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Cerebral Histopathology in Acute Toxicity Test of Curcuma Zedoria

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Abstract: This research aims to determine the effect of the acute toxicity test of white turmeric extract (Curcuma zedoaria) on the brain organ. This research is experimental research with a Post Test Only Control Group Design. This research was conducted at the Pharmacy and Histology Laboratory. Faculty of Medicine. University of North Sumatra. The research was carried out from January to March 2023. The population in this research involved male white rats (Rattus norvegicus). The results of the acute toxicity test of white turmeric extract for 14 days showed no signs of toxicity or death in rats given the smallest to largest doses, resulting in an apparent LD50 value of white turmeric extract is >2 g/kgBW, and is classified in the practically non-toxic category. Rats that received white turmeric extract showed movement or activity, appearing more energetic by the end of the second week with increasing doses. Histopathological examination of the brain in the acute toxicity test revealed minimal changes, such as edema and congestion in line with increasing doses.

Keywords: Cerebral Hostopathology; Curcuma Zedoria; Toxicity Test.

Introduction

The environment is no less important for humans in order to fulfill people daily needs (Li et al., 2017; World Health Organization, 2019). Humans can get food, shelter, medicine and body care only from the environment (Altun et al., 2021; de Sousa Lima et al., 2020; Dua et al., 2009; Jahromi et al., 2021; Liu et al., 2022). Many people believe that herbal medicine can be utilized as a way to cure disease (Arimbawa et al.. 2020). Generally. traditional medicine is considered safer than using modern medicine as it has relatively fewer side effects than modern medicine (Dewi et al., 2022; Plotnikoff et al., 2022). However, in order to minimize side effects, which depend on the accuracy of the medication, dosage, time of use, method of administration, whether it is not misused, and the selection of medicine for a particular disease, it needs to be precise about the use of the traditional medicines (Sumayyah et al., 2017).

Currently, medicinal plants are a source of new active compounds that have pharmacological and therapeutic effects, both directly and through various extraction processes. one of which includes the curcuma plant of zedoaria or commonly called white turmeric (Subositi et al., 2019). White turmeric (Curcuma zedoaria) is the herbal plant commonly used as herbal medicine in Asia. It is able to increase the body's immunity as an anti-pain and anti-inflammatory medicine (Nicholas et al., 2022). Turmeric also contains the main ingredient, namely curcumin (Carmona et al., 2022). This herbal plant has many benefits, such as anti-fungal, anti-viral, anti-carcinogenic, anti-mutagenic, anti-bacterial, and as a neuroprotector. This neuroprotector has the function to protect, restore, and regenerate nerve cells. As a neuroprotector, curcumin works as an anti-aggregation of Aβ, a β secretase inhibitor. and is able to inhibit protein and cholinesterase activity (Gemiralda et al., 2019).

White turmeric contains a main substance called curcumin (Wardhani et al., 2022). According to research by Rika Mutiara Gemiralda and Marlaokta Marlaokta. curcumin functions as a neuroprotector is able to prevent the formation and reduce the toxicity of Aβ 42

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oligomers. In addition, curcumin has the potential as an anti-aggregation of Aβ due to its ability to inhibit the formation of Aβ fibrils originating from Aβ 40 and Aβ 42 and interfere with the stabilization of Aβ fibrils that have not been formed. In this regard, it is formed and can reduce Aβ production induced by reactive oxygen species to prevent changes in Aβ structure, which are toxic to the brain (Sarris, 2018; Shanmugam et al., 2020; Vadivel et al., 2017). Curcumin also prevents the aggregation of amyloid–β. which can penetrate the brain barrier to reach brain cells and protects neurons from various cognitive decline and improves synaptic function in mouse models (Gemiralda et al., 2019).

One of the preclinical tests in the form of stages in the development of traditional medicines is the toxicity test (Wardhani et al., 2022). This test is a test that aims to determine the effects of certain compounds and observe the activity of these compounds. This is conducted to determine whether a substance derived from natural or synthetic ingredients is safe or not. Furthermore. this test also aims to determine the ability of a compound to affect specific organs that are susceptible to the substance (Nicholas et al., 2022). This requires further testing on test animals to see whether there are toxic effects or not. It aims to provide information regarding the LD 50 and use of the correct dose and to recognize certain signs if toxicity occurs. or to ensure safety (Sulastra et al., 2020).

