Hematological and Histopathological Effects of Cyanobacteria (Blue-Green Algae) from Lake Victoria Shores of Uganda in Swiss Mice

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Abstract

This was an experimental study carried out to evaluate the hematological and histopathological effects of cyanobacteria (blue-green algae) collected from Lake Victoria shores in Swiss mice. A crude aqueous cyanobacterial extract was prepared by oven drying the cyanobacteria previously preserved in 4% formalin at 45^{o}C. First, the acute toxicity of the extract was evaluated using mice in three groups (n=5) that were orally administered with single doses of 5,000, 7,000 and 10,000 mg/kg b.wt. Next, another batch of mice in three groups (n=5) were orally dosed daily for 21 days with doses of 312.5, 625 and 1,250 mg/kg b.wt. Hematological and histopathological changes in the liver, kidney, lungs and duodenum were determined. The oral LD_{50} of the extract was higher than 10,000 mg/kg b.wt. The total white blood cell count in all treatment groups was significantly decreased ($p<0.05$) in comparison to the control group. The red blood cell count, hematocrit, and hemoglobin concentrations of the treatment groups were also significantly reduced ($p<0.05$) compared to those of the normal control group at 625 and 1250 mg/kg doses of the cyanobacterial extract. Histopathological investigations revealed tissue injury in higher doses. These results show that the cyanobacterial extract has detrimental effects on total WBC count and in lung, liver and duodenal tissue on continuous exposure.

Keywords: Cyanobacteria; Lake Victoria; hematology, histopathology, Swiss mice

1. Introduction

Proliferation of blue–green algae or cyanobacteria in many parts of Lake Victoria has become a common phenomenon. This is evidenced by the increased water turbidity, discoloration and seasonal occurrence of algal blooms along the surface of the lake (Okello *et al*., 2010). Lake Victoria lies within the Victoria basin in E.

Africa with its water shared among three countries: 6% in Kenya, 43% in Tanzania and 51% in Uganda. Industrial waste from many factories within the Lake Victoria basin finds its way into the lake. The improper disposal of human sewage, animal waste and industrial effluent has provided rich nutrients which create favorable conditions for phytoplankton growth with cyanobacteria dominating. This has led to increase in the overall phytoplankton biomass in the lake with a notable decrease in water transparency (Mugidde, 1993).

Cyanobacteria are a phylum of bacteria that obtain their energy through photosynthesis and are also known as blue-green bacteria, blue-green algae or Cyanophyta. They are abundant throughout the world and contribute significantly to global primary productivity (Garcia-Pichel *et al*., 2003). Several studies have identified many blooming cyanobacterial species and confirmed presence of cyanotoxin producing cyanobacteria in L. Victoria (Haande, 2008; Okello *et al*., 2010).

Studies on water bodies worldwide have shown that some cyanobacterial species produce cyanotoxins which are released on senescence of the algae. These toxins have negative effects on tissues and organs of aquatic and terrestrial organisms exposed to this water (Butler *et al*., 2009; Carmichael, 2012). Presence of such cyanotoxin-producing cyanobacteria in water threatens the ecosystem health of L. Victoria especially the fish which directly feed on cyanobacteria, wildlife which is exposed through drinking lake water, fishermen who are exposed through their occupation and the general public which gets exposed through recreational activities and domestic use of raw water. This study evaluated the possible toxicity effects of cyanobacteria on hematological indices and key body organs after 21-day oral exposure using Swiss mice as a model.

2. Materials and Methods

2.1 Collection, Identification and Processing of Cyanobacteria

Water samples which contained algae were obtained from Murchison and Entebbe Bay by collecting surface water into a sample bottle using horizontal hauls. Samples for identification were preserved by adding 2-3 drops of acidified Lugol's iodine in 1L of cyanobacterial containing water as described by the WHO (1999). A valid sample was submitted to National Water and Sewerage Corporation (NWSC) Bacteriological Laboratory, for identification at a concentration of $3.25x10^5$ L^{-1}. The cyanobacteria species identified were *Microcystis spp*., *Anabaena planktonica* and *Anabaena circinalis*.

