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Effects of pituitary extract, ovaprim, and bitter leaf (*Vernonia amygdalina*) on the histopathology of African catfish (*Clarias gariepinus*)

Blessing B. Ajayi[a], John O. Ogunsola[b,*], Olufemi I. Olatoye[a], Richard E. Antia[c], Samuel Agbede[a]

[a] Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan 200284, Nigeria
[b] Veterinary Teaching Hospital, University of Ibadan, Ibadan 200284, Nigeria
[c] Department of Veterinary Pathology, University of Ibadan, Ibadan 200284, Nigeria

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ABSTRACT

The development of aquaculture in Nigeria is constrained by the inadequate supply of high quality of fingerlings of African catfish (*Clarias gariepinus)*. There are conflicting reports on the reproductive efficacy of *Vernonia amygdalina* when compared with the synthetic hormone Ovaprim (OV) and Pituitary Extract (PE) on *C. gariepinus*. The histopathology of select organs in catfish treated with generic Ovaprim (OV), Pituitary Extract (CPE), and aqueous extract of *Vernonia amygdalina* (VAE) were evaluated. Sixteen female *C. gariepinus* (average weight of 1 kg) wereevenly and randomly distributed to four groups. Fish in groups A, B, C, and D were injected intramuscularly with aqueous solutions of OV (0.5 mL/kg), PE (5 mg/kg), VAE (10 mg/kg), and distilled water (DW; 0.5 ml), respectively. Histological examinations of the ovary, liver, kidney, and spleen were carried out. Histopathology revealed that OV- and PE-treated groups showed synchronous and synchronous ovarian development, vacuolar change of hepatocytes, thinning of hepatic cords, reduced renal haemopoietic compartments, tubular degeneration and necrosis, and decrease in splenic periarteriolar lymphoid sheaths (PALS). VAE-treated groups had asynchronous ovarian development with atretic ovarian follicles, widespread vacuolar change of hepatocytes, intact renal tubular and hemopoietic compartments, and preponderance of large and coalescing splenic PALS. We conclude that VAE is an ineffective spawning agent, especially when compared with OV and PE, as VAE could not induce synchronous gonadal development. However, VAE may have immunopotentiating, nephroprotective, and haemopoietic properties.

1. Introduction

The African catfish, *Clarias gariepinus,* is a highly demanded protein source in Nigeria and other parts of the world (Musa, Aura, Ngugi, & Kundu, 2012). It is the most important aquaculture fish species in Nigeria because it rarely succumbs to disease (Oladosu, Ayinla, Adeyemo, Yakubu, & Ajani, 1993), is able to survive hypoxic conditions, accepts pelleted feed, rapidly grows in captivity over a short time, and has ahigh market value (Adewolu & Adeoti, 2010).

The market demand for hybrid clariid catfish in Nigeria is increasing rapidly with an increased need for sustainability inNigeria's aquaculture industry (Ndimele & Owodiende, 2012). The availability of fast-growing fish fingerlings throughout the year remains a major constraint to farmers targeting high yields. The scarcity of fish fingerlings thus hinders the promotion and development of aquaculture in the country (Adewolu, Ogunsanmi, & Yunusa, 2008). The availability of fingerlings is dependent on spawning stock gonadal development and fecundity. This has led to the use of natural and synthetic materials to induce optimal breeding.

Induced breeding is a technique by which mature fish breed in confined waterand are stimulated by endogenous and exogenous hormone administration. In Africa, various hormonal substances, such as carp and frog pituitary extracts, human chorionic gonadotropin, and ovaprim have been used to induce breeding in fish with varying magnitudes of success (Chowdhury, Chatterjee, Mondal, & Chatterji, 2010; Karami et al., 2011; Okoro, Nwadukwe, & Ibemere, 2007).

Despite the varying magnitude of success recorded using these endogenous and exogenous hormonal inducers, there remain a few drawbacks. For example, several males must be sacrificed if catfish pituitary extract is used to induce spawning (Nwadukwe, Ayinla, & Abby-Kalio, 1993). Similarly, with ovaprim, residues in catfish may have safety complications following human consumption (FDA, 2009). Consequently, safer, natural, and healthier alternatives are sought.

The search for alternatives to these known natural and synthetic

* Corresponding author. Veterinary Teaching Hospital, University of Ibadan, Ibadan 200284, Nigeria.
E-mail address: ogunsolajo@yahoo.com (J.O. Ogunsola).

