ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(1): 203 - 207

SJMLS - 9(1) - 023

Acute Toxicity Studies on Sodium Chloride Administered Intraperitoneally in Wistar Rats

Wasagu, I.Z.[1*] Onyenekwe, C.C.[2] and Bunza, F.U.[1]
Department of Chemical Pathology, School of Medial Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria [1], Department of Medial Laboratory Science, Nnamdi Azikiwe University, Akwa, Nigeria [2]
Author for Correspondence: ibrahimzubairu7@gmail.com/ +234-706-456-5488. DOI: 10.4314/sokjmls.v9i1.23

Abstract
Salt, also known as sodium chloride (NaCl), is about 40% sodium and 60% chloride. It flavors and preserves food. It is also used as a binder and stabilizer. The human body requires a minute amount of sodium to maintain the proper balance of water and minerals, conduct nerve impulses, contract and relax muscles. It is estimated that humans need about 500 mg of sodium daily for these functions. This acute toxicity study was conducted to determine the LD100 and LD50 of sodium chloride administered intraperitoneally. A total of 34 albino rats weighing between 150-160 g were used for the study. Ten (10) rats were used for the determination of LD100 of sodium chloride administered intraperitoneally with the following doses as 0.00 g/kg, 1.68 g/kg, 3.33 g/kg, 5.0 g/kg, 6.67 g/kg, 8.33 g/kg, 10.0 g/kg, 13.33 g/kg, 15.0 g/kg and 16.67 g/kg respectively. In the acute toxicity study, 24 rats were divided into six (6) groups of 4 rats each. They were treated intraperitoneally with the following doses 0.00 g/kg, 1.68 g/kg, 3.33 g/kg, 5.0 g/kg, 6.67 g/kg and 8.33 g/kg respectively. We observed that the minimum dose that caused death (LD100) in the rats was 8.33 g/kg for intraperitoneally- treated rats. The LD50 for intraperitoneally treated rats was determined using Karber arithmetic method after LD100 determination. Signs and symptoms of sodium chloride toxicity such as sedation, respiratory distress, pigmentation, coma before death were observed. It was shown that death occurred at group 4, 5 and 6. The LD50 obtained was found to be 6.04 g/kg. Based on the LD50 obtained, sodium chloride could be classified as practically non-toxic substance according to Matsumura toxicity rating of chemical when administered intraperitoneally.

Keywords: Acute, LD50, LD100, Pilot, Toxicity.

Introduction

Salt, also known as sodium chloride (NaCl), is about 40% sodium and 60% chloride. It flavors and preserves food. It is also used as a binder and stabilizer. The human body requires a minute amount of sodium to maintain the proper balance of water and minerals, conduct nerve impulses, contract and relax muscles. It is estimated that human need about 500 mg of sodium daily for these functions (He and MacGregor, 2019; Stallings *et al*., 2019).

NaCl is the oldest spice in human history and has a multifunctional role in the modern-day food industry and biotechnology (Soto-Escageda *et al*., 2016). There has been a considerable increase in the salt content of foods due to changes in human dietary habits vis-à-vis high consumption of industrialized, processed, and fast foods (Ni Mhurchu *et al*., 2011; Korosec and Pravst, 2014; Brouillard *et al*., 2020). However, industrial suitability, gustatory delights, salt addiction, and consumer's acceptability are a few of the factors still influencing the continued demand, interest, and consumption of high salt diet worldwide (Anderson *et al*., 2010; Kloss *et al*., 2015). Sodium is involved in several trans-membrane and physiological processes and is dominantly supplied via dietary salt (Grau *et al*., 2014; Ayed *et al*., 2021). Unfortunately, uncontrolled and excessive consumption of salt

has been linked to the development of cardiovascular disorders, endothelial dysfunction, and derangement in lipid metabolism (Wang *et al*., 2015; Han *et al*., 2018 & He *et al*., 2020). Increased activities of reactive oxidative species (ROS), infiltration of immune cells, and glomerular hyper-filtration have been postulated as the likely mechanisms of high salt-induced renal damage and hypertension (Shimosawa, 2013; Mattson, 2014; Fehrenbach *et al*., 2019; He *et al*., 2020).

In toxicity study, the establishment of LD100 and LD50 in order to rate the substance of interest is of paramount importance. Moreover, most of the studies on toxicity of sodium chloride did not take that into consideration. Therefore, the aim of this study is to determine the LD100 and LD50 of sodium chloride for intraperitoneal route of administration through acute toxicity studies. The toxicity study provides the minimum lethal dose that killed 50% (LD50) of the study animals (Matsumura, 1975).

Material and Methods

Materials

Materials used in this research study include 2 ml and 5 ml hypodermic syringes, gavage tube, sodium chloride which was purchased in solute form from our local shops in Sokoto, Northwestern Nigeria.

Experimental Animals

A total of 34 Wistar rats weighing between 150-160 g were used in this study. They were allowed to acclimatize for two weeks before the commencement of the experiment. They were fed pelletized growers' feed (vital feed, Jos Nigeria) and allowed access to water ad liblitum throughout the experiment period. The experimental protocol was approved by the Ethical committee of the Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of Sodium Chloride (NaCl) for Pilot and Acute Toxicity Studies

For intraperitoneal administration, 10 g of the sodium chloride was dissolved in 40 ml of sterile water. This indicates that 1 ml of the solution contains 0.25 g of sodium chloride.

