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Original Article

In vivo micronucleus test as a biomarker of genotoxicity in free-range goats from suspected contaminated environment

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ABSTRACT

Objective: Environmental pollution and the resultant genotoxicity, has become a major livestock, public and environmental health concern with direct impact on the ecosystem. Here, application of micronucleus test and frequency score as a potential biomarker of genotoxic effect and bio-monitoring have been discussed aiming at exploring environmental polution.

Materials and methods: A total of 100 domestic goats slaughtered at the Bodija Municipal Abattoir were used in this study. Blood sample was analyzed for the quantification of the hematological parameters. The bone marrow smear was viewed microscopically for the detection of micronucleus and other nuclear abnormalities. The frequency of micronucleus was quantified to group the sampled goats into MN-positive and MN-negative groups for further analysis.

Results: MN was positive in 21% of the sampled goats with varying frequency ranging from (6-15% count per 2000 cells examined). Bi-nucleation, multi-nucleation and high mitotic index were also observed and quantified. The packed cell volume, mean corpuscular volume and neutrophil count were significantly lower ($P<0.05$) in the MN-positive groups while anemia was reported in 33.3% of the MN-positive goats.

Conclusion: The finding indicates the prevalence and frequency of micronucleus as a biomarker of genotoxicity and an indicator of exposure to environmental genotoxic subtances. Hence, this highlights the relevance of these goats as important sentinel animal model. These findings, therefore, serve as a preliminary data for further studies on the latent genotoxic environmental contaminants and their potential deleterious impact.

KEYWORDS

Environmental toxicity; Genotoxicity; Micronucleus; Sentinel animals; Toxicology biomarkers

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INTRODUCTION

The environment encompasses the arboreal, terrestrial and aquatic factors that surround and support the biotic and abiotic factors on earth and serves a key role as the habitat for humans, animals and other biological lives. This natural environment has over the years been progressively polluted as a consequence of both natural (such as volcanic eruptions) and anthropogenic activities *viz*., mining, industrial activities, smelting and refining of metals, fossil fuel combustion, incineration of municipal wastes, vehicle emissions (Tchounwou et al., 2012). These diverse human activities and their consequences have made anthropogenic activities the worse driver of environmental pollution. The emergence and the progression of industrialisation over the years have also significantly worsened the deleterious impact of anthropogenic activities on the environment (Loux et al., 2011). The crucial role the environment plays in the sustenance of the biotic and abiotic component of the earth, has therefore made the excessive levels of environmental pollution a major source of threat to the ecosystem, humans, animals and plants (Kelishadi et al., 2009). This has thus resulted in the global development of different interventions, including impact assessment, legislations and policies as a means of stymieing environmental pollution. However, despite the many efforts aimed at mitigating the scourge, environmental pollution continues to pose a ubiquitous risk and threat to human and animal health (Briggs, 2003).

The impact of environmental pollution is as varied as the different components (effluents, chemicals and substances) that constitute environmental pollutant and the different sources of the pollutant (Luzhna et al., 2013). Hence, the detrimental effects of these pollutants on both the biotic (human, animals, plants, microbes) and the abiotic factors that constitute the environment are expressed through diverse mechanisms of actions (Olchawa et al., 2006). This has thus resulted in the utilization of different biomarkers to assess the pathogenic and health impact of environmental pollution (Briggs, 2003). Some of the health problems associated with exposure to these pollutants include immunosuppression; increase the incidence of disease, reduced life expectancy, reproductive loss and cancers (Filippini et al., 2015). Aside the many global health impacts of environmental pollution, different meta-analysis and systematic review of evidence-based studies have also shown the correlation and association between cancer risk (and prevalence) and diverse environmental risk factors. The neoplastic transformation and cancers associated with environmental pollution have been ascribed to the DNA damages and resultant compromised integrity of the genetic constitution of the exposed subject due to the genotoxic effects of the pollutants (Claxton, 2015). Genotoxicity due to DNA damage sequel to pollutant exposure therefore thus serves as an important environmental pollution biomarker of effect and a measure of carcinogenesis. Some of the assays used for assessing genotoxicity as a measure of the DNA damage in cells exposed to the toxic substrates include Ames Assay, *in-vitro* and *in-vivo* Toxicology Tests, and Comet Assay, Chromosome Aberration Test, Cytotoxicity Assay and Micronucleus Assay (Claxton, 2015; Hayashi, 2016).

