Original Research Article

REPRODUCTIVE TOXICITY & BIOMARKER RESPONSE TO A DAILY DOSE OF TOOTHPASTE (CLOSE UP) IN MALE ALBINO RATS *(Rattus norvegicus)*

Abstract

This study was carried out to determine the biomarker response of male albino wistar rats (Rattus novergicus) to a daily dosage of toothpaste. Twenty four wistar rats were divided randomly into two groups and housed in wooden cages. The first group which is the test group was administered with varying doses (250ul, 270ul, 300ul) according to their body weight (0.00167mg/g body weight) per week for three weeks while on the fourth week no treatment was given. This was done to observe the rate of recuperation from effects of treatment. The second group which was the control group were given distilled water of equal measurement with the treatment given to the test rats. Several biochemical and hematologic parameters were used to evaluate the effect of toothpaste. Parameters used were; for enzyme and liver functions, alkaline aminotransferase (ALT), aspartate aminotransferase (AST), and protein, for kidney sodium (Na+), potassium (K+), chloride (Cl) and bicarbonate (HCO_3) while for hematology white blood cells (WBC), red blood cells (RBC), platelets, lymphocytes, hemoglobin and packed cell volume (PCV) and sperm count was also used. The results showed significant difference ($P< 0.05$) in the parameters when compared with the control group. These findings demonstrate that toothpaste caused detrimental effect on sperm parameters which could lead to infertility in males. There were also observed changes in liver, blood parameters and kidney which could lead to renal dysfunction when exposed to this substance for extended periods. T*he intentional or accidental ingestion of toothpaste should be avoided especially in children.*

1.0 INTRODUCTION

Toothpaste is a personal care product used by millions across the world. Despite the different brands, they all have some major active ingredients that are general to all of them and essential in making toothpaste. These ingredients are fluoride (sodium fluoride), abrasives (hydrated silica) and detergents (sodium lauryl sulphate, SLS). Other inactive ingredients present are flavor, sorbitol etc. (ADA, 2017). Sodium fluoride being a major component of toothpaste is an inorganic salt. It is a chemical compound and an odourless, colourless crystalline solid (Spellman, 2008) that came into use to prevent tooth decay in the 1940s

(Murray *et al*., 2003). It has a molecular formula of NaF. It is white to greenish in colour depending on its level of purity (Haynes, 2011; British Medical Association., 2015). It is non-combustible and corrosive to aluminium metal, it is known to be insoluble in alcohol but highly soluble in water (O'Neil, 2001). Sodium Fluoride is used not just as fluorinate in toothpaste but also in the preservation of wood, as a corrosion inhibitor, insecticide, cleaning agent, chemical reagent and in glass and metallurgy industries (Aiguesperse *et al.*, 2005). Fluoride has been studied extensively for use in the medical industry (Haguenauer *et al*., 2000). Sodium fluoride is generally safe for dental health at low concentrations but continuous ingestion of large amounts of sodium fluoride poses possible dangers to health, with short term exposures causing irritations to eyes, skin and nasal membranes (Green, 2005). Studies have shown that fluorides, especially when in solution forms (aqueous forms) are more extensively absorbed into the body and are classed as toxic by both inhalation and ingestion through oral routes (Kapp, 2005) The rate at which fluoride (as Sodium Fluoride) is absorbed is inversely related to the pH of the stomach contents (WHO, 2006). Acute exposure and toxicity can result in nausea, abdominal pain, and diarrhea. Other possible effects are muscle paralysis, extremity spasms (Whitford, 2011). Study has shown that continuous ingestion of fluoride causes deleterious effects on skeletal (Cheng *et al*., 2008), dental (Flaitz *et al.*,2000), soft tissues (brain), thyroid (Bathnagar *et al*., 2005) and testis (Wan *et al*., 2006). In a study it was observed and documented by Shashi, (2003) that fluoride exposure can induce the loss of neuronal cell bodies and damage synaptic structures in different regions of the brain (Gopalakrishna *et al*., 2002) as well as cause inhibition of enzyme activity and a decrease in expression of membrane proteins (Barbar *et al*., 2006). In the blood and liver of animals it was observed that various changes like abnormal behavioural patterns and metabolism occur after chronic administration of fluoride lesions (Ramakrishna and Saralakumari, 1991; Denbesten *et al*., 1995).