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Table 1
Summary of histopathologic findings of the ovary in African catfish (*Clarias gariepinus*) treated with ovaprim, pituitary extract, *Vernonia amygdalina* extract and distilled water.

	OV	PE	VAE	DW
Gonadal Developme-nt	Group synchronous	Synchronous	Asynchronous	Asynchronous
Previtelline Follicles	+ +	–	+	+ +
Vitelline/Mature Follicles	+ + +	+ + +	+	+
Intact Vitell. Env.	Y	Y	N	N
Post-Ovulatory Follicles	+	+ +	–	–
Atretic Follicles	–	–	+ +	–

Vitell. Env. Vitelline envelope OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water; - absent; + mild; + + moderate; + + + marked.

Table 2
Summary of histopathologic findings in the liver of African catfish (*Clarias gariepinus*) treated with ovaprim, pituitary extract, *Vernonia amygdalina* extract and distilled water.

	OV	PE	VAE	DW
Cord Atrophy	+	+	+ +	–
Vacuolar Change	+	–	+ + +	+
MMC	–	+	+ +	+
Hepatocellular necrosis	+	–	–	–
Vascular changes	+	–	+ +	+

OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water; - absent; + mild; + + moderate; + + + marked; MMC melano-macrophage centres.

spawning-inducing hormones has turned the attention of researchers to medicinal plants (Oyeyemi, Ajala, Leigh, & Adesiji, 2008). The use of plants as fertility enhancers in aquaculture is receiving more attention because they are safe to both fish and consumers and available year-round in the tropic and sub-tropical regions (Francis, Akinlolu, & Kehinde, 2013). *Vernonia amygdalina* (more commonly known as bitter leaf), a perennial shrub belonging to the family Asteraceae, is one plant that has received some attention as a possible alternative to the currently available inducers of spawning (Ajala & Owoyemi, 2016; Ijeh & Ejike, 2011).

There are, however, conflicting reports on the efficacy of this plant for boosting reproductive potential and fecundity in various animals. This plant has been reported to have a positive effect in rats (Oyedeji, Bolarinwa, & Azeez, 2013; Oyeyemi et al., 2008). Using these leaves as feed was reported to enhance fertility in male *Heterobranchus bidosarlis* broodstock (Francis et al., 2013). However, Olatoye, Ajayi, Ogunsola, and Agbede (2018) reported an inability of *V. amygdalina* to induce spawning in *Clarias gariepinus*. Literature on the pathology of reproductive organs in catfish treated with inducers of spawning is scant (Blazer, 2002; Karami et al., 2011).

The aim of this study was to evaluate the histopathology of select organs of the African catfish treated with *Vernonia amygdalina* extract (VAE) with catfish pituitary extract (PE), and Ovaprim (OV), and to suggest mechanisms by which these treatments affect the reproductive parameters of the African catfish.

