European Journal of Applied Sciences 8 (5): 277-281, 2016
ISSN 2079-2077
© IDOSI Publications, 2016
DOI: 10.5829/idosi.ejas.2016.8.5.1142

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

[1]P.N. Abara, [2]U.A. Ibiam, [3]B. Ubi and [4]A.C. Udebuani

[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 potential 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 formation of nuclear abnormalities confirmed the results of induction of MN and is 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.

Detergents have been put into other uses such as washing up dishes and utensils, vegetables and fruits such as carrots and oranges are washed with detergents to improve their appearance and acceptability by buyers. Retailers boil eggs with detergent believing that eggs boiled with detergents will not crack during boiling and the shell is easier to peel. Rural women the eastern parts of Nigeria use detergents to ferment cassava used for fufu. They believe that addition of detergent 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 detergent by the oral route.

The nucleus is the organelle in the cell that contains the genetic material (DNA) that directs normal cellular function and cellular reproduction [2]. 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 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 [3]. When this occurs, the genetic material that is not incorporated into a new nucleus may form its

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

own 'micronucleus' which is clearly visible with a microscope.

Exposure to toxic substances can damage the DNA of living cells [4]. If not repaired, these DNA lesions can initiate a series of biological consequences at the celluar, organ, whole organism and finally at the community and population levels [5].

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 (NTP).

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 [3 and 6]. 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.

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 animal specimens.

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 which were given 20 g/kg body weight cyclophosphamide were in group two and groups three through eight contained the test rats. Groups three, four and five were given 3.0 g/kg body weight, 2.0 g/kg body weight and 1.0 g/kg body weight Omo® detergent respectively, while groups six seven and eight received 3.0 g/kg body weight, 2.0 g/kg body weight 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. 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 fully 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:

$$\% \text{ frequency of MN} = \frac{\textit{Number of cells containing micronuclei}}{\textit{total number of cells counted}} \times 100$$

RESULTS

Induction of Micronuclei (MN): The induction of micronuclei measured as percentage frequency in 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 frequency of induction of MN than the negative control group. The frequency of induction of micronuclei was found to be dose and time dependent with the highest frequency

