World Engineering & Applied Sciences Journal 5 (2): 53-58, 2014
ISSN 2079-2204

DOI: 10.5829/idosi.weasj.2014.5.2.1121

Cytotoxic and Genotoxic Effects of Two Detergents on *Rattus norvegicus*

[1]P.N. Abara, [2]U.A. Ibiam, B. Ubi, [4]A.C. Udebuani and [2]P.C. Ugwu Okechukwu

[1]Department of Biological Sciences, Federal University of Technology,
P.M.B. 1526, Owerri, Imo State Nigeria
[2]Department of Biochemistry, Ebonyi State University,
P.M.B. 053, Abakaliki, Ebonyi State, Nigeria
[3]Department of Biotechnology, Ebonyi State University,
P.M.B. 053, Abakaliki, Ebonyi State, Nigeria
[4]Department of Biotechnology, Federal University of Technology,
P.M.B. 1526, Owerri, Imo State Nigeria

Abstract: In the present work, we have evaluated the cytotoxic and genotoxic potentials of Omo® and Ariel® detergents on the bone marrow erythrocyte of *Rattus norvegicus* using the micronucleus (MN) and nuclear lesion (NL) tests. The groups exposed to varying concentrations of the two detergents showed statistically significant differences in MN and NL frequencies with respect to the control. Also, a significant increase ($p < 0.001$) in micronuclei and nuclear lesion frequencies were recorded with increase in detergent concentrations and period of exposure. The formaton of nuclear abnormalities confirmed the results of induction of MN and are indications of genotoxic damage.

Key words: Cytotoxicity · Genotoxicity · Detergent · *Rattus norvrgicus*

INTRODUCTION

Detergent means any substance which has the ability to clean an object. This includes soaps, soap powders and dish washing liquids. The major components of detergents are the surface active agents known as surfactants. Most detergents contain a mixture of surfactants to boost detergency [1]. Commercial detergents also contain substances such as enzymes, brighteners, builders and dyes. The most common builder used is sodium tripolyphosphate (STPP). Since phosphates are good fertilizers, they cause the algal bloom which leads to eutrophication and subsequently, pollution [2].

In Nigeria, detergents have been deployed for many uses other than washing of household utensils and dishes; for example, vegetables and fruits such as carrots, garden eggs, mangoes and oranges are washed with detergents to improve their appearance and acceptability by buyers. This is a common sight as one travels along major highways across the nation. Also, many egg retailers boil eggs with detergent because it helps to prevent cracking of the egg shell during boiling and enhances peeling of the shell after boiling; however, it also lowers the fat-soluble vitamins, proteins, cholesterol contents and fatty acid profile [2, 3]. Currently, many rural women in the eastern parts of Nigeria use detergents to ferment cassava used for making fufu because it quickens the fermentation process. In all of these practices, residual detergents are deposited in the utensils, fruits and vegetables, eggs and the fermented cassava, exposing humans to daily intake of sub-lethal doses of detergents by the oral route. These are not without some deleterious consequences though initially unnoticed.

The nucleus is the organelle in the cell that contains the genetic material (DNA) that directs normal cellular function and cellular reproduction [4]. Chromosome shape, size and number are constant for any species of organism. During cell division, the genetic material replicates and then divides equally between the two

Corresponding Author: P.N. Abara, Department of Biological Sciences, Federal University of Technology, P.M.B. 1526, Owerri, Imo State Nigeria.

daughter cells that are produced. If the process is disrupted, or the chromosomes are broken or damaged by chemicals or radiation, then the distribution of genetic material between the two daughter nuclei during cell division may be affected and pieces or entire chromosomes may fail to be included in either of the two daughter nuclei [5]. When this occurs, the genetic material that is not incorporated into a new nucleus may form its own 'micronucleus' which is clearly visible with a microscope [6].

Exposure to toxic substances can damage the DNA of living cells [7] and if not repaired, these DNA lesions can initiate a series of biological effects (consequences) at the celluar, organ, whole organism and finally at the community and population levels [8].

A growing interest in genotoxicity caused by genotoxic agents has led to the development of several techniques to detect directly the DNA damage and identify such agents. These include measurement of micronuclei and other nuclear lesions frequencies, the presence of DNA adducts, chromosomal aberrations and DNA strand break [9].