Beyond Sodium Fluoride, Sodium lauryl Sulfate (SLS) is also another major constituent of toothpaste; Sodium lauryl sulfate (SLS), also known as sodium dodecyl sulfate, is an anionic surfactant commonly used as an emulsifying cleaning agent in household cleaning products (laundry detergents, spray cleaners, and dishwasher detergents) (Cara *et al.,* 2015), it's low cost and desirable action as a foaming agent has led to its use in the formulations of toothpaste (Lippert, 2013). Like all detergents, SLS has been shown to cause skin and eye irritation and cause more skin related damage especially with prolonged exposure (Cara, *et al.,* 2015). A research carried out by Cosmetic Ingredient Review (2015) on the health and safety of the SLS chemical using rats as test subjects showed that SLS is harmful by the oral

route, while using rabbits and guinea pigs as test subjects it was found to be harmful in the dermal route. SLS was also reported to irritate the respiratory tract and cause irritation in both skin and eye of rabbits. No gross lesions or microscopic abnormalities were found in a chronic oral feeding study in rats given 0.25%, 0.5% and 1.0% of SLS in their diet for two years (Fitzhugh and Nelson, 1968) and the same result was observed in using a different test subject in a chronic oral one-year oral toxicity study using beagle pups with 0%, 0.67%, 1.0%, or 2.0% SLS. This study is aimed at evaluating the possible effects of toothpaste ingestion (accidentally or intentionally) on hepato-renal functions, hematological and sperm parameters in male albino rats and associating such effect on humans.

2.0 MATERIALS AND METHODS

2.1 *Experimental setup*

24 albino wistar rats (*Rattus norvegicus)* were used. The animals were weighed and randomly allocated into two experimental groups. Close Up toothpaste, a popular brand of toothpaste used here in Nigeria was administered to the rats in mimicking concentrations commonly used daily. 1ml of the toothpaste was dissolved in 100ml distilled water to make a solution. The estimated average daily human dosage of toothpaste used was calculated and measured and the same dosage was administered to the rats. They were calculated using the weights of the rats and the dosage administered ranged depending on the change of the weekly body weights of the rats. The oral rout was used for administration, using a 1ml syringe. The experiment was carried out for four (4) weeks. The treatment was administered to the test group for three weeks while on the fourth week no treatment was given to the test group. This was done to observe how their body adapts and tries to recuperate and handle the effects from the treatment substance. Three (3) rats from the test group were sacrificed weekly. While three (3) from the control group were sacrificed weekly. This was done to enable us collect blood and sperm samples for analysis and to allow for careful observation of the specific organs of the rats. Before each sacrifice each rat was weighed and its final body weight was recorded after overnight starvation. The animals were sacrificed by jugular puncture while under chloroform anaesthesia. Blood samples collected were taken with both EDTA and Heparin bottles for laboratory analysis while the testes were collected for sperm analysis which was done using an electron microscope.

105 2.2 *Biochemical Analysis*

106 Standard procedures were ensured during the collection of the blood, sperm and liver samples
107 prior to biochemical analysis. The plasma activity of Alkaline Phosphatase (ALP) was
108 determined using Radox kit (colorimetric method) of Rec (1972). Biuret method was used to
109 determine the level of total protein in the samples according to the method of Flack and
110 Woollen (Flack and Woollen, 1984). The plasma activity of aspartate transaminase was
111 determined using Reitman and Frankel method (Reitman and Frankel, 1957). The serum
112 electrolytes were determined using ISO 4000 Automated electrolyte analyser. SFRI, France.
113 The plasma activity of alanine transaminase was determined using Reitman and Frankel
114 method (Reitman and Frankel, 1957). The sperm motility, viability and abnormalities were
115 determined using one step eosin method and the epididymal sperm count was done with
116 Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light
117 microscope at 40× magnifications.

118 2.3 *Data Analysis*

119 Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software
120 Version 7.7 en (2017).

121

122 **3.0 RESULTS**

123 The effects of oral administration of Close Up toothpaste on the Hepato-renal parameters in
124 male albino rats are presented in Table 3.1. The result showed significant difference in the
125 levels of electrolytes and hepatocyte parameters between the Test and Control across each
126 week and between the Test and average control (four week) in each week. Results from the
127 first week revealed a higher value of sodium (Na) on test compared to the control with a
128 significant difference ($P< 0.05$) but no significant difference ($P >0.05$) among the test of
129 potassium (K), chlorine (Cl), ALT, AST and their respective control. On the second week,
130 there was no significant difference ($P >0.05$) among the test and the respective controls of
131 sodium (Na), potassium (K), bicarbonate, AST and ALT. While on the third week, the
132 analyzed result showed non-significant difference ($P >0.05$) among sodium (Na), potassium