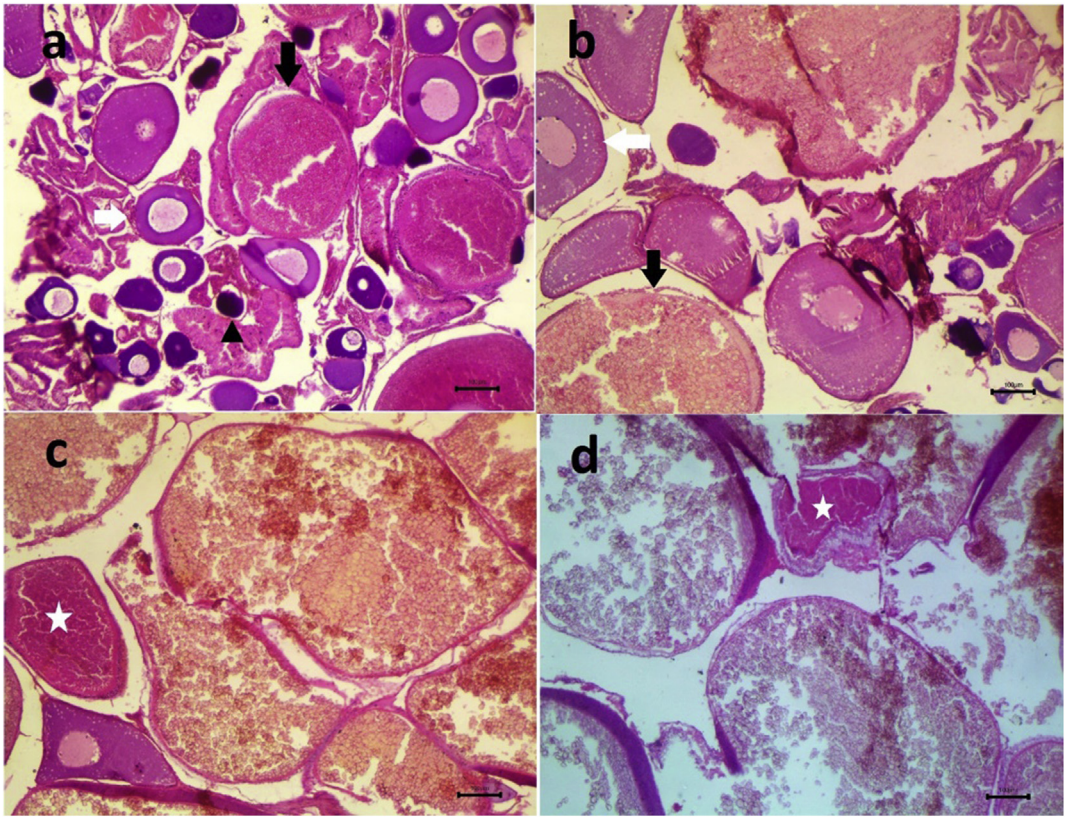

Fig. 1. African catfishovary. **(a)** OV-treated: There are numerous developing (black arrows) and mature (white arrow) follicles. Some pre-vitellogenic follicles are necrotic (arrow heads) **(b)** PE-treated: There are numerous late vitellogenic (black arrows) and mature (white arrow) follicles **(c)** VAE-treated and **(d)** DW-treated. There are mostly irregular mid-vitelline follicles with egg-yolk globules. Note the atretic misshapen follicles (star). H&E Bar equals 100 μm; OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water.

