Sub-acute toxicity of hand sanitizer dermal exposure on albino rat (*Rattus norvegicus*)

Inno-Anaemeje, P. N. [1]*, Dike, C. S. [1, 2], and Babatunde, B. B. [1, 2*]
[1]Department of Animal and Environmental Biology, University of Port Harcourt, Rivers State.
[2]Africa Centre of Excellence, Centre for Public Health and Toxicological Research (ACE-PUTOR) University of Port Harcourt
*Corresponding author: bolaji.babatunde@uniport.edu.ng

Abstract

The COVID-19 pandemic resulted in unprecedented awareness and use of hand sanitizer among the populace than seen before. This study used dermal sub-acute toxicity to assess the effect of triclosan-containing hand sanitizer on *Rattus norvegicus* skin. The research was conducted due to the toxicological and biochemical claims on the effect of triclosan. The study was a 14-day sub-acute dermal toxicity test following the Organization for Economic Cooperation and Development (OECD) guideline on animal testing with preferred test strategy by rubbing to emulate normal hand sanitizer use. The result revealed that triclosan-containing hand sanitizer induced no observable effect on the skin of rats, there was no induced liver dysfunctions, Oestrogen (E_2) and Luteinizing hormone levels were normal as in the control. Based on the parameters studied, the hand sanitizers tested is considered safe on skin for consumer use in hand hygiene. It is recommended that more toxicological researches should be carried out on chronic exposures to hand sanitizers, to ensure safety of the populace in compliance with the Sustainable Development Goals.

Keywords: Triclosan, dermal exposure, hormones

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Introduction

The use of sanitizers has increased over time due to better knowledge of microbiology, recent pandemic and other environmental health challenges because these chemical substances exhibit effectiveness as antibacterial agents. There are at least 10,000 organisms per cm^2 of normal skin, including pathogenic transitory flora (Carter 2000), and hands are one of the main hotspots for infection transmission. Sanitizers are commonly used to prevent pathogenic germs from spreading, they are used for disinfection, asepsis, and sterilization. They contain alcohols, glutaraldehyde, chlorhexidine, phenolics, and triclosan (Rutala and Weber 2019).

The outbreak of COVID-19 (Coronavirus Disease-2019) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has risen to a global public health challenge due to the contagious nature as it persists on infected surfaces for up to nine days (Kampf *et al* 2020). The World Health Organization and the Nigerian Centre for Disease Control issued preventive guidelines to break the transmission chain, which include routine hand washing and the use of sanitizers for hand disinfection in the absence of water (WHO 2020a).

There are health concerns about the use of sanitizers produced with active ingredients and additives from various chemical groups, which could be toxic. Some have been reported to cause adverse effect aside their antimicrobial activity. For instance, glutaraldehyde a high-level disinfectant (HLD) used in primary care centres is classified as toxic and sensitizing. It onsets asthma, skin and contact dermatitis (González *et al* 2013). Triclosan an active ingredient for disinfectant and hand sanitizer, is a synthetic, lipid-soluble, anti-microbial agent with broad spectrum activity. It was found in 93% of liquid, gel and foam soaps in the United States (FDA, 2013) and is absorbed and retained following dermal and oral route of exposure due to its lipophilic nature, making it bio-accumulate in users with traces found in urine, blood and human breast milk; producing bacterial resistance to antibiotics (Fang *et al* 2010; Weatherly and Gosse 2017). Triclosan has been classified as an endocrine disruptor in numerous species (Louis *et al* 2017; Wang and Tian 2015), as well as in humans (Louis *et al* 2017). Triclosan exposure was also linked to a drop in sperm count in fish (Raut and Angus 2010) and development of cancer (Kim *et al* 2014; Lee *et al* 2014; Wu *et al* 2015), albeit the evidence is inconclusive (Sadowski *et al* 2014).

