Mutagenic Potentials of the Sterilizing Fluid – Puritil on Root Tip Mitosis of *Allium cepa*

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Abstract

The mutagenic potentials of puritil which is used for sterilizing babies feeding utensils were assessed using the Allium cepa root meristem assay. The sterilizing fluid was found to exhibit both mitoclassic and chromatoclassic effects and thus induced a number of abnormalities. These abnormalities included disturbed interphase involving nuclear lesions and nuclear dissolutions, micronuclei and binucleate cells, sticky metaphase, disturbed anaphase involving unequal distribution of chromosomes and sticky bridges, and precocious chromosomes. These increased with increase in concentration and duration of treatment. Thus the mutagenic potentials of puritil exist and it should be of great concern since babies are exposed to it for at least the first two years of life.

Keywords: Mutagenic, Potentials, Puritil, Root Tips, *Allium cepa*

Introduction

The use of some liquid chemicals to sterilize babies feeding utensils is a common culture among urban dwellers in Nigeria. The most common sterilants are Puritil and Milton. Both have sodium hypochlorite (NaOCl) as the active ingredient according to the manufacturers. However, the present study is on puritil and emphasis will be on its active ingredient since ATSDR (2006) reported that the toxic effects of sodium hypochlorite are primarily due to the corrosive properties of the hypochlorite moiety. Sodium hypochlorite is a clear greenish yellow liquid chemical with the formula NaOCl. Its synonyms include: Clorox, bleach, liquid bleach, chlorox, Milton, 13% active chlorine (ATSDR, 2006). It has an odour of chlorine. According to Wikipedia (2007), the European Union classified it as being corrosive and dangerous for the environment.

Rothwell (1993) observed that human cancer and other diseases sometimes occur as a result of exposure to some environmental factors such as chemicals and radiation.

The specific uses of sodium hypochlorite are many. It is used as laundry bleach in homes, to sterilize smooth surfaces in brewing industry, as disinfectants in hospitals and for waste water purification systems, swimming pools and for drinking water (ATSDR, 2007; Wikipedia, 2007). However, there has been some debate over the use of this chemical to disinfect drinking water (Wikipedia, 2007). The point of disagreement is due to the fact that organic contaminants in the water can be oxidized to trihalomethanes which are carcinogenic (Wikipedia, 2007).

Monarca *et al*., (2005) actually provided evidence of possible genotoxic effects of three drinking water disinfectants namely: sodium hypochlorite (NaOCl), chlorine dioxide (ClO_2) and peracetic acid (PAA). From their results, they concluded that "since the test concentrations of disinfectants are typical of those used in the biocidal treatment of tap water, and similar concentrations are consumed daily by a large number of people, the genotoxicity of these compounds may constitute a significant public health concern".

It has been documented that sodium hypochlorite affects health in many ways. According to ATSDR (2006), "hypochlorite powder, solutions and vapour are irritating and corrosive. Swallowing hypochlorite or contact with the skin or eyes produces injury to any exposed tissue. Exposure to gases released from hypochlorite may cause burning of the eyes, nose, and throat; cough; and damage to the airway and lungs. Generally, the more serious the exposure, the more severe the symptoms".

Wikipedia (2007) noted that the burning effect observed on contact of the skin with strong hypochlorite solution is due to defatting and saponification of skin oils and destruction of tissue and that the slippery feel of bleach on skin is due to this process.

There is paucity of information on further health effects of sodium hypochlorite. This is confirmed by the report of ATSDR (2007) that they do not know if exposure to the chemical can result in reproductive effects, birth defects or other developmental effects. However, it has been reported that some drugs, plant extracts and chemicals can cause limited cytological abnormalities in such organisms as *Allium cepa, Vicia faba* and *Lens esculenta* (Mercykutty and Stephen, 1980, Ene-Obong and Amadi, 1987: Zhang and Yang, 1994). Rothwell (1993) also observed that some chemical mutagens for instance mustard gas, produce a delayed effect in order words inducing mutation not in the cell that was exposed but in a descendant of that cell and thus making such mutagens very dangerous.

The effects of these substances could be described as mitodepressive, mitopromotive, mitoclassic and chromatoclassic. The cytological abnormalities included accumulation of prophase, stickiness of chromosomes, sticky bridges, precocious chromosomes, polyploidy, binucleate cells, and pycnosis.

Since a baby's body is still very tender and very receptive to substances in the environment, the authors decided to investigate the possible cytological effects of the recommended dose of the chemical for sterilizing baby's feeding utensils since no further washing is required once you remove the feeding utensils from the sterilizer. Furthermore, a baby is continuously exposed to this chemical for at least the first two years of his/her life. The effects were tested on bulbs of *Allium cepa* since the *Allium cepa* root meristem assay is considered widely as a practical reliable system for the screening of environmental mutagens and carcinogens (Fiskesjo, 1985; INVITTOX, 1989). In fact, the *Allium cepa* test is protocol number 8 in INVITTOX directory of standard laboratory protocols for toxicity screening.