It is also found in the research by Rika Mutiara Gemiralda and Marlaokta's research that only around 30% of subjects experienced mild toxicity, such as headaches, diarrhea, and rashes. which serious side effects were considered invisible and not related to dose itself (Gemiralda et al., 2019). According to the description of problem identification above, this research aims to determine the effect of the acute toxicity test of white turmeric extract (Curcuma zedoaria) on the brain organ.

Method

This research is experimental research with a Post Test Only Control Group Design (Cresswell, 2017; Dhee, 2020). This research was conducted at the Pharmacy and Histology Laboratory. Faculty of Medicine. University of North Sumatra. The research was carried out from January to March 2023. The population in this research involved male white rats (Rattus norvegicus). The sample in this research is the entire population used as the research sample (total population). Sample determination was carried out using simple random sampling. Equipment used included: glassware, 1 ml 26 G injection syringe (Terumo®, Japan), 1 ml 30 G injection syringe (Terumo®, Japan), scapel and bladder, tweezers. oral syringe, measuring flask, surgical scissors, wax board, volume pipette and fixation tool, sale pot, cup and mortar, beaker glass, Erlenmeyer tube, micro pipette (Socorex®, Switzerland), stirrer, analytical balance (Chyo® Jupiter C3, 100 MD). electric gram scale (PJ. Precisia® Junior, Switzerland), binocular microscope (Olympus@, Japan), camera (Olympus®, Japan), gloves, and documentation tools. This research used test material derived from the white turmeric rhizome plant obtained from UPT Materia Medica Batu, East Java. Making Ethanol Extract of White Turmeric Rhizome (Curcuma zedoary).

White turmeric rhizomes were washed thoroughly with running water, drained, dried in indirect sunlight, and covered with a dark cloth. After drying the white turmeric, it continued with blending process, made powder, and sifted it until getting Curcuma zedoaria powder. A total of 500 grams of powder was extracted by maceration using 1.5 liters of 96% ethanol solvent. Stirring process was done twice, in the morning and evening, and filtering was carried out after 3 x 24 hours. The waste was macerated again with 1.5 liters of 96% ethanol solvent. Maceration was carried out three times. The filtrate obtained was collected, precipitated, filtered, and then evaporated under reduced pressure using a rotary evaporator until a thick extract was obtained. The dosage of ethanol extract of white turmeric rhizomes is 250 mg/kgBW, 500 mg/kgBW, 750 mg/kgBW, and 2000 mg/kgBW.

Test animals of male Wistar rats were kept in the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, University of North Sumatra, and coded 1 to 30. The rats in this study were placed individually in a clean room at a temperature of 22°-25° C. with a relative humidity of 30 -70%, and lighting was 12 hours light and 12 hours dark. Mice were fed at night and kept in ventilated cages made of stainless steel with a base area of 148.4 cm2 and a height of 17.8 cm. Each cage was labeled on the top door. The label contained the test animal code, arrival date, initial body weight, copper induction date, treatment group, and termination plan. Adaptation of mice to a new place took at least five days. Mice were weighed every three days until their body weight met the research requirements. The feed given was pelleted chicken feed with the pellet 511 brand and drinking water in distilled water, which was provided ad libitum.

A sample of 30 male rats was grouped randomly into six groups, namely Group 1, Normal control group; Group 2, Negative control group, with CMC Na; Group 3, Treatment group 1, with a dose of 250 mg/kg BW; Group 4; Group treatment 2, with a dose of 500 mg/kg BW; Group 5: Treatment group 3, with a dose of 750

mg/kg BW; and Group 6: Treatment group 4, with a dose of 2000 mg/kg BW.

Observations included clinical symptoms and the number of animals died. Physical observation of toxic symptoms was carried out mainly in the first three hours after administration of white turmeric extract (Curcuma zedoari) and continued every day for 15 days.

Results and Discussion

From the results of research on the toxicity of white turmeric ethanol extract, it was found that there are different histopathological images in the brain organs given varying doses in each treatment group.