(K), bicarbonate, AST and ALT and their respective control, except chlorine (Cl), which showed a significant difference ($P< 0.05$). Finally, on the fourth week, the result showed that there was significant difference ($P< 0.05$) among sodium (Na), potassium (K), chlorine (Cl), bicarbonate, ALT, AST and their respective control. The result also showed the various significant differences between the Test and the average control. The result on Sodium showed no significant difference between week one, week two, week three against the average control but showed significant difference ($P>0.05$) in week four. The result on Potassium (K) showed no significant difference between week one, week two against the average control at ($P>0.05$) but shows significant difference ($P< 0.05$) between the tests of week 3 and week 4. The result on chlorine (Cl) revealed there were no significant difference ($P>0.05$) between week one, week two, week three against the average control, but there were significant difference ($P< 0.05$) in the fourth week. The result on bicarbonate showed there were no significant difference ($P>0.05$) between week one, week two, week three, week four and the average control. The result on ALT, showed significant difference between week one, week two, week three, week four and the average control at ($P<0.05$). Finally, the result on AST showed significant difference between week one, week two, week three, week four and the average control at ($P<0.05$).

TABLE 3.1: RESULT SHOWING THE EFFECT OF TOOTHPASTE ON SODIUM, POTASSIUM, CHLORIDE, BICARBONATE, AST AND ALT

		Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	HCO3 (mmol/L)	AST (UI/L)	ALT (UI/L)
	Control	133.67 ± 2.50^{a}	4.05 ± 0.25^{a}	100.67 ± 4.5^{a}	23.67 ± 0.5^{a}	17.67 ± 3.50^{a}	10.67 ± 1.50^{a}
WEEK 1	Test	$143.00 \pm 4.00^{b,A}$	$3.60 \pm 0.20^{a,AB}$	$99 \pm 1.00^{a,A}$	$23 \pm 0.00^{a,AB}$	$27.33 \pm 8.50^{a,B}$	$11.67 \pm 0.50^{a,B}$
	Control	157.67 ± 22.50^{a}	7.25 ± 2.55^{a}	109.67 ± 18.50^{a}	23.67 ± 1.50^{a}	34.67 ± 3.50^{a}	10.0 ± 2.00^{a}
WEEK 2	Test	$138.67 \pm 12.50^{a,A}$	$4.38 \pm 0.05^{a,AB}$	$95 \pm 7.00^{a,A}$	$25 \pm 4.00^{a,A}$	$29.67 \pm 1.50^{a,AB}$	$6.67 \pm 0.50^{a,C}$
	Control	136.67 ± 10.50^{a}	5.0 ± 0.60^{a}	86.67 ± 4.50^{a}	24.67 ± 3.50^{a}	23.67 ± 5.50^{a}	11.0 ± 4.0^{a}
WEEK 3	Test	$129.0 \pm 1.00^{a,AB}$	$3.9 \pm 0.30^{b,AB}$	$85 \pm 1.00^{a,ab}$	$19.67 \pm 0.50^{a,B}$	$30.33 \pm 3.51^{a,AB}$	$12.67 \pm 0.5^{a,B}$
	Control	149.67 ± 0.50^{a}	5.10 ± 0.10^{a}	106 ± 1.00^{a}	23.0 ± 1.00^{a}	23.0 ± 1.00^{b}	13.06 ± 1.0^{b}
WEEK 4	Test	$111.67 \pm 3.50^{b,B}$	$2.9 \pm 0.20^{b,B}$	$76.66 \pm 4.50^{b.B}$	$20.0 \pm 1.00^{b,AB}$	$45.0 \pm 4.00^{a,A}$	$24.67 \pm 1.5^{a,A}$
AVERAGE CONTROL	Control	142.50 ± 11.83^{A}	5.43 ± 1.13^{A}	98.83 ± 9.16^{A}	23.83 ± 1.83^{AB}	25.16 ± 4.16^{B}	10.50 ± 2.5^{B}

[a-b] Different letters in the same column indicate significant difference ($P<0.05$) within each week

[A-B] Different letters in the same column indicate significance difference ($P<0.05$) across the weeks

For the haematological parameters, on the first week, there was a higher value on the test samples compared to the control in the Packed Cell Volume (PCV), haemoglobin (Hb) and red blood cell (RBC) with a significant difference ($P< 0.05$) between the test and control while for White blood cells (WBC), Platelets and Lymphocytes showed non-significant differences ($P> 0.05$) between the test and average. The second and third week both showed no significant difference ($P> 0.05$) among Packed Cell Volume(PCV), Haemoglobin(Hb) and Red blood cell (RBC), White blood cells (WBC), Platelets while Lymphocytes showed a significant difference ($P < 0.05$) between the test and control. In the fourth week there were significant difference in all hematological parameters except Red blood cells (RBC).

