Oxidative Stress Induced DNA Damage and Reproductive Toxicity in Male Albino Mice Orally Exposed to Sorbitol

Okunola A. Alabi, Lanre R. Oladimeji, Adewale A. Sorungbe and Yetunde M. Adeoluwa*

Department of Biology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

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**Corresponding author*

Okunola A Alabi, E-mail: aaalabi@futa.edu.ng; alabiokunola@yahoo.com

Tel: +234 8034416394

Abstract

In this study, the potential DNA damage and reproductive toxicity of sorbitol was investigated using bone marrow micronucleus (MN), sperm morphology, and sperm count in mice. Five doses of 90, 45, 20, 10 and 1 mg/kg/day, defined by allometry, and approximately corresponding to 1.5g, 750mg, 330mg, 165mg and 16mg of sorbitol daily consumption by a 70kg human, respectively, were used. MN analysis showed a dose-dependent induction of micronucleated polychromatic erythrocytes and other nuclear abnormalities across the treatment groups. Assessment of sperm shape showed a significant ($p < 0.05$) increase in sperm abnormalities with significant ($p < 0.05$) decrease in mean sperm count in treated groups. The result of the oxidative stress biomarkers showed induction of significant ($p < 0.05$) increase in liver catalase, MDA and serum ALT and AST activities with concomitant decrease in SOD activities in exposed mice. A significant increase in weight of exposed mice were recorded when compared with the negative control. The results of this study showed the genotoxicity and reproductive effects of sorbitol.

Keywords: Sorbitol; bone-marrow micronucleus; sperm morphology; genotoxicity; oxidative stress.

1.0 Introduction

Constant eating of sugar and sugar products is responsible for the emergence of some health effects. Consumption of sugar in high quantities is significantly associated with cardiovascular diseases, dental caries, obesity, high blood sugar level and can be deleterious in diabetic patients (Howard and Wylie-Rosete 2002). Therefore, there is a growing demand for sugar-free substitutes. Foods that are sugar-free are sweetened by non-nutritive sweeteners (also known as alternative or low calorie sweeteners) as sugar substitutes. Such sugar substitutes generally contain very few or no calories and therefore induce little or no glycemic response even though they taste like sugar. Sugar substitutes are now freely used in the production of processed foods like puddings, jellies, baked goods, jams, carbonated beverages, candy and powder drink mixes (Hobbs et al. 2012).

Sorbitol (also called glucitol) is a sugar alcohol which undergoes a slow metabolism in human system. Diet foods, cough syrups, toothpaste, mints, sugar-free chewing gums and mouthwash are all sweetened with sorbitol (Hobbs et al. 2012). Although, sorbitol has a minimal influence on the glycemic level and few calories when compared with sugar, yet, consumption of large quantities of sorbitol can cause diarrhea (Shafirir 1991). Sorbitol has also been found to induce DNA fragmentation in Chinese Hamster ovary cells (Johannes et al. 1992). Regular consumption of additives in food induces cytotoxic, genotoxic and carcinogenicity (Zengin et al. 2011; Hobbs et al. 2012; Saad et al. 2014). Damage to the DNA by food additives is dependent on several factors among which are their transportation across the nuclear and/or nuclear membranes, deactivation and activation of processes involving intracellular enzymes, the level of scavenging radicals and the mechanism(s) of repair possessed by the exposed cells (Howard and Wylie-Rosete 2002). Despite the regular use of sorbitol in food, there is paucity of information about its genotoxic, mutagenic and carcinogenic effects. Thus, it is of public health importance to study the potential mutagenicity and genotoxicity of this food additive so as to safe guard the health of the populace consuming it.

