Original Research Article

Hematological, genotoxic and oxidative stress responses in *Oreochromis niloticus* (Nile tilapia) exposed to varying concentrations of ethanolic extract of *Commelina benghalensis*

ABSTRACT

This study evaluated the hematological, genotoxic and oxidative stress biomarkers of *Oreochromis niloticus* exposed to different concentrations of ethanolic extract of *Commelina benghalensis* (Dayflower). Sublethal exposure at 1% and 10% LC_{50} was done for twenty-one (21) days with physico-chemical parameters of cultured water and behavioral responses assessed. Hematological, genotoxic, antioxidant enzyme and non-enzyme activities studies were analyzed using standard methods. Results showed increase in the levels of Red blood cells, heamoglobin and white blood cells across extract concentrations compared to control. The number of micronucleated cells increased compared to control, though not significant difference ($p>0.05$). Superoxide dismutase (SOD), reduced glutathione (GSH) and glutathionde-S-transferase (GST) activities were inhibited by the plant extract, while the level of lipid peroxidation as expressed by malondialdehyde (MDA) increased compared to control. Skin colour change, erratic swimming and mortality were observed at both higher concentration (10%) and lower concentration (1%) of the extract, though behavioral response at 10% extract concentration was higher than 1% extract concentration. In conclusion, the plant *C.benghalensis* has the potential to exert stress and promote cellular damage in contrast to its ability to boost the blood status of *O. niloticus* at varying concentration.

Keywords: Commelina benghalensis, hematological, genotoxic, antioxidant

1. INTRODUCTION

The ethno-social lives of different tribes of people throughout the world have seen to be inevitably reliant on plants and their products for therapeutic purposes. In many indigenous cultures, knowledge and practice of using herbal medicine has been passed from generation to generation. According to the World Health Organization (WHO), 70– 80% of the world population depends exclusively on herbs for their primary health care[1][2][3].

Dayflower also known as ***Commelina benghalensis*** is a perennial herb native to tropical Asia and Africa. It has been widely introduced to areas outside its native range, including to the neotropics, Hawaii, the West Indies and to both coasts of North America. It has a long flowering period, from spring to fall in subtropical areas, and throughout the year closer to the equator [4]. It is often associated with disturbed soils. *Commelina benghalesis* is genus of the family, *commelinaceae*. It is regarded as the largest genus of the family. They are annual and perennial herbs. *Commelina benghalesis* commonly known as dayflower or wandering jew is a weed of cultivated, waste and roadsides and it is also a pan-tropical weed.

Plant toxicity study is essential in fisheries by providing answers to physiological, ecological and environmental concerns associated to such aquatic plants. Clearly, past report revealed acute toxicity test of this plant (*Commelina benghalensis*) on shrimps, hence became necessary to determine the cytotoxicity of the plant under sublethal conditions in fish. This study would provide a baseline information on the toxicity prowess of *C. benghalensis* on frequently consumed fish (*O. niloticus)* in water

bodies, which could have multiplier effects on consumers of such fish.

Commelina benghalensis is a spreading, annual herb, which is sparsely and shortly pubescent, sometimes bearing subterranean runners with self-pollinating, reduced flowers and thin roots. Its leaves are ovate, up to ±80 × 30 mm, shiny, pale apple-green, with apex that is obtuse to acute and the base abruptly narrowed into a sheathing petiole beset with long, red, or rarely colourless, several-celled setae (bristles), at the mouth.

Commelina benghalesis produces very attractive small flowers with deep ink-blue petals. Inflorescences are subsessile spathes, clustered at apices of branches, obliquely fused, triangular, 10 × 15 mm; apex short, acute. Cymes 2, the upper one often suppressed later in the season. It has 5- seeded capsules and flowers from August to June.

Nile tilapia (*Oreochromis niloticus* Linnaeus 1757) is native to Africa, ranging from the upper Nile River south to the equator and west to the Atlantic coast[6]. It is one of the aquatic organisms affected by heavy metals and used as metal biological marker in toxicological studies[7]. The intensive farming of *O. niloticus* is rapidly expanding and is the second most widely farmed fish in the world, after carps. Several species of tilapia are cultured commercially, but *O. niloticus* is the predominant cultured species worldwide[8]. It presents rusticity, good growth rate, and adaptability to confinement, producing a tasty white-colour meat[9]. The ease of reproduction of the *O. niloticus* encourages farmers to acquire the species and populate their tanks at a low investment cost. Furthermore, the fish is hardy, occurs in a wide range of environmental variations, tolerating extreme limits of temperature and oxygen, as well as the presence of various pollutants[10].

2. MATERIAL AND METHODS

Fingerlings of Nile tilapia were exposed to ethanolic extract of day flower over 4 days and 21 days to determine their acute and sublethal toxicity effects, respectively in the laboratory. A control also containing fingerlings of Nile tilapia (from the same batch that was exposed) was simultaneously maintained for the same period and under same laboratory conditions. After exposure to high concentrations during the acute toxicity studies, 96 hours median lethal dose (LC50) was determined using probit analysis. The result of the LC50 was used in fractions of 1% and 10% concentration and exposed to Nile tilapia during the chronic toxicity test to determine possible sub lethal effects of the exposure. The 96 hours LC50 is the concentration which results in mortality of 50% of the catfish after 96 hours of exposure.

