

Carcinogenic and Noncarcinogenic Health Risk Assessment of Heavy Metals on Some Selected Traditional Herbal Drugs

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Abstract

Herbal drugs have gained significant popularity as alternative medicines among residents in the northern region of Nigeria, capitalizing on the abundant medicinal plants available in the area. This study aimed to investigate the levels of five selected heavy metals (Cd), (Hg), (Ni), (Pb), and (Zn) in five herbal drugs sold in Andaza Town, Kiyawa Local Government Area of Jigawa State. The preparation of the samples was carried out using a wet digestion method with nitric acid (HNO₃) and perchloric acid (HClO₄), and the heavy metals were quantified using an Atomic Absorption Spectrophotometer (AAS). The health risk assessment (HRA) was performed for both adults and children using metrics such as estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and target cancer risk (TCR). On a dry weight (dw) basis, the concentrations of heavy metals were as follows (mean ± SD, mg/kg dw): Cd (0.156-0.312, 0.221 ± 0.065), Hg (8.58-16.7, 12.7 ± 3.78), Ni (0.960-2.74, 1.67 ± 0.698), Zn (4.26-10.1, 6.17 ± 2.34), and Pb (0.876-1.91). Lead was not detected in two out of the five samples, and therefore, the mean ± SD for Pb was not computed. A comparison with the WHO maximum permissible limits revealed that Hg levels were consistently above the recommended limits, while two samples exceeded the Cd limit. However, the concentrations of Ni, Zn, and Pb were within the permissible limits established by WHO. The HRA results indicated that the THQ values for all metals, except Hg, were below 1 for both adults (70 kg body weight) and children (24 kg body weight), suggesting that the metals themselves do not present a significant non-carcinogenic risk. However, Hg had relatively high THQ values for both demographics, potentially posing health risks. The percentage non-carcinogenic risk (NCR) analysis showed that Hg was responsible for more than 95% of the HI, highlighting its dominant role in the overall non-carcinogenic risk. Additionally, the TCR analysis indicated that Pb exposure could lead to a moderate to high cancer risk, whereas Cd poses a high to very high cancer risk if the herbal medicines are consumed over extended periods. In conclusion, it is crucial to monitor and regulate the consumption of these herbal medicines to prevent potential health issues for consumers. Strengthening regulatory frameworks and ensuring safe practices in the cultivation and processing of herbal medicines are essential for safeguarding public health.

Keywords: Herbal drugs, Heavy metals, Target Hazard Quotient, Hazard Index, Health Risk

1. Introduction

The use of herbal drugs and alternative medicines has seen a notable increase globally, including in Nigeria (Mustapha et al., 2024). These remedies are employed in the prevention and management of various diseases such as rheumatism, hypertension, ulcers, cough, sexual impotency, headaches, and fertility issues. Unlike conventional pharmaceuticals, herbal medicines are often not regulated for purity and potency, which poses significant risks to human health due to potential contamination with heavy metals and toxins (Atinafu et al., 2015). The nature and origin of herbal drugs can make them susceptible to contamination by heavy metals, toxins, and pesticides, leading to serious health issues for consumers. Ingestion of heavy metals can result in their bioaccumulation in the body, causing health problems such as organ dysfunction, stunted growth, and immune deficiencies (Muhammad et al., 2024).

Many people believe that herbal drugs are inherently safe due to their natural origins (Sulaiman et al., 2024). However, studies have shown that some herbal preparations contain heavy metals in concentrations exceeding the limits set by regulatory agencies such as the World Health Organization (WHO) (Samali et al., 2017). Exposure to heavy metals has become a primary concern among researchers (Ma'aruf et al., 2024), health professionals (Durumin-Iya et al., 2023), and nutrition experts due to its public health implications (Otitoju et al., 2012). Heavy metals are naturally occurring components of the earth's crust but can also be distributed through various human activities. These metals are non-biodegradable and can persist in the environment for extended periods (Ara et al., 2018). Even at low concentrations, heavy metal pollution and their cumulative health effects are significant global health concerns. Contaminated soil can transfer heavy metals to plants, which then enter the food chain, posing potential risks to human and animal health (Mustapha et al., 2024). Due to their non-biodegradable nature and long biological half-lives, heavy metals can accumulate in different parts of the body and are highly toxic even at low concentrations (m et al., 2024). Herbal medicine is increasingly popular, especially among local populations in developing countries like Nigeria. However, the quality, efficacy, and safety of herbal medicines containing potentially toxic metals need critical assessment before these products can be marketed (Wang et al., 1996). Traditional herbal medicine is a vital yet often underestimated health resource with many applications, particularly in preventing and managing chronic diseases and meeting the health needs of aging populations (WHO, 2019).