The test algae samples were transferred to the Pharmacology and Toxicology Research Lab of the College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB). The samples were filtered using a 100 µm sieve as described by Graham and Johns, (2007) and the residue obtained was preserved in 10% formalin. The sample was then oven dried at 45^{o}C and ground manually using a mortar and pestle to form a powder. The powder (1g) was dissolved in 5 ml of normal saline (0.9 % w/v) to form an aqueous crude extract and complete dissolution was achieved by use of a vortex mixer into a uniform solution. Freshly prepared aqueous crude extracts were used for the toxicity evaluation.

2.2 Animals

The experimental mice (N=40) were obtained from CoVAB animal houses and acclimatized for one week in the lab. They were fed on a standard diet of rodent pellets with clean water provided ad-libitum. The experimental animals were exposed to a 12 hour day - 12 hour night cycle and humidity of 60 ± 5%.

2.3 Evaluation of Acute Toxicity

Twenty mice (16-20g; 7 weeks old; 1:1 male: female ratio) were fasted for 16 hours prior to the study. They were divided into four groups of five mice each. A stock solution of the cyanobacterial powder was prepared in a sterile flask at a concentration of 200 mg/ml. The volume of the stock solution to be administered to the animals was calculated using the formula;

$$Volume = \frac{dose\ (mg/kg) \times weight\ (kg)}{Concentration\ of\ stock\ solution\ (mg/ml)}$$

In a preliminary toxicity study to determine the experimental doses, two mice from two groups were subjected orally to limit test doses of 2500 and 5000 mg/kg (OECD, 2008) and observed for 72 hours post treatment for mortality and any toxicity signs. Basing on the preliminary toxicity results, groups I, II, III and IV of the mice were administered orally once, using an intragastric tube with 1ml normal saline, 5,000, 7,500 and 10,000 mg/kg b.wt. of the extract respectively and any mortalities were recorded.

2.4 Evaluation of Sub-Acute Toxicity

Twenty Swiss mice of body weight 16 - 20g were divided into 4 groups of 5 mice each. The experimental animals were orally dosed once, daily using an intragastric tube (size 4) with 1ml of normal saline (Group 1) while those in groups 2, 3, and 4 received the cyanobacterial extract at doses of 312.5, 625 and 1,250 mg/kg b.wt. respectively for 21 days. The doses of the cyanobacterial extract were calculated from $^1/_4{}^{th}$, $^1/_8{}^{th}$, $^1/_{16}{}^{th}$ of the limit test dose of 5,000 mg/kg b.wt. After the 21 days, the mice were sacrificed and blood obtained by cardiac puncture using a 2ml syringe. The collected blood was stored in EDTA vacutainers and labeled. Fresh organs (liver, duodenum, kidney and lungs) were collected and fixed in 10% buffered formalin.

2.4.1 Hematological Parameters Evaluation

The full blood picture of the collected blood samples was determined using a Beckman coulter auto-analyzer (Model: LH-780). Parameters determined were: RBC count, total WBC count, Hb concentration, hematocrit, MCV, MCH, MCHC, total platelet count and differential WBC count.

2.4.2 Histopathological Evaluation

The collected organs were fixed for 2 days in 10% buffered formalin, trimmed to a suitable size and separately placed in cassettes. The cassettes containing the tissues were loaded in an automated tissue processor which further fixed the tissues in 10% formalin for 1 hour. The tissues were then dehydrated in various isopropanol baths of increasing concentrations of 70%, 80%, 90%, 96% and two changes of absolute alcohol for 1.5 hours in each bath.

This was followed by immersing the tissues in two changes of a clearing agent (xylene) for 1.5 hours in each bath to increase the refractive index. The tissues were then impregnated with paraffin wax by immersing them in two changes of molten paraffin at 56°C for 2 hours in each. The processed tissues were embedded, sectioned using a microtome and the obtained sections (5µm thickness) were mounted on glass slides. The sections were stained using H&E staining technique (Nowacek, 2010; Bancrofti & Gamble, 2011) and observed for any histopathological changes under a high power microscope at Central Diagnostic Laboratory (CoVAB).

