



Influence of *Aloe barbadensis* (Miller 1768) Extract on White Blood Cells Counts as Immunological Parameters of Adult *Heterobranchus Bidorsalis* (Geoffroy Saint-Hilaire 1809)

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ABSTRACTS

Aloe barbadensis extract was incorporated in the feeds of adult *Heterobranchus bidorsalis* at concentrations of 0.0, 25.0, 50.0, 75.0, 100.0, and 125.0 mg/Kg of feeds in triplicates. Fresh gel was mixed with feeds and sundried. The fish were fed at 6% body weight divided into two rations per day. The fish were fed for 14 days before being analysed for hematological parameters such as total white blood cell count, lymphocyte, neutrophils counts, eosinophils counts, basophils counts as well as monocyte counts. The monocytes and eosinophils were significantly lower ($p < 0.05$) in the treated fish while basophils were not encountered at all. The neutrophils and lymphocytes being the immune responsive leucocytes were increased in circulation due to *Aloe barbadensis* exposure. *Aloe barbadensis* extract is therefore recommended as fish supplements at concentration of 50.0 to 75.0 mg/Kg of feeds for adult *Heterobranchus*

Keywords: *Aloe barbadensis* extract, leukocytes, immune status, adult *Heterobranchus bidorsalis*,

The name aloe probably stems from Arabic word Alloeh meaning “shining bitter substance”. These are succulent plants that probably originated in Northern Africa. There is no naturally occurring population. It has been used in herbal medicine since the beginning of the first century AD, as it is mentioned in the New Testament of the Bible.

They are widely used in the cosmetics and alternative medicine industries today because of their rejuvenating, soothing, effectively decreasing inflammation and promotion of wound healing due to their effects on white blood cells and antioxidant property (Akinye *et al.*, 2003). Many agents have been isolated, identified and demonstrated to synergize with one another in many health remedial activities such as anti-viral, anti cancerous and boosting of immunity. It has been demonstrated to enhance the immune system's responses to cancer, promote the growth of new and healthy cells and reduce the over all viral load within the body thereby revitalizing the body in its fight against cancer (Krumar *et al.*, 2002). Its salicylic acid is aspring-like and prevents the biosynthesis of prostaglandins from arachidonic acid, which explains in part, why it reduces vasodilation and decreases the vascular effects of histamine, serotonin and other mediator of inflammation (Yagi and Machil, 2005).

Aloe barbadensis is one of the most widely used healing plants in the history of mankind for the treatment of disorders such as arthritis, gout dermatitis, peptic ulcer and burns. It contains Anthraquinones, aloin, endin and saponins found exclusively in the plant sap, which like vitamins, are useful in small quantities. They aid absorption from the gastrointestinal tract and have anti-microbial and pain killing effects. Saponins form about 3% of the Aloe gel. Cholesterol, campesterol, b sisioterol and lupeol are plant steroids and are important as anti-inflammatory agents. Aloe gel provides 20 of the 22 amino acids needed in human body and seven out of the 8 essential amino acids (Pittman *et al.*, 2002).

Good health is a resultant of both internal and external environments and involves absence of pathogenic organism. Internal environment include antioxidant agents to combat free radicals and direct stimulation of immune system cells that include white blood cells and immunological parameters such as lymphocytes, monocytes and microphages.

Haematological variables are used for clinical diagnosis of fish physiology, which is determined by the effects of external stressors and toxic substances as a result of the close association between the circulatory fluid and the external environment (Adeyemo 2005 and Fischer *et al.*, 2006). Bacterial parasitic infections equally alter the haematological parameters of fish (Martins *et al.*, 2004; and Martins *et al.*, 2008).

Baker *et al.* (2001) pointed that packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) mean cell volume (MCV) vary with age and health status of the individual. Factors which increase the MCV include megaloblastic and chronic haemolytic anaemias in human. Similar observations were made experimentally by toxicity test of Diaxinon on common carp by Svoboda *et al.* (2001).

