Testiculotoxicity activities of ethyl alcohol-based hand sanitizer in adult male Wistar rats

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SUMMARY

Chronic exposure to ethyl alcohol-based hand sanitizer (EABHS) is associated with some systemic toxicities such as respiratory distress, nervous injury, cardiac arrest, and liver damage, but no report on its impact on sperm test functions. This study aims to evaluate the impact of EABHS toxicity on the male reproductive functions in Wistar rats. The rats were randomly divided into four groups, with five rats each. The treatment of all groups lasted for 8 weeks (gavage administered). Group A received 5 ml/kg normal saline; Group B rats received 5 ml/kg EABHS; Group C received 10 ml/kg EABHS; Group D received 20 ml/kg EABHS for 56 days. Body weight increased significantly in all treated groups compared to the control. Testicular volume decreased significantly in groups C and D, while testes weight did not decrease significantly in groups B-D compared to group A. Significant changes were seen in the testis weight to body weight ratio in groups C and D compared to group A. Chronic exposure to EABHS results in a significant decrease in sperm count and motility and an increase in dysmorphology in groups C and D when compared to group A. Excessive use of EAHBS can exacerbate impairment of sperm parameters and testicular toxicity in male reproduction.

Key words: Hand sanitizer – Semen analysis – Oxidative stress – Testicular toxicity

INTRODUCTION

The advent of the coronavirus disease-2019 (COVID-19) global pandemic led to the increased use of ethyl alcohol-based hand sanitizer (EABHS) to limit the spread of COVID-19 in the general population (Saha et al., 2021). The efficacy of EABHS in reducing the transmission of pathogenic microorganisms is well-established; however, their correct use is critical for achieving optimal microbial killing and preventing potential hazards (Toney-Butler et al., 2023). For safe and effective use of EABHS, it is crucial to have proper knowledge of hand hygiene techniques, how to select the right product, and safe handling practices (Saha et al., 2021; Toney-Butler et al., 2023). This is necessary to avoid adverse effects like allergies, skin irritation, lung injury, fire hazards, and toxicities (Saha et al., 2021) The World Health Organization (WHO) endorsed two formulations for the local production of EABHS. These preparations

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are based on either ethanol or isopropyl alcohol as the primary antiseptic agents, supplemented with hydrogen peroxide and glycerol (WHO, 2009, Saha et al. 2021). The hydrogen peroxide is included to inactivate any bacterial spores that may be present in the solution, while the glycerol acts as a humectant to prevent skin dryness (Saha et al., 2021; Villa et al., 2021).

Hand sanitizer ingredients include alcohol and are designed to kill germs on the surface of the skin. One key ingredient in some sanitizers and other personal care products is triclosan (TCS). Research has shown that TCS can act as an endocrine disruptor, by interfering with the body's hormone system and fertility (Saha et al., 2021; Bhatt et al., 2024). In contrast, one study reported that TCS-based hand sanitizer has no detrimental effect on the skin, liver and hormonal functions (Inno-Anaemeje et al., 2022). Chronic exposure to EABHS is associated with some systemic toxicities such as respiratory distress, nervous injury, cardiac arrest, and liver damage (Saha et al., 2021). Still, no study has reported on its impact on sperm functions. This study aims to evaluate the impact of EABHS toxicity on the male reproductive functions in Wistar rats.

MATERIALS AND METHODS

Animals' procurement and acclimatization

This study was conducted in the animal house of the Faculty of Basic Medical Sciences, Department of Anatomy, Ekiti State University, Ado Ekiti. The animals were housed in hanging stainless-steel wire cages kept in an isolated room at a controlled temperature (22°C ± 2°C) and humidity (40-60%). Lighting was maintained on a 12h cycle (12 h light/12 h dark and 22°C ± 2°C). Each group of rats was allowed to have free access to water (ad libitum) and standard rat chow. The acclimatization period lasted for 2 weeks. Ethics approval was received from the Ethics and Research Committee, Department of Anatomy, College of Medicine, Ekiti State University, Ado-Ekiti (ERCANA/2025/02/001). All procedures guiding the use of animals were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NRC, 2011).

Chemical

An EABHS (CAREX) containing 70% alcohol manufactured by PZ Cussons International, 3500 Aviator Way, Wythenshawe, Manchester M22 5TG, United Kingdom.