The micronucleus (MN) test is a part of the many of tests that new products must go through before they are introduced into the market. It is a mutagenic test system for the detection of chemicals which induce formation of small membrane bound DNA fragment [10]. In Nigeria today, people handle detergents carelessly because they are ignorant of the consequences of exposure to this chemical substance. There is no doubt that detergent poses a health risk, but the extent of risk is not well known in Nigeria. Indeed, the toxicity of detergents to humans and the environment has received limited attention in Nigeria, as in many countries in Africa.

Evaluation studies of DNA damage using PCR and other methods is very expensive, hence there is need to explore cheaper alternatives like micronucleus and nuclear lesion tests.

Therefore, the present study was undertaken to evaluate the genotoxic potential of sub-lethal concentrations of Omo® and Ariel® detergents to the albino wistar (*Rattus, Norvegicus*) using the micronucleus and nuclear lesion tests.

MATERIALS AND METHODS

Test Substance: For this study, commercial-grades of Omo® detergent, manufactured by Lever Brothers PLC and Ariel® detergent, manufactured by Proctor and Gamble were procured from the local market.

Animal Husbandary: Eighty healthy specimens of *R. norvegicus* were procured from the animal house of the University of Nigeria Nsukka in May, 2014. Their weight was between 60 to 120 g. The animals were housed in wooden cages and allowed to acclimatize for two weeks before commencing the research. Pelleted Growers® feed and water was freely given to the animals.

Methods: The albino rats were grouped into eight of ten rats each. Exactly 40 g of each detergent was weighed out and dissolved in 100 ml of distilled water. The control rats were in group one, while the positive control rats which were given 20 g/kg body weight Cyclophosphamide were placed in group two. Groups three, four and five were given 3.0, 2.0 and 1.0 g/kg body weight Omo® detergent, respectively, while groups six, seven and eight received 3.0, 2.0 and 1.0 g/kg body weight Ariel® detergent solution, respectively. The rats were exposed to the detergent solutions daily for 100 days. Three rats from each concentration were sacrificed by cervical dislocation after 30 days, 60 days and 100 days of exposure. From each rat, five slides were prepared for micronucleus and nuclear lesion tests (based on the method of Utulu and Bakare, 2010) [11]. From each animal, 1000 cells were scored under x 1000 objective. The two femurs were removed with the aid of surgical blades and cleaned. The epiphysis was cut off and the bone marrow flushed with 1.0 ml fetal bovine serum (Sigma Aldrich Cheme GmbH, Germany), into 1.5 ml ependorf tubes that were clearly labeled to reflect the different groups and centrifuged at 2000 rpm for 5 minutes. The supernatant was removed and the pellet resuspended in another 1.0 ml fetal bovine serum (FBS) in the ependorf tubes, mixed properly and centrifuged again at the same speed. The supernatant was again removed and 0.5 ml of FBS was added to the pellet, mixed properly and smears made in grease free frosted slides, fixed in methanol for 10 minutes and left left to air dry at room temperature and finally stained with 0.4% May-Gruenwald stain for 4 minutes, followed by 5% Giemsa stain for 5 minutes. After dehydration through graded alcohol and clearing in xylene, slides were mounted in DPX. Micronuclei frequency was calculated as follows:

$$\%\ frequency\ of\ MN = \frac{Number\ of\ cells\ containing\ micronuclei}{total\ number\ of\ cells\ counted}$$