The WHO has defined traditional medicine, including herbal drugs, as therapeutic practices that have been in existence for centuries before the development and spread of modern medicine (Sulaiman et al., 2024). Many patients turn to herbal therapies, citing a “sense of control” and “mental comfort from taking action,” especially when dealing with chronic or incurable diseases like diabetes, cancer, arthritis, or AIDS. In such cases, patients often feel that conventional medicine has failed them. For acute, self-limiting conditions like colds, sore throats, or bee stings, home remedies are often used due to the unavailability, inconvenience, cost, or time-consuming nature of professional care (Winslow and Kroll, 2015). In rural areas, cultural factors further encourage the use of botanicals, with beliefs that plants from an area can cure diseases prevalent

in that area (Winslow and Kroll, 2017). In India, vast sections of the rural population lack access to modern medicine, with primary health centers in rural areas often lacking staff, diagnostic facilities, and adequate drug supplies (Mudur, 2013).

Globally, there is a growing preference for higher intake of fresh vegetables and fruits over red meat for better health, as they significantly reduce the incidence of chronic diseases like diabetes, cancer, cardiovascular diseases, and other age-related diseases (Prakash et al., 2012). However, unsafe food consumption due to heavy metal contamination remains a severe problem, caused by anthropogenic activities such as industrial emissions, automobile exhaust, excessive use of metal-based fertilizers, and pesticide application (Cui et al., 2014). Some heavy metals like arsenic (As), cadmium (Cd), and lead (Pb) have no known beneficial role in human metabolism and are considered chemical carcinogens even at very low exposure levels (Naseer et al., 2024). Heavy metals present in minute quantities in the environment can enter food chains through biomagnification, increasing to toxic levels in humans and other organisms. Dietary intake of lead, copper, and chromium through food can sometimes exceed permissible limits, particularly in urban areas (Yebpella et al., 2011).

Northern Nigeria, due to its geographical location, is home to various herbs. However, there is limited data on the toxic heavy metals in herbal drugs produced and consumed in this region. This study aims to investigate the toxicity of lead (Pb), mercury (Hg), zinc (Zn), cadmium (Cd), and nickel (Ni) in five herbal drugs available in Andaza town, Kiyawa Local Government Area of Jigawa State, Nigeria. The samples were coded as HD-1, HD-2, HD-3, HD-4, and HD-5

2. Material and Method

The herbal drug samples, in powdered form, were purchased from various shops in Andaza town, Kiyawa Local Government Area of Jigawa State, Nigeria. These samples were sealed in small polythene bags and covered with sachet papers. Upon receipt, the samples were sieved separately using a 2mm sieve to obtain fine particles. For analysis, five grams of each dried herbal drug sample was accurately weighed using a balance and transferred into digestion flasks. To each flask, 8 mL of trioxonitrate (V) acid (HNO_3) and 4 mL of perchloric acid (HClO_4) were added. The flasks were placed on a hot plate set at 80°C , with the temperature gradually increased to 120°C until complete digestion was achieved. After digestion, the samples were filtered, diluted with distilled water, and made up to a final volume of 100 mL. The concentrations of heavy metals (Pb, Hg, Ni, Cd, and Zn) in the samples were determined using a PerkinElmer PinAAcle 900H Atomic Absorption Spectrophotometer (Usman et al., 2024) at the Central Laboratory, Bayero University, and Kano, Nigeria.

2 Carcinogenic and Non-carcinogenic Risk Assessment Methodology

2.1 Estimated Daily Intake (EDI) of the Heavy Metals

EDI of the heavy metals was calculated using the following equation (Uddin *et al.*, 2019)

$$EDI = \frac{C_m \times R_f}{B_w} \dots \dots \dots (1)$$

Where C_m is the concentration of heavy metals (mg kg^{-1} dry weight), R_f represents the daily intake of food in kg per person per day ($0.015\text{kg/person/day}$) and B_w is the average body weight in kg (24kg for children; 70kg for adults).