In most countries, genotoxicity testing has become an integral component in the evaluation of toxicity. Regulatory bodies have recommended the use of both *in vitro* and *in vivo* assays in studying genotoxic effects because no individual assay can detect all the relevant end-points in genotoxicity. Among the several standardized *in vivo* assays, sperm morphology and bone marrow micronucleus assays are commonly used. Sperm morphology assay is carried out to identify agents with potential to alter the process of spermatogenesis. It is highly sensitive in detecting known germ-cell mutagens and therefore, has always been carried out to identify new mutagens in germ cells (Topham 1980). Also, sperm morphology assay in mouse can be used to expose animals to a mutagen and study the relationship(s) that exists between mutagenicity and carcinogenicity (Wyrobek et al. 1983).

An alternative assay that can be used to measure a gross damage to the chromosome is micronucleus (MN) assay. In this method, cells that have completed mitosis can possess fragments of chromosome due to breakage, or as a result of rearrangement and has not been fully inserted into the daughter cell's nuclei can appear inform of small

secondary nuclei (micro nuclei) in the cytoplasm. Since MN are easier to score than chromosome preparations, it is generally preferred for the evaluation of chromosome damage. Furthermore, knowing that there is no incorporation of whole chromosomes into the main nuclei, therefore, this assay can easily be used for scoring aneuploidy events, even though, an extra procedure for staining is required for the identification of MNs with whole chromosomes (Schmid 1975). *In vitro* and *in vivo* MN assay is recommended for the routine assessment of chemical and waste toxicity (Cimino 2006). *In vitro* MN assays is very important in the assessment of toxicity, however, *in vivo* MN is more relevant in the assessment of genotoxicity in humans because it considers human's pharmacokinetics, metabolism and other biological activities.

This work was designed to assess the possible cytogenotoxic and mutagenic effects of sorbitol on somatic and germ cells using mouse bone marrow MN, sperm morphology assays and sperm count. The potential mechanism of observed cytogenotoxicity via oxidative stress was also studied.

2.0 Materials and Method

Biological materials and sorbitol purchase

Sorbitol solution was purchased from Sigma-Aldrich, Germany (CAS No.: 50-70-4; Purity = 98%; vapor density <1; vapor pressure <0.1 mmHg (25°C); mp 98-100°C). Male Swiss mice (albino: *Mus musculus*) between 6-10 weeks old and have been inbred for several generations, were purchased from Department of Physiology, University of Ibadan, Nigeria. They were kept in a well-ventilated animal house, free of pathogens at the Department of Biology, Federal University of Technology, Akure for 2 weeks acclimatization. They remained in this room till the end of the study. They were supplied with Ladokun® pelleted feed and clean water to drink. The mice were later divided into 2 categories: for MN assay, mice of 8 weeks old were used, while in sperm morphology assay, mice of 12 weeks old were used because spermatogenesis in mice is stable from 12 weeks old. All the mice were catered for in accordance with standard guidelines (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011). The weight of the animals were recorded at the beginning and end of the experiments.

Micronucleus (MN) assay

Mice of seven groups (6 mice per group) were used in this assay. Each mouse/group was orally administered 0.3 mL sorbitol equivalent to a daily dose of 90, 45, 20, 10 and 1 mg/kg/day, defined by allometry, and approximately corresponding to 1.5g, 750mg, 330mg, 165mg and 16mg of sorbitol daily consumption by a 70kg human, respectively. The recommended daily limit of sorbitol by human is 50g (USFDA, 2018). The oral exposure was once per day for 30 consecutive days as against negative (distilled water) and positive (cyclophosphamide, 20 mg/kg bw) controls. Bone marrow was prepared for MN assessment following the procedure of Schmid (1975, 1976). Briefly, mice were sacrificed through cervical dislocation, their femurs removed and the bone marrow flushed from the bones with Foetal Bovine Serum (Sigma Aldrich Cheme GmbH, Germany). At 2000 rpm, the cells were centrifuged for 5 min and prepared slides were stained with both May-Grunwald and Giemsa stains. Minimum of 4000 cells/mouse were scored to assess the frequency of MN in polychromatic erythrocytes

(MNPCE). Relative size of the erythrocytes and differential staining of PCEs (bluish-purple) and normochromatic erythrocytes (NCEs, pinkish-orange) were used as indices for differentiation between the cells. Other nuclear abnormalities (NA) were also analyzed.