Acute Toxicity Test Results

Table 1. Data on the Number of Deaths Based on the Test Animals after the Acute Toxicity Test of White Turmeric Extract

Treatment	Group	Number of Rats	Number of Deaths
Normal Control (K1)	Aquadest	5	0
Negative Control (K2)	CMC	**5**	0
Dose I (K3)	250 mg/ kgBW	5	0
Dose II (K4)	500 mg/ kgBW	**5**	0
Dose III (K5)	750 mg/ kgBW	5	0
Dose IV (K6)	2000 mg/ kgBW	**5**	0

The Results of Brain Histopathological Observations of White Rats (Rattus norvegicus) Based on Congestion and Edema Lesions in All Treatment Groups are Presented in the Following Table (Yustisia et al., 2020).

Table 2. Observations of White Rats (Rattus norvegicus) Based on Congestion and Edema Lesions

Treatment	Group	Edema Score				Congestion Score			
		0	1	2	3	0	1	2	3
Normal Control (K1)	Aquadest	-	4	1	-	5	-	-	-
Negative Control (K2)	CMC	-	3	2	-	5	-	-	-
Dose I (K3)	250 mg/ kgBW	2	3	-	-	1	4	-	-
Dose II (K4)	500 mg/ kgBW	4	1	-	-	5	-	-	-
Dose III (K5)	750 mg/ kgBW	2	3	-	-	4	1	-	-
Dose IV (K6)	2000 mg/ kgBW	-	3	2	-	3	2	-	-

Information: Score 0: no changes occur; score 1: focal (mild) changes occur; Score 2: multifocal (moderate) changes; Score 3: diffuse (severe) changes.

Results of initial body weight measurement and body weight in rats after 14 days of white turmeric extract administration and organ weight table.

Table 3. Comparison of Average Initial and Final Body Weights of Rats after 14 Days

Group	Initial Body Weight (g)	Final Body Weight (g)	P Value
Group 1	154.60 ± 7.266	165.80 ± 12.617	0.134
Group 2	164.25 ± 21.639	181.00 ± 20.445	0.004
Group 3	196.00 ± 18.762	186.00 ± 21.743	0.238
Group 4	173.00 ± 22.858	181.20 ± 20.873	0.044
Group 5	164.80 ± 20.657	171.00 ± 17.479	0.419
Group 6	188.40 ± 21.408	191.60 ± 27.098	0.493

Based on the observations of average body weight, there is a significant difference in initial and final body weight between groups 2 and 4 (p-value < 0.05). Meanwhile in groups 1, 3, 5, and 6, there is no significant difference between initial and final body weight (p-value > 0.05).

Table 4. Statistical Table of the Toxicity Test of White Turmeric Extract

		Brain Edema						
		None		Mild		Moderate		p value
		n	%	n	%	n	%	
Group Test	Group 1	-	0.0	4	80.0	1	20.0	0.467
	Group 2	-	0.0	3	60.0	2	40.0	
	Group 3	2	40.0	3	60.0	-	0.0	
	Group 4	4	80.0	1	20.0	-	0.0	
	Group 5	2	40.0	3	60.0	-	0.0	
	Group 6	-	0.0	3	60.0	2	40.0	
	Total	8	26.7	17	56.7	5	16.7	

From the results above, it does not meet the requirements of the Chi-square test. Eighteen cells (100.0%) were found and the minimum expected count is 83. Therefore, an alternative test was carried out by combining cells. The following is a table that combines cells in the form of none and mild level (focal) with moderate level (multifocal) (Sopiyudin. 2014).

Table 5. Combines Cells

		Brain Edema				P-Value
		None		Mild + Moderate		
		n	%	n	%	
Group Test	Group 1	-	0.0	5	22.7	0.342
	Group 2	-	0.0	5	22.7	
	Group 3	2	25.0	3	13.6	
	Group 4	4	50.0	1	4.5	
	Group 5	2	25.0	3	13.6	
	Group 6	-	0.0	5	22.7	
	Total	8	100.0	22	100.0	

The Chi-square test still does not meet the requirements even though cell merging has been carried

out. Thus, the analysis test continues with the alternative Man-Whitney test by combining cells. Through the Mann-Whitney test, a p-value of 0.342 was obtained. Because the p-value is >0.05, there is no significant difference between the six doses of white turmeric extract.