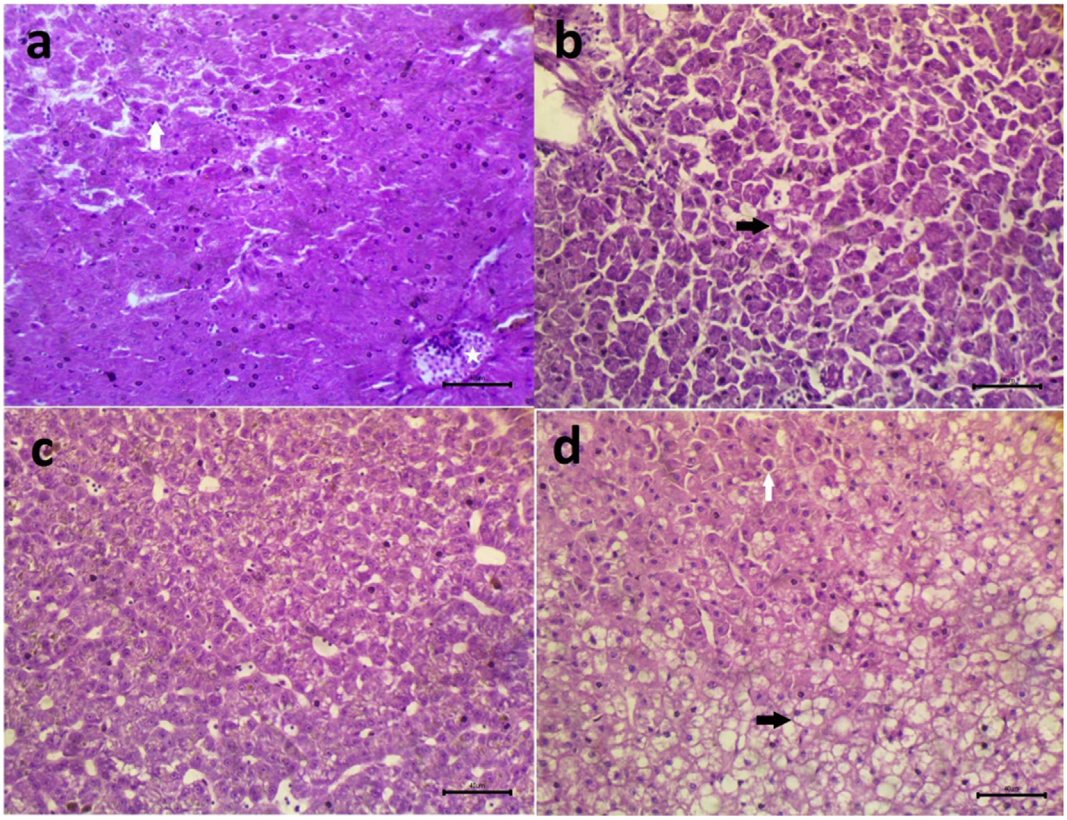

Fig. 2. African catfishliver. **(a)** OV-treated: There are a few foci of single-cell hepatocellular necrosis (black arrow) and thinning of hepatic cords. Note the congested blood vessel (star) **(b)** PE-treated: There are a few foci of mild vacuolar change (white arrow) and slightly distended hepatic sinusoids **(c)** VAE-treated: Hepatocytes generally have a finely reticulated cytoplasmic appearance **(d)** DW-treated: There are locally extensive foci of vacuolar change of hepatocytes (white arrow) as well as a few foci of random single-cell hepatocellular necrosis (black arrow). H&E; Bar equals 40 µm; OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water.

Table 3
Summary of histopathologic findings in the kidney of African catfish (*Clarias gariepinus*) treated with ovaprim, pituitary extract, *Vernonia amygdalina* extract and distilled water.

	OV	PE	VAE	DW
Glomerular changes	–	–	–	–
Haemopoietic compartment	+	+ +	+ + +	+ +
Tubular necrosis	–	+	–	+
Cloudy swelling of tubular epithelium	+	+ + +	–	+ +
MMC	+	+	+ +	+ +
Vascular changes	+ + +	+	+	+

OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water; - absent; + mild; + + moderate; + + + marked; MMC melano-macrophage centres.

2. Materials and methods

2.1. Experimental fish

Twenty African catfish brood stock, 4 males and 16 females, weighing an average of 1.0 kg, were purchased from a fish breeding farm. They were weighed and acclimatized for 3 days in makeshift well-aerated plastic tanks. The same source of water was for each tank and their water was changed daily. Physico-chemical properties (pH, dissolved oxygen) and ambient temperature were optimal as described by Adewolu et al. (2008) and consistent across all groups. The fish fasted for 24 h prior to the treatment, spawning, and organ harvesting. The experiment was repeated in triplicate at the Fish Diseases Laboratory unit of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria.

2.2. Synthetic hormone

Ovaprim (OV) was purchased as a ready-to-use product. The solution contains an analogue of 20 µg of Salmon gonadotropin releasing hormone (sGnPHa) and a dopamine antagonist, domperidone, at 10 mg/mL and administered at the dosage of 0.5 mL/kg.

2.3. Pituitary gland extraction

The four male catfish were euthanized and the pituitary gland was extracted with a sterilized needle. The pituitary gland was crushed and 0.9% normal saline solution was added to make the pituitary extract (PE) suspension and a final concentration of 5 mg per kg body weight of treated fish. Each preparation was done minutes before injection.

2.4. Vernonia amygdalina extraction

Leaves of *Vernonia amygdalina* were obtained from a small-scale farm in Ibadan, Oyo state and were identified and authenticated at the Herbarium Unit, Botany Department, University of Ibadan with identification number UIH-22618. The plants were sun-dried, pulverized, sieved, and weighed.0.9% normal saline was added to specific weight of VAE to obtain a final concentration of the VAE aqueous extract as 20 mg/mL.