Sperm morphology assay

The procedure of Wyrobek et al. (1983) as modified by Bakare et al. (2005) was followed for the sperm morphology assay. Same concentrations, route of exposure and duration as in MN assay were used for this assay. Five mice were treated for each sorbitol concentration. Sperm cells were obtained from the caudal epididymes at 5 weeks from the first day of exposure, because spermatogenesis in mice completes in approximately 34.5 days according to Bartke et al. (1974). Mice were sacrificed by cervical dislocation, their epididymes were removed surgically, and two sperm suspensions were prepared from each of the testis by using physiological saline to mince the caudal. Smears were prepared on grease-free slides after staining for 45 mins with 1% Eosin Y. The air dried slides were coded and observed microscopically under oil immersion at 1000x. For each mouse, 1000 sperm cells were analyzed for possible morphological abnormalities following the criteria described by Wyrobek and Bruce (1975).

Sperm counts

Caput epididymes of the testes of each mouse used for the sperm morphology assay were removed surgically and minced with physiological saline. Numerical counting of sperms was made after preparing their suspension using Neubauers' hemacytometer at 400x (Alabi et al. 2013). Pooled sperm count from the mice in each group was expressed as mean sperm count per milliliter of suspension.

Biochemical test

Two mice per group from the MN assay were used for this test. At 24 h post exposure and overnight fasting, mice were lightly anesthetized and blood collected through cardiac puncture into lithium coated serum separator tubes. Liver was removed surgically, placed on ice bath to remove excess blood and weighed before it was used for biochemical analysis. This was followed by homogenization in ice cold isotonic phosphate buffer of 7.4 pH and centrifuged (10,000 g for 15 min, 4°C). Resultant supernatant was refrigerated at -70 °C before biochemical analysis was carried out (Agnihotri et al. 2007). Coagulated blood of the mice was centrifuged for 10 min at 3000 g to obtain serum (supernatant) which was then refrigerated at -70 °C until analyzed. The catalase activity was measured following the method of Sinha (1972), while superoxide dismutase (SOD) was analyzed following the procedure of Misra and Fridovich (1972). Determination of protein content followed Biuret method (Gornall et al. 1949) and reduced glutathione (GSH) determination was according to the procedure described by Habig et al. (1974). Malondialdehyde (MDA), an indication of lipid peroxidation, was determined according to the report of Shokunbi and Odetola (2008). Finally, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were measured following the report of Reitman and Frankel (1957) using Randox kits (Randox Laboratories diagnostic Ltd, UK).

Statistical analysis

The SPSS® 20.0 statistical package was used in the analysis of the data. The data were reported in terms of percentage frequency and

mean±standard error. To test the significance at the different dose-level of each assay, Dunnett t- test was employed. The difference between negative control and individual dose-group was analyzed at probability level of 0.05.

3.0 Results

Weight

The weight of the mice before and at the last day of exposure to different doses of sorbitol is shown in Table 1. The data showed a significant increase in weight in mice treated with 10-90 mg/kg/day of sorbitol compared to the control group in both MN and sperm morphology assays.

MN assay

Generally, the mice did not show any change in their food and water intake throughout the exposure period, however, there was weight gain across the groups at the end of the exposure period which is not statistically significant compared to the negative control (data not shown). The results of PCE, MNPCE, NCE and MNCE observed in the bone marrow of mice exposed to different doses of sorbitol is shown in Figure 1. Compared to the negative control, the results showed a significant increase ($p < 0.05$) in MNPCE at 20 mg/kg/day and above which was dose-dependent. A dose-dependent significant rise ($p < 0.05$) in NCE (i.e. PCE:NCE) (Table 2) and MNCE compared to the negative control was also recorded at 20, 45 and 90 mg/kg/day of sorbitol (Figure 1). Different nuclear abnormalities were observed in mice exposed to different doses of sorbitol such as macrocytes, microcytes, oval, helmet, tear drop and Cabot's ring cells (Figure 2). The nuclear abnormalities had the highest occurrence in the mice exposed to 90 mg/kg/day sorbitol.