2.1 Test Organisms

Fingerlings *Oreochromis niloticus* with average weight of 3.46g were procured from a fish farm in Ogun state Nigeria and transported to the environmental biology laboratory, Yaba college of technology in plastic bags half full with pond water. The fish were acclimatized to laboratory conditions in 100L plastic tanks.

2.2 Preparation of Test Compounds

The test compound was ethanolic extract of *Commelina benghalensis* (day flower). The extraction process was carried out using soxhlet apparatus. Begin by building a rig using stands and clamps to support the extraction apparatus. Following this, the solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 16 hours.

Figure 1: picture of commelina benghalensis.

2.3 Acute Toxicity Testing

Range finding of the extract concentration was done to determine the concentration suitable for the acute toxicity. The 96 hours acute toxicity of *C. banghalensis* ethanolic extracts on fingerlings of *O. niloticus* was conducted following static bioassay procedures described by Yadav and Singh (2010). Four test concentrations of 500mg/L, 650mg/L, 800mg/L, 950mg/L and 0.00mg/L (control) were prepared and replicated twice for each treatment in a 10L transparent plastic container. Each of the tanks was stocked with 10 fish at different concentrations of the *C. banghalensis*. Throughout the 96 hours test, the test fish were not fed and the mortality in each tank was monitored and recorded every 24 hours till the end of the test. Dead fish were immediately removed to avoid contamination.

2.4 Chronic Toxicity Testing

Sub-lethal test was done for 21 days using the LC_{50} gotten from the acute toxicity from *C. banghalensis* ethanolic extracts on fingerlings. $1/10^{th}$ value and $1/100^{th}$ value of the LC_{50} was considered the sub-lethal concentration. Two test chronic concentrations and control were prepared and replicated twice for each treatment in a transparent plastic container. Each of the tanks was stocked with 10 fish at different concentrations of the *C. banghalensis*. Throughout the 21 days test, the test fish were fed every 2 days including the change of water and introducing another concentration and the mortality in each tank was monitored and recorded every 24 hrs till the end of the test. Dead fish were immediately removed to avoid contamination.

2.5 Hematological Studies

After the sublethal, which is 21 days some fish were sacrificed and their blood samples was collected with EDTA bottles and place in the refrigerated centrifuges, to obtain plasma and then analyzed in the laboratory to assess levels of hematological indices. Hematological parameters such as red blood cell, white blood cell, packed cell volume, hemoglobin, red blood cell indices and white blood cell differential counts were carried out following standard procedures using Automated hematology analyzer.

2.6 Micronucleus Assay

Blood samples were obtained from the caudal vein of the fish using a syringe [11]. The blood was then smeared on glass slides per concentration. According to the method used by[12], the smeared slides were air-dried overnight at room temperature, then, fixed in absolute ethanol and allowed to dry for 15 minutes. They were stained with May-Grunwald stain for 10 minutes, and then counter stained with 5% Giemsa for another 10 minutes, rinsed very slightly through tap water and allowed to dry. The slides were analyzed at 100x (oil immersion) for micronucleus and other nuclear abnormalities under a light microscope (Olympus CHC Model). The micronuclei were characterized by the presence of a small cell inclusion detached from a larger definite nucleus, while those with two joined nuclei of equal size were defined as binucleated. Other cells with eight-shape and notched were also observed. The cells were counted using a hand-held counter (Counter Compass: No. 7777, China).

2.7 Antioxidant Enzymes and Non-enzyme Analysis

2.7.1 Homogenizing Sample

The dissected liver and intestine were removed and weighed. The organs were homogenized with 0.1phosphate buffer (PH 7.2) putting the organ each into the mortar and was blended with a pestle together. The resulting homogenate was centrifuged at 2500RPM for 15minutes. The supernatant was decanted and stored at -20°C.

2.7.2 Determination Of Catalase Activity

According to Quinlan, et al (1994), Catalase (CAT) was assayed colorimetrically at 620nm and expressed as moles of hydrogen peroxide (H202) consumed/min/mg protein. 1.0ml of 0.01M pH 7.0 phosphate buffer, 0.1ml of Plasma and 0.4ml of 2M H,O, made up the reaction mixture (1.5ml). The addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio) stopped the process.

2.7.3 Determination Of Reduced Glutathione (GSH)

The method of Ellman, G. L. (1959) was used to determine reduced glutathione (GSH) was determined. 10% TCA (equal volume) was added to the homogenate added and centrifuged. 0.5 ml of Ellmans reagent (19.8 mg of 5, 5°-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0) were added to 1.0 ml of supernatant. At a wavelength of 412 nm, the absorbance was measured.

2.7.4 Determination Of Glutathione Peroxidase (GPx)

0.2ml of 0.4M phosphate buffer pH 7.0, 0.1ml of 10mM sodium azide, 0.2ml of plasma, 0.2ml of glutathione salt (GSH) and 0.1ml of 0.2mM Hz02 were added to evaluate glutathione peroxidase (GPx) activity. The mixture was incubated at 37°C for 10mins. The reaction was arrested by 0.4ml of 10% TCA, and centrifuged. Ellman's reagent was used to measure the glutathione content of the supernatant.

2.7.5 Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA- TBA-HCI reagent (thiobarbituric acid 0.37%, 0.24N HCI and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56 x 105 Mi 'CM-1.