Table 6. Toxicity Test Statistics of White Turmeric Extract Against Brain Congestion, Brain Histopathology of White Rats (Rattus Morvegicus) (Sopiyudin, 2014)

		Brain Congestion				P-Value
		None		Mild		
		n	%	n	%	
Group Test	Group 1	4	80.0	1	20.0	0.243
	Group 2	0	0.0	5	100.0	
	Group 3	1	20.0	4	80.0	
	Group 4	5	100.0	0	0.0	
	Group 5	4	80.0	1	20.0	
	Group 6	3	60.0	2	40.0	
	Total	17	56.7	13	43.3	

From the results above, it does not meet the requirements of the Chi-square test. Where twelve cells (100.0%) were found and the minimum expected count is 2.17. Thus, the analysis test was continued with the alternative Man Whitney test. Through the Man-Whitney test, a p value of 0.243 was obtained. Because the p-value is >0.05, there is no significant difference between the six doses of white turmeric extract.

Results of Observing Toxicity Signs

In observations carried out every day for 14 days, it was found that the skin, fur, and eyes of the rats appeared normal. They did not experience lethargy, convulsions, tremors, diarrhea, and died in the first week. Likewise, in the second week, the rats did not appear to experience toxic symptoms. The movement or activity of the rats at the end of the second week increased so that they looked more enthusiastic.

Figure 1. Rats activity in the second week of treatment group at dose of 2000 mg/kgBW

Results of Histological Observations of Rats' Brain

The brain histology of five white rats (R. norvegicus) in the control treatment is normal (K1) and the sample results are only found to be in a state of focal or mild edema with a score of 1.

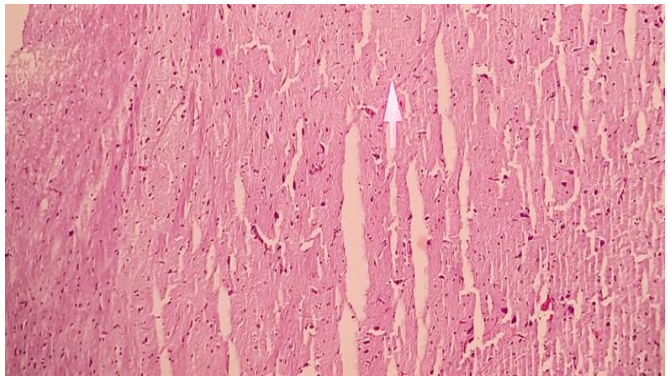

Figure 2. Brain histology of putth rats (Rattus norvegicus) treated with Normal Control (K1), (HE, 400 times). There is visible focal edema around the pyramidal cells of the brain as indicated by the arrow

It can be seen that the negative control treatment (K2) is obtained and the sample results are found to be in a state of focal or mild edema and a state of focal congestion with each having a score of 1.

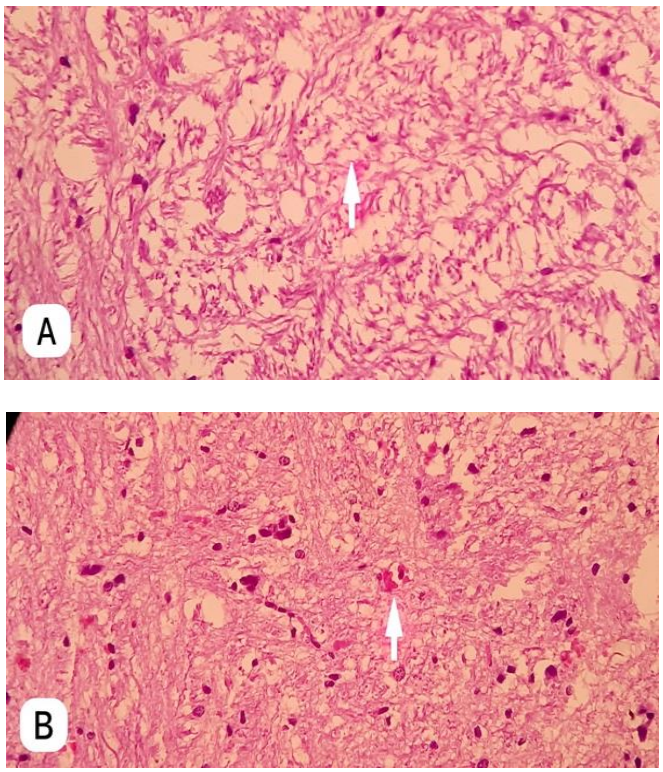

Figure 3. Histopathological image of the large brain of putth mice (R. norvegicus) treated with Negative Control (K2), (HE, 400 times). There is a focal edema lesion (A) and focal congestion (B).