Sperm morphology assay

Different doses of sorbitol induced significant ($p < 0.05$) dose-dependent abnormal sperm cells at all doses except at 1mg/g body weight (Table 2) compared to negative control. The negative and positive controls showed 3.32% and 21.17% abnormal sperm cells, respectively. Sorbitol doses of 1, 10, 20, 45 and 90 mg/kg/day induced 4.21%, 5.91%, 6.06%, 12.58% and 14.37% abnormalities, respectively. Different abnormalities such as double tail, amorphous head, kidney shaped head, knobbed hook, short hoot etc were observed in exposed mice (Figure 3).

Sperm count

A decrease in a dose-dependent manner was observed in the mean sperm count across the different dose of sorbitol used (Table 3). However, a statistical significant ($p < 0.05$) decrease of mean sperm count was observed at 20, 45 and 90 mg/kg/day sorbitol. The mean sperm count in the negative and positive controls were $5.10 \times 10^7/\text{mL}$ and $19.00 \times 10^5/\text{mL}$, respectively. The decrease in mean sperm count was significant with respect to inter-dose variations but not significant with respect to inter-animal variations.

Biochemical test

The effects of sorbitol treatment on oxidative stress biomarkers in mice are shown in Table 4. The data showed a significant ($p < 0.05$) increase in the liver catalase, MDA and serum ALT and AST, with

concomitant decrease in the liver SOD activities of the treated mice (Figures 4 and 5). A significant ($p < 0.05$) increase in liver catalase and serum ALT and AST activities were observed at 10, 20, 45 and 90 mg/kg/day sorbitol compared to the control group, while a significant The result of soil physicochemical parameters tabulated below:

($p < 0.05$) increase in MDA was recorded from 20 mg/kg/day dose of sorbitol upward.. However, 45 and 90 mg/kg/day dose of sorbitol induced significant ($p < 0.05$) decrease in liver SOD activities compared to the control (Figure 5).

Table 1: Summary of the average weight changes in mice exposed to different doses of sorbitol.

Dose (mg/kg/day)	Micronucleus assay		Sperm morphology assay	
	Initial weight	Final weight	Initial weight	Final weight
Distilled water	32.02±0.10	41.06±0.04	40.01±0.11	49.40±0.08
1	32.51±0.04	47.19±0.12	40.51±0.06	52.10±0.11
10	33.01±0.08	57.21±0.05*	40.92±0.03	62.00±0.03*
20	33.28±0.10	59.08±0.06*	41.37±0.22	64.37±0.09*
45	33.52±0.03	62.17±0.01*	41.55±0.16	64.94±0.02*
90	33.91±0.06	64.42±0.22*	41.92±0.02	65.39±0.17*
Cyclophosphamide (20mg/kgbw)	32.04±0.11	38.92±0.04	40.99±0.10	50.50±0.03

*significant at $p < 0.05$.

Table 2: Summary of morphologically abnormal sperm cells and polychromatic and normochromatic erythrocytes ratio induced in mice exposed to different doses of sorbitol.

Dose (mg/kg/day)	Number of Sperms	% Frequency of	PCE/NCE ratio
	counted	abnormality	
Distilled water	20000	3.32	4.25
1	20000	4.21	4.18
10	20000	5.91	3.97
20	20000	6.06*	2.81*
45	20000	12.58*	2.17*
90	20000	14.37*	2.01*
Cyclophosphamide (20mg/kgbw)	20000	21.17*	1.38*

*significant at $p < 0.05$. PCE=polychromatic erythrocytes; NCE=normochromatic erythrocytes

Table 3: The mean (mL) sperm count of mice exposed to the different doses of sorbitol.