Weeks *et al.* (1986) has described a marked increase in the number of macrophages in fishes living in polluted waters. Phagocytosis of bacteria by monocytes and neutrophils is increased in the increase presence of these foreign cells. Baker *et al.*

(2001) explain that increased neutrophil population could be detected in circulating blood during stress. This is because the emarginated pool (the population of neutrophil which role along the walls of the blood vessels) is released into the circulating pool to enhance more frequent phagocytosis. It was observed that chitinolytic and bacteriolytic enzymes (lysosomes) which may give protection against chitin containing parasite are rich in oesophageal granulocyte – producing tissues (Osman and Caceci, 1991).

A shift from polymorphic white blood cell to monomorphic types signifies that the older cells (polymorphic) are being destroyed (Barker *et al.*, 2001). The monomorphic types, which are younger cells, are on increase production to fight the agent of blood cell destruction. This shows increase in their percentage. On the other hand, if new white blood cells are not produced, there would be increase in the percentage of old cells with polymorphic nuclei, which is in the case in a specimen whose immune system is not disturbed.

Clariids are the second most cultured group of fishes in Nigeria (Adekunle, 2011, Oluyemi *et al.*, 2008 and Offem *et al.*, 2010). They are promising candidate as fish products that will narrow the gap between fish demand and supply. *Heterobranchus* and other clariids have no standardized blood pictures in literature as it is for other species (Ellis, 1977; Palikova *et al.*, 1999; Santhakumar *et al.*, 1999; Oluah and Mgbenka, 2004; Ibiwoye *et al.*, 2006; Pealic *et al.*, 2013) in spite of their high advantage as a culture species. They have good eating quality, command high price, rarely dies due to transportation stress, grow fast in captivity due to its acceptance of artificial feeds and can endure harsh environmental conditions. But the present lag between demand and supply is disturbing. Means of improving growth in fish is needed. One way of doing this is to improve the health status of the fish by investigating into factor that improves growth for fast production of animal protein biomass. One such and very important factor is the blood picture.

MATERIALS AND METHODS

Aloe extract

Aloe barbadensis (fresh) were collected and identified using keys of (Dutta, 1981, Random access identification key by Burke Museum of natural history and culture 2003). The leaves were washed with water and cut transversely into pieces. The thick epidermis was removed and the gel in the center of the leaf was homogenized. The mucilage was mixed with feeds in measured amounts for oral administration. The mixture was sun dried to allow the extract be absorbed into the feeds. The fish were fed at 6 % of their body weight for 14 days. The feeds of pellet size 3 to 4 mm were obtained from COPPENS, www.coppens.eu. The daily ration was divided into two (3 % body weight) and fed at 10 a m and 4 p m (Gilbert, 1996 and Ajani *et al.*, 2007).

Fish specimens

Heterobranchus bidorsalis of length 243.64 ± 20.31 g and weight 40.56 ± 0.97 cm were obtained from the Fish farm of Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Forestry, Cross River University of Technology Nigeria. These were acclimatized for two weeks in the laboratory of The Department of Fisheries and Aquatic Sciences (CRUTECH). The fish were divided into groups of ten fish per group. Three groups each were assigned to a particular treatment for the purpose of replication. Plastic drums of 44 cm surface diameter and 100 cm height were filled with stream water up to 45 cm level giving a volume of $\delta r^2 h = 152$ litres of water per tank. Pond water was used in culturing the fish for fourteen days.

Collection of blood samples

At the end of the 14 days administration with the plants extract, fish were randomly sampled for blood. The laboratory was kept dark and the fish were netted individually with the aid of a torch light to avoid stress due to struggle, because of its influence on the blood picture (Pickering *et al.*, 1982 and Rahkonen and Pasternack, 1999). A needle fixed to a heparinised syringe was used to remove blood by puncturing dorsal blood vessel lying below the vertebral column (Lewbert, 2001). About 5 ml of blood was taken from one fish each from the various tanks and kept in EDTA bottles waiting for haematological analysis. The sample bottles were vigorously shocked to ensure that bloods were properly mixed with anticoagulants to avoid clotting of blood samples. Haemoglobin was estimated using a *haemoglobinometer*. A Shali graduated tube was filled with N/100 HCl acid up to 2.0 ml mark, to which 0.02 ml of blood was added. Distilled water was added drop by drop with gentle shaking to mix. This continued until colour changed to match a standard. The amount of mixture in the tube gives haemoglobin concentration in percentages (Baker *et al.*, 2001).