Triclosan was banned in soap products (liquid, gel, foam and bar) by the FDA in September 2016, but it is still allowed in hand sanitizers. It was also banned from all human hygiene biocidal products by the European

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Union (EU) in January, 2017 (Juncker 2016). Triclosan-containing soaps were found to provide no additional skin-sanitizing benefits when compared to soaps that did not include triclosan (Kim *et al* 2015). Triclosan was also reported to cause bacterial resistance as a result of target site alteration, which reduced the chemical's inhibitory impact (Mcbrain *et al* 2002). In clinical settings, increased triclosan resistance is linked to an increase in resistance to a variety of other antibiotics (Chen *et al* 2009). Sanitizer brands usually contain additives for scent, which are synthetic fragrances derived from petrochemicals, these chemicals include derivatives of benzene, aldehydes, phthalates, which are capable of causing cancer, birth defects, nervous-system disorders, allergies and endocrine disruptors (Bom *et al* 2019). These additives are usually not fully listed on labels and in some cases, they are summarized as fragrance but they could constitute serious health problems.

Although some ingredients were recommended for use over others by health organizations such as alcohol-based hand sanitizers (ABHS), regular and increased use as mandated by the COVID-19 safety protocol (WHO 2020a), increases dermal exposure to the ingredients, which can cause skin irritation; fissures, contact dermatitis (Lachenmeier 2008), gastrointestinal discomfort and nausea.

Brand misinformation and misrepresentation could cause consumer-user related problems (Berardi *et al* 2020a). Although the Food and Drug Administration and the World Health Organization issued guidelines and standards for the formulation of hand sanitizers (WHO 2020b; FDA 2019), but it is possible that with increased demand some products may not adhere strictly to these guidelines. When cosmetic product standards are violated on a regular basis, it impacts negatively on the skin.

Although frequent use of hand sanitizers may expose users to skin problems, unavailability may expose users to germs (Berardi *et al* 2020a). An ideal sanitizer, among other qualities, should work quickly, have a broad spectrum, be safe for human users, be water soluble, pose no risk to the environment, and its residual concentration should have no long-term harmful effect (Rutala and Weber 2019). This study was conducted in response to the frequent use and the need for toxicological research to assess the safety of chemical substances used as ingredient in sanitizer formulation. The aim of this study was to assess the effect of triclosan-containing sanitizer on the skin and some biochemical parameters of Albino rats.

Materials and methods

Animal handling

Thirty-six female adult Albino Rats (*Rattus norvegicus*) weighing between 160-200kg, nulliparous, non-pregnant, with healthy and intact skin were purchased and from Department of Animal and Environmental Biology, University of Port Harcourt. The animals were acclimatized in the animal house to the experimental conditions for 3 weeks, under room temperature of 22±3ºC and 12hours light and dark periods. Standard laboratory hygienic conditions and practice were ensured. Animals were feed with hybrid feed and deionized water *ad libitum*. Using group-caging for welfare reasons, they were grouped into 6 with 5 animals in each.

Experimental design

The fur of the rats was removed from the dorsal area by carefully shaving with a clipper about 24 hours before the test with care taken to avoid abrading the skin. After shaving, de-ionized water was applied to clean the skin surface. All groups, including the control, were subjected to these procedures. Individual weights of animals were measured on the day of application and thereafter.

First guard sanitizer containing triclosan used for the study was obtained from a retail consumer store and the dermal toxicity test was conducted in accordance to the Organisation for Economic Co-operation and Development (OECD) guideline (OECD 2002). The sanitizer for this study was used directly as a consumer product without additional preparation. It was applied uniformly over 4cm^2 of the exposed shaved skin surface of each rat in each group, by rubbing with hands covered with clean medical gloves; to emulate normal human application on hands. Groups 1, 2, 3, 4 and 5 were rubbed 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of the hand sanitizer, respectively while Group 6 (control) received non. The treatment was applied at 24 hours interval (daily) for 14 days. The test chemical was administered to the animals in a sequential manner with 5 animals used for each step per group. Skin changes were evaluated at 24 hours-time intervals. During the exposure period, the animals were caged singly and according to the treatment dose. The dosage was determined based on skin irritation reported at 6.0 mg/animal/day and the NOAEL value in rats of 3.0 mg/animal/day (Burns 1997).

Macroscopic observation

Animals were physically observed for dermal reaction immediately after dosing for the first 30 minutes and then at 1-hour interval, daily throughout the study duration. The dermal reaction/signs looked out for were oedema, fissure, colouration and patches. All observations were recorded for each animal.