2.7.6 Determination Of Superoxide Dismutase (SOD) activity.

Total SOD activity in tissue homogenates assessed using a modified version of the Marklund and Marklund technique. The approach is based on SOD's ability to inhibit the autoxidation of pyrogallol. In 970uL of buffer (100mMTris-HCI, ImM EDTA, pH &,.2), 10uL of homogenates and 20uL pyrogallol (13Mm) were combined. At 25°C, assay was carried out in thermostated cuvettes and variations of absorbance were measured using a spectrophotometer set to 480nm. The amount of enzyme can inhibit the auto-oxidation of 50% the total pyrogallol in the process is defined as one unit of SOD activity.

2.8 Behavioral Studies

The behavioral responses of *Oreochromis niloticus* fingerlings to ethanolic extract of *Commelina benghalensis* in the treatment groups and the mortality rate was observed and recorded every 24 hours for 21 days. The responses observed were aggressiveness, bloated when dead, erratic swimming pattern, blood stain around gill, loss of reflex, discoloration, moulting, gulping of air, restlessness, spiral movements and excessive mucus secretion.

2.9 Statistical Analysis

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp 2011). Mean values were compared using one-way analysis of variance (ANOVA). Results were presented as Mean±Standard deviation. Post hoc test was done using the Student-Newman-Keuls (SNK). P – value less than 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis Of *Commenlina Benghalensis* (Day Flower)

3.1.1 Qualitative Screening of Phytochemical Analysis of *Commenlina benghalensis* (Day Flower)

The result below is the phytochemical analysis of *Commenlina benghalensis* (day flower) shows the qualitative screening of different phytochemical constituent that are present in the aqueous ethanol extract and the powdered specimens using standard procedures to identify the constituents as described by Edeoga *et al.,* with slight modification. The present constituent are represented with (+) while those absent are represented with (-).

Table 1: Qualitative phytochemical screening of *Commelina benghalensis*

Phytochemicals	*Commelina benghalensis* plant extract
Saponins	+
Alkaloids	+
Flavonoids	+
Tannins	+
cardiac glycoside	+
Steroid	-
Terpernoids	-
Phlobatanin	-

3.1.2 Quantitative Screening of Phytochemical Analysis of *Commenlina benghalensis* (day flower)

The result below (table 2) is the phytochemical analysis of *Commenlina benghalensis* (day flower) shows the quantitative screening of different phytochemical constituent that are present in the aqueous ethanol extract and the powdered specimens using standard procedures to identify the constituents as described by Edeoga *et al.,* with slight modification. The present constituent are represented in (%).

Table 2: Result of the quantitative phytochemical constituent of ethanol extract of *Commelina banghalensis* plant

Phytochemical constituents	*Commelina benghalensis* plant extract (%)
Saponins	1.5
Alkaloids	1.2
Flavonoids	5.4
Tannins	1.37
Phenolic	0.23
Steroid	N.D
Phlobatanin	N.D
Terpernoids	N.D

3.2 Physicochemical Parameters Of Culture Water

The physicochemical parameters of the culture water recoded at experimental day 7, day 14 and day 21 are shown in Table 3. Result showed significantly highest level of water hardness in the water samples at experimental day 21. There was however no significant difference in the level of water hardness recorded in the water samples at day 7 and day 14. Level of water nitrate recorded in the water samples collected during the three experimental days were less than 0.001.

Similarly, levels of water temperature, pH, dissolved oxygen and total dissolved solids were observed to increase with increase in the experimental days. These were however not significantly different in the water samples obtained at day 7, day 14 and day 21. Also, levels of biochemical oxygen demand, chemical oxygen demand, sulphate, phosphate, turbidity, chloride, calcium and conductivity recorded in the water samples obtained at experimental day

7, day 14 and day 21 were not significantly different.

Table 3: Physicochemical parameters of culture water obtained at experimental days 7, 14 and 21

	Day 7	Day 14	Day 21
Temperature (^{o}C)	25.60± 0.85^{a}	26.00± 1.41^{a}	26.40± 0.57^{a}
pH	6.95±0. 07^{a}	7.25±0. 35^{a}	7.40±0. 57^{a}
Dissolved oxygen (mg/L)	6.90±0. 14^{a}	6.85±0. 07^{a}	6.95±0. 07^{a}
Hardness (mg/L)	12.00± 0.71^{b}	12.00± 1.13^{b}	14.00± 0.85^{a}
Biochemical oxygen demand (mg/L)	7.00±0. 57^{a}	8.60±0. 85^{a}	7.80±1. 13^{a}
Chemical oxygen demand (mg/L)	27.40± 0.57^{a}	26.50± 0.71^{a}	27.50± 0.71^{a}
Nitrate (mg/L)	<0.001	<0.001	<0.001
Sulphate (mg/L)	15.00± 1.41^{a}	13.00± 0.71^{a}	15.00± 1.13^{a}
Phosphate (mg/L)	1.08±0. 04^{a}	0.90±0. 07^{a}	1.23±0. 04^{a}
Turbidity (mg/L)	31.70± 0.99^{a}	32.30± 0.42^{a}	31.50± 0.71^{a}
Chloride (mg/L)	38.31± 0.44^{a}	38.10± 0.14^{a}	38.56± 0.79^{a}
Calcium (mg/L)	3.21±0. 30^{a}	3.56±0. 08^{a}	3.45±0. 07^{a}
Conductivity (mg/L)	247.00 ±2.83^{a}	256.00 ±4.24^{a}	252.00 ±2.83^{a}
Total Dissolved solid (mg/L)	123.50 ±0.71^{a}	123.80 ±1.13^{a}	124.00 ±4.24^{a}

^{abcd}Means (±Standard deviation) with similar superscripts in the same row are not significantly different ($p > 0.05$).