$$\text{Haemoglobin} = \frac{\text{value obtained} \times 17.2 \text{ g/100 ml}}{100}$$

Identification and counting of fish white blood cells

White blood cells of fish were identified using description of Arnold (2009) and Palikova *et al.* (1999). A dry micropipette was used to suck in 0.2 ml – 0.5 ml blood from the EDTA bottle and mixed with 0.4 ml of Turk-fluid in a test tube. A smear was prepared and stained with Feushaman stain and examined under low power of (X10) using an oil immersion Microscope (model Olympic microscope, Japan 395864). The counting chamber- haemocytometer model Galman Hawksley Ltd England was used for blood cell counting. The blood (a mixture of blood and Turks-fluid) was counted as:

Number of cell counted x depth x area x dilution factor ($10^4/\text{mm}^3$) (Oluyemi *et al.*, 2008).

Statistical analysis

The physicochemical and haematological parameters were analysed using analysis of variance (ANOVA) at 0.05 % alpha level by SPSS, version 13.0. The *post hoc* comparison of means for physicochemical parameters was carried out using Duncan’s multiple range test (Ayotunde *et al.*, 2010 and Shallangwa and Auta, 2008).

RESULTS AND DISCUSSION

The prevailing temperature of the laboratory was 26.61 ± 0.9 °C throughout the experiment; and dissolved oxygen ranged from 5.80 ± 0.39 mg /L to 6.83 mg /L. The pH of the tanks waters ranged from 6.29 ± 0.31 to 7.47 ± 0.25 during the fourteen days experimental period. These values are expressed in Table 1. There was increase in haemoglobin concentration in fishes that were fed with Aloe extract. As seen in Figure 1, the increase showed that there is an optimum at concentration of 75 mg/Kg of feeds. Eosinophils number was significantly higher at higher concentrations 100.00 mg/kg of feeds and 125.0 mg/kg of feeds). There were significant increases in the number of white blood cells such as lymphocytes, neutrophils and eosinophils. The number of lymphocytes and neutrophils dropped down at very high concentration of the extract (see Figure 2). The population of monocytes significantly dropped on exposure of fish to Aloe extract.

Table 1: Showing the physicochemical properties of water exposed to different *Aloe barbadensis* extract for a period of 14 days. Mean values carrying same letters as superscripts are statistically the same while those carrying different letters are statistically different.

Dose(mg/Kg)	Dissolved oxygen	Acid pH	Temperature
0.0	6.83 ± 0.36^a	7.47 ± 0.25^a	27.04 ± 0.23^{ab}
25.0	6.66 ± 0.19^a	7.26 ± 0.25^{ab}	25.57 ± 0.66^b
50.0	6.49 ± 0.36^b	7.20 ± 0.17^{abc}	25.57 ± 0.66^b
75.0	6.19 ± 0.19^b	7.20 ± 0.20^{abc}	26.61 ± 0.44^b
100.0	5.79 ± 0.45^c	7.11 ± 0.26^{bc}	27.48 ± 0.38^a
125.0	5.80 ± 0.39^c	6.92 ± 0.31^c	27.38 ± 0.58^a

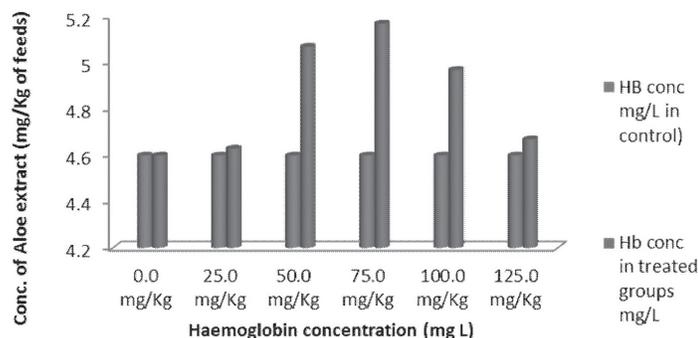


Fig. 1: Showing influence of Aloe extract on the haemoglobin concentration of adult *Heterobranchus bidorsalis*. Haemoglobin concentration was seen to be significantly different between control and the treated groups ($p \leq 0.05$)