2.5 Data Analysis

The means ± SEM of the hematological parameters were determined and statistical analysis was carried out using GraphPad Prism 5.Inc by One-Way ANOVA to compare mean differences among the treatment groups. The differences between means of the treatment and the normal control were tested for significance using the Multiple Comparison Post-hoc Dunnet test. The results were considered statistically significant at 95% level of significance ($p<0.05$).

3. Results

The oral LD_{50} of the crude aqueous cyanobacterial extract was higher than 10,000 mg/kg b.wt. since no mortality was observed at this dose. There was a statistically significant ($p<0.05$) difference in the total WBC count between the control group and the treatment groups (Table 1). Furthermore, the RBC count, hematocrit, and haemoglobin concentrations of the treatment groups were also significantly reduced ($p<0.05$) compared to those of the normal control group at the higher doses of the cyanobacterial extract (Table 1). The basophil and eosinophil levels of all the treatment groups were elevated in comparison to those of the normal control group though the increase was not statistically different ($p>0.05$) as shown in Table 1.

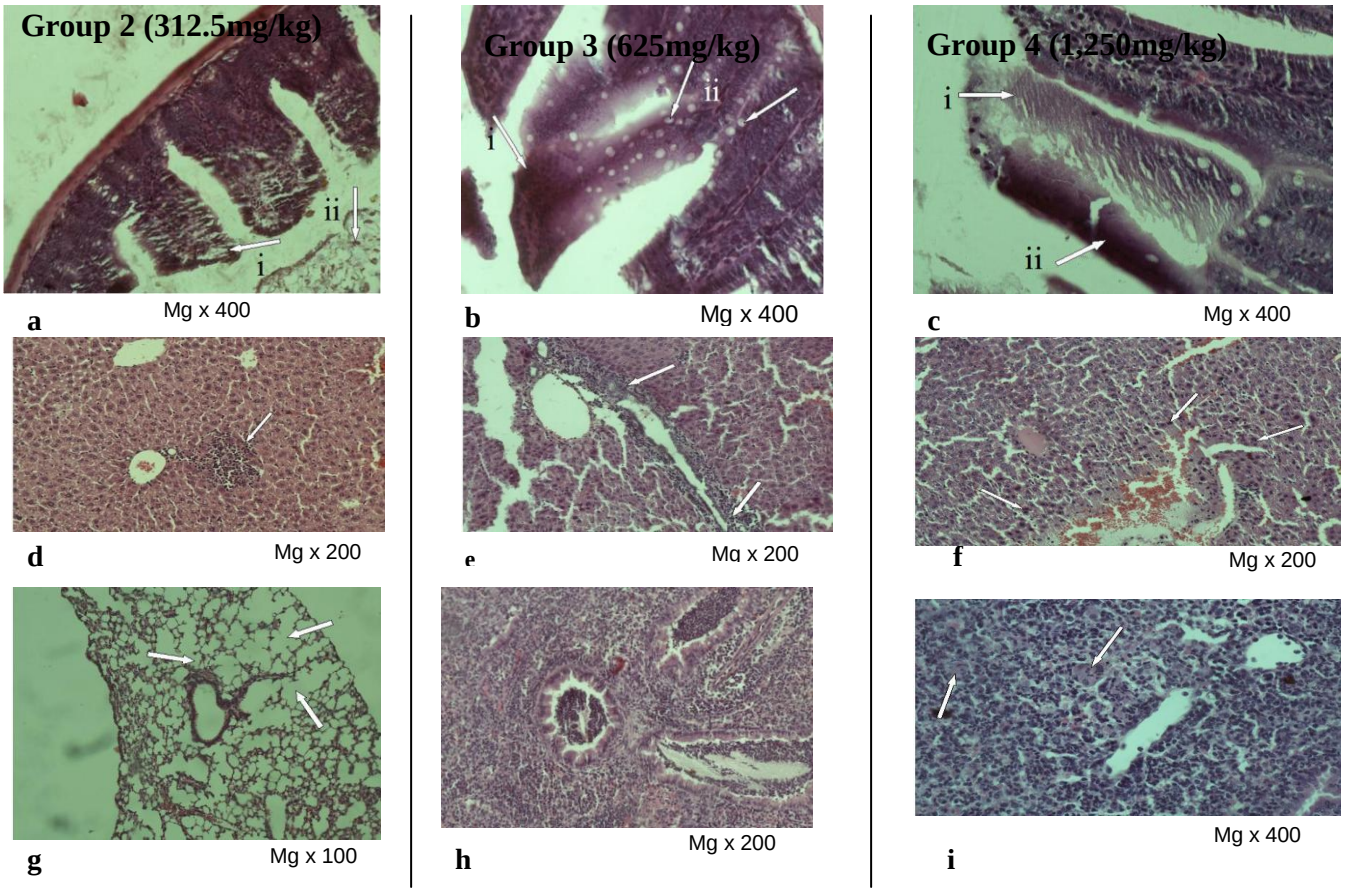
Histopathological evaluation with H&E staining revealed treatment-related tissue changes which intensified with increase dose of the crude cyanobacterial extract (Figure 1). Tissue injuries observed included: squamous metaplasia in the duodenum, vacuolar degeneration in the kidney and hepatic necrosis. In the lungs, there was emphysema, haemorrhage, neutrophilic and lymphocytic infiltration, congestion and broncho-pneumonia. Lung injury observed was predominantly in bronchi and parenchyma in animals dosed with 625 mg/kg b.w of the extract and more diffuse in animals dosed with 1,250 mg/kg b.w of the extract.