Materials and Methods

Fresh and healthy onion (*Allium cepa*) bulbs were grown in a mixture of sawdust and water. When the roots were about 2 – 5cm after 4 – 6 days of planting, the rooting bulbs were transferred to beakers containing tap water and left for 24 hours. This is in order to allow time for recovery in case there were any abnormalities caused by the sawdust culture.

Three different concentrations of Puritil were used. These were half the recommended dose (1/2R), the recommended dose (R) for sterilizing babies feeding utensils and double the recommended dose (2R). In other words, the concentrations used were 1/2R, R, and 2R where R is the recommended dose which is one tablespoonful to every litre of water. The bulbs were transferred from the beakers containing tap water to those containing the test solution. Some bulbs were left in tap water to serve as the control. The durations of treatment ranged from 6.ooam – 12noon (6hours); 6.ooam – 6.oopm (12 hours) and 6.ooam – 6.ooam (24 hours). Each of the concentrations was applied for the three different time durations.

Subsequently, 6 –10 root tips were cut off from each bulb, washed 2 – 3 times in tap water and fixed in Carnoy's solution (1:3 acetic acid and absolute alcohol). The fixed materials were stored in the refrigerator for at least 24 hours. Hydrolysis of the root tips was best achieved using 0. IN HCl at 60^{o} C for 7 – 8 minutes and staining was done using lactopropionic orcein (2% LPO). Three slides were prepared for each treatment combination and a total count of 1200 cells made. Various types of abnormalities induced by each treatment at various stages were recorded. The 1200 cells were made up of number of dividing cells and number of non dividing cells. Cells with abnormalities were expressed as a percentage of the total number of dividing cells per treatment (Ene-Obong *et al*., 1991). Analysis of variance (ANOVA) was used to analyse the data obtained for the abnormal cells.

Results

Table 1 shows the analysis of variance of abnormal cells. It could be seen that the durations of treatment, the concentrations of treatment and the interactions between them induced highly significantly different number of abnormal cells. Table 2, shows that 24 hours of treatment of onion root tips with the sterilizing fluid of various concentrations induced the highest number of abnormal cells. It was followed by 12 hours. The least number was observed at 6 hours of treatment.

Table 1: Analysis of variance of abnormal cells

Source of Variation	DF	SS	MS	VR
Duration (D)	2	41. 1667	20. 5833	39. 00 ***
Conc. (C)	3	76. 5556	25. 5185	48. 35 ***
D X C	6	18. 6117	3. 1019	5. 88 ***
Residual	24	12. 6667	0. 5278	-
Total	35	149. 0000	-	-

*** = *Very highly significant at 0.01% probability*

Table 2: Mean effects of durations on the number of abnormal cells

Durations	Means
6 HRS	0.8 ± 0.20
12 HRS	2.4 ± 0.28
24 HRS	3.3 ± 0.28
LSD 0.05	0.61

Table 3: Mean effects of concentrations on the number of abnormal cells

Concentrations	Means
Control	0 .0
½ R	1. 9 ± 0. 24
R	2. 8 ± 0. 25
2R	4. 0 ± 0.35
LSD 0.05	0.71

Table 4: Interactions between Durations and Concentrations (D x C) on the Number of Abnormal Cells Induced in Root Tip Mitosis of *Allium cepa*

Duration	Control	1/2R	R	2R
6 HRS	0.0	0.3 ± 0.17	1.3 ± 0.33	1.3 ± 0.49
12 HRS	0.0	2.3 ± 0.48	2.7 ± 0.48	4.7 ± 0.42
24 HRS	0.0	3.0 ± 0.34	4.3 ± 0.40	6.0 ± 0.52
LSD 0.05	1.22			

Table 3 reveals that double the recommended dose (2R) of puritil induced the highest number of abnormal cells. Its effect was followed by the recommended dose (R) and half the recommended dose (1/2R). The control root tips did not show any abnormal cells.

The interactive effects of durations and concentrations are shown in Table 4. It can be observed that: for 6 hours, double the recommended dose (2R), the recommended dose (R) and half the recommended dose (1/2R) did not differ significantly in the number of abnormal cells they induced. However, the number of abnormal cells induced by them was significantly different from that of the control.

- For 12 hours, (2R) produced the highest number of abnormal cells and those of (R) and (1/2R) followed its effect. However, there were no significant differences between the effects of (R) and (1/2R). All the dosages of puritil produced significantly more abnormal cells than the control treatment.
- For 24 hours, the number of abnormal cells decreased in the following order :
2R > R >1/2R > the control. The different abnormal cells induced by the treatments include – disturbed interphase involving nuclear lesions and nuclear dissolutions, micronuclei, binucleate cells at various stages of division, abnormal metaphase cells involving sticky metaphase and prophase – metaphase where early metaphase chromosomes still retain their prophase positions, disturbed anaphase involving unequal distribution of chromosomes and anaphase bridges, and precocious chromosomes.