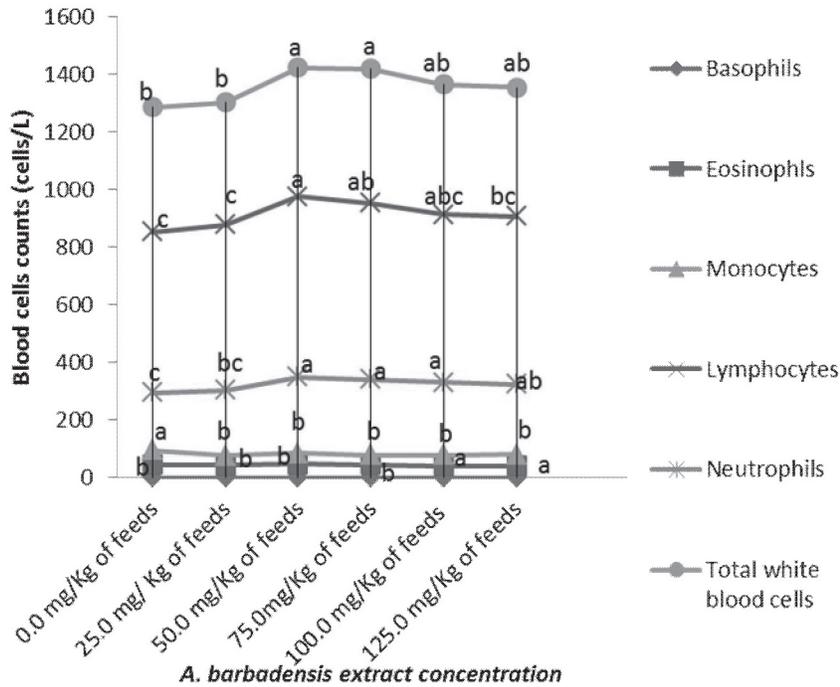


Fig. 2: Showing graphs of different cells counts of *Heterobranchus bidorsalis* at different concentration of *Aloe barbadensis* extract concentrations (mg/kg of feeds). Mean cell counts carrying same letters were statistically the same while those carrying different letters were statistically different ($p < 0.05$).

The temperature was not influenced by the introduction of extract to the culture environments. It was rather been determined by the environment. However, dissolved oxygen and pH were influenced by the extract. These parameters were higher in the *Aloe barbadensis* feed tanks than in the control tanks. All these physicochemical parameters are capable of hampering fish health at their abnormal ranges. However, the values observed here were still conducive for normal health of fish (Driscoll et al., 1989; Vangenechten et al., 1989; Offem and Ayotunde, 2008)

Aloe plant is rich in minerals which are essential for the development of cells and tissues in low concentrations (Grand and Rubel, 2004). Aloe mucopolysaccharides, are long chain sugars, interject themselves into cell membranes resulting in an increase in the fluidity and permeability of the membranes allowing toxins to flow out of the cell more easily and nutrients to enter the cells. This results in improved cellular metabolism throughout the body and an overall boost of energy production. These sugars are taken whole from the gut by pinocytosis and appear in the blood stream in exactly the same form. In this form, they are able to exert their immuno-

