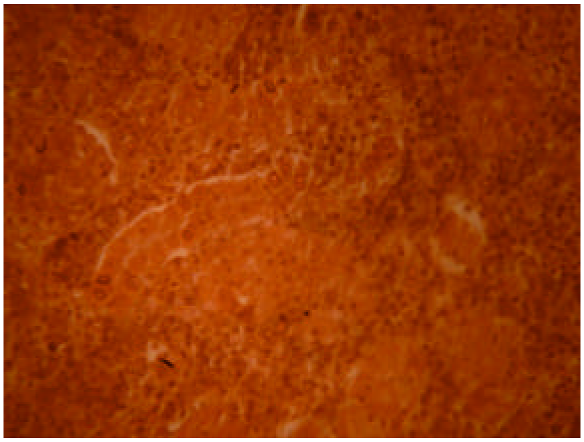

Fig. 9: Normal histology of spleen as observed in the control (H and E x 600)

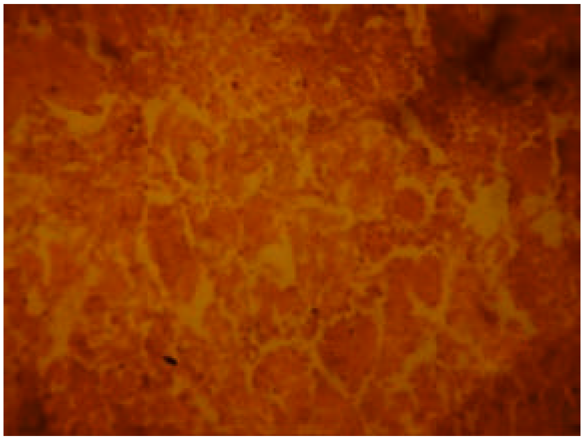

Fig. 10: Massive lymphoid depletion in the spleen of formalin exposed broodstock (H and E x 400)

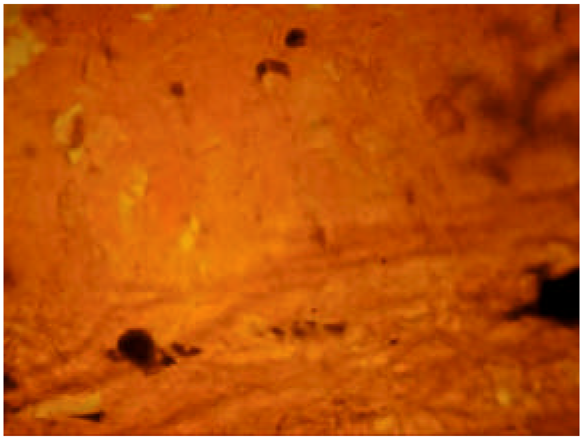

Fig. 11: Normal histology of skin as observed in the control (H and E x 600)

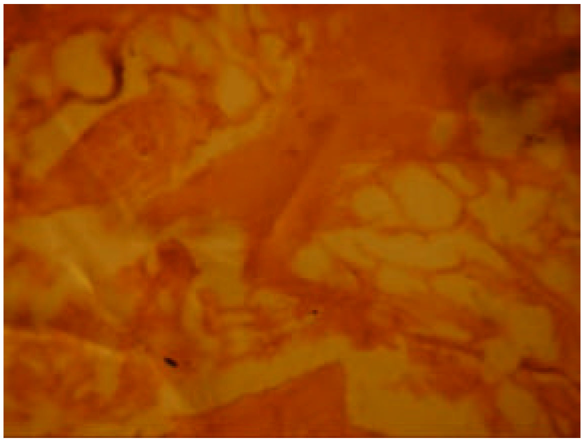

Fig. 12: Necrosis and vacuolation of the skin of formalin exposed broodstock (H and E x 400)

Histological examination of the skin revealed generalized massive vacoulation in formalin-exposed broodstock. which agrees with the findings of Buchmann *et al.* (2004) in which previous studies demonstrated extensive damage to epithelial structure of fin and change in skin composition due to formalin exposure. Williams and Wootten (1981) found cytoplasmic degeneration in the liver of Rainbow trout exposed to 200 mg $L^{-1}$ formalin for 96 h; hepatocyte and fatty degeneration was also observed in the liver of silver barb fry exposed to formalin at a concentration of 83.0 mg $L^{-1}$ for 96 h (FAO, 1988), similar to the multifocal necrosis of hepatocytes observed in formalin exposed broodstock in this study. There were no significant differences in the water quality parameters of culture water of control and formalin exposed brood stocks. However, $CO_2$, chloride and hardness was higher in the control.

## CONCLUSION

Formalin usage for prophylactic therapeutic treatment caused alteration of tissue histology. Further study is hereby recommended to determine its mechanism of action and withdrawal period after usage of formalin to avoid the reprotoxic effect observed in this study.

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