3.3 Behavioural Response of *Oreochromis niloticus* to Varying Concentration of *C. benghalensis*

Table 4 shows the behavioural responses of *Oreochromis niloticus* to varying concentrations of *C. benghalensis*. The result shows that *Oreochromis niloticus* exposed to the *C. benghalensis* extract showed behavioural patterns such as erratic movement, loss of reflex and hyperventilation. The death of the fingerlings was directly concentration dependent in relation to exposure time. At 10% extract concentration, there was loss of reflex, erratic swimming and hyperventilation mortality increased with respect to exposure days(>7days) while the 1% extract concentrations show minor impact on the reflex, respiration and swimming. Mortality recorded increased at longer exposure days(>14days).

TABLE 4: Sub-lethal test Behavioural response of *Oreochromis niloticus* to aqueous extract of *C. benghalensis*

Behavioural response	CONCENTRATION(Mg/L)						
	Control			**1%**		**10%**	
		7days	**14days**	**21days**	**7days**	**14days**	**21days**
Loss of reflex	-	-	-	+	+	+	++
Hyperventilation	-	-	-	+	+	+	++
Erratic swimming	-	-	-	+	-	+	++
Mortality	+	+	++	+++	++	+++	+++

Key : -Absent, +Low, ++ Mild, +++ High.

3.4 Hematological Parameters

The hematological parameters of tilapia fish exposed to ethanolic extract of day flower plant, *Cmmelina benghalensis* are shown in Table 5. Result showed significantly higher ($p < 0.05$) level of white blood cells in the tilapia fishes exposed to 10% ethanolic extract of day flower plant. This was followed by the control and lowest in those exposed to 1% ethanolic extract of day flower plant.

Similarly, hemoglobin level was significantly highest in the tilapia fishes exposed to 1% ethanolic extract of day flower plant. However, hemoglobin levels recorded in the control tilapia fish and those exposed to 10% ethanolic extract of day flower plant were not significantly different ($p > 0.05$). Also, red blood cell counts and levels of hematocrit recorded in the control tilapia fish and those exposed to 1% and 10% ethanolic extract of day flower plant were not significantly different.

3.4.1 Levels of Blood Differentials

Table 5 shows the levels of blood differentials of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*. Levels of Mean cell volume, Mean cell hemoglobin and Mean cell hemoglobin concentration were significantly highest in the control tilapia fish. This were observed to significantly reduce in the tilapia fish exposed to ethanolic extract of day flower plant with increase concentration of exposure. These were lowest in the group exposed to 10% ethanolic extract of day flower plant.

Levels of platelets, lymphocytes, eosinophils, basophils % and monocytes % were significantly higher in the tilapia fish exposed to 10% ethanolic extract of day flower plant than those of the other experimental groups. Level of neutrophils recorded in the control tilapia fish and those exposed to 10% ethanolic extract of day flower plant was not significantly different. These were higher than that of tilapia fish exposed to 1% ethanolic extract of day flower plant. Similarly, level of eosinophil % was significantly lowest in the tilapia fishes exposed to 10% ethanolic extract of day flower plant. Eosinophil % level recorded in the control group and those 1% ethanolic extract of day flower plant was not significantly different. On the other hand, levels of Red blood cell distribution width standard deviation, red cell distribution width with a coefficient of variation, platelet distribution width, mean platelet volume, platelet large cell ratio, platelet hematocrit or platelet volume ratio, monocytes, basophils, neutrophils %, lymphocytes %, IG and IG % recorded in the control tilapia fish and those exposed to 1% and 10% ethanolic extract of day flower plant were not significantly different.

Table 5: Hematological parameters of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*

	Control	1% Day flower	10% Day flower
White blood cells $\times 10^9$/L	4.91±0.01^{b}	2.01±0.01^{c}	6.83±1.13^{a}
Red blood cells $\times 10^{12}$/L	1.62±0.03^{a}	1.01±0.01^{a}	1.89±0.13^{a}
Haemoglobin g/L	61.70±0.99^{b}	78.30±0.42^{a}	63.90±1.27^{b}
Haematocrit g/L	0.25±0.01^{a}	0.18±0.03^{a}	0.21±0.01^{a}

^{abc}Means (±Standard deviation) with similar superscripts in the same row are not significantly different ($p > 0.05$)

Table 6: Levels of blood differentials of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*