2.5. Experimental procedure

The female fish were divided into four groups of four fish each. Fish

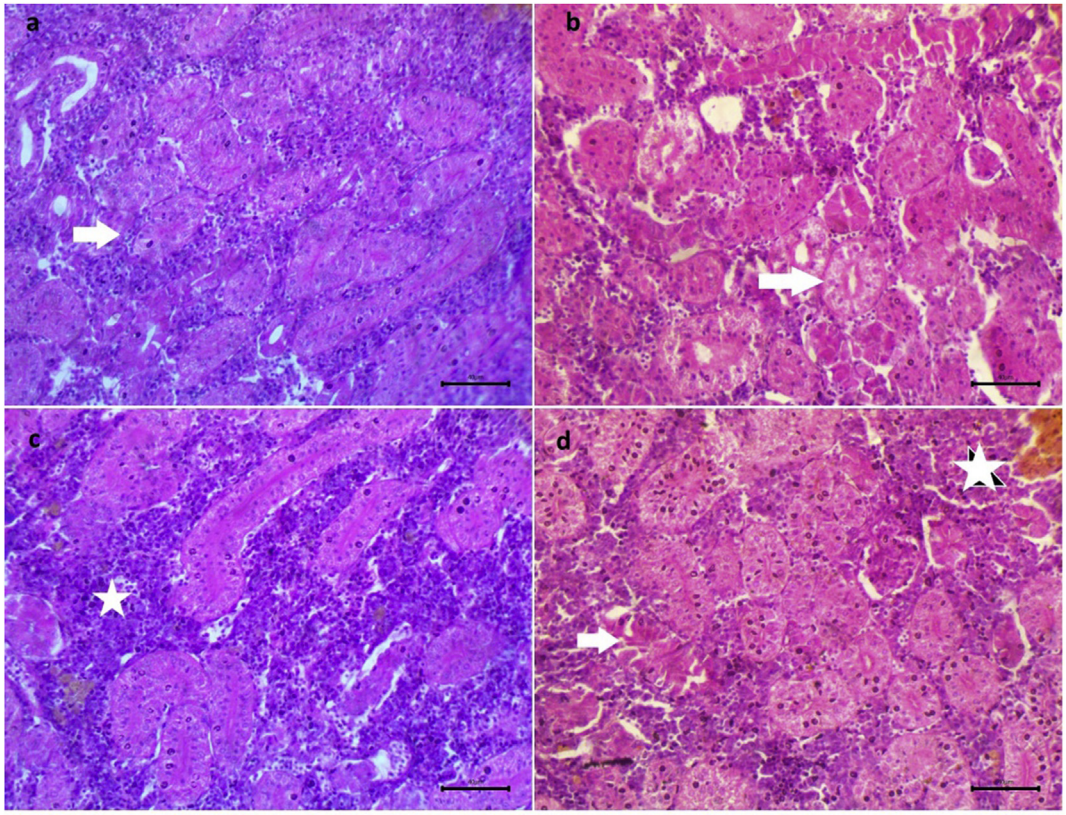

Fig. 3. African katfishkidney. **(a)** OV-treated: A few foci of tubular degeneration (arrow) **(b)** PE-treated: Multiple foci of tubular degeneration and necrosis (arrow) **(c)** VAE-treated: Tubular compartment is devoid of lesions while the haemopoietic compartment (star) is relatively increased when compared with *a, b* and *d* **(d)** DW-treated: Locally extensive foci of mild cloudy change of tubular epithelial cells. H&E; Bar equals 40 µm; OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water.

Table 4
Summary of histopathologic findings in the spleen of African catfish (*Clarias gariepinus*) treated with ovaprim, pituitary extract, *Vernonia amygdalina* extract and distilled water.

	OV	PE	VAE	DW
PALS	+	+	+ + +	+
MMC	+	+ +	+ +	+ +
Vascular congestion	+	+ +	+ +	+ +

OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water; - absent; + mild; + + moderate; + + + marked; PALS periarteriolar lymphoid sheath; MMC melanomacrophage centres.

in Group A were injected with 0.5 ml OV (0.5 mL/kg) each, fish in Group B were injected with 0.5 mL PE (5 mg/kg), fish in Group C were injected 0.5 ml of VAE (10 mg/kg), and fish in Group D (which served as controls) were injected with 0.5 mL distilled water (DW) each. The intramuscular injection was made above the lateral line toward the dorsal section and pointed towards the ventral side as described by Olatoye et al. (2018). The brood stock fish were reared in a water tank at room temperature for 12 h prior to euthanization.

2.6. Histopathology

Ovary, liver, kidney, and spleen samples from catfish in each group were collected and fixed for 24 h in Bouin's fluid, then transferred into a 10% formalin solution. The tissues were processed in an automatic tissue processor and blocked in molten paraffin wax. Five micrometer thin sections were cut with a rotary microtome. The sections were then stained in haematoxylin and eosin (H&E) and mounted on slides with Canada balsam. Slides were then viewed under the light microscope and representative photomicrographs were obtained with the aid of an Amscope [R] digital camera fitted to the microscope. The lesions observed were scored based on severity as absent, mild, moderate and marked by two independent examiners/histopathologists.