Dose (mg/kg/day)	Mean (/ml)
Distilled water	5.10×10^7
1	5.76×10^7
10	4.86×10^7
20	$2.79 \times 10^{7*}$
45	$1.32 \times 10^{7*}$
90	$1.01 \times 10^{7*}$
Cyclophosphamide (20mg/kgbw)	19.00×10^6

***significant at $p < 0.05$.**

Table 4: The effects of sorbitol on the liver lipid peroxidation (MDA), catalase, superoxide dismutase (SOD), reduced glutathione (GSH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.

Dose (mg/kg/day)	Catalase ($\mu\text{m}/\text{mg}$)	MDA ($\mu\text{mol}/\text{ml}$)	GSH ($\mu\text{m}/\text{g tissue}$)	SOD (U/ml/Min)	AST (U/ml)	ALT (U/ml)
Normal	75.18 $\pm$ 0.15	4.98 $\pm$ 0.04	9.02 $\pm$ 0.10	5.72 $\pm$ 0.33	40.29 $\pm$ 0.06	18.47 $\pm$ 1.00
1	82.37 $\pm$ 0.32	4.93 $\pm$ 0.10	9.37 $\pm$ 0.90	5.02 $\pm$ 1.02	49.30 $\pm$ 1.00	24.20 $\pm$ 0.02
10	91.21 $\pm$ 0.07*	7.32 $\pm$ 0.44	8.18 $\pm$ 0.49	4.24 $\pm$ 0.67	52.22 $\pm$ 0.20*	31.17 $\pm$ 0.50*
20	96.08 $\pm$ 0.22*	8.21 $\pm$ 1.02*	8.00 $\pm$ 1.00	4.00 $\pm$ 0.90	56.17 $\pm$ 0.08*	42.29 $\pm$ 0.07*
45	102.21 $\pm$ 0.72*	8.38 $\pm$ 0.58*	7.93 $\pm$ 1.32	3.30 $\pm$ 0.50*	68.72 $\pm$ 1.00*	46.04 $\pm$ 1.02*
90	105.03 $\pm$ 0.18*	9.82 $\pm$ 0.82*	7.32 $\pm$ 0.43	2.72 $\pm$ 0.50*	73.72 $\pm$ 0.04*	49.33 $\pm$ 0.33*

***significant at 0.05. The data is presented as mean $\pm$ SEM.**

Figure 1: The mean of (A) polychromatic erythrocytes (PCE), (B) micronucleated PCE (MNPCE), (C) normochromatic erythrocytes (NCE) and (D) micronucleated NCE (MNNCE) induced in mice exposed to different concentrations of sorbitol. *significant at 0.05 compared to distilled water; NC – negative control (distilled water); PC – Positive control (cyclophosphamide).

Figure 2: Micronucleus and other nuclear abnormalities induced in bone marrow of mice exposed to sorbitol. Four representative images, May-Grunwald and Giemsa stains, (magnification $\times 100$). a-polychromatic erythrocyte (PCE); b-macrocyte with two micronuclei; c-helmet cell; d-cabot's ring; e-oval PCE; f-micronucleated PCE; g-oval microcyte; h-micronucleated microcyte; i-cone PCE; j-normochromatich erythrocytes (NCE); k-microcyte; l-cabot's ring; m-tear drop macrocyte.

Figure 3: Abnormal sperm morphologies induced in mice exposed to different doses of sorbitol (a) normal sperm cells (b) short hook (c) knobbed hook (d) kidney head (e) misshapen head with wrong tail attachment (f) banana shaped head (g) hook at wrong angle (h) knobbed hook (i) amorphous head (j) short hook with amorphous head (k) double tail (l) wrong tail attachment. (1% Eosin Y stain; $\times 100$).