2.2 Noncancer Risk

This was investigated by calculating the Target Hazard Quotient (THQ) using the following formula (Uddin *et al.*, 2019):

$$THQ = \frac{EDI \times E_f \times D_e}{D_f \times T_{avncar}} \dots \dots \dots (2)$$

Where, THQ denotes non-cancer risks, E_f represents the exposure frequency ($365 \text{ days year}^{-1}$), and D_e represents exposure duration (65 years); D_f denotes reference dose. D_f of Pb, Hg, Ni, Cd and Zn are 0.004, 0.0003, 0.02, 0.001 and 0.30 (mg/kg/day) respectively (USEPA, 2021) and T_{avncar} represents average time for non-carcinogens ($365\text{days year}^{-1} \times D_e$) (USEPA, 2011).

2.3 Chronic hazard index (HI)

Chronic hazard index is the sum of more than one hazard quotient for multiple toxicants or multiple exposure pathways (Ekhaton *et al.*, 2017). This was determined using the equation:

$$HI = \sum THQ \dots \dots \dots (3)$$

2.4 Target Cancer Risk (TCR)

TCR was estimated by using the formula (USEPA, 2011):

$$TCR = THQ \times Sepo \dots \dots \dots (4)$$

$Sepo$ = carcinogenic potency slope. Pb and Cd reference values are 0.0085 and 6.10 mg kg⁻¹ bw day⁻¹ respectively (USEPA, 2015).

3 Result and Discussion

The sample codes for the traditional herbs are shown in Table 1. The codes were KD-1 through KD-5. Table 1 displayed the samples' composition and range of traditional usage. A couple of the samples' compositions were comparable to one another.

Table 1: Local herbal medicines: codes, composition, and traditional uses

Sample Code	Composition	Traditional use	References
HD-1	<i>Balanites aegyptiaca</i> , <i>Ziziphus mauritiana</i> , <i>Psidium guajava</i>	Malaria, yellow fever and abdominal pain	(Lee et al., 2024)
HD-2	<i>Ziziphus spina</i> , <i>Moringa oleifera</i> , <i>Ziziphus mauritiana</i>	Breast cancer, skin cancer and leukemia	(Wang et al., 2024)
HD-3	<i>Perkia biglobosa</i> , <i>Balanites aegyptiaca</i> , <i>Psidium guajava</i>	Typhoid fever, malaria and vomiting	(Pore et al., 2023)
HD-4	<i>Ziziphus spina</i> , <i>Psidium guajava</i> , <i>Balanites aegyptiaca</i>	Cold, skin rashes and body inflammation	(Koniecznyński et al., 2017)
HD-5	<i>Moringa oleifera</i> , <i>Psidium guajava</i>	Body pain, headache and body weakness	(Izzo et al., 2012)

Table 2: Mean Concentration (mg/kg dw) of Heavy Metals in Local Herbal Drugs

Sample ID	Cd	Hg	Ni	Pb	Zn
HD-1	0.180 ± 0.001	9.37 ± 0.065	0.96 ± 0.012	ND	4.26 ± 0.001
	0.179-0.181	9.304-9.434	0.949-0.972	NC	4.259-4.260
HD-2	0.264 ± 0.001	16.3 ± 0.170	1.79 ± 0.013	0.876 ± 0.003	5.14 ± 0.004
	0.263-0.265	16.350-16.490	1.778-1.803	0.872-0.898	5.1351.143
HD-3	0.156 ± 0.001	8.58 ± 0.122	1.73 ± 0.006	ND	4.90 ± 0.003

	0.155-0.157	8.456-8.708	1.724-1.736	NC	4.898-4.903
HD-4	0.312 ± 0.001	16.7 ± 0.103	2.74 ± 0.014	1.91 ± 0.015	10.1 ± 0.003
	0.311-0.313	16.589-16.808	2.726-2.754	1.896-1.924	10.095-10.101
HD-5	0.192 ± 0.002	12.4 ± 0.066	1.14 ± 0.011	1.21 ± 0.011	6.43 ± 0.002
	0.190-0.194	12.320-12.460	1.130-1.150	1.198-1.222	6.427-6.435
Mean ± SD	0.221±0.065	12.670±3.78	1.672±0.698	NC	6.166±2.34
CV%	29.4	29.8	41.8	NC	37.9
Range	0.155-0.313	9.304-16.808	0.949-2.754	0.872-1.924	4.259-10.101