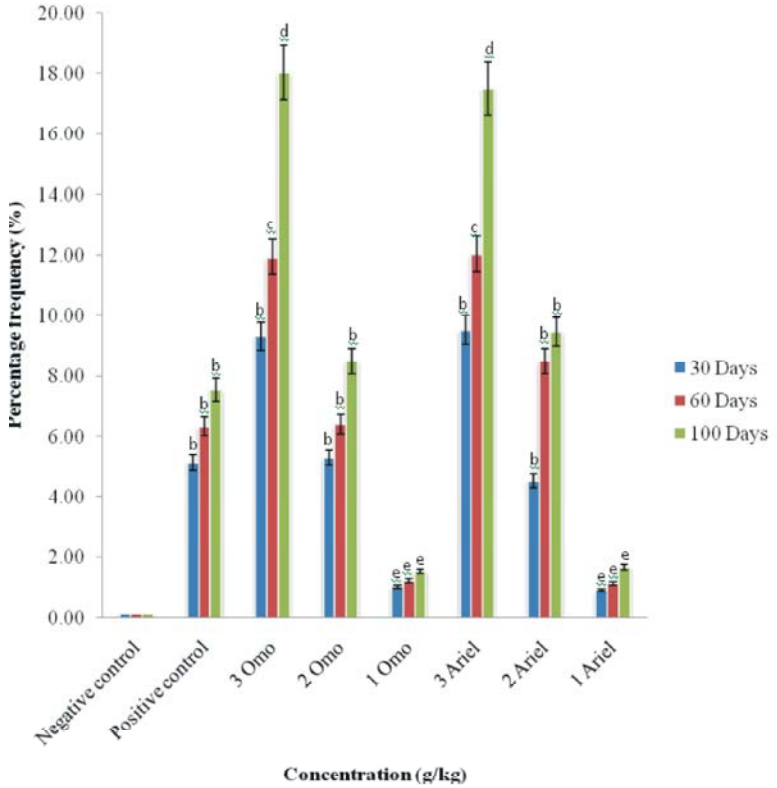

Fig. 1: Percentage frequency of induction of micronuclei Bars with different alphabets are significantly different

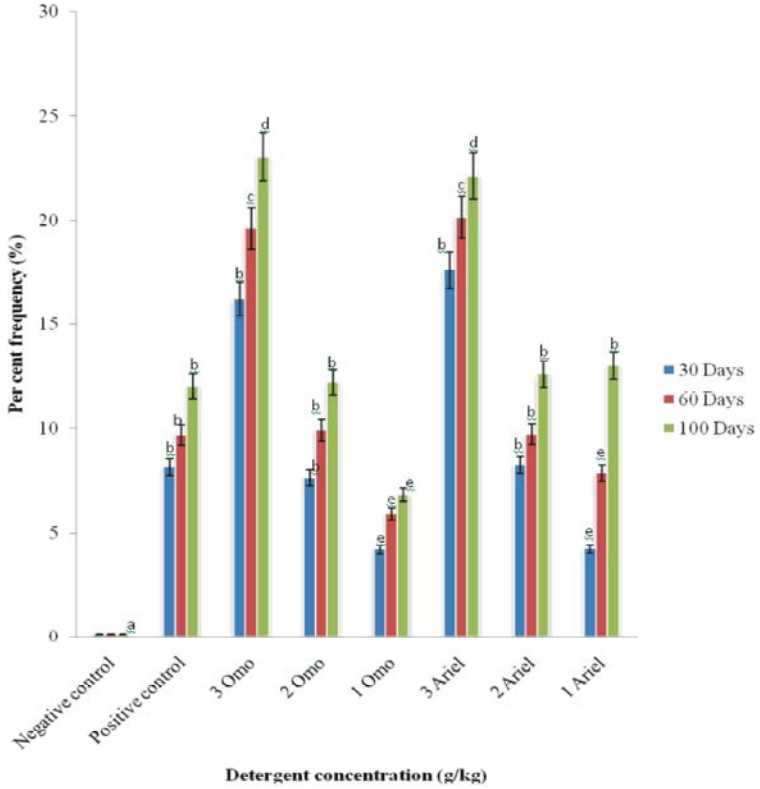

Fig. 2: Percentage formation of nuclear abnormalities Bars with different alphabets are significantly different.

Table 1: MN frequencies in the bone marrow erythrocyte of *R. norvegicus* exposed to Omo® and Ariel® at different concentrations and exposure times

		Length of exposure (Days)		
Detergent	Concentration (g/kg)	30	60	100
Cyclophospamide	20.0	5.15±5.03	6.33±4.16	7.57±3.22
Omo	0.0	0.13±0.58[1,a]	0.13±1.16[a]	0.13±1.53 [1, a]
	1.0	1.03±2.52[1,a]	1.23±1.53 [1, a]	1.53±2.52 [1, a]
	2.0	5.30±7.00[2, b]	6.40±3.46 [2, b]	8.50±2.00 [2, b]
	3.0	9.33±7.10 [3, b]	11.93±4.04 [4, c]	18.03±11.50 [5, d]
Ariel	1.0	0.90±2.00 [1,a]	1.13±1.12 [1, a]	1.67±1.53 [1,a]
	2.0	4.53±3.06 [2,b]	8.50±4.36 [3, b]	9. 47±5.51 [3, c]
	3.0	9.53±3.06 [3, c]	12. 03±3.06 [4, c]	17. 50±6.56 [5, d]

Values with different numeric superscript differ significantly (P < 0.001) between durations within concentrations
Values with different alphabetic superscript differ significantly (P < 0.001) between concentrations within durations