Table 1: Hematological Parameters for the Experimental Groups

Hematological parameter	Means ± SEM			
	Group 1 *(Control)*	Group 2 *(312.5 mg/kg)*	Group 3 *(625 mg/kg)*	Group 4 *(1250 mg/kg)*
WBC (10^3/µL)	4.03±0.89	1.60±0.51*	1.18±0.28**	1.10±0.33**
RBC (10^6/µL)	6.22±1.12	3.12±0.40	2.60±0.99*	2.30±0.89*
HGB (g/dL)	10.5±2.08	6.77±0.66	4.57±1.08*	3.10±0.92**
HCT (%)	27.6±8.28	22.10±0.60	18.3±1.86*	16.7±1.49*
MCV (fL)	44.3±1.52	45.2±3.20	44.0±2.58	44.0±2.91
MCH (pg)	17.1±0.61	16.7±0.46	15.4±8.6	13.3±7.46
MCHC (g/dL)	38.8±2.08	37.2±2.13	39.7±4.96	30.7±17.1
Platelets (10^3/µL)	480±91	260±89	235±58.2	227±50
Neutrophils (%)	30.5±8.91	33.1±6.16	28.9±3.34	35.5±3.78
Lymphocytes (%)	54.7±20.2	46.0±8.14	54.8±9.94	47.5±6.83
Monocytes (%)	2.63±1.45	2.57±1.56	2.42±0.42	2.72±1.35
Eosinophils (%)	5.50±2.67	6.95±4.44	6.55±3.97	6.72±3.59
Basophils (%)	6.67±3.61	8.30±3.19	7.32±2.65	7.58±3.39

Key: SEM-standard error of the mean; n=5; ** ($p \leq 0.05$); ** P(0.01)

Fig 1: Histopathological Findings for the Experimental Groups



a) i- Atrophy of the villi, ii- Cellular debris in the lumen. b) i- Focal squamous metaplasia, ii- Increased number of goblet cells in the villi. c) i- Necrosis of intestinal villi. ii- entirely necrotic tissue forming cellular debris in the intestinal lumen. d) Multifocal perivascular necrosis. e) Multifocal hepatocyte necrosis around the blood vessels. f) Diffuse necrosis in the liver. g) Emphysema. h) Emphysema, extensive infiltration by neutrophils and lymphocytes and mild bronchopneumonia. i) Diffuse infiltration by leukocytes, hemorrhage and lung congestion.