ND = Not detected; NC = Not computed

The concentrations of heavy metals (mg/kg) are presented in Table 2. The mean values of cadmium (Cd) ranged from 0.156 to 0.312 mg/kg, with the highest concentration found in sample HD-4 (0.312 mg/kg), close to the WHO maximum permissible limit of 0.3 mg/kg (2007). These levels were lower than those found in vegetables from Bangladesh: spinach (0.44-0.85 mg/kg), kholrabi (0.32-0.96 mg/kg), and papaya (0.23-1.05 mg/kg) (Uddin et al., 2019). Cd was undetected in herbal preparations from Ethiopia (Meseret et al., 2020), Jordan (Alhusban et al., 2019), and Bangladesh (Zamir et al., 2015). Cd is carcinogenic at high concentrations, with potential to cause renal impairment, weak bones, and lung problems (Mahurpawar, 2015).

The lead (Pb) concentrations ranged from 0.876 mg/kg in HD-2 to 1.91 mg/kg in HD-4, while Pb was undetected in HD-1 and HD-3. These results are well below the WHO limit of 10.0 mg/kg for local herbal preparations and lower than those reported in other studies, such as vegetables in Bangladesh (1.62-13.4 mg/kg) (Uddin et al., 2019) and traditional herbal preparations in Northeast Ethiopia (3.00-4.00 mg/kg) (Meseret et al., 2020). Similarly low levels were observed in herbal drugs consumed in Saudi Arabia (Maghrabi, 2014). Pb toxicity can result in vision impairment, kidney and brain damage, and reproductive disorders, making its avoidance crucial (Dghaim et al., 2015).

The mercury (Hg) concentrations in this study ranged from 8.58 to 16.7 mg/kg, significantly exceeding the maximum limits set by regulatory agencies: European Union (0.02 mg/kg, 2006), Chinese Department of Preventive Medicine (0.20 mg/kg, 1994), and WHO/FAO (0.02 mg/kg, 2007). This indicates heavy contamination of the environment where the herbs were grown. Hg can be transformed into methyl mercury, a highly toxic and biomagnified substance, by microorganisms in aquatic environments. It can transfer from mother to fetus and infants through diet and breastfeeding, posing severe health risks, including decreased motor function, memory loss, neurological disorders, and reproductive issues (O'Rourke et al., 2024). The zinc (Zn) concentrations ranged from 4.26 to 10.1 mg/kg, lower than those reported in some powdered herbal medicines in Lagos, Nigeria (10.4-38.9 mg/kg) (Onwordi et al., 2015) and below the maximum

permissible limits set by the Chinese Department of Preventive Medicine (50.0 mg/kg, 1994) and WHO/FAO (60 mg/kg, 2007). Zn is essential for the body's metabolic processes, including cholesterol and carbohydrate metabolism (Adesina et al., 2020). However, excessive Zn intake can have negative health effects. Nickel (Ni) concentrations ranged from 0.960 to 2.74 mg/kg, within the permissible limit of 10.0 mg/kg set by WHO (1996). These levels are lower than those reported for contaminated vegetables from Varanasi, India (10.45-39.25 mg/kg) (Singh et al., 2010). While trace amounts of Ni are biochemically useful, high concentrations are toxic (Ameen et al., 2019).

The estimated daily intake (EDI) values for adults (70 kg body weight) and children (24 kg body weight) are presented in Table 3. For adults, the EDI values (mg/kg/day) were: Cd (3.34E-5 - 6.69E-5), Hg (1.84E-3 - 3.49E-3), Ni (2.06E-4 - 5.87E-4), Pb (1.88E-4 - 4.09E-4), and Zn (9.13E-4 - 2.16E-3). For children, the EDI values were: Cd (9.75E-5 - 1.95E-4), Hg (5.36E-3 - 1.04E-2), Ni (6.00E-4 - 1.71E-3), Pb (5.48E-4 - 1.19E-3), and Zn (2.66E-3 - 6.31E-3). The coefficient of variation percent (CV %) for each metal was low (<45%), indicating consistent results. The mean value for Pb was not computed due to its undetected levels in HD-1 and HD-3, indicating no contamination in these samples.