Figure 4: The effects of different concentrations of sorbitol on the liver lipid peroxidation (MDA), catalase and reduced glutathione (GSH) activities in mice. *significant at 0.05

Figure 5: The effects of different concentrations of sorbitol on the superoxide dismutase (SOD), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in mice. *significant at 0.05

4.0 Discussion

In the last decade, there was a growing public demand for sugar substitutes in food and drug industries due to the perceived health benefits of sugar-free products over sugar-containing products. This public demand has therefore made sorbitol to become an important industrial raw material which is now extensively used in industries such as chemical, pharmaceutical, food and light. Although sorbitol is used and consumed in large quantities worldwide, little but contradictory reports exist on its impact on the genetic materials and reproduction of the consumer. In the present study, we have orally exposed mice to different doses of sorbitol, doses below the daily allowed dose for human consumption. Our study has shown that sorbitol is genotoxic, thus, inducing significant amount of micronucleus and other nuclear abnormalities in somatic cells, and abnormal sperm morphology with concurrent decrease in sperm number in germ cells.

The result of our study showed a significant increase in the weight of sorbitol exposed mice compared to the control. This could be as a result of accumulation of visceral fat as epidemiological reports have proven association between increased visceral fat and consumption of artificial sweeteners like sorbitol (Cardoso et al., 2016).

The data from the micronucleus assay showed that sorbitol is clastogenic, inducing both chromatid and chromosome breakage; and aneugenic, inducing chromosome lagging and chromosome loss, as a result of the dysfunction of the mitotic apparatus in somatic cells (Savage 1988; Alabi et al. 2019). Also, the result showed a significant difference in the PCE:NCE ratio in exposed mice and the control, which is concentration-dependent. The ratio of PCE to NCE between test agent-exposed mice and control mice indicates a cytotoxicity index of the bone marrow (Krishna and Hayashi 2000), hence, the observed results showed that sorbitol caused an increase in the rate of aging of PCE to NCE, thereby decreasing their normal life span and increasing the risk of genotoxicity. It is also an indication of a probable suppression of bone marrow proliferation resulting in bone marrow toxicity, cell depression, aging and reduction of the cell's normal life span (Singh et al. 2013; Lozovska et al. 2015).

The study further showed that sorbitol induced significant increase in nuclear abnormalities in the treated mice. The presence of microcytic hypochromic erythrocytes indicated a deficiency of haemoglobin synthesis leading to chronic anaemia. The other nuclear abnormalities observed further confirmed induction of varieties of anaemia. While the presence of oval macrocytes is an indication of megaloblastic anaemia,²⁹ teardrop indicates myelophthisic anaemia. The presence of cabbot's ring is an indication of severe anaemia, because these are the remnants of nuclear membrane.

The data of the sperm morphology assay indicated that sorbitol is a possible germ cell mutagen especially at high dose studied, since higher doses exerted greater mutagenic effect on the mouse germ cells. The results further showed the ability of sorbitol to alter the process of spermatogenesis by the induction of several morphological abnormalities such as sperm cell with distal droplet, sperm cells with projecting filament from midpiece, banana head, double tail, kidney head, wrong tail attachment, long hook, short hook, amorphous head, hook at wrong angle, microcephaly, double head, folded sperm, swollen hook, knobbed hook, no hook, fused neck with tail and looped tail. The induced abnormalities are indication of alteration in the quality of the genetic material and can affect the overall function and motility of these sperm cells. Sperm shape abnormalities such as abnormal head defects (banana, amorphous, kidney shaped, double head) do not seem to affect the motility of spermatozoa but can significantly reduce their *in vitro* and the *in vivo* fertilizing capacity (Jeyendran et al. 1986). Also, the sperm head defects may be markers for other sperm defects that significantly impair fertility (Nikolettos et al. 1999). The induction of abnormal head may be as a result of the mutagenic effect of sorbitol on the genes for expression of acrosomes characteristics (Topham 1980). The sperm tail defects might be due to sperm aging while bended midpiece might have grown from wrong centrioles (Menkveld et al. 1990). Abnormalities with the hook are believed to be due to disorganization of the acrosomal membrane, which often leads to a change of the nuclear shape. Significant increase in the number of morphologically abnormal sperm has been associated with infertility (Wyrobek et al. 1984). Induction of abnormal sperms has been assumed to be due to an abnormal chromosome (Bruce et al. 1974), minor alteration in testicular DNA (Giri et al. 2002), point mutation (Narayana et al. 2002, Alabi and Bakare 2015) and mistakes in the spermatozoa-differentiating process during spermatogenesis (Odeigah et al. 1997, Bakare et al. 2005). The studies of Odeigah et al. (1997) and Agunbiade et al. (2012) reported that abnormalities in sperm heads might occur by physiological, cytotoxic or genetic mechanisms or alterations in testicular DNA, which in turn disrupts the process of differentiation of spermatozoa.