In the treatment dose of 250mg/kgBW (K3) and the sample results were found to be in a state of focal or mild edema with a score of 1.

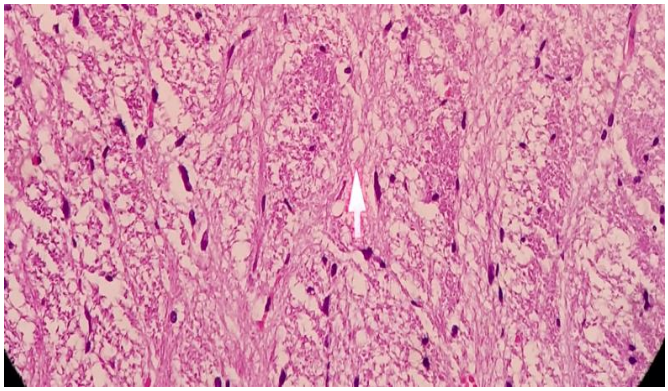

Figure 4. Histopathological image of the large brain of a white rat (R. norvegicus) (K3), (HE, 400 times). A focal edematous lesion is visible (A).

With a treatment dose of 500mg/kgBW (K4), changes in five rat brain tissues are observed and the sample results are found to be in normal condition with a score of 0.

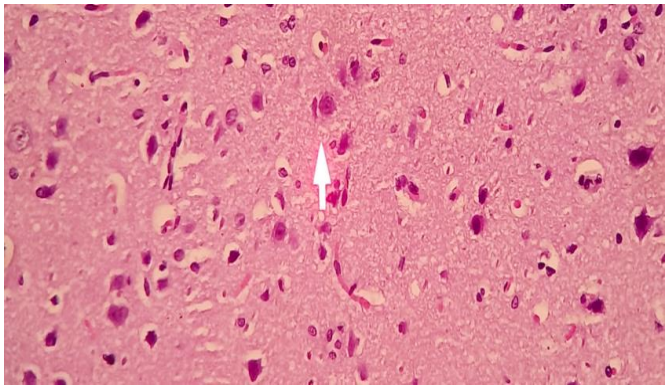

Figure 5. Brain histology of putth rats (Rattus norvegicus) treated with a dose of 500mg/kgBW (K4), (HE, 400 times) and looks normal.

There is also a treatment dose of 750mg/kgBW (K5), the histological changes in the large brain of white rats (R. norvegicus) are observed and the results of the samples are found to be normal with a score of 0.

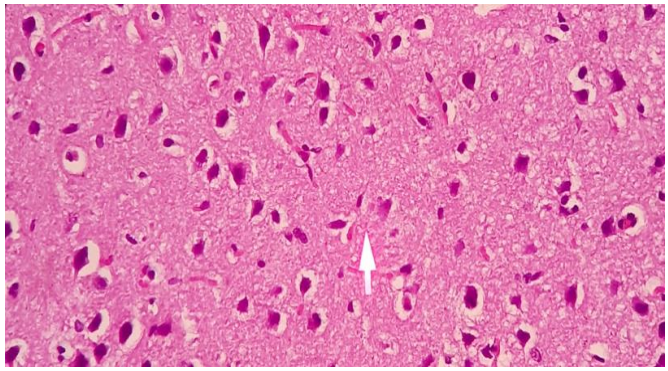

Figure 6. Brain histology of white rats (Rattus norvegicus) treated with a dose of 7500mg/ kgBW (K5), (HE, 400 times) and looks normal

The description of edema and congestion at the treatment dose of 2000mg/kgBW (K6) is observed for histological changes in the large brain of white rats (R. norvegicus) and the results obtained from 5 samples are found to have focal or mild edema with a score of 1.