Sample collection and biochemical analysis

Animals were euthanized at the conclusion of the macroscopic observations. Blood samples were taken from the rats on day 14 using the cardiac puncture procedure after they were euthanized with diethyl-ether and serum biochemical parameters were estimated from the serum samples using clinical chemistry analyser. Estradiol (E2) test was done following the Tiets NW method with pg/ul as test unit and Luteinizing Hormone (LH) test was done following the Layman *et al* (1992) method with mlu/ml as the test unit. Aspartate transaminase and Alanine transaminase (AST/ALT) were tested using Reitnam and Frankel method with u/l as the test unit. Alkaline Phosphate (ALP) test was done following Kochmarand Moss (1976) method. Total Protein (TP), albumin (ALB) and total bilirubin (TB) and conjugated bilirubin (B) were analysed using the biuret, bromocresol green and Bianchi-Bosisio (2005) methods, respectively.

Statistical analysis

Result from this study were summarized and the mean of the different groups tested using a one- way Analysis of Variance (ANOVA). All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS v 20.0.7)

Results

Dermal Observation

In the sub-acute dermal toxicity study, no appreciable skin effect was observed in the treated and control rats throughout the experimental period.

Effects on hormones

The result of the effect of the sanitizer on serum oestrogen (E2) and luteinizing hormones are presented in Figure 1. Estradiol (E2) levels of animals treated with 0, 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer were 64.50±2.12, 71.50±10.61, 78.50±7.78, 68.00±9.90, 56.50±2.12 and 65±8.49, respectively. Variation between the different treatment groups was not significantly different ($p>0.05$). In contrast, luteinizing hormone was significantly higher ($p<0.05$) in the rats administered with 1.2ml (1.16±0.40) and 1.5ml (0.855±0.29) of sanitizer compared with control (0.65±0.30) and the 0.9ml (0.34±0.12Miu/ml) groups (Figure1a).

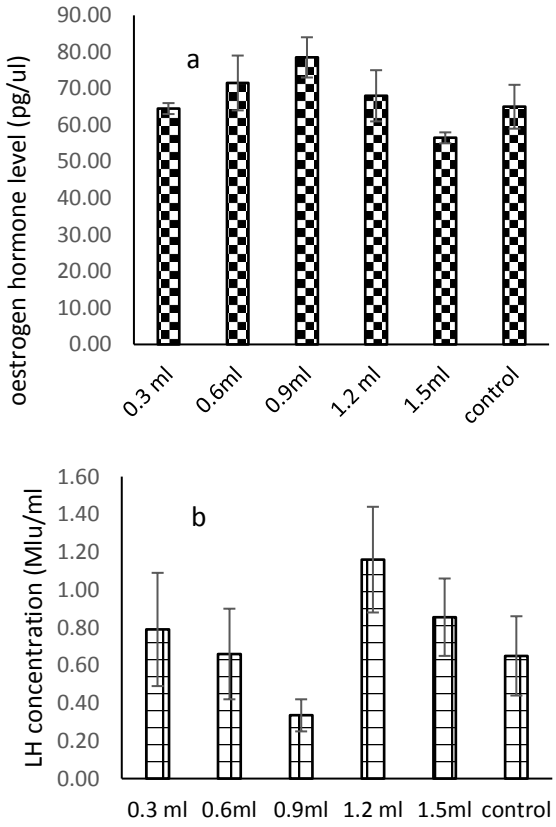

Figure 1. Effects of treatment on (a) Oestrogen (E2) and (b) Luteinizing hormones levels on female rats.

Liver function

The results showed that there was no significant difference ($p>0.05$) in the concentration of AST, ALT and ALP in all the groups when compared with the control. Mean values of AST for animals treated with 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control were 34.50±0.71, 30.00±1.41, 42.50±6.36, 40.00±8.49, 37.00±4.24 and 37.00±8.49 (Figure 2). The mean value of ALT level in animals treated with 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control were 10.45±1.34, 13.65±3.32, 12.2±2.12, 12.7±4.67, 13.05±0.64 and 13.35±2.90, respectively. Animals treated with 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control had mean ALP of 33.00±12.73, 43.0±8.49, 42.0±1.41, 38.5±4.95, 39.5±10.61 and 37.5±0.71, respectively.

