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Micronucleus as a Biomarker of Genotoxicity in Village Weaver Bird (*Ploceus cucullatus*)

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ABSTRACT

Environmental impact assessment studies highlight the harmful effect of toxicants such as pesticides, heavy metals and industrial wastes on target and non-target species including birds. The effect of toxicant exposure on birds was used as an assessment of environmental contamination. The presence of micronucleus was investigated as a biomarker of genotoxicity in free-ranged village weavers captured within Ibadan Metropolis, Nigeria. Micronucleus assay was done using bone marrow collected from tibia and femur post-mortem. The clinical, haematologic, and biochemical changes were also monitored. The haemocytometric method was used to evaluate the erythrogram and leucogram changes. Microscopy was used for histopathology. It was discovered that 20% of the village weavers were positive for micronucleus while 80% were negative for micronucleus. The tissue changes observed in this study also underscore toxicity to possible environmental toxicants. In this study, micronucleus assay was used to evaluate the possibility of exposure of village weavers to genotoxic substances.

Key words: Avian, Clinical Pathology, Genotoxicity, Micronucleus, *Ploceus cucullatus*

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INTRODUCTION

Avifauna and wildlife play significant role in the ecosystem. However, un-natural activities have made these species very vulnerable to environmental intoxication (Mitra et al., 2011). This present increase in the consideration of these animals has been borne from the important role of these animals in the ecosystem and their potential as sentinels in the monitoring of environmental toxicity (Guitart et al., 1994). A number of toxic agents abound both in nature and as synthetic products of anthropogenic activities. Some of these toxic agents included agro-chemicals such as pesticides, herbicides, heavy metals, and industrial wastes which vary in their comparative toxicity. Exposure to these toxicants may produce genotoxic effects in avifauna and/or wildlife in general. Genotoxicity described the property of chemical agents that induce changes to the structure or number of genes via chemical interaction with DNA and/or non-DNA targets (Maurici et al., 2005). There are three primary effects that genotoxins can have on organisms; these effects can either be carcinogenic, mutagenic or teratogenic affecting the genetic material of an organism (Maurici et al., 2005). Genotoxicity assay plays an important role in the safety evaluation of chemicals and other environmental toxicants (Makoto et al., 2007).

The purpose of genotoxicity testing was to ascertain if animals have been exposed to xenobiotics or environmental toxicants that have genotoxic property or that can affect the genetic material of an organism. It is well known that there are in-vitro and in-vivo assay systems for evaluation of chemical genotoxicity on different endpoints (Makoto et al., 2007). In-vivo tests are important when high levels of organismal exposure are likely and micronucleus assay is one of the in-vivo testing used for assessing exposure to genotoxins in animals (Tice et al., 2000). Micronuclei (MN) are small fragments of nuclear content that appear in the cytoplasm of nucleated cells such as red blood cells of amphibians, birds, fish, and reptiles (Davis and Floyd, 2013), and these micronuclei are formed when cells undergo incomplete division or experience damage to their nuclei (genotoxic damage). They originate from acentric chromosome fragments or whole chromosomes that were not included in the main daughter nuclei during metaphase or anaphase of cell division (Lindberg et al., 2007). Micronuclei reflect chromosome damage and may thus provide a biomarker of early-stage genotoxicity (Hitoshi et al., 2003). The micronucleus assay or test was one of the most widely applied short term test used in genotoxicity and has apparently become one of the most important tests implemented by the regulatory entities of different countries to evaluate mutagenicity of, and sensitivity to, environmental contaminants (EPA, 1998). Wild animal models are recently being used as experimental models for these evaluations though the species employed to monitor the potential genotoxic effect were considered as a source of variability as certain genotoxic agents have been described as species-specific (Da Silva et al., 2000). The presence and frequency of micronucleus in bone marrow smear serves as an important guide in the assessment of genotoxicity of birds. The presence of village weavers in the tropic environment makes them good sentinels to environmental toxicant and exposure.

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Given the potential for contamination by environmental toxicants and the importance of birds as bio-monitors of contamination, this study is proposed to determine genotoxic effects of environmental toxicants in village weavers within Ibadan metropolis, Nigeria using the micronucleus as a biomarker.