	Control	1% Day flower	10% Day flower
MCV FL	136.15±4.24[a]	129.03±2.87[b]	122.49±0.69[c]
MCH Pg	45.63±0.89[a]	43.41±0.58[b]	41.80±1.13[c]
MCHC g/L	335.00±7.07[a]	295.00±2.83[b]	275.00±5.66[c]
PLT ×10^9/L	89.00±2.83[c]	95.50±3.54[b]	110.00±5.66[a]
RDW-SD FL	141.86±1.50[a]	135.21±3.13[a]	139.03±2.87[a]
RDW-CV %	16.26±0.37[a]	16.00±1.41[a]	15.81±1.15[a]
PDW FL	7.48±0.11[a]	7.10±0.14[a]	7.96±1.27[a]
MPV FL	6.94±0.06[a]	7.20±0.28[a]	8.00±0.85[a]
P-LCR %	16.48±0.68[a]	16.59±0.13[a]	17.18±0.25[a]
PCT %	0.26±0.01[a]	0.21±0.01[a]	0.32±0.03[a]
NEUT 10^9/L	0.21±0.01[a]	0.10±0.03[b]	0.26±0.04[a]
LYM ×10^9/L	2.88±1.13[b]	1.22±0.03[c]	3.40±0.57[a]
MON ×10^9/L	0.21±0.01[a]	0.18±0.01[a]	0.29±0.03[a]
EOS ×10^9/L	0.11±0.00[b]	0.05±0.00[c]	0.38±0.01[a]
BAS ×10^9/L	0.70±0.07[a]	0.61±0.01[a]	0.90±0.28[a]
NEUT %	2.98±1.27[a]	3.20±0.28[a]	2.71±0.01[a]
LYMPH %	41.62±0.88[a]	39.60±0.85[a]	42.01±2.84[a]
MONO %	10.07±0.10[b]	7.08±0.11[c]	12.40±0.57[a]
EOS %	0.54±0.06[a]	0.72±0.03[a]	0.10±0.01[b]
BASO%	0.10±0.01[b]	0.30±0.04[b]	1.20±0.28[a]
IG ×10^9/L	0.10±0.03[a]	0.13±0.04[a]	0.18±0.03[a]
IG %	0.30±0.07[a]	0.10±0.03[a]	0.50±0.14[a]

[abcd]Means (±Standard deviation) with similar superscripts in the same row are not significantly different ($p > 0.05$); WBC = White Bloob cell; RBC = Red blood cell; HGB = Haemoglobin; MCV = Mean cell volume; MCH = Mean cell haemoglobin; MCHC = Mean cell haemoglobin; concentration; PLT = platelet; NEUT = Neutrophils; LYMP = Lymphocytes; MONO = Monocytes; EOS = Eosinophils; BAS = Basophils; RDW = SD = Red blood cell distribution width standard deviation; RDW = CV = red cell distribution width with a coefficient of variation; PLT = platelet distribution; PDW = platelet distribution width; PLCR = platelet large cell ratio; MPV = mean platelet volume; PCT = platelet haematocrit or platelet volume.

3.5 MICRONUCLEUS ASSAY

3.5.1 Numbers of Micronucleated Cells

The mean numbers of micronucleated cells of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis* are shown in Figure 2. Mean number of micronucleated cells was significantly highest ($p < 0.05$) in the tilapia fish group exposed to 10% ethanolic extract of day flower plant. This was followed by those exposed to 1% ethanolic extract of day flower plant. Mean number of micronucleated was however observed to significantly increase in the tilapia fish groups with increase in the level of the extract exposure.

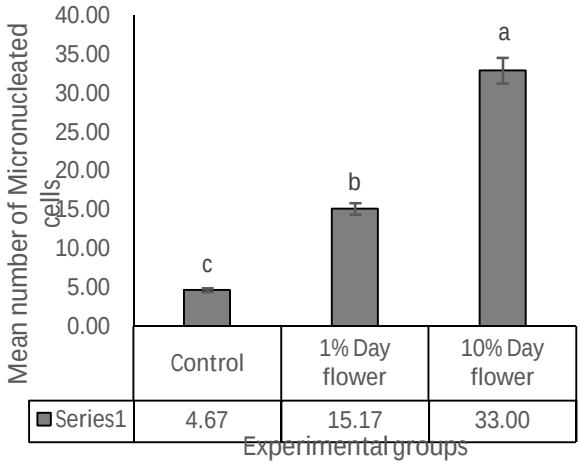

Figure 2: Mean numbers of micronucleated cells of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; [abc]Bars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation

3.5.2 Mean Numbers of Bi-nucleated Cells

Figure 3 also showed the mean number of bi-nucleated cells of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*. Mean number of bi-nucleated cells was also observed to significantly increase in the tilapia fish groups with increase in the level of ethanolic extract of day flower plant extract exposure. This was significantly highest

in the tilapia fish group exposed to 10% ethanolic extract of day flower plant. There was however no significant difference ($p > 0.05$) in the mean number of bi-nucleated cells recorded in the control fish and those exposed to 1% ethanolic extract of day flower plant.

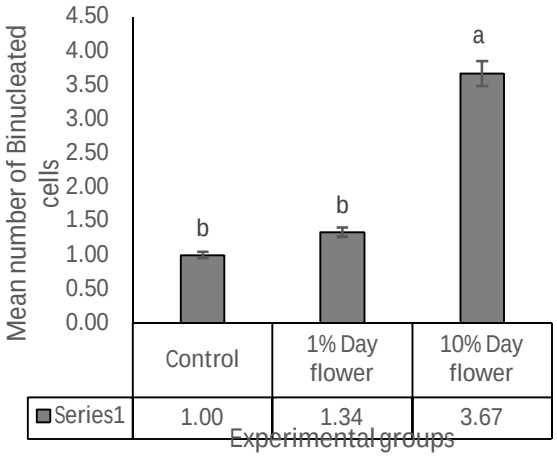

Figure 3: Mean numbers of bi-nucleated cells of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; [abc]Bars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation

3.5.3 Mean Numbers of Eight-Shaped Cells

The mean number of eight-shaped cells in tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis* are represented in Figure 4. Mean number of eight-shaped cells was observed to significantly increase in the tilapia fish groups with increase in the level of the ethanolic extract of day flower plant exposure. Mean number of eight-shaped cells was significantly highest in the tilapia fishes exposed to 10% ethanolic extract of day flower plant. This was followed by those exposed to 1% ethanolic extract of day flower plant and significantly lowest in the control tilapia fish group.