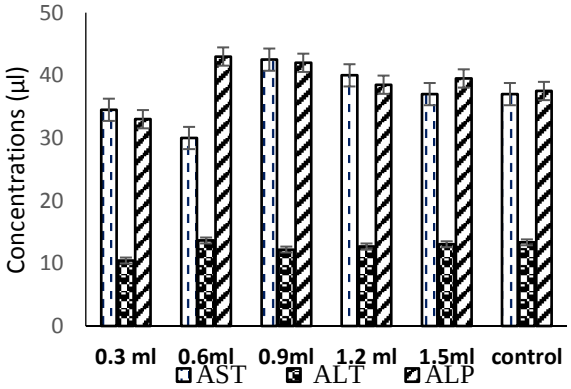

Figure 2. Effects of treatment on serum aspartate transaminase, alanine aminotransferase and alkaline phosphatase concentration (u/l) in female rats

The results also showed that there was no significant difference ($p>0.05$) in the concentration of TP and ALB between the groups when compared with the control. Animals treated with 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control had average TP levels of 56.50±0.71, 66.50±2.12, 78.00±4.24, 66.50±7.78, 81.00±1.41 and 57.00±2.83g/l, respectively (Figure 3). The mean value of ALB level of animals treated with 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control were 40.50±0.71, 44.00±1.41, 46.00±1.41, 47.00±1.41, 48.50±2.12 and 39.00±1.41.

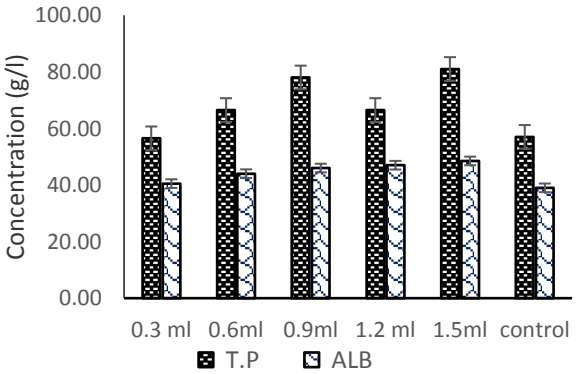

Figure 3. Effects of treatment on serum total protein and albumin concentration in female rats

The result showed that there was no significant difference ($p>0.05$) in the concentration of TB and CB between the different groups and the control. Animals treated with

0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control had average TB levels of 7.40±0.57, 6.15±0.21, 8.10±0.57, 8.05±1.77, 6.7±0.99 and 7.45±1.77, respectively (Figure 4). The average CB level of animals treated with 0.3ml, 0.6ml, 0.9ml, 1.2ml, 1.5ml of sanitizer and the control were 4.80±0.14, 4.25±0.35, 5.65±0.78, 6.35±0.21, 4.95±1.06 and 4.80±1.41, respectively.

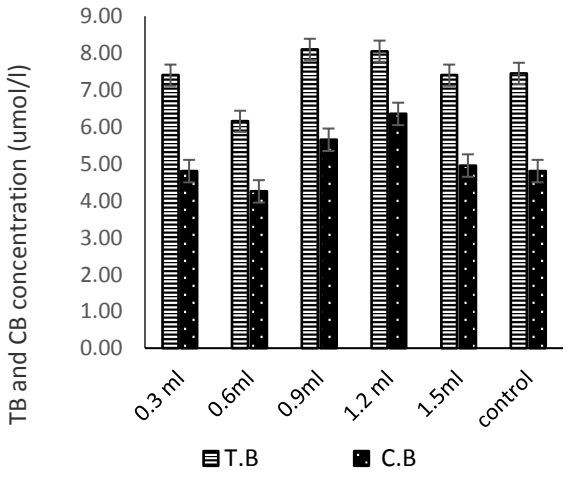

Figure 4. Effects of treatment on serum total bilirubin and conjugated bilirubin concentration in female rats

Discussion

The result of the macroscopic observation suggests that the sanitizers despite containing triclosan had no observable negative dermal effect on the rats, which suggests that the doses administered to the rats are safe. A similar study involving ten participants, in a single application of triclosan (0.3%) on a patch resulted in no discomfort (Barkvoll and Rlla 1994). In a repeated washing test with soap containing only 2% triclosan, one research showed irritation, but it was unclear whether this was due to triclosan or the soap base (Bendig 1990). In another study where triclosan was applied to the shaved back skin of Pirbright white guinea pigs at concentrations of 0.1, 0.5, 1.0, and 5.0% to a $2cm^2$ area, skin irritation was not observed in concentrations below 5% (Sachsse and Ullmann 1975). However, in studies where higher doses were applied, dermal irritation was observed in triclosan doses of 12.5, 25, 62.5, 125 and 250 mg per kg body weight per day in acetone (Burns *et al* 2001a) and propylene glycol (Burns *et al* 2001b) after day-4 exposure. Bhargava and Leonard (1996) and DeSalva *et al* (1989) also reported that triclosan caused skin irritation at doses of 15mg and 30mg.