2.7. Ethical consideration

All experiments were carried out in accordance with the animal welfare care of experimental animals and approved by the Animal Care Research Ethics Committee (ACUREC), University of Ibadan, Nigeria.

3. Results

3.1. Histopathology of the ovary

Ovarian stage of follicular development ranged from asynchronous in VAE-treated and DW-treated fish to group synchronous in OV-treated fish PE-treated fish. Details of the ovarian histopathologic features in African catfish treated with OV, PE, VAE, and DW are presented in Table 1 and Fig. 1a – d. Numerous developing and mature follicles and a few necrotic pre-vitellogenic follicles were present in the ovaprim-treated groups and numerous late vitellogenic and mature folliclesin PE-treated fish. In both VAE-treated and DW-treated fish, there were mostly irregular mid-vitelline follicles with egg-yolk globules. A few atretic misshapen follicles were frequently encountered.

3.2. Histopathology of the liver

The histopathologic findings in the liver were mostly subtle and mild. The lesions included thinning of hepatic cords with resultant dilated sinusoids, vacuolar change of hepatocytes, and variation in the

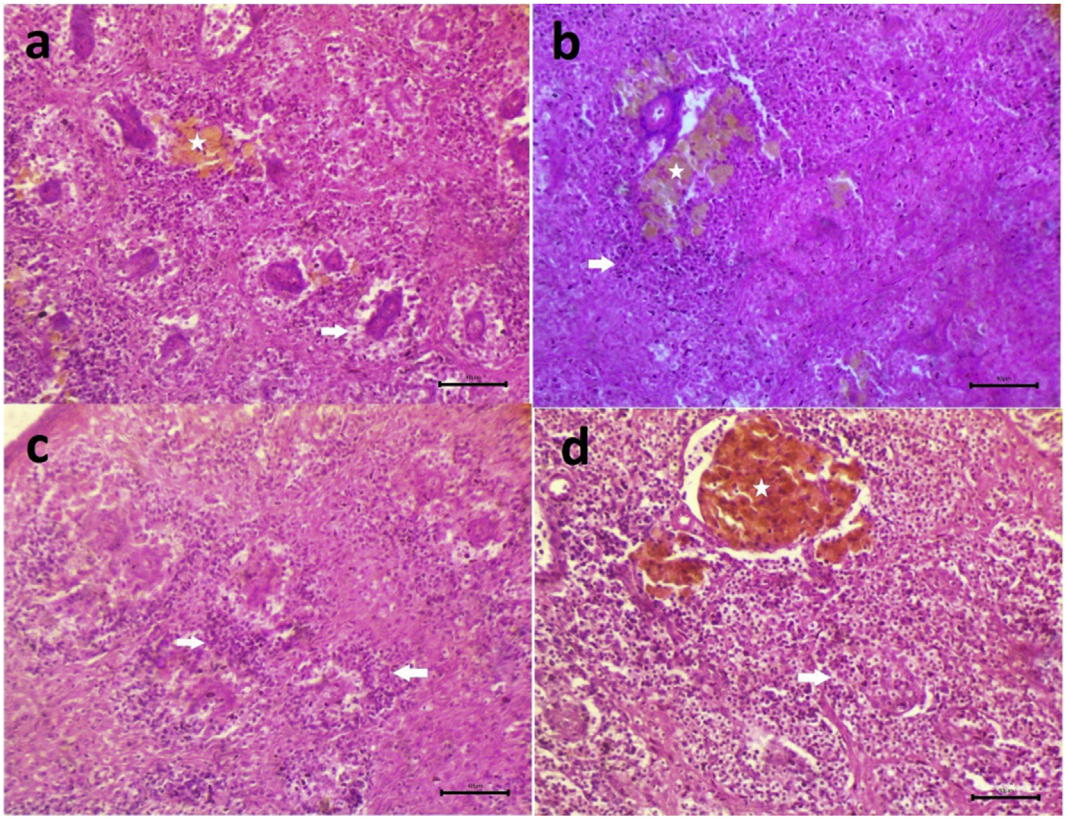

Fig. 4. African catfishspleen. The periarteriolar lymphoid sheaths are depleted in **(a)** OV-treated and **(b)** PE-treated; coalescing in **(c)** VAE-treated; and moderate in **(d)** DW-treated. Note the melanomacrophage centres (star) that are present in *a, b,* and *d.* H&E; Bar equals 40 µm; OV ovaprim; PE pituitary extract; VAE *Vernonia amygdalina* extract; DW distilled water.

quantity and size of melano-macrophage centres (MMCs). Details of the hepatic histopathologic features in African catfish treated with OV, PE, VAEs, and DW are presented in Table 2 and Fig. 2a – d.