Micronucleus (MN) assay is one of the most sensitive markers for detecting DNA damage and has been used to investigate the genotoxicity, clastogenicity and aneugenicity of a variety of chemicals (Morita et al., 2011). This has been extensively used as an important investigative tool for hazard screening, exposure-based risk assessments and in investigating the effects of clastogens and aneuploidogens in occupational and environmental exposure in human epidemiological studies (Ishikawa et al., 2003).

Different criteria have been used for the scoring of micronucleus as a biomarker for genotoxicity. As described by Luzhna et al. (2013), MNs are smaller nucleus (one or more) often found in association with a main nucleus in a cell and are typically 1/3 to 1/6 the size of the main nucleus. In comparison with the main nucleus, MN is characteristically round to oval and bears comparable staining intensity and texture with the nucleus. MN detection is a physiological finding in the bone marrow due to the high hemopoietic cells replicative activities, hence a baseline MN frequency of 5% is adjudged as normal. This frequency score threshold is thus used for adjudging detection of MN in micronucleated polychromatic erythrocytes either as a physiological or pathological occurrence (Luzhna et al., 2013). In addition to MN detection, the presence of other nuclear abnormalities such as binucleation, multinucleation, high (aberrant) mitotic index has also been used as indices of genetic change and damage (Osman, 2014). Hence the combination of these nuclear abnormalities can be used as supporting evidence to corroborate the evidence of the detection of a high micronucleus frequency score as a pathological change.

Domestic animals due to their interaction with the environment are exposed to different pollutants leading to a resultant occurrence of toxic changes including DNA damage, nuclear changes and other diseases in the population (Tchounwou et al., 2012; Corredor-Santamaría et al., 2016). These animals could, therefore,

be used as sentinel animals for the assessment of the biomarkers and in the early detection of the effect of environmental pollutions on public health due to their cohabitation with humans. The trophic level of some of these domestic animals such as small ruminants (goats) makes them important sentinels due to their ability to bioaccumulate and biomagnify environmental pollutants present in the ecosystem hence predisposing them to toxic changes (Hosseini et al., 2013). Furthermore, in developing countries, the semi-intensive husbandry system and the cohabitation of small ruminants in close proximity to humans also make them especially useful as sentinel animals (Reif, 2011). The objective of our study was to access the detection of micronucleus as a genotoxicity biomarker for the evaluation of the impact of environmental pollution on goats slaughtered in Ibadan, Nigeria.

MATERIALS AND METHODS

Study Area, time and animal: The study was carried out in Ibadan (7°23'47"N 3°55'0"E), Oyo State from October 2015 - January 2016. A total of 100 adult male goats were selected for this study from the goats slaughtered at the Bodija Municipal Abattoir, Ibadan. The selected goats were all Red Sokoto breed and the weight ranged from 12 to 18 Kg. Antemortem examination was conducted for the goats for obvious clinical signs and to ascertain their health status.

Blood and bone marrow collection: Blood samples (2-3 mL) were collected by Jugular venipuncture into EDTA bottles and transported using cold packs to the laboratory for complete blood cell count (Latimer et al., 2003). The femoral bones of the slaughtered goats were collected post-mortem and the bone marrow smears were made immediately to ensure optimal retention of marrow cytomorphology (Valli et al., 2002)

Hematology: Complete blood cell count was carried out on the collected blood sample for a quantitative quantification of the red cell, leukocyte and platelet parameters using routine method as described by Duncan and Prasse (Latimer et al., 2003).