This present study clearly showed that dermal contact with triclosan containing hand sanitizer on a daily dose of 0, 0.3mg, 0.6mg. 0.9mg, 1.5mg caused no observable skin effect. Although, the concentration of triclosan in the hand sanitizer consumer products is unknown, it is probably too low to trigger a serious skin reaction. The concentration of triclosan in the test hand sanitizer product was not indicated, but according to studies, 0.1-2% is employed in the formulation of hand hygiene products along with excipients and other antiseptic ingredients (WHO 2009).

In this study, estradiol and luteinizing hormones for all groups were not significantly affected ($p>0.05$). However, a previous study showed that triclosan decreased thyroid hormone and impacted oestrogen-mediated responses in pubertal and weanling female rats (Stocker *et al* 2010). Other studies showed that hormone levels such as progesterone, oestrogen, testosterone and thyroxine (T4) were reduced, when triclosan was administered orally (Feng *et al* 2016; Louis *et al* 2017). Thyroid hormone disruption was linked to low doses of triclosan (0.03µg/l) in a research, due to its near resemblance to certain oestrogens; triclosan inhibits oestrogen sulfotransferase in the sheep placenta, an enzyme that helps digest the hormone and transport it to the developing foetus (Owoicho *et al* 2021). It is thought that triclosan could be harmful to a pregnant rat if enough of it reaches the placenta and affects the enzyme.

Enzyme activities are often regarded as sensitive biochemical indicators for determining the level of tissue damage in exposed organisms (Hemmadi 2017). Despite the difference in the concentration of AST, ALP and ALT, the values were still within a normal range. Contrarily, Aswathy *et al* (2021) found an increase in the activities of alanine and aspartate aminotransferases, implying that triclosan exposure could damage liver tissues**.** Serum AST, ALP and ALT did not show any significant alterations to the triclosan-containing sanitizer applied on female rats when compared to the respective control group animals, indicating that the test compound did not have any effect on the AST and ALT activities.

Serum elevations of ALT activity are rare and if elevated indicates parenchymal liver disease, since ALT is a more liver-specific enzyme. Serum ALT did not show any significant alterations to the triclosan-containing sanitizer applied on female rats. Low levels of ALP are associated with hyperthyroidism, with rare condition of idiopathic hypophosphatasia associated with rickets; the levels in reduction were not much and not elevated, which entails normal liver function. After sub lethal triclosan exposure for 96 hours, Dar *et al* (2020b) found a similar increase in the activities of the alanine and aspartate aminotransferase enzymes demonstrating the effectiveness of these biomarker enzymes in assessing the stress response of fish in contaminated environments**.**

Other liver function parameters (total protein, protein albumin, albumin, total bilirubin and conjugated bilirubin) were compared with the CDC (2010) range and they were all within normal range despite the difference in the concentration. This means that, exposure of the rats to triclosan-containing sanitizer did not alter the proper function of the hepatic system. Low total protein values suggest dysfunction in liver and kidney, metabolic, nutritional disorder or dehydration, which may be attributed to experimental conditions. Yueh *et al* (2014) reported that, triclosan could increase hepatocyte proliferation, fibrogenesis and oxidative stress, which could be the driving force behind the development of advanced liver disease in mice. Serum protein, albumin, total bilirubin and conjugated bilirubin did not show any

significant alterations to the triclosan-containing sanitizer applied on female rats when compared to the respective control group animals, indicating that the test compound did not have any effect on serum biochemical parameters estimated.

Conclusion

The study revealed that, the triclosan-containing hand sanitizer with an unknown triclosan concentration was too low to trigger a serious skin reaction or alter proper hepatic function in albino rats. In addition, the serum biochemical parameters tested did not show any appreciable change in response to the treatment. Therefore, it could be concluded from the present study that despite the alteration and difference in the liver function and hormonal levels, the parameters measured were within normal range. Therefore, the test material does not pose any toxic effect on the skin, oestrogen hormone, luteinizing hormone and liver function of female Albino rat.

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