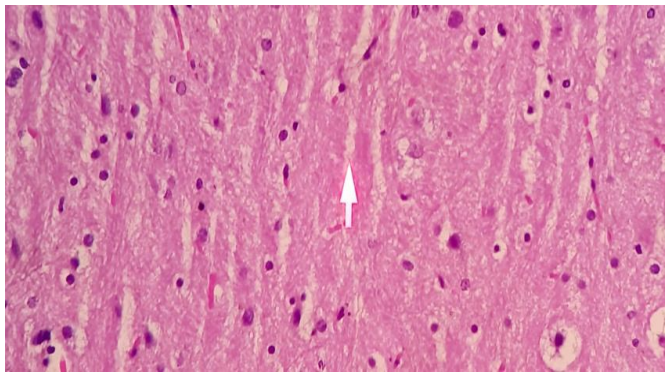

Figure 7. Brain histology of white rats (Rattus norvegicus) treated with a dose of 2000mg/kgBW (K5), (HE, 400 times). There is visible focal edema (B).

Discussion

Acute toxicity test is a test to detect symptoms of toxicity that can be produced from the results of toxicity tests using animals test and can be used as an indication of relative toxicity if exposure to humans occurs (BPOM RI. 2014). In order to state whether there is acute toxicity or not, the LD 50 value is commonly used. LD 50 is the dose that statistically can kill 50% of experimental animals. LD 50 is determined by providing the medicine in varying or graded doses to a group of experimental animals and each animal was provided a single dose (Sulastra et al., 2020).

The accute toxicity test if white tumeric extract was conducted to assess the safety of the herbal substance when consumed repeatedly at specific intervals, with a focus on identifying any toxicity changes, such as brain edema and congestion. Pathophysiologically, brain edema can be localized depending on the characteristics and extent of the injury, either in focal areas, such as

abscesses or neoplasms or it can also occur in a generalized manner, for instance, in cases of encephalitis and hypertensive crises or obstruction in cerebral venous outflow as described in the positive control group treatment (K1), negative control (K2), dose of 250 mg/kgBW (K3), and dose of 2000 mg/kgBW (K6). Macroscopically, in generalized edema, the brain may experience swelling accompanied by narrowed sulci and bulging gyri. This indicates signs of increased intracranial pressure pressing on the rigid cranium (Johnson, 2003).

In contrast to congestion, it is a passive process due to disruption of venous blood return from a tissue. This process can occur systemically or locally with the occurrence of separate venous obstruction. The tissue in question will become cyanotic. particularly when congestion worsens to the point where deoxygenated hemoglobin accumulates in the affected tissue (Johnson, 2003), where it can be clearly seen as depicted in the negative control group (K2).

In brain histopathology, the groups with unexpected changes often occur when experimental animals are used that are not specifically pathogen free (SPF) (Sudira et al., 2019). In addition, excision of a piece of tissue from the brain, either in the operating room or laboratory, can cause the cells in the tissue to die. This indicates a shift in ion and water content between the extracellular and intracellular spaces, which is a characteristic feature of cytotoxic edema. However, the tissue does not swell or become heavier, and will not show ionic edema, vasogenic edema, or hemorrhagic conversion because there is no new source of water, ions, and blood (Husna et al., 2017).

Congestion and edema generally occur simultaneously because congestion in the capillaries is closely related to the appearance of edema. For example, another cause of infarction that occurs is compression of blood vessels due to edema or traumatic rupture of blood vessels and in cases of venous thrombosis, infarction can occur although more often it only induces obstruction and venous congestion. Both of these toxic effects can cause infarction (Johnson, 2003).

Conclusion

The results of the acute toxicity test of white turmeric extract for 14 days showed no signs of toxicity or death in rats given the smallest to largest doses, resulting in an apparent LD50 value of white turmeric extract is >2 g/kgBW, and is classified in the practically non-toxic category. Rats that received white turmeric extract showed movement or activity, appearing more energetic by the end of the second week with increasing doses. Histopathological examination of the brain in the acute toxicity test revealed minimal changes, such as edema and congestion in line with increasing doses.

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Author Contributions
Conceptualization: F.M.W., data curation: A.L., F.M.W., E.T., A.T.L, funding acquisition: A.L., F.M.W., E.T., A.T.L., methodology: F.M.W, visualization: A.T.L., writing – original draft: A.L., F.M.W., E.T., A.T.L., writing – review & editing: A.L., F.M.W., E.T., A.T.L.

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Conflicts of Interest
No Conflicts of interest.

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