There were no significant difference ($P> 0.05$) in Packed cell volume (PCV) in week1, week2,week3 when compared with the average control but there was a significant difference ($P< 0.05$) in the week 4. No significant difference ($P> 0.05$) was seen in the fourth week for Haemoglobin between the test and average control but significant difference ($P< 0.05$) was noted all through the first three weeks. No significant difference was seen in both Red Blood Cells (RBC) and White blood cells (WBC) through the four weeks when the test was compared with the average control. Platelets showed significant difference ($P< 0.05$) across all four weeks when the test and average control were compared. Lymphocytes showed no significant difference all through the four weeks when the test is compared to the average control. In the result for semen analysis, results from week 1 to week 4 all had a lesser value of sperm count on the test when compared to the control, with a significant difference ($P<0.05$) between the control and the treatment although the result showed no significant difference ($P > 0.05$) between the test and the average control across the four weeks.

TABLE 3.2: RESULT OF THE EFFECT OF TOOTHPASTE ON PROTEIN, PACKED CELL VOLUME, HEMOGLOBIN, RED BLOOD CELLS, WHITE BLOOD CELLS, PLATELETS, LYMPHOCYTES

		PROTEIN (g/L)	PCV (%)	Hb (g/dl)	RBC (X^{12})	WBC (X^9)	PLATELETS (X^9)	LYMPHOCYTES (X^9)
	Control	67.70 ± 12.19^{a}	26.50 ± 1.50^{b}	9.00 ± 0.30^{b}	4.35 ± 0.15^{b}	9.00 ± 2.50^{a}	270 ± 0.00^{a}	70 ± 5.00^{a}
WEEK 1	Test	$59.01 \pm 1.57^{a,A}$	$39.50 \pm 0.50^{a,A}$	$13.13 \pm 0.15^{a,A}$	$6.23 \pm 0.25^{a,A}$	$10.73 \pm 1.25^{a,A}$	$310 \pm 40.0^{a,BC}$	$70 \pm 0.00^{a,B}$
	Control	72.31 ± 3.36^{a}	32.55 ± 2.95^{a}	9.90 ± 0.90^{a}	5.68 ± 0.89^{a}	9.85 ± 5.65^{a}	335 ± 105.0^{a}	84 ± 1.40^{a}
WEEK 2	Test	$66.01 \pm 8.84^{a,A}$	$35.15 \pm 2.05^{a,AB}$	$10.85 \pm 0.75^{a,AB}$	$6.43 \pm 0.67^{a,AB}$	$12.0 \pm 3.20^{a,A}$	$333 \pm 108.5^{a,B}$	$72 \pm 1.55^{b,B}$
	Control	69.23 ± 2.15^{a}	32.84 ± 3.95^{a}	10.36 ± 1.15^{a}	6.04 ± 0.64^{a}	7.4 ± 2.85^{a}	423 ± 108.0^{a}	78 ± 1.40^{b}
WEEK 3	Test	$63.75 \pm 2.55^{b,A}$	$26.23 \pm 3.85^{a,CD}$	$8.15 \pm 1.35^{a,CD}$	$4.38 \pm 1.01^{a,B}$	$4.36 \pm 2.50^{a,B}$	$127 \pm 62.50^{a,C}$	$86 \pm 0.65^{a,A}$
	Control	73.27 ± 2.15^{a}	39.05 ± 2.35^{a}	13.83 ± 0.45^{a}	6.90 ± 1.60^{a}	6.25 ± 0.05^{a}	416 ± 3.50^{b}	84 ± 0.70^{a}
WEEK 4	Test	$62.90 \pm 3.84^{b,A}$	$22.50 \pm 1.30^{b,D}$	$6.50 \pm 0.90^{b,D}$	$4.36 \pm 0.15^{a,B}$	$4.33 \pm 0.11^{b,B}$	$615 \pm 61.0^{a,A}$	$51 \pm 2.55^{b,C}$
AVERAGE CONTROL	Control	69.07 ± 5.9^{A}	$30.63 \pm 2.8BC$	9.76 ± 0.78^{BC}	5.31 ± 0.5^{AB}	8.76 ± 3.67^{AB}	342.83 ± 71^{B}	77.53 ± 2.6^{AB}