Experimental design

This study design is experimental. Twenty adult male rats of the Wistar strain were used for this study, with a body weight of 204-235 g. The experimental animals were randomly divided into four groups of five rats each (n = 5). The treatment of all groups lasted for 8 weeks.

Group A rats were gavagely exposed to 5 ml/kg body weight of normal saline (NS) for 56 days.

Group B rats were gavagely exposed to 5 ml/kg body weight of EABHS for 56 days.

Group C rats were gavagely exposed to 10 ml/kg body weight of EABHS for 56 days.

Group D rats were gavagely exposed to 20 ml/kg body weight of EABHS for 56 days.

All the groups were euthanized on day 57 for sperm analysis and biochemical assay.

Epididymal sperm collection, processing and analysis

At the end of the treatment period, animals were euthanized under anaesthesia, and the reproductive organs were carefully dissected. The cauda epididymis was minced in pre-warmed phosphate-buffered saline (PBS) to release spermatozoa. The sperm suspension was incubated at 27°C for 10-15 minutes before analysis (Wang, 2002).

Sperm Motility

Sperm motility was assessed under a light microscope at X400 magnification. At least 200 spermatozoa were counted per sample and classified as motile or immotile. Results were expressed as a percentage of total sperm. The sperm motility percentage was calculated using the number of the live sperm divided by the total number of sperm cells (Naidu et al, 2024).

Sperm Count

Sperm counts were determined using a Neubauer hemacytometer under a phase-contrast microscope. A dilution factor was used, and results were expressed as million sperm/mL (Ogedengbe et al., 2016).

Sperm Morphology

A smear of sperm suspension was stained with eosin-nigrosin stain. Morphological abnormalities (e.g., head, midpiece, tail defects) were analyzed under oil immersion at X1000 magnification. At least 200 sperm per sample was evaluated, and abnormalities were expressed as a percentage (Jegede et al., 2017).

Biochemical parameters estimation

The oxidative stress parameters were evaluated following established protocol for lipid peroxidation measurement of malondialdehyde (MDA) by Buege and Aust (1978) and reduced glutathione (GSH) level in the testicular tissue by Griffith (1980). The concentrations were expressed as nmol mg-1 protein.

Statistical analysis

A one-way analysis of variance (Anova) was used to compare changes between the control group and the treatment groups. A $P < 0.05$ value was considered a statistical difference. Data was expressed as Mean ± SD. All statistical analyses were completed using BlueSky Statistics, version 10.3.4 (GA) for Windows (2323 W Dickens Ave, Chicago, IL 60647, USA).

RESULTS

Body weight, testicular weight, and volume

Across all experimental groups' body weight decreased between the initial and final except group A. The changes in groups B-D body weight decreased significantly when compared to group A. The testis weight did not decrease significantly in groups B-D when compared to group A. The testis volume in groups C and D decreased significantly when compared to group A. The ratio of testis weight:body weight in groups C and D decreased significantly ($p<0.05$) when compared to group A (Table 1).

Sperm analysis

Following EABHS administration, the sperm count decreased significantly in groups C and D when compared to group A, while group B did not decrease significantly when compared to group A. Following EABHS administration, the sperm motility decreased significantly in groups C and D when compared to group A. While group B did not decrease significantly when compared to group A. Following EABHS administration, the sperm dysmorphology increased significantly in groups C and D when compared to group A, while group B did not increase significantly when compared to group A (Table 2).

Oxidative stress levels

Following EABHS administration, the GSH levels decreased significantly in groups C and D when compared to group A, while group B did not decrease significantly when compared to group A. Following EABHS administration, the MDA levels increased significantly in groups C and D when compared to group A. At the same time, group B did not increase significantlywhen compared to group A (Table 3).

DISCUSSION

Hand sanitizer plays a key role in preventing the transmission of pathogenic microorganisms, but its inappropriate use may harm the human body. The ingredients in EABHS preparations may cause serious health problems if not used carefully. In addition, tolerability varies from one individual to another. Chronic exposure to EABHS has been reported to cause systemic toxicity, including respiratory distress, nervous injury, cardiac arrest, and liver damage (Saha et al. 2021). Yet no study has been done on its impact on testicular function. This study aims to evaluate the impact of EABHS toxicity on the male reproductive function in Wistar rats.