Bone marrow examination and micronucleus frequency: The bone marrow smear was made using the femoral bones according to the method described by Viegas et al. (2010). The slides were dried at room temperature overnight and then fixed and stained using routine Giemsa stain. The stained smears were examined using an Olympus light microscope (CX21) attached to a digital computerised camera (AmScope, MU900), (1000× magnification), at medium magnification to examine the cell morphology, spread of the cells and the staining quality of the slides. The visual detection of micronucleus, other nuclear abnormalities (such as abnormal mitotic index, binucleation and multinucleation) and the assessment of the bone marrow count to evaluate the count of myeloid series, erythroid series and the myeloid-erythroid ratio was then carried out at higher magnification.

The quantitative count of the micronucleated erythrocyte was obtained by counting the number of micronuclei observed per 2000 polychromatic erythrocytes (PCE) and this was used to compute the percentage micronucleus frequency as described by Viegas et al. (2010). The detection of more than 5% micronucleus frequency was used as the threshold benchmark for pathology and such samples were reported as MN-positive as described by Aquino et al. (2011). The hematology of the MN-positive and MN-negative goats was also compared.

Statistical analysis: The statistical analysis of the study data was carried out using SPSS for Windows Ver. 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, New York: IBM Corp.). Student's *t*-test was used to compare two means and Fisher exact test was used for comparison of proportions between groups where appropriate. A *P*-value of <0.05 was accepted as statistically significant.

RESULTS

Genotoxicity

From this study, 21% of the sampled goats were MN-positive with MN frequency score ranging between 6-15%. The microscopic examination of the bone marrow smear for micronucleus detection and scoring also revealed varying abundance of other nuclear abnormalities such binucleation, multi-nucleation and high mitotic index (**Figure 1-2**). A high prevalence of aberrant high mitotic index was recorded as the most prevalent nuclear abnormalities compared to multinucleation which was the least observed of the other nuclear abnormalities (**Figure 3**).

Hematological variables

The hematological changes in the MN-positive and MN-negative goats are represented in **Table 1**. The erythrocyte and neutrophil count as shown in the MN-positive goats was significantly lower ($P<0.05$) compared to the MN-negative goats. In terms of the erythrocyte

Table 1: Mean±SD of the hematology of the MN-positive and MN-negative goats

Hematological	MN positive	MN Negative
Packed Cell Volume (%)	25.3±1.2*	28.2±1.8
Hemoglobin Conc. (g**m**/dL)	8.4±2.3	9.3±2.4
Red Blood Cell Count ($\times10^3/\mu L$)	9.6±1.8	10.1±1.9
Mean Cell Volume (fL)	25.9±3.6*	28.5±8.6
MCHC (g**m**/dL)	33.1±1.9	32.9±3.6
Platelet ($\times10^5/\mu L$)	158.5±85.8	181.2±96.0
White Blood Cell Count ($\times10^3/\mu L$)	8.7±3.2	8.1±3.3
Lymphocyte Count ($\times10^3/\mu L$)	4.6±2.5	4.4±2.5
Neutrophil Count ($\times10^3/\mu L$)	3.9±1.6*	6.7±1.9
Monocyte Count ($\times10^3/\mu L$)	0.1±0.1	0.2±0.3
Eosinophil Count ($\times10^3/\mu L$)	0.0±0.1	0.0±0.1