Table 3. Estimated daily intake (EDI) of the herbal drugs for adults and children

Sample	Adults (70kg body weight, bw)					Children (24kg body weight, bw)				
	Cd	Hg	Ni	Pb	Zn	Cd	Hg	Ni	Pb	Zn
HD-1	3.86 E-5	2.01 E-3	2.06 E-4	ND	9.13 E-4	1.13 E-4	5.86 E-3	6.00 E-4	NC	2.66 E-3
HD-2	5.66 E-5	3.49 E-3	3.84 E-4	1.88 E-4	1.10 E-3	1.65 E-4	1.02 E-2	1.12 E-3	5.48 E-4	3.21 E-3
HD-3	3.34 E-5	1.84 E-3	3.71 E-4	ND	1.05 E-3	9.75 E-5	5.36 E-3	1.08 E-3	NC	3.06 E-3
HD-4	6.69 E-5	3.47 E-3	5.87 E-4	4.09 E-4	2.16 E-3	1.95 E-4	1.04 E-2	1.71 E-3	1.19 E-3	6.31 E-3
HD-5	4.11 E-5	2.66 E-3	2.44 E-4	2.44 E-4	1.38 E-3	1.20 E-4	7.75 E-3	7.13 E-4	7.56 E-4	4.02 E-3
Mean	4.73 E-5	2.69 E-3	3.58 E-4	NC	1.32 E-3	1.38 E-4	7.91 E-3	1.04 E-3	NC	3.85 E-3
SD	1.39 E-5	7.80 E-4	1.49 E-4	NC	4.99 E-4	4.05 E-5	2.35 E-3	4.35 E-4	NC	1.46 E-3
CV%	29.4	29.0	41.8	NC	37.8	29.4	29.8	41.8	NC	37.9

NC = Not computed

The carcinogenic and non-carcinogenic risk assessment for the consumption of the herbal products is shown in Table 4. The target hazard quotient (THQ) (for adults and children respectively) were Cd (0.033 – 0.067 and 0.098 – 0.195); Hg (6.13 – 11.6 and 17.9 – 34.7); Ni (0.010 – 0.029 and 0.030 – 0.086); Pb (0.047 – 0.102 and 0.137 – 0.298); Zn (0.003 – 0.007 and 0.009 – 0.021). According to the New York State Department of Health (NYSDOH) (2007), if EDI/reference dose (RfD) \leq RfD, the health risk will be very low; if EDI/RfD $>$ 1-5RfD, the risk will be low; if EDI/RfD $>$ 5 -10RfD, there will be moderate risk; If EDI/RfD $>$ 10RfD, there will be high risk. In this study, the ratio of EDI/RfD was greater than RfD in each of the samples in both 70kgbw and 24kgbw categories for Pb. For Cd, it was greater in all samples (in both categories); for Zn, it was less than RfD in all samples in both 70kgbw and 24kgbw categories and for Ni, it was greater for all samples in 24kg bw and for only HD-4 in 70kgbw category. It is worthy of note that the ratio of EDI/RfD of Hg was more than thousand times greater than RfD for all samples in both categories indicating high potential health risk.

Table 4. Target hazard quotient, hazard index and target cancer risk for adults and children

Metals	Adults (70 kg bw)					Children (24 kg bw)				
	HD-1	HD-2	HD-3	HD-4	HD-5	HD-1	HD-2	HD-3	HD-4	HD-5
Cd	0.039	0.057	0.033	0.067	0.041	0.113	0.165	0.098	0.195	0.120
Hg	6.70	11.6	6.13	11.6	8.87	19.5	34.0	17.9	34.7	25.8
Ni	0.010	0.019	0.019	0.029	0.012	0.030	0.056	0.054	0.086	0.036
Pb	NC	0.047	NC	0.102	0.061	NC	0.137	NC	0.298	0.189
Zn	0.003	0.004	0.004	0.007	0.005	0.009	0.011	0.010	0.021	0.013
HI	6.75	11.7	6.19	11.8	8.99	19.7	34.4	18.1	35.3	26.8
TCR _(Pb)	NC	0.001	NC	0.001	0.001	NC	0.001	NC	0.003	0.002
TCR _(Cd)	0.238	0.348	0.201	0.409	0.250	0.689	1.01	0.598	1.19	0.732

HI = Hazard quotient; TCR = Target cancer risk; NC = Not computed

The acceptable value for THQ, which measures the probability of having non-carcinogenic health issues is \leq 1.0 (USEPA, 2011) and if the THQ value of any metal exceeds its permissible limit, it might cause non-carcinogenic health problems for humans (Ambedkar and Maniyan, 2011). The results in this study showed that THQ of 4 out of 5 heavy metals (representing 80%) were less than the tolerable limit of 1.0. However, extremely high levels of THQ for Hg implies that it could pose non-carcinogenic health risk to the consumers.