Dosages and Administration of Sodium Chloride for Pilot Studies

A total of ten (10) rats were used for the determination of LD100 of sodium chloride administered intraperitoneally. The doses were as follows; group 1 received 0.0 g/kg, group 2 received 1.67 g/kg, group 3 received 3.33 g /kg, group 4 received 5 g/kg, group 5 received 6.67 g/kg, group 6 received 8.33 g/kg, group 7 received 10.0 g/kg, group 8 received 13.33 g/kg, group 9 received 15.0 g/kg and group 10 received 16.67 g/kg respectively.

Dosages and Administration of Sodium Chloride for Acute Toxicity Studies

A total of twenty-four (24) rats for acute toxicity studies in intraperitoneally- treated rats were divided into six (6) groups of four (4) rats each. The doses were as follows; group 1 received 0.0 g/kg, group 2 received 1.67 g/kg, group 3 received 3.33 g/kg, group 4 received 5 g/kg, group 5 received 6.67 g/kg and group 6 received 8.33 g/kg respectively.

Determination of LD50 of Sodium Chloride

The LD50 of sodium chloride administered intraperitoneally was obtained using the arithmetic method of Karber after determining the LD100 from the pilot study in intraperitoneally treated rats. The arithmetic method of Karber for calculating LD50 was as follows.

LD50 = LD100 - (Sum of dose diff. x mean dead/No of rats)

Results

After the administration of the sodium chloride in the pilot study, the treated rats were monitored within 24 hrs for signs and symptoms of sodium chloride toxicity such as sedation, respiratory distress, pigmentation, coma before death occurred.

From the pilot study carried out, the minimum dose that caused death (LD100) in the animals was 8.33 g/kg for intraperitoneally treated rats (Table 1).

Table 1: Determination of minimum Dose that Cause 100% Death (LD100) of Sodium Chloride Intraperitoneally Treated Rats

Groups	No of Rats	Volume (ml)	Dose (g/kg)	Dead
1	1	0.0	0.00	NO
2	1	1.0	1.67	NO
3	1	2.0	3.33	NO
4	1	3.0	5.00	NO
5	1	4.0	6.67	NO
*6	1	*5.0	*8.33	YES
7	1	6.0	10.0	YES
8	1	8.0	13.33	YES
9	1	9.0	15.0	YES
10	1	10.0	16.67	YES

*Minimum Dose that cause 100% Death (LD100)

The LD50 for intraperitoneally treated rats were determined using Karber arithmetic method after LD100 determination. Signs and symptoms of sodium chloride toxicity such as sedation, respiratory distress, pigmentation, coma before death were observed. It was shown that death occurred at group 4, 5 and 6.

By applying the arithmetic method of Karber, the LD100 is 8.33 g/kg, the sum of dose difference x mean of dead = 9.16, number of rats per group = 4. Therefore, LD50 = 8.33 - (9.16/4) = 8.33 - 2.29 = 6.04 g/kg (Table 2).

Table 2: Determination of Median Lethal Dose (LD50) for Sodium Chloride Intraperitoneally Treated Rats

Groups	Dose (g/kg)	No of Rats	Dose Diff.	No of Dead	Mean of Dead	Dose Diff. x Mean of Dead
1	0.00	4	0.00	0	-	-
2	1.67	4	1.67	0	-	-
3	3.33	4	1.66	0	-	-
4	5.00	4	1.67	1	0.5	0.84
5	6.67	4	1.67	2	1.5	2.51
6	8.33	4	1.66	4	3.5	5.81
						Total = 9.16

Discussion

From the pilot study carried out, the result obtained was 8.33 g/kg as LD100 for intraperitoneal routes. According to the result, the LD100 indicates the least dose that caused 100% death of the experimental rats as shown in table 1. In the other hand, LD50 of sodium chloride (NaCl) administered intraperitoneally was 6.04 g/kg by the use of Karber's arithmetic method (Table 2).

Therefore, according to (Matsumura, 1975; Elekima *et al*., 2017) LD50 rating of chemicals toxicity, sodium chloride given intraperitoneally could be rated as practically non-toxic substance. The results obtained agree with the findings of Gaunt *et al*. (1967), which had 3.8g/kg as LD50. The results of the acute study revealed signs and symptoms of sodium chloride toxicity. The severity of the signs and symptoms were dosage dependent. In other words, the more the dosages

were increased, the more severity of the signs and symptoms of toxicity. This research agreed with the finding of (Gaunt *et al*., 1967; Matsumura, 1975; Ai-mashhedy, 2016; Elekima *et al*., 2017).

Conclusion

The LD100 of sodium chloride from the results obtained was 8.33 g/kg for intraperitoneal route while the LD50 of sodium chloride (NaCl) administered intraperitoneally was 6.04 g/kg. Therefore, the LD50 of sodium chloride given intraperitoneally could be rated as practically non-toxic substance. It is recommended that other substances should be conducted for pilot and acute toxicity studies in order to ascertain their toxic effect before starting supplementation with it.

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Citation: Wasagu, I.Z., Onyenekwe, C.C., Bunza, F.U. Acute Toxicity Studies on Sodium Chloride Administered Intraperitoneally in Wistar Rats***. Sokoto Journal of Medical Laboratory Science; 9(1): 203 - 207.*** DOI: 10.4314/sokjmls.v9i1.23