[a-b] Different letters in the same column indicate significant difference (P<0.05) within each week

[A-B] Different letters in the same column indicate significance difference (P<0.05) across the weeks

TABLE 3.3: RESULT OF THE EFFECT OF TOOTHPASTE ON SPERM COUNT

		SPERM COUNT ($X10^6$)
	Control	475 ± 125^{a}
WEEK 1	Test	$455 \pm 5^{b,A}$
	Control	575 ± 25^{a}
WEEK 2	Test	$225 \pm 225^{b,A}$
	Control	450 ± 150^{a}
WEEK 3	Test	$125 \pm 125^{a,A}$
	Control	650 ± 50^{a}
WEEK 4	Test	$250 \pm 250^{b,A}$
AVERAGE CONTROL	Control	500 ± 100^{A}

$^{a-b}$ Different letters in the same column indicate significant difference ($P<0.05$) within each week

$^{A-B}$ Different letters in the same column indicate significance difference ($P<0.05$) across the weeks

4.0 DISCUSSION

ALT and AST are general traditional biomarkers used widely for detecting drug induced liver injury (Yukuta *et al*., 2004). In this study, increase or decrease in the levels of these biomarkers is defined by comparing the values obtained from the test animals with the control. The liver enzyme assay showed a gradual increase in the serum levels of AST and ALT with a significant difference in AST ($P < 0.05$) while there was also a significant difference in ALT ($P < 0.05$) the increase in the level of serum AST and ALT is an indicator of increased activity of the liver possibly due the abnormal presence of sodium fluoride (NaF), Sodium lauryl sulphate (SLS) and other components of the toothpaste that are foreign to the body system. The results also showed that there was a slight significant difference ($P< 0.05$) in protein and there was a decrease in the protein levels of the test rats as compared to

the control, this might be due to possible negative effect on NaF on the Liver. This decrease although inconsistent with the work of (Green, 2005) is consistent with the work of Debensten *et al.,* (1995), Barbar *et al. (*2006) and Anamika *et al,* (2012). A more recent study done by Imtithal and Baraa, (2017) indicated that sodium fluoride caused a significant decrease in serum protein and albumin concentrations. The general low value of sodium (Na), potassium (K), bicarbonate in the test compared to the control respectively with a significant difference ($P< 0.05$) on the last week and also when comparing the weeks with the average control might be because of increased secretion of the electrolytes from the body during urine formation. The toothpaste components may cause abnormal inhibition of release of hormones (Anti-Diuretic Hormone) that regulates electrolyte balance. This is because fluoride has been shown to negatively affect the thyroid gland that plays a major role in controlling our body metabolism and internal homeostasis (Bhathnagar *et al*., 2005), and exposure to it according to Gopalakrishna *et al*., (2002) can induce the loss of neuronal cell bodies and damage synaptic structures in different regions of the brain. The low level of leukocytes (WBC) recorded on the third and fourth week when compared to the control might be linked to the inflammatory effects of Sodium Fluoride on lymphatic organ, this is in agreement with Maryam *et al*, (2017). The gradual decrease in PCV, Hb and RBC from week two to week four indicates that NaF has a negative effect on blood when introduced into the system over a long period of time although the difference wasn't significant since Maryam *et al*, (2017) also reported a significant lower blood indices in their experiment. For the sperm count, Results from week 1 to week 4 all had a lesser value of sperm count on the test when compared to the control, with a significant difference ($P<0.05$) between the control and the treatment, this significant negative effect of NaF is in agreement with the work of Wang *et al*., (2006) who reported a deleterious effect of fluoride on the testis which is the site for sperm production, and also agreed with work done by Chinoy and Sequira, (1989) and Arora *et al.,* (2010) who

observed in their experiment that there was a significant decrease in the epididymal sperm count when sodium fluoride which is a major component of toothpaste was administered to rats.

5.0 CONCLUSION

The results from the study clearly points out that a prolonged ingestion of toothpaste generally affects the function of the liver, kidney and also the semen (for males) negatively which might lead to renal dysfunction and infertility in men. Based on this efforts should be made to prevent the accidental ingestion of toothpaste especially in children.

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