MATERIAL AND METHODS

Preliminary survey, Study design and animals

Personal interviews were conducted with the captors of these animals in three markets within Ibadan (Bode, Oranyan and Sasha), Nigeria. It was discovered that these birds were trapped from farmlands (roosting sites) within Ibadan Metropolis. They were often captured at night with net traps. These birds were granivores feeding mostly on seeds of sorghum, millet, rice, maize grains, other cereals and insects. Thirty village weaver-birds (*Ploceus cucullatus*) with an average weight of 32g were captured. Since the animals were not aggressive, they were restrained manually with gloves

Collection and Analysis of blood and tissue samples

Two ml blood samples were collected via jugular venipuncture with the aid of a 23 gauge hypodermic sterile needle and a sterilized 2mL syringe into residue-free lithium heparinized sample bottles for haematology and non-heparinized sample bottles for serum biochemistry. The serum samples were separated by centrifugation at 1200 rpm for 15 minutes into plastic vials. The procedure for sample collection was in strict adherence to the FAO manual of sample collection from wild birds. Haematological parameters such as Packed Cell Volume (PCV), Haemoglobin concentration (Hb) and Red Blood Cell (RBC) were determined using standard techniques as already described by Coles, 1986 and Omonona and Emikpe, 2011. The biochemical parameters (Aspartate Aminotransferase and Alanine Aminotransferase) were determined using the haemocytometric method as described by (Bishay et al., 2000). Bone marrow smears from the tibia and femur bones of the birds were used for micronucleus assay. It was fixed in 99% methanol and stained with Romanowsky-Giemsa stain. The presence and frequencies micronucleus were scored for the presence of micronucleus using an oil immersion objective (100×) Olympus CX21 light microscope. The birds were divided into two groups, based on the presence and frequency of micronucleus (Countryman and Heddle, 1976). The first group (positive) was six in number while the second group (negative) was twenty-four in number. Also, tissues from the lung, liver and kidney were collected, immersed in 10% buffered formalin and processed routinely for histopathology (Cooper, 2002). Microscopic evaluation of tissue changes was similarly done using a light microscope (Olympus CX21).

Statistical analysis

Average statistical mean ± standard deviation was used for the description of the result while subjecting it to T-test using Statistical Package for Social Sciences (SPSS version 20). Significant differences were assumed at $P \leq 0.05$.

RESULTS

Micronucleus assay

The micronucleus assay revealed that 20% of the village weavers were positive for micronucleus while 80% were negative as shown in table 1. The micronucleus assay result was used to separate the birds into two groups. Figures 1 and 2 show micronucleus in polychromatophilic red cells in the bone marrow smear (Giemsa stain 100× magnification) in an adult village weaver-bird.

Haematology

It was observed that the means of Hb, RBC, MCH and MCHC showed a reduction in values in the Micronucleus Positive (MNP) compared to the Micronucleus Negative (MNN) birds. Reduction in values of the mean PCV and MCV were statistically not significant ($P \geq 0.05$) in the MNP birds compared with the MNN birds. The mean PCV of the MNP and MNN though showed variations, both were within the reference range for normal signifying absence of anaemia, while individually, very few birds were anaemic and some showed possible signs of dehydration or polycythemia. The means of the PCV and MCV showed higher values in the MNP compared to the MNN though not statistically significant ($P \geq 0.05$). Furthermore, the means of White Blood Cell (WBC), Platelet count (PLT) and Heterophil (HETERO) showed a reduction in values in the MNP birds compared to the MNN birds. The mean WBC of the MNP and MNN though showed variations, were above the reference range for normal signifying leucocytosis, while the mean PLT of the MNP and MNN too though showed variations, were above the reference range for the normal indicative of thrombocytosis.

Serum Biochemistry

It was observed that the means of ALT showed a reduction in values in the MNP when compared to the MNN birds ($P \geq 0.05$). The variation in the mean values of the liver enzymes was suggestive of damage to the liver (hepatocellular injury).

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Histopathology

The findings showed that there were varying degrees of atrophy to loss of villi, necrosis of enterocytes, and cryptal hyperplasia to necrosis with minimal inflammation in the intestine (Figure 3). Predominant changes observed in the liver include centrilobular to diffuse degeneration of hepatocytes and necrosis in peri-portal zones of the liver (Figure 5) while vascular changes were observed in the lungs (Figure 4) of the village weaver-birds. In the kidney, there were chiefly degenerative changes in the renal tubular epithelium to a few showing necrosis and minimal dystrophic calcification (Figure 6).