In summary, for the various concentrations the number of abnormal cells increased in the following order: 1/2R<R<2R while for the durations, the number of abnormal cells increased in the following order: 6hours<12hours<24hours. These cells are shown in Plates 1 to 5. The normal mitotic cells are shown in plates 6 to 9 and they can be compared with plates 1 to 5.

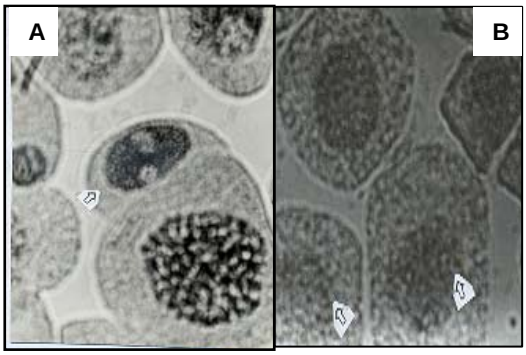

Plate 1: Disturbed interphase cells (a) arrow shows nuclear lesions (b) arrow shows nuclear dissolutions. Mag. X1000

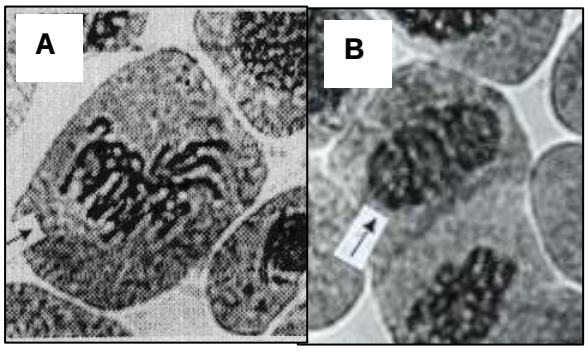

Plate 2: Abnormal metaphase cells (a) sticky metaphase and abnormal alignment of chromosomes. A cell with micronuclei is also shown in this photograph (b) prophase-metaphase where early metaphase chromosomes still retain their prophase position. Mag. X1000

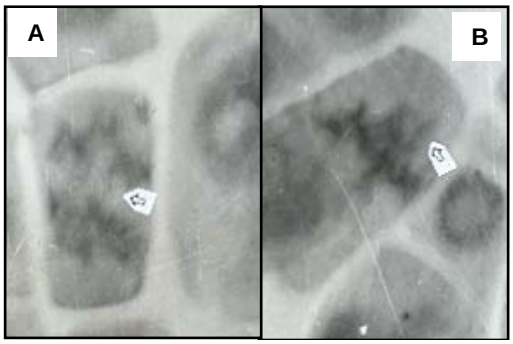

Plate 3: Disturbed anaphase cells (a) arrow shows anaphase bridges (b) arrow shows precocious chromosomes. Mag. X1000

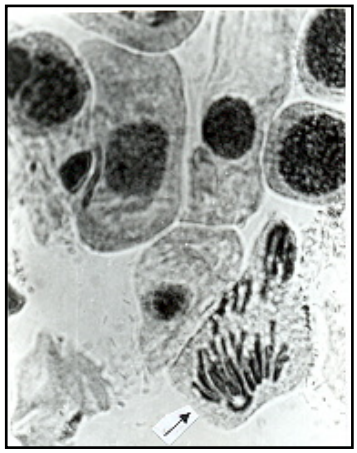

Plate 4: Abnormal anaphase cell-unequal distribution of chromosomes or multipolar spindle. Mag. X1000

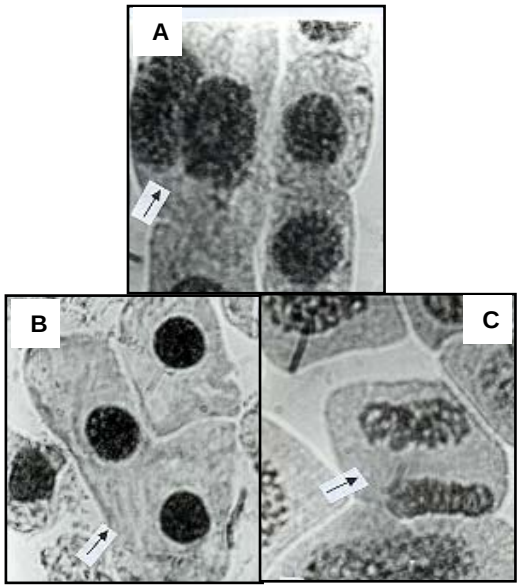

Plate 5: Binucleate cells (a) both at very early prophase (b) both at interphase (c) both at early metaphase. Mag. X1000

Discussion

According to INVITTOX (1989), the *Allium* test provides a rapid screening procedure for chemicals, pollutants, contaminants etc, which may represent environmental hazards.