regulatory function on the blood. Some of these polysaccharides are not absorbed but stick to cells lining the gut and form a barrier preventing absorption of unwanted materials thereby, helping to prevent a “leaking” gut syndrome. Digestion and assimilation are enhanced as the mucopolysaccharides increase tolerance for allergenic food from gut and causes optimum production of white blood and others cells of the immune system. Many workers observed that toxins at low concentrations (sub lethal) cause increase in number of leucocytes (Weeks, *et al.*, 1986; Santhakumar *et al.*, 1999; Oluah and Mgbenka, 2004; Ibiwoye *et al.*, 2006). However, it should be noted that increased population of white blood cells in circulation does not automatically mean increase haemopoiesis. Influx of marginated population to fight toxin could result in increase white blood cells counts in circulation. Four factors have been named as responsible for changes observed in the population of white blood cells in circulation, namely: increased productions, egress from the circulation, demargination, and released from storage compartments. Increase in lymphocytes and neutrophils suggest that aloe gel extract may have stimulated the humoral immunity. Hence, it can be concluded that the Aloe Gel extract may be a potential candidate in several immuno-suppressed clinical conditions.

There was significant increase in the cells counts such as neutrophils, lymphocytes and eosinophils. The cells count however became less for neutrophils and lymphocytes when the extract concentration was further increased to more than 75.0 mg/Kg of feeds. That shows that the concentration may have exceeded the optimal concentration of 50.0 mg/Kg of feeds to 75.0 mg/Kg of feeds. All biological processes have optimal and tolerant ranges. Higher concentrations than optimal therefore, could overwhelm the cells leading to their mortality. Total white blood cell counts followed the pattern of lymphocytes and neutrophils because their populations dominate other types of white blood cells. But the monocytes rather were significantly lower in aloe extract fed fish. Their reduced populations in circulation could be due to mortality. Higher concentration of the extract could have led to higher oxygen demand and reduced visibility in water, resulting in stress. Optimal oxygen availability and normal pH range are key factors in the maintenance of normal health of organisms.

Atherton (2011) reported that Green in 1993 demonstrated the efficacy of *Aloe barbadensis* in horses suffering from post viral debility, where white blood cell (WBC) counts, that were low in ill animals to almost fetal levels, had returned to normal after *Aloe barbadensis* treatment.

Leukemia is a cancer of the blood and bone marrow brought by the rapid production of abnormal white blood cells (WBC). These abnormal white blood cells are not able to fight infections and yet impair the ability of the bone marrow to produce red blood cells and platelets (Foon *et al.*, 2005). Other workers have rather observed reduced white blood cell counts in fishes exposed to toxins (Gill and Pant, 1985; Oluah and Nwosu, 2003; Mgbenka *et al.*, 2003). Rahkonen and Pasternack (1999) saw no change in total white blood cell count of brown trout but on differential

counts. Differential cell count rather than total cell count was observed because immune cells are specifically produced for particular purposes (Baker *et al.*, 2001).

Aloe gel contains glucomannan, a special complex polysaccharide composed largely of the sugar, mannose. It interacts with special cell-surface receptor on fibroblasts, stimulating them to grow faster. Aloe gibberellins, a hormone, accelerate healing by stimulating cell replication. This occurs as a result of many active ingredients within aloe extracts coming together and exerting their own distinct effects simultaneously, to produce the overall effects of enhancing immunological parameters (Yagi and Machil, 2005). *Aloe* is rich in all vitamins except vitamin D. Vitamin A (beta-carotene), C and E and traces of vitamin B₁₂ (one of the very few plant sources of this vitamin). There is evidence that aloe plants have anti oxidant properties that assist in protecting the body from free radicals, thus reducing the risk of developing a variety of health problem.

Neutrophils quantitative abnormalities such as Neutropenia could be due to decrease production of granulocytes. Extensive radiation therapy produces neutropenia almost invariably. Drugs such as Phenothiazines, phenylbutazone and allopurinol have been seen to induce neutropenia through idiosyncratic reactions. Viral and bacterial infections can lower the polymorphonuclear count. The risk of infection increase when the absolute granulocyte count falls below 1000 per micro liter.

Neutrophil qualitative abnormalities include functional defects in chemotaxis, phagocytosis, and bacterial killing. They can be due to extrinsic or intrinsic abnormalities of the granulocyte. Basophils were absent. They have been seen to be between 1-2 % of the white blood cell population in many land vertebrates and could be completely void in many species of fishes (Palikova *et al.*, 1999, Oluah; Mgbenka, 2004; Arnold, 2009).

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