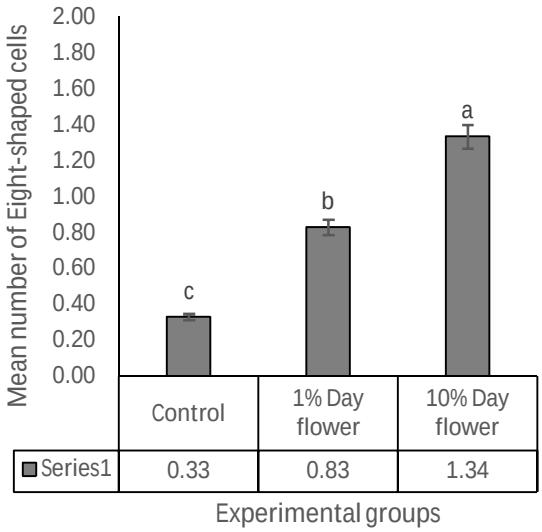

Figure 4: Mean numbers of eight-shaped cells of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; [abc]Bars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation

3.5.4 Mean Numbers of Notched Cells

Figure 5 presents the mean number of notched cells in tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*. Mean number of notched cells recorded in the control tilapia fish group and those recorded in the tilapia fish exposed to 1% ethanolic extract of day flower plant was not significantly different. On the other hand, mean number of notched cells was significantly highest in the tilapia fishes exposed to 10% ethanolic extract of day flower plant.

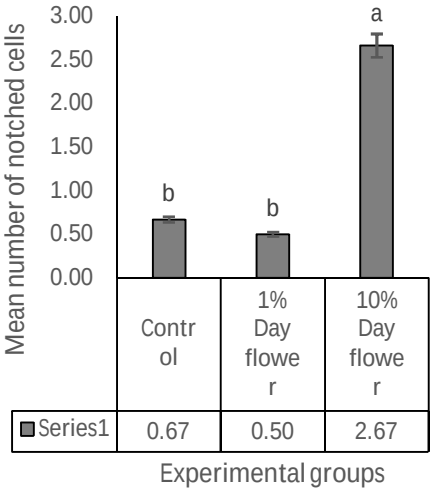

Figure 5: Mean numbers of notched cells of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; [abc]Bars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation.

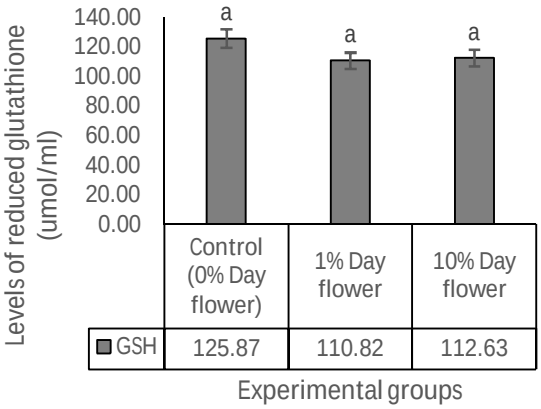

Figure 6: Levels of reduced glutathione (GSH) in the liver of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; abcBars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation

3.6 Levels Of Oxidative Stress Markers

3.6.1 Reduced glutathione (GSH)

The levels of reduced glutathione (GSH) in the liver of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis* is shown in Figure 6. Result showed no significant difference ($p > 0.05$) in the levels of GSH recorded in the control tilapia fish and those recorded in the groups exposed to 1% and 10% ethanolic extract of day flower plant. However, GSH level was highest in the control tilapia fish. This was followed by those exposed to 10% ethanolic extract of day flower plant and 1% ethanolic extract of day flower plant respectively.

3.6.2 Superoxide dismutase (SOD)

Figure 7 shows the levels of superoxide dismutase (SOD) recorded in the liver of tilapia fishes exposed to ethanolic extract of day flower plant. Level of SOD was significantly highest in the control tilapia fish group than those exposed to 1% and 10% ethanolic extract of day flower plant. On the other hand, the level of SOD recorded in the tilapia fish groups exposed to 1% ethanolic extract of day flower plant and those exposed to 10% ethanolic extract of day flower plant was not significantly different.

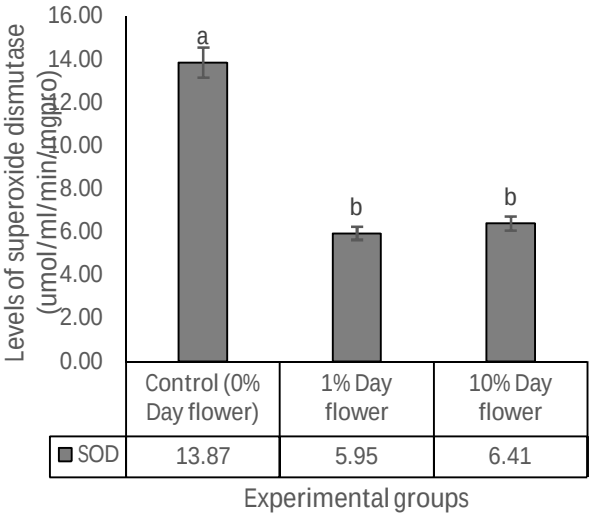

Figure 7: Levels of superoxide dismutase (SOD) in the liver of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; abcBars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation.