Table 1. Micronucleus prevalence and red blood cell indices in adult weaver-birds

MN	Number	PCV	Hb	RBC	MCV	MCH	MCHC
Positive	6	47.2 ± 3.06	8.7 ± 1.02	3.2 ± 0.44	149.6 ± 32.91	27.4 ± 4.84	18.5 ± 2.04
Negative	24	44.7 ± 6.54	9.4 ± 0.82	3.3 ± 0.35	138.3 ± 26.38	29.1 ± 4.59	21.5 ± 4.09

MN: Micronucleus, PCV (%): Packed Cell Volume, Hb (g/dl): Haemoglobin Concentration, RBC (*103/µL): Red Blood Cell, MCV (fl): Mean Cell Volume, MCH (%): Mean Cell Haemoglobin, MCHC (pg): Mean Cell Haemoglobin Concentration

Table 2. Micronucleus prevalence and white blood cell indices in adult weaver-birds

MN	Number	WBC	PLT	LYM	HETERO	MONO	EOSINO	BASO
Positive	6	1.6 ± 3.0	1.4 ± 4.6	1.0 ± 1.9	3.9 ± 1.5	5.8 ± 2.9	7.1 ± 2.6	52.6 ± 84.89
Negative	24	1.5 ± 2.2	1.4 ± 4.3	9.9 ± 1.5	4.9 ± 1.7	4.9 ± 1.9	6.6 ± 3.4	45.4 ± 73.24

MN: Micronucleus, WBC (*10^3/µL): White Blood Cell, PLT (*10^5/µL): Platelet Count, Lympho: Lymphocytes, HETERO: Heterophil, Mono: Monocytes, Esino: Eosinophils, Baso: Basophils-

Table 3. Micronucleus prevalence and serum biochemistry indices in adult weaver-birds

MN	Number	AST	ALT
Positive	6	209.7 ± 35.83	23.2 ± 6.46
Negative	24	194.5 ± 28.49	24.5 ± 4.49

MN: Micronucleus, AST: Aspartate Amino transferase (U/l), ALT: Alanine Aminotransferase (U/l).

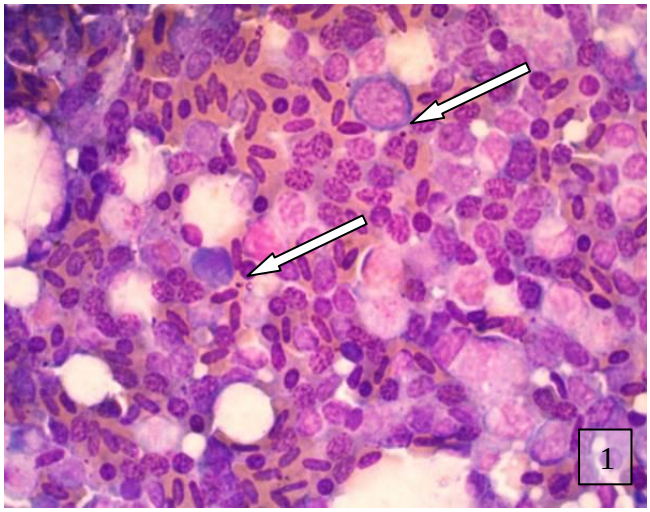

Figure 1. Cells with micronucleus (white arrow) in bone marrow smear stained with Giemsa stain (x 100 magnification) in an adult village weaver-bird.

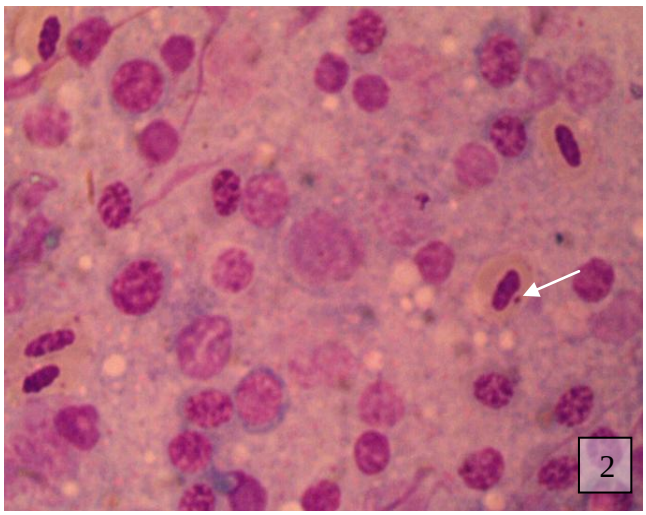

Figure 2. Polychromatophilic red cells with micronucleus (white arrow) in bone marrow smear stained with Giemsa stain (100× magnification) in an adult village weaver-bird.