4. Discussion

Studies show that blue-green algae possess toxins that have potentially detrimental effects on fish, wildlife and people exposed to them. In this study, the hematological and histopathological effects of a crude aqueous cyanobacterial extract were determined using a mice model. The crude extract provides conditions that closely mimic the situation in the environment (WHO, 1999). The significant reduction ($p<0.05$) in the WBC count between the control group and treatment groups suggests that the crude cyanobacterial extract caused leucopenia and this could be due to an immunosuppressive effect of the blue-green algal extract in mice (Henderson *et al*., 1998). A study conducted in China using BALB/c mice found that exposure to cyanobacterial bloom extract administered by IP injection for 14 days at three sub-lethal doses of 16, 32, 64 mg/kg b.w resulted in immunosuppression in mice (Shen *et al*., 2003). The results of this study are in agreement with those of Yuan *et al*., (2012) that showed a significant decrease of WBC counts with continuous exposure of rabbits to microcystin, a major toxin in blue green algae of *Microcystis spp*.

In this study, RBC count, hematocrit and hemoglobin concentrations were significantly reduced ($p<0.05$) in higher extract doses when compared with the normal control group, thus indicating anaemia. A study by Sukenik *et al*., (2006) reveals that the anaemia produced by the cyanobacteria is due to deformation of red blood cells, which transform into acanthocytes. This transformation of the red blood cell structure is caused by liver dysfunction which affects heam synthesis. This is concomitant with histopathological observations made during the study that indicated liver damage. This also agrees with findings by Zhou *et al*., (2013) who also found significant decrease of RBC counts, Hb and hemacrit in mice on 30 day exposure to microcystin which they attributed to disturbed hematopoietic growth factors and bone marrow cell apoptosis.

The histopathological findings revealed that the degree of tissue injury produced by the extract increases with the crude cyanobacterial extract dose. However, in the kidney tissue injury was minimal and was only observed at the 625 mg/kg extract dose. This could have arisen due to intra-individual species differences, which was also observed by Zhang *et al*., (2002). The squamous metaplasia and vacuolar degeneration observed in the duodenum tissue at the 625 mg/kg dose indicates that prolonged exposure to crude cyanobacterial extract could induce formation of precancerous lesions (Butler *et al*., 2009). Studies by Falconer (2008) also showed similar findings. Other studies indicate that when an irritant causes cell injury like persistent metaplasia, cells may tend to progress to dysplasia, and then to malignant neoplasia or cancer (Kumar *et al*., 2004).

In the lungs, there was progression from emphysema to broncho-pneumonia, and to diffuse pneumonia with increase in dose. Emphysema can be attributed to the necrotic effect of the absorbed cyanobacterial extract circulating in blood on the alveolar walls (Picanço *et al*., 2004). Similar histopathological findings were also observed in studies by Gupta *et al*., (2003) where injury to the bronchi and lung parenchyma was predominant. A study by Soares *et al*., (2007) in mice which were injected intraperitoneally with microcystin-LR found similar results and these included alveolar collapse, interstitial edema and inflammatory cell infiltration. The pneumonia observed can be attributed mainly to liver dysfunction since pathological pulmonary manifestations such as those observed in this study are often caused by alterations in the production or clearance of circulating cytokines and other mediators by the liver (Meyer *et al*., 1999; Zhou *et al*., 2013). This indicates that the crude cyanobacterial aqueous extract induces development of hepato-pulmonary syndrome, a condition associated with end-stage liver disease. An intraperitoneal injection of microcystins has been demonstrated to cause immediate death in laboratory animals by hepatocyte disassociation, loss of sinusoidal structure and death either by intrahepatic hemorrhage or by pulmonary embolism caused by disassociated hepatocytic debris circulating in blood (WHO, 1999; 2003). Cyanotoxins such microcystins have been demonstrated to cause hepatotoxicity and this leads to increase in serum levels of liver enzymes in mice (Deore and Bansal, 2013). There were no major histopathological changes in kidney tissue and this agrees with studies by Carvalho *et al*., (2004).

Conclusion

We have demonstrated that while the cyanobacterial aqueous extract has limited acute toxicity, it can cause deleterious effects on key body organs on continuous exposure. Therefore, there is need to sensitize the public about the potential dangers to human health that could occur due to exposure to cyanobacterial contaminated water for a prolonged period. However, there is need for more detailed chronic exposure studies in mice and other animal models.

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