Table 2: Percentage frequency of formation of nuclear abnormalities

		Length of exposure (Days)		
Detergent	Concentration (g/kg)	30	60	100
Cyclophospamide	20.0	8.13±6.11	9.67±6.11	12.00±2.00
Omo	0.0	0.13±0.58 [1,a]	0.13±1.53 [1, a]	0.13±0.58 [1, a]
	1.0	4.17±3.51 [2, b]	5.87±5.51 [2, b]	6.80±6.00 [2, b]
	2.0	7.60±7.21 [3, c]	9.90±8.54 [3, c]	12.27±6.43 [3, d]
	3.0	16.20±9.17 [4, e]	19.60±10.00 [4, f]	23.03±4.51 [4, g]
Ariel	1.0	4.33±7.57 [2, b]	6.20±6.00 [b]	8.23±7.51 [c]
	2.0	8.23±3.06 [3 c]	9.70±5.00 [3,c]	12.57±5.51 [3, d]
	3.0	17.60±8.00 [4, e]	20. 13±4.51 [4, f]	22.13±6.51 [4, g]

Values with different numeric superscript differ significantly (P < 0.001) between durations within concentrations
Values with different alphabetic superscript differ significantly (P < 0.001) between concentrations within durations

RESULTS

Induction of Micronuclei (MN): The induction of micronuclei measured as percentage frequency of the bone marrow erythrocyte of the control and treatment groups (Table 1) indicated that the rat specimens exposed to different concentrations of Omo® and Ariel® detergents exhibited significantly higher (P<0.05) frequency of induction of MN than the negative control group. The frequency of induction of micronuclei was found to be both dose and time dependent, with the highest frequency occurring in the group exposed to **Omo®** and **Ariel®** of concentrations of 3.0 g/kg body weight, followed by 2.0 g/kg body weight and then 1.0 g/kg body weight. The lowest MN frequency was observed at day 30 and there was a gradual linear increase in the frequency of induction of MN as the time of exposure increased.

Formation of Nuclear Abnormalities in (NL) the Bone Marrow Erythrocyte: In addition to the micronuclei as a malformation, the two detergents also induced four kinds of nuclear lesions, lobed nuclei, irregular nuclei, heart shaped nuclei and kidney shaped nuclei. The frequencies of the NL in the treatment groups were also found to increase significantly (P < 0.001) with increase in dose and exposure time.

DISCUSSION

Notwithstanding the increasing rate of exposure to detergent in Nigeria, there are no available literatures concerning the evaluation of the genotoxic potential of detergent. On the other hand, there are a lot of literatures on the toxicity of detergents in fish [12-15], Yahaya *et al*. [12] also studied the effect of detergent on the blood of the wistar rat (*R. norvegicus*). The MN induction is a well known biomarker for assessing the toxicity of exposure of organisms to substances [16-20]; it has been used in different animals. Some pesticides such as carbamate and dithiocarbamate have been reported to induce MN formation in animals [21, 22]. In the present study, all the concentrations of detergents significantly (P < 0.001) induced MN compared to the negative control and the frequency of MN induction increased with increase in concentration and duration of exposure. However, our result deviated from the findings of Utulu and Bakare [11], who observed a no dose dependent response in rats

treated with caffeine. The frequency of formation of micronuclei obtained in this study showed that the detergents Omo and Ariel caused some damages to the DNA of *R. norvegicus*. The increase in the frequency of MN in the bone marrow erythrocyte of rat recorded in this study is believed to be as a result of the disruption of the DNA repair process in the rapidly multiplying bone marrow cells by the detergents.

Nuclear lesions are well established indicators of cytotoxicity. Ayllon and Garcia-vazquez [4] and Pachoco and Santos [21] have shown an association between the frequency of such lesions and exposure to genotoxic agents. Hose *et al.* [16] and Bombil *et al*. [5] reported a higher than normal nuclear lesion (NL) frequencies in fish inhabiting contaminated water or exposed to toxicants in the laboratory.

In this study, the groups exposed to Ariel® and Omo® detergents showed a significant ($P < 0.001$) increase in NL frequency, which was both concentration and time dependent also. This result suggests that the nuclear lesions found in this study are considered indicators of genotoxicity, in addition to the micronuclei formed and should be included to the routine tests for toxicological experiments.