*MCHC: Mean Cell Hemoglobin Concentration. *Significance at P<0.05*

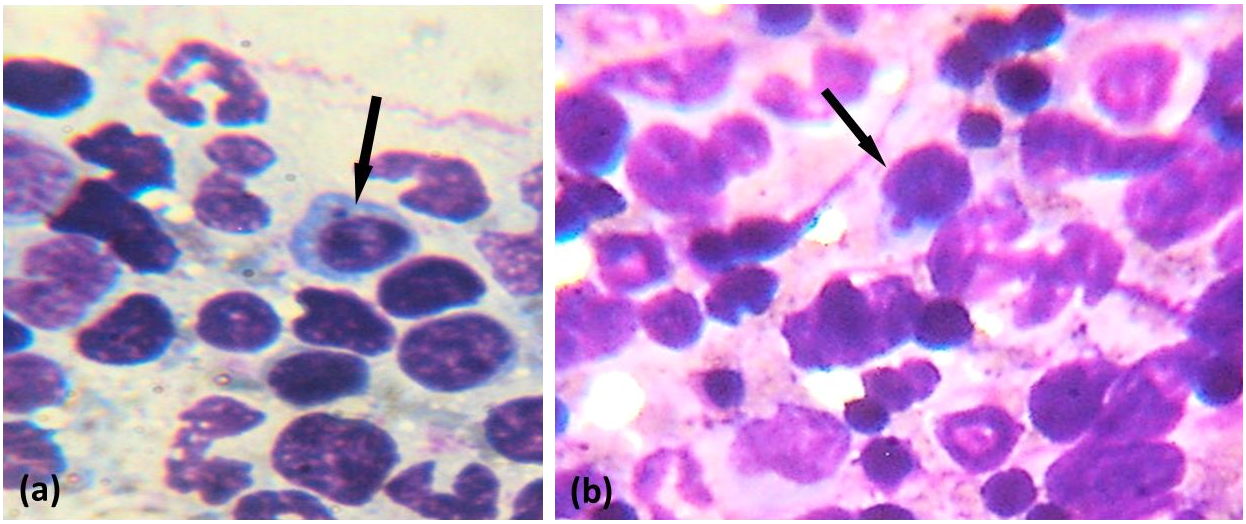

Figure 1: Micronucleus in polychromatophilic erythrocytes (Giemsa stain ×100). (a) micronucleus in early polychromatophilic erythrocyte (black arrow) in bone marrow smear. (b) micronucleus in a polychromatophilic erythrocyte (black arrow) in bone marrow smear.

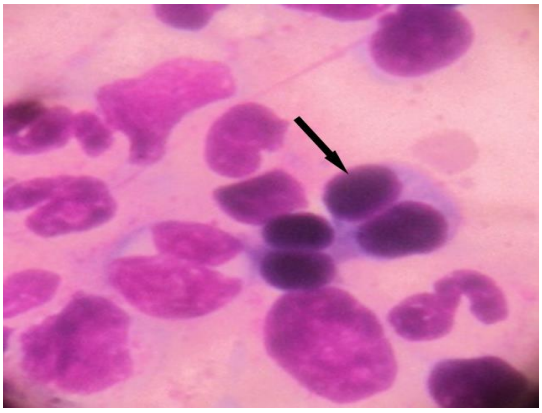

Figure 2: Binucleated cells (black arrows) in bone marrow smear stained with Giemsa stain ×100 magnification.

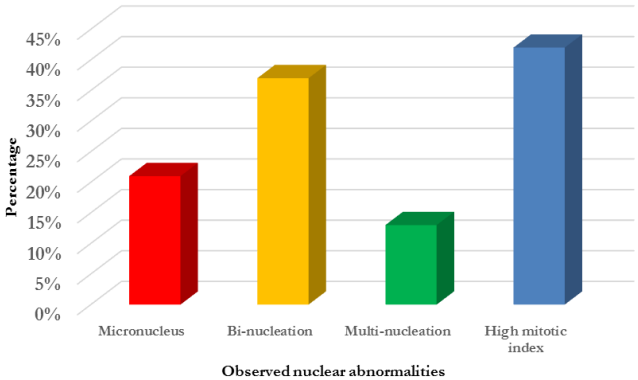

Figure 3: Percentage of MN-positive goats and other nuclear abnormalities in the bone marrow of the goats

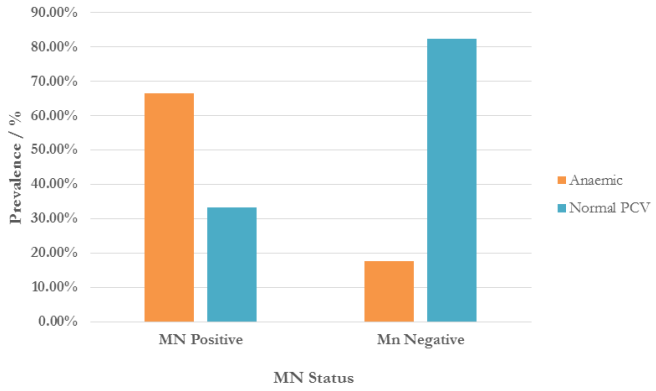

Figure 4: Relationship between the anemic statuses of the sampled goats based on their MN status.

parameters, the PCV and MCV values of the MN-positive goats (25.3±1.2% and 25.9±3.6 fL respectively) were significantly lower than the values observed in the MN-negative goats. The mean values of these erythrocyte parameters were in the lower range of the normal which aligned with the anemia reported in some of the sampled goats. Furthermore, an analysis of the difference in the prevalence of anemia status was conducted in the two MN groups (Figure 4) with a significantly higher (P<0.05) prevalence of anemia observed in the MN-positive compared to the MN-negative groups.