3.6.3 Catalase (CAT)

Levels of catalase recorded in the liver of tilapia fish exposed to ethanolic extract of day flower plant are represented in Figure 8. Catalase level was highest in the tilapia fish group exposed to 10% ethanolic extract of day flower plant. However, there was no significant difference in the level of catalase recorded in the control group and those exposed to 1% and 10% ethanolic extract of day flower plant.

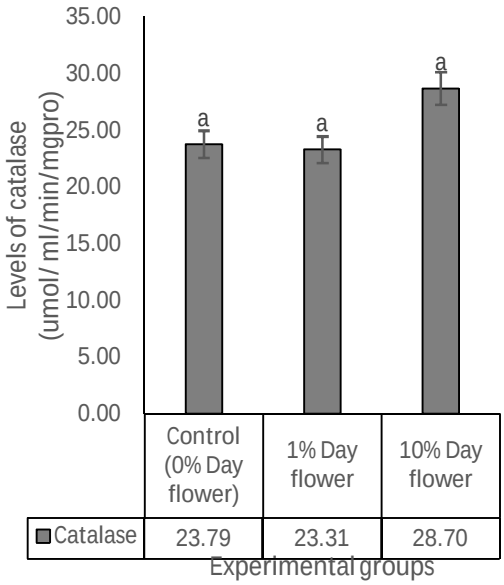

Figure 8: Levels of catalase in the liver of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; abcBars with similar alphabets are not significantly different ($p > 0.05$); Error bars represents standard deviation

3.6.4 Superoxide-S-transferase (GST)

Figure 9 shows the levels of superoxide-S-transferase (GST) recorded in the liver of tilapia fishes exposed to ethanolic extract of day flower plant. Liver GST level was significantly highest in the control tilapia fish group than those exposed to 1% and 10% ethanolic extract of day flower plant. However, the level of GST recorded in the tilapia fish groups exposed to 1% ethanolic extract of day flower plant and those exposed to 10% ethanolic extract of day flower plant was not significantly different.

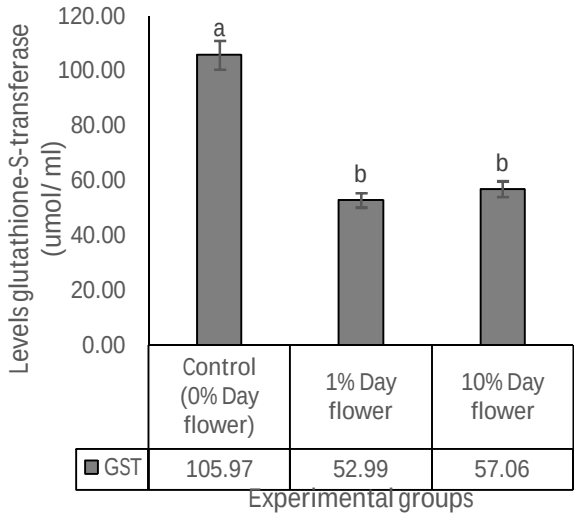

Figure 9: Levels of glutathione-S-transferase (GST) in the liver of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis*; abcBars with similar alphabets are not significantly different (p > 0.05); Error bars represents standard deviation

3.6.5 Malondialdehyde (MDA)

Level of malondialdehyde (MDA) was observed to be significantly higher in the tilapia fish group exposed to 1% and 10% ethanolic extract of day flower plant (Figure 10). This was significantly highest in the tilapia fish exposed to 1% ethanolic extract of day flower plant. This was followed by those exposed to 10% ethanolic extract of day flower plant. Level of MDA was significantly lowest in the control tilapia fish group.

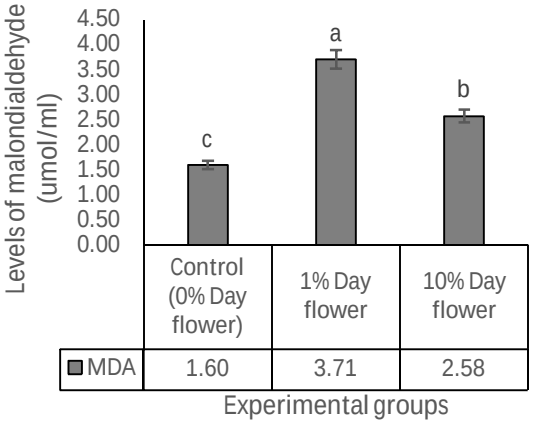

Figure 10: Levels of malondialdehyde (MDA) in the liver of tilapia fish exposed to ethanolic extract of day flower plant, *Commelina benghalensis;* abcBars with similar alphabets are not significantly different (p > 0.05); Error bars represents standard deviation