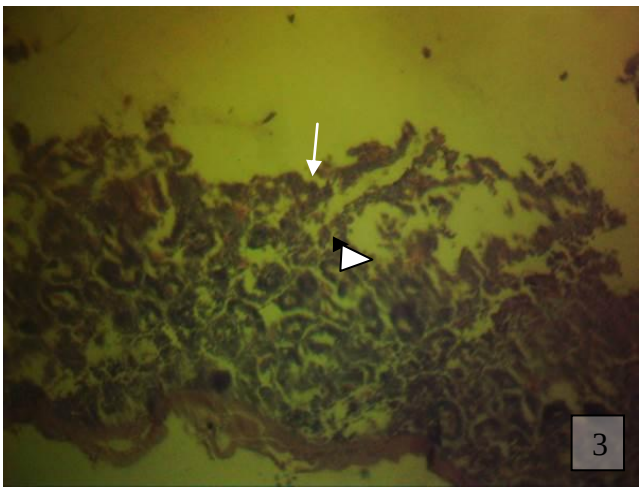

Figure 3. Necrosis of intestinal crypts (arrow) in an adult village weaver-bird (100× magnification).

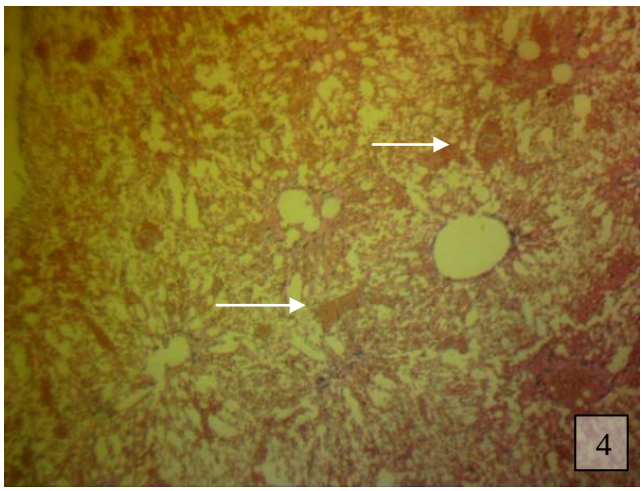

Figure 4. Congestion and haemorrhage in air spaces (arrow) of lungs in an adult village weaver-bird. (100× magnification)

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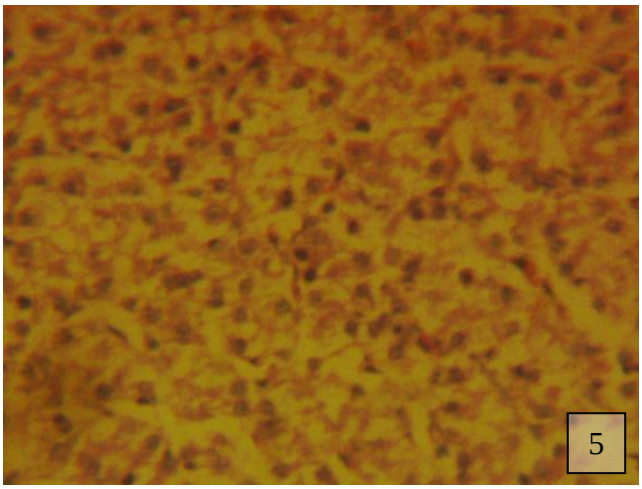

Figure 5. Diffuse degeneration of liver hepatocytes in adult village weaver-bird (100× magnification).

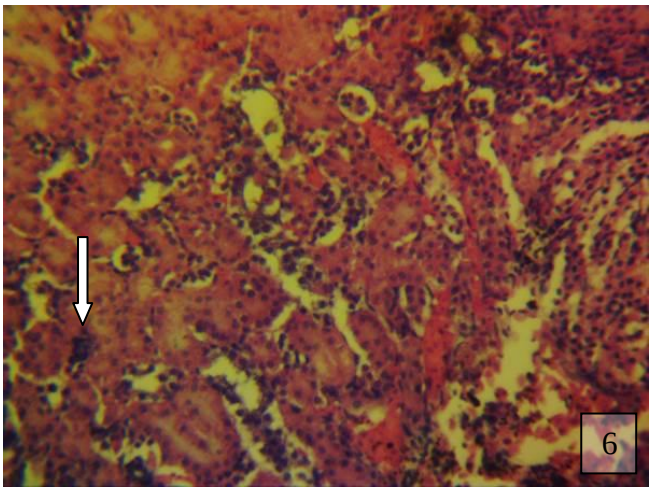

Figure 6. Necrosis of renal tubular epithelium of kidney in adult village weaver-bird (100x magnification).