A significant reduction (P<0.05) was also observed in the neutrophil count of the MN-positive goats (3.9±1.6 $\times 10^3/\mu L$) compared to the MN-negative goats. There was, however, no significant difference in the platelet count and the other white blood cell parameters between the two MN groups.

DISCUSSION

Micronucleus assay and the detection of an elevated frequency of micronucleated polychromatic erythrocytes above the baseline in field exposures or above the control subjects in treated animals has been used as an indication of induced chromosome damage (Terradas et al., 2010, Luzhna et al., 2013).

From this study, 21% of the sampled goats were MN – positive with micronucleus frequency score above the physiological threshold (5% MN frequency score) as recommended by Fenech (2000). The detection of MN frequency above this threshold has thus been attributed to significant DNA damage/genotoxic and chromosomal damages caused by exposure to cytotoxic agents. Similar observation has also been made by other researchers in correlating the detection and a high frequency score of micronucleus to DNA damage and genotoxicity (Lau et al., 2009; Watters et al., 2009; Lal and Ames, 2011) The occurrence and frequency of micronucleus detected in this present study can therefore be associated with the exposure of the sampled goats to genotoxic agents in the environment in which they were raised. This finding is corroborated by the detection of similar high frequency of micronucleus and other nuclear abnormalities in fishes caught from polluted water bodies (Corredor-Santamaría et al., 2016).

The presence of other nuclear abnormalities found in this study also further supports the evidence of the detected high micronucleus frequency as pathological changes. According to different studies, bi-nucleation, multi-nucleation and high mitotic all serve as important indices of DNA damage and genotoxic changes associated with toxicological exposures (Osman, 2014). For instance, toxicological studies have shown a significant correlation between binucleated cells with MN and low to medium high blood lead level thus further reinforcing the importance of the nuclear abnormality found in this study as indicators of toxicological changes and exposure (Vaglenov et al., 2001).

The significantly lower (P<0.05) PCV and MCV detected in the MN-positive study goats along with the higher of anemia in the MN-positive goats also serves as an important indicator of the pathological impact of the inciting toxicant and the associated genotoxicity in the MN-positive goats. This finding is also consistent with the report of similar reduction in hematological parameters from different toxicological exposure studies and the association of such findings with genotoxicity and high micronucleus frequency in other studies (Corredor-Santamaría et al., 2016). This reduction along with the drop in the neutrophil count can be ascribed to damage to the bone marrow causing a reduced hematopoietic activity of the bone marrow and the modification of the hemopoietic cell division and maturation (Corredor-Santamaría et al., 2016). Other potential pathogenetic changes that can be responsible for the hematological parameter changes include alteration of the hemopoietic mechanisms, increased blood cell reduction and shortened life cycle, and bone marrow depression due to the effect of pollutants exposure (Valli et al., 2002).

CONCLUSION

This present study on the detection and frequency of micronucleus, a genotoxic change in the goats, therefore, points to the exposure of these animals to genotoxic agents (environmental) and raises the concern of the potential threats of the exposure to the goats and other

inhabitant of the environment from which the goats were sampled. The knowledge from this study is thus intended to serve as a basis for future studies on the prevalence, sources, effects and possible control of environmental pollutants in terrestrial habitats.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AJJ did the study conception and design, acquisition of funding, analysis and interpretation of data, drafting of manuscript and critical revision. OOT contributed by working on the acquisition of data, acquisition of funding, analysis and interpretation of data and drafting of manuscript. AAA contributed by running the analysis and interpretation of data and also took part in drafting of manuscript and critical revision.

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