REFERENCES

1. Crawford, C. and M.D. Zirwas, 2014. Laundry detergentgs and skin irritancy-a comprehensive review. Skinmed, 12(1): 23-31.
2. Ogundiran, M.A., O.O. Fawole, S.O. Adewoye and T.A. Ayandiran, 2010. Toxicological impact of detergent effluent on juvinal of African catfish (*Clarias gariepinus)*. Agriculture and Biology journal of North America, 1(3): 330-342.
3. Al-sabti, K., 1986. Comparative micronucleated erythrocyte cell induction in three cyprinids by five carcinogenic-mutagenic chemicals. Cytobioscience, 47: 147-154.
4. Ayllon, F. and E. Garcia-Vazquez, 2000. Induction of micronuclei and other nuclear abnormalities in Europe minnow *Phoxinus phoxinus* and mollie *Poecilia latipinna*: an assessment of the fish micronucleus test. Mutation Research, 467: 177-186.
5. Bombail, V., D. Aw, E. Gordon and J. Batty, 2001. Application of the comet and micronucleus assay to butterfish (*Pholis gunneilus*) erythrocytes from the Firth of Forth, Scotland. Chemosphere, 44: 383-392.
6. National Toxicology Program (NTP): http://ntp.niehs.nih.gov/go/9401.
7. Campbell, N.A., J.B. Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky and R.B. Jackson, (2008). Biology 8th edition. Pearson publishers New York, pp: 98-104.
8. Cavas, T. and S. Ergene-Gozukara, 2005. Induction of Micronuclei and Nuclear Abnormalities in *Oreochromis nilotica* following exposure to petroleum refinery and chromium processing plant effluents. Aquatic Toxicology, 74: 264-271.
9. Chauhan, L.K.S., N. Pant, S.K. Gupta and S.P. An Srivastava, 2000. Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbosulfan exposure. Mutation Research, 465: 123-129.
10. Cid, M.G., D. Loria and E. Matos, 1990. Genotoxicity of the pesticide propoxur and its nitroso derivation, NO-propoxur on human lymphocytes *in vitro*. Mutation Research, 232: 45-48.
11. Utulu, S. and A.A. Bakare, 2010. DNA damage in the germ and bone marrow cells of mice by caffeine. Research Journal of Biological Sciences, 5(8): 536-541.
12. Yahaya, T., J. Okpuzor and E.O. Oladele, 2011. Investigation of toxicity of detergents. Journal of Environmental Science and Technology, 4(6): 638-645.
13. Das, N. and R. Nanda, 1986. Induction of micronuclei in peripheral erythrocyte of fish *Heteropneustes fossilis* by mitomycin C and paper mill effluent, Mutation Research, 175: 67-71.
14. Fenech, M., W.P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi and E. Zeiger, 2003. Micronucleus project. "HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutation Research., 534(1-2): 65-75.
15. Giri, S., A. Giri, G.D. Sharma and S.B. Prasad, 2002. Mutagenic effects of carbosulfan, a carbamate pesticide. Mutation Research, 519: 75-82.
16. Hose, J., J. Cross, S. Smith and D. Dario, 1987. Elevated circulating erythrocyte micronuclei in fish from contaminated sites of southern California. Marine Environment Research, 22: 167-176.
17. Lee, R.F. and S. Steinert, 2003. Use of single cell- gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and fresh water) animals. Mutation Research, 544: 43-64.

18. Nwani, C.D., W.S. Lakra, N.S. Nagpure, R. Kumar, B. Kushwaha and S.K. Srivastava, 2010. Mutagenic and genotoxic effect of carbosulfan in freshwater fish, *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food and chemical toxi cology, 48: 202-208.
19. Ogundiran, M.A., O.O. Fawole, S.O. Adewoye and T.A. Ayandiran, 2009. Pathologic Lesions in the Gills Structures of *Clarias gariepinus* on exposure to sub lethal concentrations of soap and detergent effluents. Journal of Cell and Animal Biology, 3(5): 078-082.
20. Osman, A., E. Ali, M. Hashem, M. Mostafa and J. Mekkany, 2010. Genotoxicity of two pathogenic strains of Zoosporic fungi (*Achlya klebsiana* and *Aphanomyces laevis*) on erythrocytes of Nile tilapia Oreochromis niloticus niloticus Ecotoxicology and Environmental safety, 73: 24-31.
21. Pacheco, M. and M. Santos, 2002. Naphthalene and beta-naphthalene effects on *Anguilla Anguilla* L. Hepatic metabolism and erythrocytic nuclear abnormalities. Environmental Interactions, 28: 285-293.
22. Schmid, W., 1975. The micronucleus test. *Mutation Res*earch, 31: 9-15.