Notably, the body weight of all the experimental groups decreased significantly after exposure to EAHBS when compared to the control group. However, the weight of the testes did not decrease significantly across the experimental groups when compared to the control group. The

Table 1. Mean and standard deviation (Mean ± SD) of the body weight, testis weight and testis volume among experimental groups

GROUPS	Initial body weight (g)	Final body weight (g)	Testis weight (g)	Testis volume (mL)	Testis weight/body weight ratio
GROUP A	206.0±2.00	210±1.00	1.3±3.10	1.2±0.30	0.006
GROUP B	220.41±3.20	200±1.20*	1.20±0.60	1.17±0.20	0.006
GROUP C	220.5±2.50	190±1.50*	1.05±0.70	1.0±0.40*	0.005*
GROUP D	230.3±5.00	181±3.00*	0.59±0.50	0.5±0.30*	0.003*

Group A: Control group received 5 ml/kg NS, Group B: 5 ml/kg EABHS, Group C: 10 ml/kg EABHS, Group D: 20 ml/kg EABHS.
Mean ± SD, *p-value: <0.05 is significant compared Group A.

Table 2. Effect of ethyl alcohol-based hand sanitizer on sperm analysis in Wistar rats

Treatment Groups	Sperm count (x 106/mL)	Sperm motility (%)	Morphology Abnormal (%)
GROUP A	145.4± 3.0	95.6 ± 6.5	19.5 ± 5.8
GROUP B	120 ± 3.2	70.3± 4.5	20.7 ± 3.7
GROUP C	95.6 ± 1.9*	60.2 ± 2.4*	27.7 ± 1.4*
GROUP D	74.3 ± 1.2*	50.2 ± 1.2*	49.23 ± 2.5*

Group A: Control group received 5 ml/kg NS, Group B: 5 ml/kg EABHS, Group C: 10 ml/kg EABHS, Group D: 20 ml/kg EABHS.
Mean ± SD, *p-value: <0.05 is significant compared Group A.

Table 3. Effect of ethyl alcohol-based hand sanitizer on glutathione (GSH) malondialdehyde (MDA) levels in Wistar rats

GROUPS	GSH (nmol mg^{-1} protein)	MDA (nmol mg^{-1} protein)
GROUP A	0.9 ± 0.04	0.3 ± 0.01
GROUP B	0.89 ± 0.03	0.39 ± 0.03
GROUP C	0.4 ± 0.02*	0.4 ± 0.11*
GROUP D	0.37 ± 0.12*	0.45 ± 0.02*

Group A: Control group received 5 ml/kg NS, Group B: 5 ml/kg EABHS, Group C: 10 ml/kg EABHS, Group D: 20 ml/kg EABHS.
Mean ± SD, *p-value: <0.05 is significant compared Group A.

ratio of testis weight:body weight in groups C and D decreased significantly when compared to the control group. In a recent study, rats exposed to triclosan showed no significant gain in body and organ weights (Montagnini et al. 2021). Chronic consumption of alcohol causes disruption of metabolism and absorption of nutrients, resulting in weight loss (Kolota et al., 2019). The alcohol constituent of EABHS is the plausible cause of loss in body weight due to its ability to permeate the blood-testis barrier (Edenfield et al., 2025).

Disruption of this barrier can lead to the exposure of sperm to toxins and immune factors, which may damage sperm cells and impair spermatogenesis. In our study, the sperm count and motility decreased significantly in EABHS-exposed groups C and D, while sperm dysmorphology increased significantly in groups C and D when compared to the control. A similar trend was reported in the sperm characteristics of herb-based alcohol beverages in Sprague-Dawley rats (Biney et al., 2020). Lower serum testosterone levels were attributed to these findings. Decreased testosterone can impair sperm lineage cells in rats (Biney et al., 2020).

Previous studies indicated that alcohol ingestion increases the level of reactive oxygen species (ROS) in rats resulting in the increase of lipid peroxidation (MDA) and decrease of antioxidants such as the GSH level (Kolota et al., 2019; Gireesh et al., 2022). We reported significantly elevated MDA levels and reduced GSH levels in groups C and D when compared to the control group. An increase in ROS reduces the protection of antioxidants, leading to damage to sperm cells.

In conclusion, EABHS has a protective role against microbes by denaturing the protein cell wall, reducing hand-to-hand transmission at low doses. Caution is needed to dissuade excessive use of EABHS to minimize impairment of sperm parameters and testicular toxicity in male reproduction. Where possible, alcohol-free hand sanitizer should be recommended instead of EABHS.

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