3.3. Histopathology of the kidney

Histopathologic changes in the kidney included variation in the relative proportions of haemopoietic and tubular compartments, varying degrees of tubular degeneration and necrosis, variation in the size and quantity of the renal MMCs, as well as vascular changes. Details of the renal histopathologic features in African catfish treated with OV, PE, VAE, and DW are presented in Table 3 and Fig. 3a – d.

3.4. Histopathology of the spleen

Histopathologic changes recorded in the spleen across the four groups included variations in the size (ranging from small, to large, to coalescing) of the peri-arteriolar lymphoid sheaths (PALSs), variations in size and abundance of the melanophage centres (MMCs), as well as varying degrees of vascular congestion. Details of the splenic histopathologic features in African catfish treated with OV, PE, VAE, and DW are presented in Table 4 and Fig. 4a – d.

4. Discussion

Literature on the effects of VAE on reproductive organs of the African catfish is scant (Ajala, Adedoyin, & Aina, 2015). However, it is replete with studies on the histology of the ovary in various species of fish. Histologically, development of oocytes have been described by Blazer (2002) as synchronous (where majority of oocytes are in same stage of development), group synchronous (where there is a pre-vitellogenic resting stage as well as another uniform stage of oocyte maturation), and asynchronous (where developing oocytes can be in as many as four stages). In this study, fish treated with VAE and DW had asynchronous gonadal development. Fish treated with OV and PE had group synchronous and synchronous gonadal development, respectively. VAE-treated fish had disrupted vitelline envelope of the oocytes as well as atretic follicles. This would suggest that VAE injection did not aid folliculogenesis in the African catfish. This might be responsible for the inability to spawn even after application of mechanical pressure reported by Olatoye et al. (2018). Conversely, known spawning agents, such as OV and PE, revealed numerous synchronously-developing ovarian follicles at histology, similar to those by Chowdhury et al. (2010), Karami et al. (2011). Although no direct comparison has been made between the effect of OV and PE on the spawning ability of catfish, the finding that PE-treated fish had synchronous gonadal development (compared to the group synchronous development in OV-treated fish) and relatively higher numbers of post-ovulatory follicles would suggest that PE is a better inducer of folliculogenesis and spawning than OV.

In the liver, fish treated with VAEhad widespread vacuolar change of hepatocytes. Although diet can influence the histologic appearance of the hepatocytes in fish, finding cytoplasmic vacuolation with the nuclei in an eccentric position characterizes vacuolar or fatty degeneration of hepatocytes, similar to Ajala et al. (2015). Interestingly, VAE appears to be highly antigenic. Fish treated with VAE had numerous splenic coalescing PALSs. This is suggestive of lymphoid proliferation following antigenic stimulation. This might suggest an immuno-potentiating effect of VAE. Fish treated with OV and PE on the other hand, did not induce the formation of coalescing PALSs in the spleen.

VAE effects might not be limited to inducing lymphoid proliferation as seen in the spleen. It does cause a relative increase in the haematopoietic compartment in the kidney. This would suggest a role for VAE in haematopoiesis. Results obtained in this study is similar to the

findings of Eyo et al. (2013), who reported VAE to cause an increase in leukocytes of albino rats through many cytokines regulations. Also, tubular degeneration and necrosis which was present in controls and fish treated with OV and PE, was absent in VAE-treated fish. This would suggest a nephron-protective role for VAE.

5. Conclusion

VAE could not induce synchronous gonadal development in *Clarias gariepinus*. This would explain why VAE is ineffective as a spawning agent, unlike OV and PE. Despite its ineffectiveness in synchronous gonadal development, VAE appears to be strongly antigenic, immunopotentiating, nephro-protective, and an agonist for haematopoiesis.

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