There was significant reduction in the mean sperm count of mice exposed to the different concentrations of sorbitol, which implies the damaging effect of sorbitol on the sperm production by altering the sperm quality during spermatogenesis and the quantity of sperm cells produced. Reduction in sperm number is also an indication of damage of the sertoli cells, since the number of sertoli cells determines the volume of sperm cells produced. Reduction in the number of viable sperm cells has been suggested to be responsible for infertility in males (Alabi et al. 2013).

Sorbitol cytogenotoxicity and mutagenicity in somatic and germ cells in this study is of public health concern. This is because the highest dose utilized in this study (90 mg/kg/day) suggests that the daily allowable intake of 50 g/per day/72kg for human consumption (FDA, 2015) might be cytogenotoxic and mutagenic, especially in children. In literature, the dose of sorbitol in sweets, chewing gum, horseradish sauce and jams is as high as 350-950, 400, 110, and 80-100 mg/mL, respectively (Food Intolerance 2017). Even though the Joint FAO/WHO Expert committee on Food Additives (JECFA) and the European Union's Scientific Committee recommended that this sweetener is safe for consumption, the results of this study showed that sorbitol is cytotoxic, genotoxic and mutagenic. The genotoxicity of sorbitol reported in this study is in accordance with the report of Findikli and Turkoglu (2014), which showed that sorbitol is genotoxic. Also, Johannes et al. (1992) reported that sorbitol induced DNA damage in Chinese hamster ovary (CHO) cells, and Cardoso et al. (2016) reported that exposure to sorbitol during lactation causes metabolic alteration and genotoxic effect in rat offspring. This therefore calls for further studies, especially in humans, to ascertain the safety of sorbitol for consumption.

The possible mechanism of induced cytogenotoxicity by sorbitol was studied via oxidative stress biomarkers. The data showed that sorbitol induced a significant increase in the liver catalase and serum ALT and AST, with concomitant decrease in the liver SOD activities of the treated mice. Increased catalase activity is an indication of increased oxidative stress with potential to cause more damages to the DNA, protein and lipids (Sarkar and Sil 2014; Ferreira et al. 2015). Decrease in SOD activities, which is supposed to be the first line of enzyme defense against free radicals by catalyzing the dismutation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Abdelhalim et al. 2015), can subsequently lead to the accumulation of these free radicals in the biological system and hence be responsible for the observed cytogenotoxicity. Indeed, the ability of sorbitol to generate reactive oxygen species have been reported by Aquilano et al. (2007). The significant increase in serum ALT and AST by sorbitol are indication of hepatocellular necrosis, which indicates the presence of hepatic injury (Friedman et al. 1996), cell membrane damage and leakage (Kaplan 1993) in exposed mice.

In conclusion, this study reports the ability of sorbitol to induced cytotoxic, genotoxic and mutagenic effects in both somatic and germ cells of mice after oral exposure for 30 days. The studies further showed that oxidative stress is a possible mechanism of action of the observed cytogenotoxicity. The report of this study calls for further study on human subjects so as to arrive at a scientific conclusion on the health effect of consumption of sorbitol by the public.

Author's contribution

Conception: AOA

Design: AOA, AAS

Execution: LRO, YMA, AAS, AOA

Interpretation: YMA, LRO, AOA

Writing the paper: LRO, YMA, AAS, AOA

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Declaration of conflicting interest

The authors report no conflict of interest.

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