3.7 DISCUSSION

This study is aimed to investigate the effect of *C. benghalensis* extract on some physiological indices of *O. niloticus* and to determine the genotoxic, oxidative stress and hematological parameter of this plant extract on Nile tilapia. Phytochemicals generally is known as secondary breakdown product of plants biogenesis, and are essentially utilized by the plans of several occasions as well as guarding and protection (Bosh's *et al.,* 2018). These Phytochemicals include phenols, terpenoids, flavonoid, tannins, cardiac glycosides etc. (Evans, 2009). In the study, it can be seen that *Commelina benghalensis* extract contains most of these constituents. The phytochemical analysis of *Commenlina benghalensis* in this present study revealed the presence of saponins, alkaloids, flavonoids, tannins, cardiac glycoside while steroids is not detected in the plants extract. Previous research has also shown that the tannin containing plant extracts could be used as anti-inflammatory, antioxidant and hemostatic pharmaceuticals[13]. Alkaloids have been shown to have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, anticancer actions and antimalarial activity. Similarly, flavonoids have been shown to possess antimicrobial and anti-inflammatory properties[14]. This study has shown that exposure of tilapia fish (*Orechromis niloticus*) to ethanolic extract of day flower plant does not pose negative effects on the hematological parameters of the fish. For instance, the red blood cell count and hematocrit levels were not significantly affected. Previous study has shown that hematological parameters (especially PCV, total and differential leukocyte counts in the blood) provide an indication of the health status

of the fish[15]. [16] also associated hematological evaluation as a useful tool in assessing the health status of fish and monitoring their stress responses. According to[17], the red blood cells are a nucleate blood cells containing hemoglobin which is important for distribution of oxygen to the various body cells and taking carbon dioxide from the same body cells. Low PCV has been associated with stress and anemic condition in the fish [16] and could lead to a disease situation and reduction in feeding level [18]. Hence, exposure of tilapia fish to ethanolic extract of day flower plant does not pose negative effect on the health and stress level of the fish. However, exposure to ethanolic extract of day flower plant significantly increase white blood cell count and hemoglobin level in tilapia fish. White blood cells are also important part of the body's immune system responsible for destroying infectious agents and removal of aberrant cellular debris and any other foreign substances [19]. High level of hemoglobin also indicates enhanced oxygen uptake by the fish [17]. Hence, increased levels of these essential blood components show that exposure of the tilapia fish to this plant extract could aid homeostasis and body immune response.

This study also evaluates the genotoxicity effect of exposure to ethanolic extract of day flower plant, *Commelina benghalensis* on tilapia fish. In this study, exposure of tilapia fishes exposed to the ethanolic extract of day flower plant was able to induce higher numbers of micronucleated cells and other cell abnormality such as bi-nucleated cells, eight-shaped cells and notched cells. According to [20], micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei has been used as an index of genetic damage. Thus, higher significantly higher numbers of micronucleated cells recorded in the tilapia fish exposed to ethanolic extract of day flower plant could be an indication that this plant extract could cause genetic damage in exposed tilapia fishes.

Although, the mechanism of the observed genotoxicity in the tilapia fish exposed to day flower plant used in this study is not clear, it could be as a result of a component of the plant. In a previous study,[21] reported a high level of hydrogen cyanide in different parts of *Commenlina benghalensis* plant. High level of hydrogen cyanide has been described to be toxic to human and animal systems.

Again, this study also shows that the exposure of tilapia fish to ethanolic extract of day flower plant, *Commelina benghalensis* has the potential oxidative stress effect to reduce liver levels of superoxide dismutase and glutathione-S-transferase. These enzymes were previously classified as antioxidant enzymes responsible for the protection of the body cells against the harm caused by reactive oxygen species [22]. [24] explained that a reduction in the level of SOD could be associated with increasing amount of reactive oxygen and hydroxyl radicals that inactivate the chemical structure of SOD and ultimately result into loss of enzyme activity. This is because the superoxide dismutase is the frontline enzymes that act on superoxide radicals of the reactive oxygen species and converting it to hydrogen peroxide (H_2O_2) [22]. Similarly, glutathione S-transferase (GST) as a cytosolic liver enzyme which is also an essential marker of hepatocyte damage caused by conditions such as hemorrhagic shock, ischemia and reperfusion, liver transplant rejection and paracetamol overdose [23][25]. The role of the enzyme glutathione-S-transferase has been described to catalyze the formation of conjugates between glutathione and a wide variety of electrophilic compounds such as carcinogens, toxins, and drugs [26]. The reduced level of superoxide dismutase and glutathione-S-transferase recorded in the liver of tilapia fish exposed to ethanolic extract of day flower plant could be an indication that exposure of tilapia fish to this plant extract has could pose oxidative stress on the liver.

Lower levels of superoxide dismutase and glutathione-S-transferase could have resulted in the increased level of lipid peroxidation [malondialdehyde (MDA)] in the liver of tilapia fish exposed to ethanolic extract of day flower plant. [27] had earlier reported that lipid peroxidation results from the release of free radicals and can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes to form malondialdehyde. It is therefore possible that the extract of day flower plant used in this study has some component that has the ability to initiate oxidative stress.

4. CONCLUSION

The findings in this study clearly demonstrate the threat and beneficial effect of the exposure of ethanolic extract of *Commelina benghalensis* (day flower) on *Oreochromis niloticus* (Nile tilapia). Based on the findings of this study, exposure of tilapia fish to ethanolic extract of day flower plant has the potential of disrupting the antioxidant system of the liver, leading to increased lipid peroxidation, the result showed that the selected plant (*Commelina banghalensis*) contains appreciable number of Phytochemicals such as saponins, alkaloids, flavonoids, tannins and cardiac glycoside which are significant to the pharmacological activities of the plants. This study also shows that exposure of tilapia fish to ethanolic extract of day flower plant, *Commelina benghalensis* could be beneficial to the fish health by improving its haematological parameters such as the white blood cells, platelets, lymphocytes, eosinophils, monocytes and basophils. And also, from the results of this study, exposure of tilapia fish to ethanolic extract of day flower plant, *Commelina benghalensis* could be genotoxic by causing some genetic damage and stress exertion.

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