Hazard index, (HI) is known to be the combined influence of all heavy metals under consideration. HI value less than 1.0 implies that the combined effects of heavy metals could not cause long term non-carcinogenic health problems for the consumers and values above might have adverse health challenges. HI levels in this study were well above 1.0 in both adults and children categories which is an indication of long term non-carcinogenic health risk.

The levels of TCR in this study for 70kg and 24kg body weights were Pb: 0.001 and 0.001-0.003 respectively; Cd: 0.201-0.409 and 0.598-1.19 respectively. It has been reported that when $TCR \leq 10^{-6}$, there is low carcinogenic risk; when $TCR = 10^{-5} - 10^{-3}$, the carcinogenic risk is moderate; when $TCR = 10^{-3} - 10^{-1}$, it implies high risk and when $TCR \geq 10^{-1}$, the risk is very high (NYSDOH, 2007). In this report, the TCR of Pb will pose moderate to high cancer risk whereas, TCR of Cd will pose very high cancer risk when the herbal drugs are consumed over a long period of time.

Table 5. Percentages of non-carcinogenic risk of heavy metals in the herbal drug samples

Metals	Adults (70kg bw)					Children (24kg bw)				
	HD-1	HD-2	HD-3	HD-4	HD-5	HD-1	HD-2	HD-3	HD-4	HD-5
Cd	0.578	0.487	0.533	0.568	0.456	0.592	0.480	0.541	0.552	0.458
Hg	99.3	99.1	99.0	98.3	98.7	99.7	98.8	98.9	98.3	98.5
Ni	0.148	0.162	0.307	0.246	0.133	0.152	0.163	0.298	0.244	0.137
Pb	NC	0.402	NC	0.864	0.679	NC	0.398	NC	0.844	0.721
Zn	0.044	0.034	0.065	0.059	0.056	0.046	0.032	0.055	0.059	0.050

NC = Not computed

The percentage contribution of each metal to non-carcinogenic risk were computed and shown in Table 5. The percentages were in the range (%): Cd (0.456-0.578); Hg (98.3-99.3); Ni (0.133-0.307); Pb (0.402-0.864); Zn (0.034-0.065) for 70kg bw category and Cd (0.458-0.592); Hg (98.3-99.0); Ni (0.137-0.298); Pb (0.398-0.844); Zn (0.032-0.059) for 24kg bw category. It is evident from the results that Hg is the major contributor to the overall non-carcinogenic risk values with more than 95% contribution in each sample for both adults and children categories, showing high levels of contamination of the traditional herbal samples with Hg.

Conclusion

This study investigated the toxicity levels of five samples of herbal medicines. The results showed that many of the metals were within the World Health Organization's maximum permissible levels. However, mercury (Hg) was found in the samples at concentrations far above the recommended maximum limits set by regulatory agencies. This contamination could originate from the soil where the medicinal plants were cultivated, the processing methods, or the surrounding environment. The Target Hazard Quotient (THQ) results for both adults and children indicated that only Hg would pose a non-carcinogenic health risk to consumers, as the levels for other metals were below the maximum limit of 1.0. However, the high Hazard Index (HI) values in virtually all the samples suggested that the combined effects of these heavy metals could pose cumulative health challenges when consumed over a long period. The study also highlighted the potential cancer risks associated with these herbal medicines. Moderate to high cancer risks could develop from lead (Pb) exposure, whereas very high cancer risks could arise from cadmium (Cd) exposure

if the samples are consumed over a long period. The high levels of Hg present a serious health concern, likely indicative of pollution.

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Conflict of Interest

The authors declare that there are no conflicts of interest, including financial interests that could influence the results or interpretation of this research paper.

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