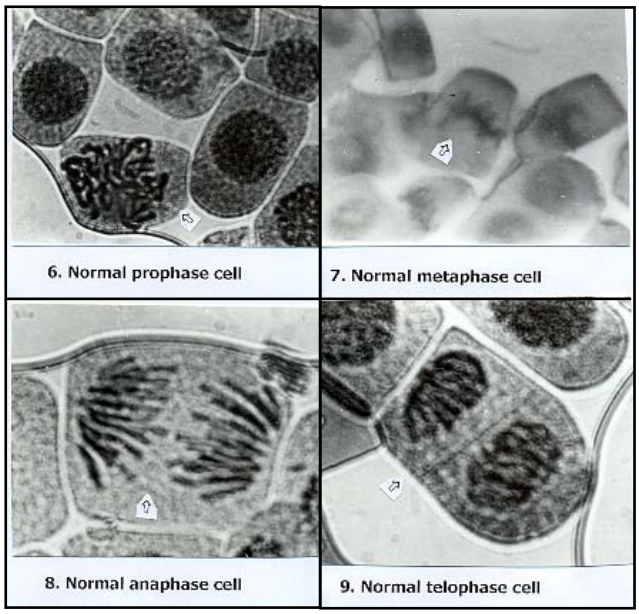

Plate 6-9: Arrow shows normal prophase, metaphase, anaphase and Telophase cells respectively Mag. X1000

Toxicity is measured by both macroscopic parameters(for example, growth inhibition) where the degree of damage is used to assess the toxic status of the chemical tested and microscopic parameters where the rate of chromosome damage and cell division disturbances may be used to predict mutagenesis.

Exposure of *Allium cepa* root tips to various concentrations of Puritil induced many cell division disturbances as earlier listed in the results section. Mercykutty and Stephen (1980) noted that the presence of nuclear lesions and nuclear dissolution offer cytological evidence for the inhibitory action on DNA biosynthesis. Sarbhoy (1980) reported that binucleate cells arise as a result of the failure of cytokinesis or cell plate formation at telophase stage. And according to Partanen (1963), increase in chromosome number can appear by the presence of more than one nucleus per cell.

The presence of micronuclei, bridges, unequal distribution or multipolar separations can directly lead to the production of aneuploid cells (Novak, 1981). Ghareeb and George (1997) further reported that these abnormalities occur as a result of partial spindle failure. Amer and Ali (1974) had earlier noted that micronuclei result from lagging chromosomes at anaphase or telophase stages.

Sticky metaphase and sticky anaphase bridges which were observed in the present study have been attributed to many causes. Quoting several authors, Mercykutty and Stephen (1980) reported that stickiness may be the result of depolymerisation of DNA, partial dissolution of nucleoproteins, breakage and exchanges of the basic folded fibre unit of chromatids or the stripping of the protein covering of DNA in chromosomes. Raj and Reddy (1971) noted that stickiness of chromosomes may be a potential source of translocations, which consequently could cause changes in nuclear set up and that such translocations may be due to chromosome fragmentation resulting from the stress of anaphase movement.

Based on the results of this study, mutagenic potentials of the active ingredient of puritil exist. This inference is based on the report of Ennever *et al*., (1988). The authors wrote that "a positive result in *Allium* test system should be taken to indicate a potential biological hazard. They further noted that it has been rare to obtain false negatives, in either the *Allium* test or other similar plant tests. They thus concluded that whenever a compound is tested and a negative result is obtained, that compound can be reliably considered to be nonmutagenic."

Fiskesjo (1985) also reported that "when the Allium test was compared with other short – term alternative toxicity test systems that made use of eukaryotic and prokaryotic organisms, the results from the Allium test compared favourably with those from these test systems". However, INVITTOX (1989) noted that the criteria for comparing different test systems and subsequently extrapolating to humans are that, results from many replications of the tests should be obtained and also that the metabolic pathways of the compound of interest should be considered.

The health effects of sodium hypochlorite which is the active ingredient in puritil have already been documented in the introduction to this paper. It is important to note the report of ATSDR (2006) where they wrote that children may be more vulnerable to corrosive agents than adults because of the small diameter of their airways. Puritil is corrosive. This is seen in the eating away of the label on the container in which the chemical is stored. The authors also observed that constant exposure of the feeding bottles to the sterilizing fluid also led to the bleaching of the calibrations on the bottles. Again, the sterilizing water containing the recommended one tablespoonful of puritil always smelled heavily of chlorine. The results of the study have clearly revealed its potential mutagenicity.

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