DISCUSSION

From this study, it was discovered that 20% of the village weavers were positive for micronucleus (MNP) while 80% were negative for micronucleus, which showed some level of toxicity or chromosomal changes in these birds. This is consistent with the report of Fenech and Morley (1989) who reported that micronucleus could signify chromosomal damages caused by cytotoxic agents. Also some researchers have associated the presence and frequency of micronucleus to DNA damage and subsequent genotoxicity (Watters et al., 2009).

The presence of micronuclei in the bone marrow smear is a normal finding in most cases however the relative measure of the frequency of micronucleus serves as a basis for evaluating the normal physiological occurrence and pathological presentation (Countryman and Heddle, 1976). In the study of Countryman and Heddle (1976) the presence of 10 cells (5%) with micronucleus in a count of 200 cells is indicative of a pathological DNA alteration. The positive samples are indicative of exposure to genotoxins (any of the environmental pollutants), which have been reported to cause chromosomal damages as reported by (Sirois, 1995). MN can also be adduced to the defect in the cellular DNA repair mechanisms and the resultant accumulation of DNA aberrations and chromosomal alterations occurring post-exposure to gentotoxic agents (Watters et al., 2009).

As reported by Sears and Udden (2011) the presence of micronucleus, its frequency and other nuclear abnormalities such as bi-nucleation and mitotic figures were used for the evaluation of genotoxic effects in this study. The presence of bi-nucleation also in the present study further corroborates the pathological evidence of micronucleus seen in bone marrow smears of the adult village weaver-birds stained with Giemsa. The presence of all these nuclear abnormalities and changes is also in conformity with the report by Bishay et al., 2000 that highlighted the use of bi-nucleation, multi-nucleation and high mitotic index as indices of genetic change and damage.

Free-ranging wild animals can be exposed to different environmental pollutants that may contribute to the prevalence of genetic damage and others diseases in the population (Šutiaková et al., 2004). This study has been able to affirm this assertion through the levels of genotoxicity seen in 20% of the randomly sampled village weaver-birds used for this study. The occurrence and frequency of micronucleus as an indicator of genotoxicity in these weaver-birds is assumed to be associated with the presence of genotoxicity-inducing agents, chemicals or compounds in the environment in which they were captured. This is of significance considering the feeding and behavioral ecology of the birds.

Exposure to environmental pollution has also been associated with a lot of deleterious damages to human, animal and environmental health in general (Anand et al., 2008). Some examples include increase in incidence and mortality of cardiopulmonary diseases; immunosuppression and increase in susceptibility to diseases; organ disturbances and failure; reduction in life expectancy and increases in cancer incidence (Cohen et al., 2005; Kargarfard, 2011 and Coogan et al., 2012). The tissue changes observed in this study also underscore toxicity to possible environmental contaminants, which may not be unconnected to chronic exposures and bioaccumulation of such xenobiotic substances. The range of degenerative to inflammatory changes seen in the histopathology of the study animals also serves as proof of the pathologies associated with exposure to environmental pollutants. The summative effect of these histopathologic changes may result in deleterious sequela such as immunosuppression, organ failure, and neoplastic changes with concomitant animal and biodiversity loss.

The observed changes in the haematological, serum chemistry and histopathological indices in this study serve as an indicator of the state of health of these birds. These observed pathologic changes serves as an important evidence of the clinical haematological and clinical biochemical changes associated with exposure to environmental pollutants and the concomitant genotoxic changes (Sirois, 1995).

The outcome of this study forms a preliminary data on the health effect of environmental contaminants using village weavers as sentinels to detect genotoxicity and comparing with the haematological, biochemical indices (Opara and Fagbemi, 2008). The knowledge from this study can be used as a basis for adjunct and/or future studies of prevalence, types, effects and possible control of environmental pollutants especially the genotoxic pollutants. Furthermore due to the relative abundance of these birds in the environment and their close proximity to human

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settlements, this study serves to provide a basis for the relative importance of these birds as biomonitoring sentinels for ascertaining environmental toxicity threats to public health.

In conclusion, micronucleus assay in this study has been used to evaluate the possibility of exposure of the Village-weavers to any of the wide range of genotoxic susbstances found in the environment in which they are captured. It is therefore recommended that further studies of residue analyses be done to ascertain the different environmental contaminants responsible for the genotoxicity seen in the study birds as shown by the presence of micronucleus.

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