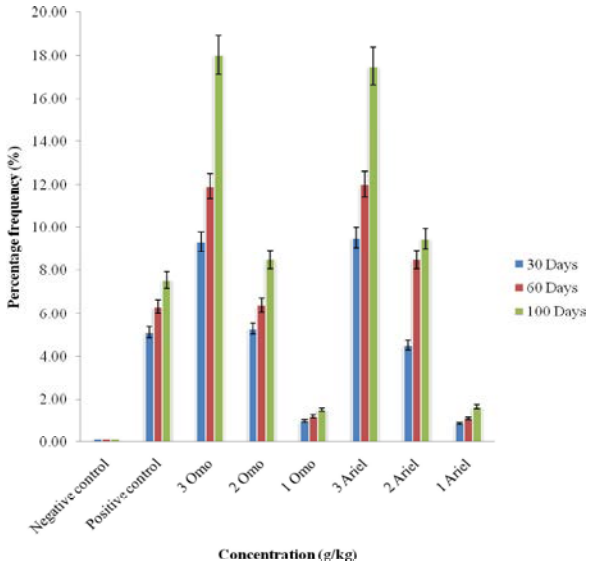

Fig. 1: Percentage frequency of induction of micronuclei

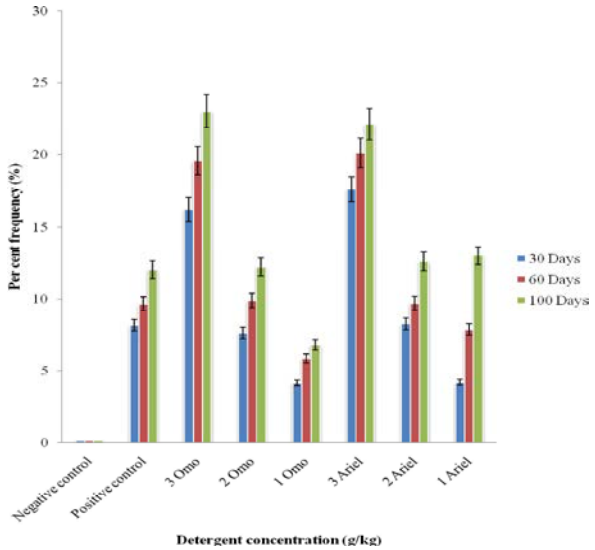

Fig. 2: Percentage formation of nuclear abnormalities

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[a]
	1.0	1.03±2.52[1,a]	1.23±1.53	1.53±2.52[a]
	2.0	5.30±7.00[2, b]	6.40±3.46[b]	8.50±2.00[b]
	3.0	9.33±7.10[3]	11.93±4.04[c]	18.03±11.50[d]
Ariel	1.0	0.90±2.00 [1,a]	1.13±1.12[a]	1.67±1.53[a]
	2.0	4.53±3.06 [2,b]	8.50±4.36[b]	9. 47±5.51[c]
	3.0	9.53±3.06[3]	12. 03±3.06[c]	17. 50±6.56[d]

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

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

occurring in the group exposed to 3.0 g/kg body weight, followed by 2.0 g/kg body weight and then 1.0 g/kg body weight. A significant effect of duration of exposure ($P < 0.001$) was observed in rat specimen exposed to Omo and Ariel detergents. 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 experiment progressed.

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.

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	0.13±1.53	0.13±0.58
	1.0	4.17±3.51	5.87±5.51	6.80±6.00
	2.0	7.60±7.21	9.90±8.54	12.27±6.43
	3.0	16.20±9.17	19.60±10.00	23.03±4.51
Ariel	1.0	4.33±7.57	6.20±6.00	8.23±7.51
	2.0	8.23±3.06	9.70±5.00	12.57±5.51
	3.0	17.60±8.00	20. 13±4.51	22.13±6.51

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 [7, 8 and 9], also studied the effect of detergent on the blood of the wistar rat (*R. norvegicus*).

The MN test has the possibility of detecting substances and has been used in different animals. Some pesticides such as carbamate and dithiocarbamate have been reported to induce MN formation in animals [10-12]. In the present study, all the concentrations of detergent induced significantly ($P < 0.001$) MN than the negative control and its frequency increased with increase in concentration and duration of exposure. This result deviated from the findings of [13], 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 were able to damage 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 [14] and Pachoco and Santos [15], have recorded an association between the frequency of such lesions and exposure to genotoxic agents. Four different kinds of nuclear lesions were observed in the bone marrow erythrocyte of *R. norvegicus* in this study. They are: irregular nuclei, heart shaped nuclei, kidney shaped nuclei and lobed nuclei. Many other studies reported similar nuclear malformations. Osman *et al*. [4], observed five different nuclear malformations namely: blebbed nuclei, lobed nuclei, irregular nuclei, heart shaped nuclei and kidney shaped nuclei in *Oreochromis niloticus* exposed to zoosporic cysts suspension of two pathogenic fungal species. Al-sabti [16], Das and Nanda [17], Hose *et al.* [18] and Bombil *et al*. [19], also recorded 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® as well as those exposed to Omo® detergents showed a significant ($P < 0.001$) in NL frequency. This increase was observed to be both concentration and time dependent also. This result suggest that the nuclear lesions found in this study should be 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. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. Yahaya, T., J. Okpuzor and E.O. Oladele, 2011. Investigation of toxicity of detergents. Journal of Environmental Science and Technology, 4(6): 638-645.
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. Giri, S., A. Giri, G.D. Sharma and S.B. Prasad, 2002. Mutagenic effects of carbosulfan, a carbamate pesticide. Mutation Research, 519: 75-82.
12. 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.
13. 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.
14. 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.

15. 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.
16. Al-sabti, K., 1986. Comparative micronucleated erythrocyte cell induction in three cyprinids by five carcinogenic – mutagenic chemicals. Cytobioscience, 47: 147-154.
17. 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.
18. 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.
19. 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.